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Formation Stable Heavy Hydrocarbon/ Water Emulsion by Bioemulsifiers Produced by Bacillus Licheniformis

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INTRODUCTION

With the combination of an increase in world energy demand and the decline of conventional oils, heavy crude oils have been presented as a relevant hydrocarbon resource for use in the future [1]. The decrease in worldwide conventional oil reserves and the increase in global fuel demand have driven continuous innovation in the petroleum industry and have spurred the development of new production and transportation technologies for heavy oils. In addition, forecasts report that heavy oils will be the world's primary fossil energy resource in the near future. This predicted reliance on heavy oils is accompanied by a predicted increase in the market value of these oils [2]. Although the heavy oil represented at least half of the recoverable oil resources of the world, demand for heavy and extra heavy oil has been marginal because of its high viscosity and composition complexity which make it difficult and expensive to produce, transport and refine [3]. Different methods have been used to resolve this problem such as dilution with alcohols and light crudes, heating, upgrading, core annulus flow, and forming oil/ water (O/W) emulsions stabilized by surfactants [1,2]. Another favorable pipeline technique is the transport of viscous crudes as concentrated oil-in-water (O/W) emulsions [4]. The flow of viscous oil in the form of oil-in-water emulsions is an attractive route for the hydraulic transport of heavy oils because emulsification can reduce viscosity to values of 50-200 cP. Furthermore, emulsion technology can improve residual oil removal from mature fields which are not as efficiently recovered by traditional methods that apply heat or diluents [5].

Emulsion consists of two phases: the internal or

discontinuous phase of finely divided droplets and External or continuous phase. The external or continuous phase is the matrix which keeps droplets in suspension. The interphase consists of an emulsifier or stabilizer, which keeps the emulsion stable, binding the internal and external phase together and preventing droplets from approaching each other and coalescing [6].The most important part of an emulsion is the emulsifier which is usually expensive enough to affect the economy of emulsification process [7]. Moreover, emulsifiers are a subclass of surfactants which stabilize dispersions. Bioemulsifiers are amphipathic molecules secreted by microorganisms to facilitate uptake of insoluble substrates [8]. Bioemulsifiers have been recently attracting the industrial community as natural and promising candidates for the replacement of synthetic commercial surfactants due to their intrinsic properties such as lower toxicity, higher biodegradability, higher foaming capacity, and higher activity at extreme temperatures, pH levels and salinity [9]. Moreover, emulsions can be produced in high pressure systems, membrane systems, ultrasonic systems, rotor-stator systems, and disc systems. A further differentiation into continuous and discontinuous processes can be undertaken. In the different processes, different break-up mechanisms are responsible for the droplet disruption [10]. The rotor–stator assembly consists of a rotor housed concentrically inside the stator with two or more blades and a stator with either vertical or slanted slots. As the rotor rotates, it generates a lower pressure to draw the liquid in and out of the assembly, resulting in circulation and emulsification [11]. In this paper, heavy oil in water emulsion which has been produced by using raw bioemulsifier and reducing viscosity and stability has been considered by us.

MATERIALS AND METHODS

MATERIALS

The crude oil sample used in this study was Mazut (2520 cP). Moreover, strain Bacillus Licheniformis (ACO4) as seen in Fig. 1 was provided by the Research Institute of Petroleum Industry (RIPI). These strains were the halothermotolerant gram-positive spore-forming rodshaped bacterium, isolated from petroleum reservoirs in Iran, and identified by phenotypic characterization and 16S rRNA analysis. Medium composed of K_2HPO_4, NaCl, $NaNO_3$, glucose, urea, $FeSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$ all chemicals used in this study were purchased from Merck (Darmstadt, Germany). The Stabiram emulsifier was prepared from CECA Company (France).

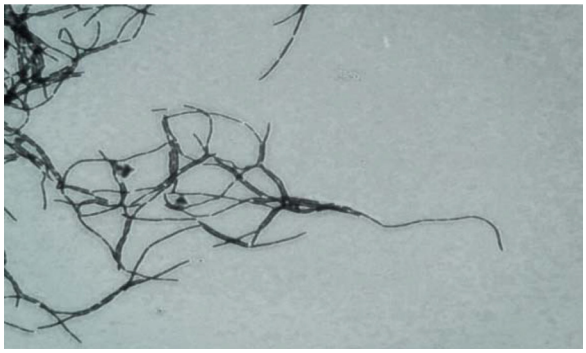

Figure 1: Microscopic Image of the Bacterial Strain Bacillus licheniformis.

METHODS

MEDIA AND CULTURING CONDITIONS

The culture was prepared in 1L flasks containing 500 mL of a medium composed of 1.8 g L^{-1} K_2HPO_4,1.2gL^{-1} KH_2PO_4, 0.1 g L–1NaCl, 4gL–1$NaNO_3$, 1.5 g L^{-1} glucose, 0.05 g L^{-1} urea, 0.01 g L^{-1} $FeSO_4 \cdot 7H_2O$, and 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$. K_2HPO_4, KH_2PO_4, NaCl, $NaNO_3$, glucose, and urea were autoclaved at 121 °C for 15 min (solution A).

$FeSO_4 \cdot 7H_2O$ and $MgSO_4 \cdot 7H_2O$ were prepared separately in sterile water (solution B) and mixed with solution A. For preparation of the solid medium, agar with an initial concentration of 20 $g.L^{-1}$ was added to the liquid medium [3]. The culture was incubated at 30 °C for 72 h with shaking at 180 rpm [3].

BIOEMULSIFIER PREPARATION

The raw bioemulsifier (Fig. 2) was extracted from bacterial culture by the method of Farahbakhsh et al. The cells were disrupted at 121 °C for 15 min, and centrifuged at 10000 rpm and 4 °C for 20 min. Then a crude bioemulsifier preparation was obtained to remove the cells. Three volumes of cold ethanol were added to the supernatant and kept at 4 °C overnight. Also, crude bioemulsifier precipitate was collected after centrifugation at 13,000 g for 20 min and was washed with distilled water.

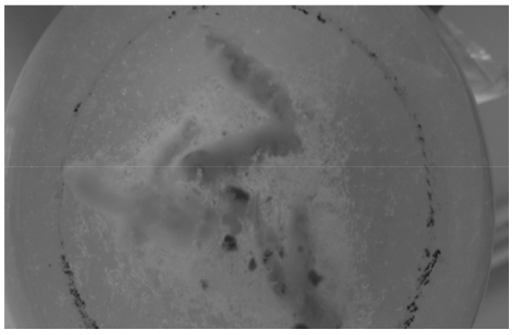

Figure 2: Raw bioemulsifier.

FORMATION OF HEAVY OIL-IN-WATER EMULSION

In the emulsification process, 500 gr of heavy hydrocarbon emulsions in water which was produced with mixing of the heavy oil sample were heated to 65 °C with continuous stirring by 60%, 65%. In addition, 70% ratio of it in aqueous solution were prepared by dispersing emulsifier mixture (bioemulsifier+Stabiram=1.32%) in distilled water at 50 °C [3] and were mixed with a rotor-stator system. Then the heavy oil was added to aqueous phase and mixed with colloid mills stirring at 5000 rpm for 5 seconds. To ensure maximum stability of the emulsion during transportation, the bond between water and oil was reinforced by exposing the mixture to a cold shock of ice bath to reach 37 °C. The viscosity of emulsions was measured by a Brook Field DV-II+Pro. Also, a decrease in emulsion viscosity was an indicator of the formation of the oil-in-water emulsion.

STABILITY OF HEAVY OIL EMULSION IN WATER

100 cc of the emulsion is closed in a graduated cylinder, and then closed for 24 hours at room temperature. Afterwards, the height of the separated water under the cylinder is measured. Then the stability of the emulsion is calculated by Eq. 1:

$$Stability = \frac{Separated\,Water}{Total\ Height} \times 100 \qquad (1)$$

TESTING DESIGN

In this paper, the most suitable model was selected based on the number of selected factors, the tested levels and the large number of experiments to optimizing and simpliffing analysis of the results while maintaining the relative quality of the Taguchi model analysis and Qualitek-4 software was used.

To calculate the effect of the error, all the experiments were performed with three repetitions, and the results were recorded. Results are analyzed by Signal-to-Noise (S/N) and ANOVA analysis methods. To evaluate the distribution of the results, the standard deviation function of the formulas 2 and 3 is used (as seen

in Eqs. 2 and 3):

$$S/N = 10\log(msd) \quad (2)$$

$$MSD = [(y_1)^2 + (y_2)^2 + ...(y_n)^2]/n = [Avg.(y_i)^2] = Y^2_{exp} \quad (3)$$

S/N is error rate, n is number of tests, y_i is results of each experiment, and Y_{exp} is response of the software in optimal conditions. Due to the fact that the purpose of emulsion formation is to reduce viscosity, the result of this project is the type of calculation and analysis "Smaller better". Moreover, the effective factors in the formation of O/W emulsion and their levels are summarized in Table 1.

Table 1: Effective factors in the formation of O/W .emulsion and their levels

Effective factor	level		
temperature	25	35	45
Raito of O/W(%)	60	65	70
Raito of BE/E (%)	70	80	90

RESULTS AND DISCUSSION

THE PRODUCTION OF BIO-EMULSION AND THE FORMATION OF HEAVY HYDROCARBON IN WATER EMULSIONS

The shape of the microscopic (1) bacterial strain and the shape (2) indicate the growth of the strain and the production of gross emulsifier. The use of chemical emulsion reduces viscosity significantly, but the use of emulsifying agents is essential for increasing the stability of emulsion. Microscopic image of heavy hydrocarbon is shown in Fig. 3a; in addition, the heavy hydrocarbon in water emulsion (Fig. 3b) was produced according to the design test, and the results of the viscosity of the experiments are presented in Table 2. All images are magnified 100 times.

The results are presented in Table 2 which have been analyzed by the Taguchi model based on ANOVA ANALYSIS.

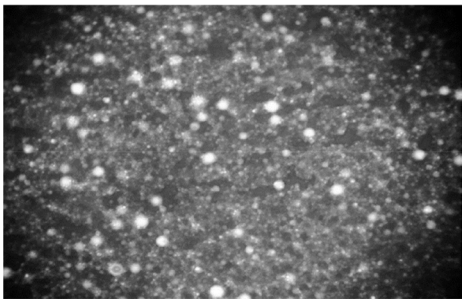

Figure 3a: Microscopic Image of Heavy Hydrocarbon.

Figure 3 b: Microscopic Image of Heavy Hydrocarbon in Water Emulsion.

Table 2: Viscosity results of experiments from Taguchi design.

Test No	Factor and level			Viscosity (cP)B.E (%)O (%)		
	T (°C)	O (%)	T (%)			
1	1	1	1	102	105	108
2	1	2	2	190	210	211
3	1	3	3	397	380	318
4	2	1	2	80	85	88
5	2	2	3	120	123.5	128
6	2	3	1	625	618	619
7	3	1	3	50	58	62
8	3	2	1	115	112	109
9	3	3	2	625	618	619
Heavy oil	1	-	-	2520	2520	2520
water	1	-	-	0.89	0.9	0.89

Moreover, the results of the experiments are presented in Table 3.

As shown in the ANOVA Table, the percentage of heavy hydrocarbons has the greatest effect on viscosity reduction. Afterwards, temperature and then the percentage of bioemulsifier have the greatest effect.

STABILITY

The results of the emulsion stability for 4 days are shown in Table 4. In addition, the stability of the emulsions was measured every 24 hours and calculated using Eq. 1.

The results show the high stability of emulsion which has been produced by Bacillus licheniformis strains.

Table 3: Analysis of ANOVA variance.

Factor		DOF	Sum of Squares	Variance	F-ratio	Pure Sum	[%] Percent
A	Temperature [°C]	2	17.876	8.893	3.085	12.021	2.947
B	Oil [wt.%]	2	368.252	184.126	63.877	362.487	88.883
C	B/E [wt.%]	2	16.017	8.008	2.778	10.252	2.513
Other/Error		2	5.764	2.882	-	-	5.657
Total		8	407.821	-	-	-	%100

Table 4: Results of the stability of heavy hydrocarbon in water emulsion.

tests	Emulsion stability			
	Frist day	Second day	Third day	Forth day
1	89.65	76.73	50.9	45
2	100	94.06	94.06	94.06
3	100	100	100	100
4	100	97.03	93.02	93.02
5	100	100	100	100
6	100	94.82	93.52	87.07
7	100	100	100	100
8	61.22	48.29	25.77	93.02
9	95.54	95.54	94.06	93.02

CONCLUSIONS

In this paper, Taguchi experimental design has been used to investigate the effect of temperature, hydrocarbon concentration, chemical emulsifier concentration and chemical properties. The viscosity results from Taguchi are presented in Table 2. The ANOVA variance for analysis of Taguchi results is associated with the participation rate of each of the factors (Table 3). Temperature, heavy hydrocarbon concentration and bio-emulsifying concentration are three effective factors in reducing the viscosity of the emulsion, but analysis of variance in Table 3 showed that heavy hydrocarbon concentration is 88.88%, as the concentration of hydrocarbons is higher than water. This result is not far from the mind, and then the temperature is 2.94%, and then the emulsifier has the highest effect (2.51%). The reduction of viscosity is the main indicator of optimal conditions in this study. The optimal conditions for the Taguchi design are given in Table 5.

The optimum conditions have been predicted by the Taguchi method correspond to the emulsion produced in the seventh experiment (Table 4). The results show that in this case the heavy hydrocarbon viscosity decreases from 2520cP to 62 cP. The stability results of this emulsion are shown in Table 4 that this emulsion is completely stable over a period of 4 days. Moreover, the results of this study show that the emulsifying composition of this bacterial strain and chemical emulsifier Stabiram 4582 has a high stability in addition to good results in reducing viscosity. Due to the high ability of this bacterial strain in the production of stable emulsions, the high purity of this emulsifier and the identification of its structure, the stability of this emulsion has been evaluated in pilot pipelines and used for salt water as a continuous phase of the effect of different salts and acidity levels of water.

The optimum conditions have been predicted by the Taguchi method correspond to the emulsion produced in the seventh experiment (Table 4).

Table 5: Optimal conditions for Taguchi test design.

column/factor	Level Description	Level	Contribution
(A) T	45	3	1.944
(B) O	60	1	6.874
(C) B	90	3	1.886
Total Contribution From All Factors……			10.613
Current Grand Average of Performance…..			-44.802
Expected Result At Optimum Condition…..			-34.188

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