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ASSESSMENT OF OIL DEGRADING ABILITY IN DRILLING MUD BY BIOSURFACTANTS OF STRAIN *BREVIBACTERIAN CELERE*

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Abstract. Environmental pollution from waste sources of oil and gas extraction industry is facing many challenges. Particularly, the drilling mud from the mining process at sea is very difficult to handle because it is generated in conducting exploratory drilling and mine development, consisting of a mixture of soil and rock contaminated with oil, chemicals, and drilling fluid. The treatment technology using biosurfactant is receiving much attention due to biosurfactant is a bipolar compound that allows the dissolution of insoluble substances in water, creating an emulsion that helps microorganisms better contact the oil and easily decomposes the contaminated oil. Therefore, this study was carried out to assessment of oil degrading ability in drilling mud by biosurfactants produced from microbial strain *Brevibacterian celere* M150. The results showed that strain *B. celere* could generate biosurfactants under conditions: pH = 7; [NaCl] = 1%; temperature 30°C and the carbon source is diesel oil. The amount of diesel and saraline added to the culture medium degraded by 82.6% and 74.5%, respectively; Surface tension also decreased from 36.5 mN/m to 29.7 mN/m for diesel and from 29.2 mN/m to 27.6% mN/m for saraline oil. This indicates that the biosurfactants produced by this strain are a potential source of materials for environmental pollution treatment in the Petroleum industry in particular and in sea environmental pollution in general.

Key word: *Biosurfactants, Brevibacterium celere, Drilling mud, Microbial strain, Petroleum industry.*

1. INTRODUCTION

Petroleum and its products are source of nessessary energy for many countries around the world in general and Vietnam in particular. The oilfield industry plays an important role in our country's economic development. Besides the great benefits, the activities in the Oil and Gas industry also cause serious ecological pollution to both water, land, and air environments. The oil and gas drilling process releases a large amount of drilling mud, it will cause serious pollution if it is not treated and discharged directly into environment [1]. Drilling mud is generated during exploratory drilling and oilfield development, and consists of a mixture of soil, rock contaminated with oil, chemicals, and drilling fluid [2]. The pollution status is even greater if drilling fluids contained oil. According to Vietnamese regular, this waste source must be brought ashore for treatment. However, is very difficult and expensive to treat them so contractors often neglect it. There are many physicochemical methods proposed to solve the pollution status derived from drilling mud discharge, but they are not very effective and are expensive. Recently, the biological method is being widely applied due to its outstanding advantages, in which the use of biosurfactant of microorganisms to decompose oil in petrolium drilling mud is being interesting of researchers.

Biosurfactants are natural hydrocarbon emulsions produced by some of bacteria fungi and yeast strains. They are extracellular polymers, with a bipolar structure consisting of two parts: hydrophobic moiety and hydrophilic moiety, forming micelles concentrated inside the surface of the liquid layer between different polars such as water and oil. Therefore, it has the ability to reduce surface tension between molecules, create hydrogen bridges and interactions between hydrophobic and hydrophilic substances, helping microorganisms have better contact with oil and more easily decomposition of contaminated oil [2, 3]. It also has antibiotic properties such as gramicidin S or polymicin and reducing the surface tension [4]. Biosurfactants have very different characteristics in both chemical structure and molecular size, from very simple structure like fatty acids to complex like polymer compounds. On the other hand, biosurfactant is easily biodegradable, non-toxic and can be produced from cheap substrates such as industrial waste, thereby completely solving the problem of pollution caused by these waste sources. Therefore, this study was conducted to evaluate the oil decomposition ability of biosurfactant produced by the microbial strain B. celere.

2. METHODOLOGY

2.1. Setup the experiments

The bacterial strain *Brevebacterium cerere* (species has been identified) received from the Petroleum Microbiology Department - Institute of Biotechnology was used for further experiments in this study.

The experimental medium is Gost 1% mineral medium with the following ingredients ($g.L^{-1}$): Na_2HPO_4 = 0.7; KH_2PO_4 = 0.3; KNO_3 = 3; $MgSO_4$ = 0.4 and tap water 1 L. Shake the solution at pH = 7.5; v = 200 rpm and continuously monitored for 5 days.

2.2. The analysis methods

2.2.1. Evaluate the biosurfactant producing capacity by Pruthi method

The capacity for producing biosurfactant was evaluated by the emulsification index E24 [5]. The emulsification index E24 characterizes the emulsification ability of metabolic products produced by microorganisms in xylene solvent after 24 h at 4°C. Take 1 ml of culture solution, centrifuge to remove cells, add 1 ml of xylene into the test tube and voltex for 1 min at 2000 rpm. Samples were kept at 4°C for 24 h and then measured the height of emulsion column. The emulsion stability was determined and calculated after 24 h according to formula as follow [4]:

E24 = (Emulsion layer (height)/Solution (height)) x 100% (Height: mm)

2.2.2. Evaluate the effect of environmental factors to create the biosurfactant

The ability to produce biosurfactant of microbial strains is influenced by many environmental factors such as pH, temperature, carbon source and salt concentration. In this study, the conditions for conducting survey experiments are conducted as follows [5]:

- Effect of temperature: The experimental medium was conducted at 1% NaCl concentration; pH = 7.5; The carbon source is DO oil with temperatures of 22, 28, 30 and 37°C.

- Effect of pH: The experimental medium was prepared with pH values of 6.5; 7; 7.5 and 8 were supplemented with 5% (v/v) DO oil, shaken at a speed of 200 rpm and temperature of 30°C.

Effect of NaCl concentration: The experimental medium was conducted with a NaCl concentration range of 0, 1, 2 and 3% with carbon source as DO, pH = 7.5, at temperature condition of 30°C.

- Effect of carbon source: The experimental medium was conducted with DO oil, saraline, glycerin, olive oil at 1% salt concentration; pH = 7.5; The temperature condition is 30°C.

After 120 hours (5 days) of shaking culture, the optimal biosurfactant production ability will be evaluated through the emulsification index E24.

2.2.3. Evaluate the oil decomposition ability of biosurfactant

a) Evaluate the oil decomposition ability of biosurfactant to number of microbial cells

The influence of biosurfactant on microorganisms is evaluated through fluctuations in the number of microbial cells. Prepare the flasks containing of Gost 1% NaCl mineral medium, add 5% saraline oil (v/v) and 0.5ml *B. celere* bacteria fluid into the flasks. Then, add biosurfactant to every flask according to ratio 1, 2 and 3ml respectively and one flask control (without adding biosurfactant). Shake all flasks at conditions: speed of 200 rpm, temperature of 30°C. Count and compare the number of microbial cells at D0 and D5.

b) Measure the surface tension and the viscosity of oil

The determining surface tension process is measured in a thermostat mechanic. The viscosity was measured by using Ostwald viscometer. These experiments were conducted in Center of additivies and petrolium products, Vietnam Institute of Industrial Chemistry

c) Analyze oil content by gas chromatography instrument system

The oil degrading ability by biosurfactant depends on the amount of oil decomposed during the shaking process. In this study, the remaining oil content in the sample after 5 days was analyzed by gas chromatography at Laboratory Chemistry Department 5 - Technical Center for Standards, Metrology and Quality 1 - General Department of Standards, Metrology and Quality.

2.3. Statistics analysis

All experiments were meticulously conducted in triplicate. Subsequently, the data obtained was processed using the software Sigmaplot 14.

3. RESULTS AND DISCUSSION

3.1. Effect of some environmental factors on the biosurfactant producing capacity of B. celere strain

3.1.1. Effect of pH on the biosurfactant producing capacity

Effect of pH on the biosurfactant producing capacity in Figure 1 showed that pH = 7.5 is the optimal value for *B. celere* strain producing biosurfactant. Especially, the emulsification index E24 of this strain achieved 72% after 72 hours. These results also indicated that pH range for bacteria growth is fairly wide. The bacteria growth at pH values 6.5; 7 and 8 was recorded but not as high as at pH = 7.5. Normally, neutral pH is the best value for the bacteria to grow. Morever, this bacteria strain is isolated from seawater so the pH 7.5 is also a suitable pH for the seawater environment. Therefore, pH 7.5 is a suitable value for the bacterial strain to grow and produce amounts of biosurfactant high.

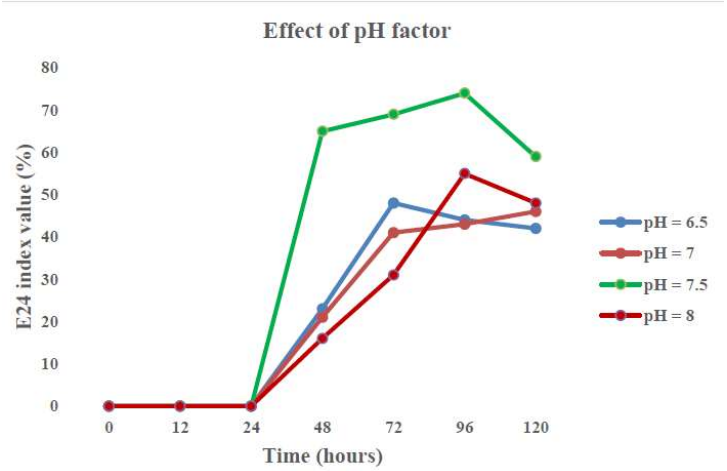

Figure 1. *Effect of pH on the biosurfactant producing capacity of B. celere strain*

3.1.2. Effect of temperature on the biosurfactant producing capacity

Similarly, the result in figure 2 showed that *B. celere* strain has a wide temperature range for biosurfactant production. The emulsification index E24 at four temperature value is stable and at the temperature 30°C reached the highest emulsification index E24 (67% after 120 hours). Therefore, the temperature of 30°C is the appropriate temperature for the bacterial strain producing amounts of biosurfactant high.

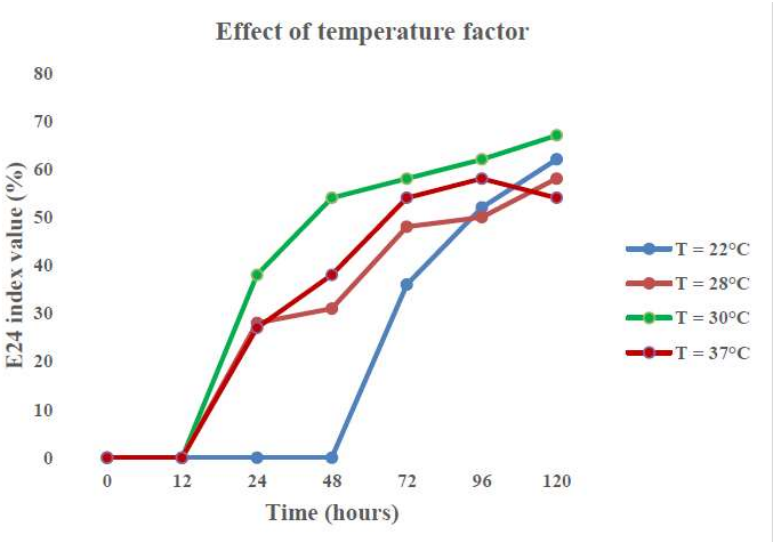

Figure 2. *Effect of temperature on the biosurfactant producing capacity of B. celere strain*

3.3.3. Effect of NaCl concentration on the biosurfactant producing capacity

The oil pollution often occurs at the sea and the experimental bacterial strain isolated from coastal areas, so testing how salinity effect bacterial growth is very important for future applications. The experimental results showed that the appropriate salt concentration for the producing biorsurfactant of 1% (Fig. 3). The *B. celere* strain can grow with a wide range of salt concentrations from 0% - 3%, however, this strain achieves the highest emulsification index of 67% at a concentration NaCl 1%. Therefore, concentration NaCl 1% is the best suitable concentration for *B. celere* strain to grow and develop.

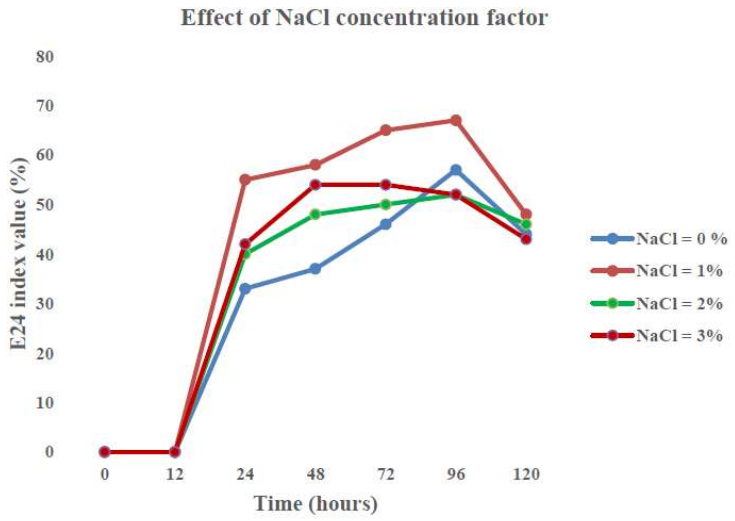

***Figure 3**. Effect of NaCl concentration on the biosurfactant producing capacity of B. celere strain*

3.3.4. Effect of carbon source on the biosurfactant producing capacity

The carbon source is also a very important factor affecting the biosurfactant producing capacity. Currently, oil spills at sea often contain saraline, DO oil and paraffin. Especially in drilling mud, saraline oil often accounts for a large amount. The technical requirements for petroleum oil exploitation are deep drilling and oblique drilling into the ground, so the drilling fluid commonly used contains the oil (saraline). Therefore, the selection of carbon sources suitable for biosurfactant production in this study is presented in Figure 4. The analysis results showed that *B. celere* strain is the ability to produce biosurfactant and decompose oil strongly, the highest decomposing carbon source is saraline and DO oil.

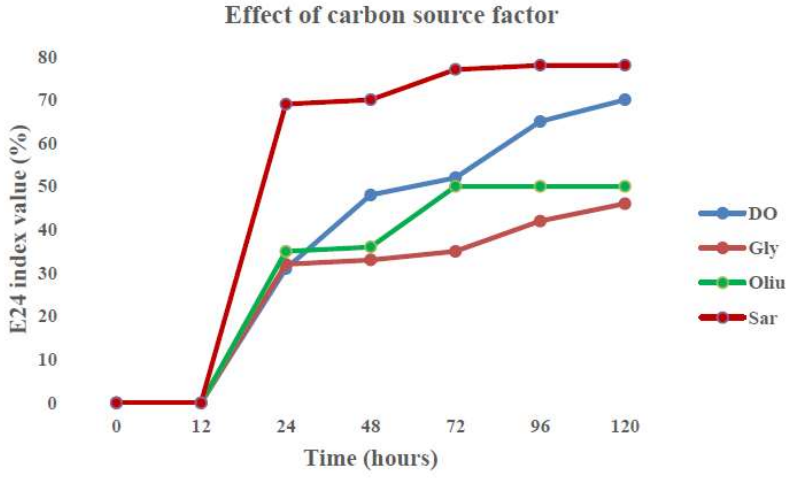

***Figure 4.** Effect of carbon source on the biosurfactant producing capacity of B. celere strain*

3.4. Evaluate the effect of biosurfactant on oil decomposition ability

3.4.1. Effect of biosurfactant on the microbial population in drilling mud

The effect of biosurfactant is evaluated through its impact on changing the number of microorganism in drilling mud. When the number of cell increases, it means that these

strains use oil as a source of nutrition and leading to reduce the amount of oil. This is a way to treat oil pollution by biostimulation method - taking the local biome as the center of the pollution treatment process [4]. The variation in the number of bacteria cell is shown in Table 1.

Table 1. Effect of biosurfactant on the number of bacteria cell in drilling mud

Volume of biosurfactant added (ml)	Number of bacteria cell (CFU/ml)	
	Before	After
0 ml	4.27×10^3	6.2×10^2
1ml	4.27×10^3	1.7×10^6
2ml	4.27×10^3	7.8×10^8
3ml	4.27×10^3	1.1×10^9

The results showed that the biosurfactant increased the number of bacteria cells in oil-contaminated drilling mud. The number of microorganisms in shaking culture flasks grow and change the medium colour from white to brown, yellow brown or red brown. Oil decomposes to form a solution and dissolves in the culture medium. There are cases where bacteria thrive and form a membrane between the oil and water phases. The number of bacteria cells on average increased from 10^3 to 10^9 cells/ml. When adding 2ml to 3ml biosurfactant, the number of bacteria cells increase significantly. The number of cells also increased greatly by 70.9% and up to 10^9 CFU/ml when adding 2 and 3 ml biosurfactant, respectively. In the control sample (without adding biosurfactant), the number of bacteria cells decreased by nearly 10 times. In addition, the number of M150 strain cells in all shaking culture flasks containing DO, glycerin, and olive oil grew well. The analysis results showed that biosurfactant of strain M150 has a stimulating effect on microorganism population in drilling mud. The number of cells increases the most obvious when the amount of biosurfactant added is 3 ml.

3.4.2. Effect of biosurfactant on viscosity and surface tension of oil

The effect of biosurfactant on viscosity and surface tension of oil is shown in Table 2. The analysis result showed that both the surface tension and viscosity of the experimental sample adding biosurfactant decreased compared to the control sample without biosurfactant.

Table 2. Results of measuring viscosity and surface tension of bacterial fluid samples

Type of oil	Samples	Temperature (25^0C)	
		Tension (mN/m)	Viscosity (cst)
DO	Control	36.5	0.93
	Add biosurfactant	29.7	0.91
Saraline	Control	29.2	0.92
	Add biosurfactant	27.6	0.91

3.4.3. Effect of biosurfactant on the ability to decompose oil in drilling mud

Conduct the experiments with M150 strain in mineral medium Gost 1% NaCl, supplemented with 5% DO and saraline oil. The bacterial culture samples after 5 days were analyzed on a gas chromatograph (Gas Chromatography - Shimazdu). The results are shown in table 3.

Table 3. Effects of biosurfactant on the composition of DO and saraline oil

No.	Before	After	Amount of oil used (%)
DO oil (g/50ml)	1.95 g	1.0231 g	82.6
Saraline oil (g/50ml)	2.075 g	0.6775 g	74.5

The result in Table 3 showed that the research strain used 82.6% and 75.4% saraline and DO oil, respectively. The obtained data demonstrated that the research strain can create biosurfactant and decompose oil in drilling mud within 5 days and the maximum decomposition efficiency reached 82.6%.

Our results have a number of similarities with Shah et al., (2016), Barakat et al., (2017), Joshi et al., (2014) findings [6-8]. Shah et al., (2016) showed that the biossurfactant produced by *Pseudomonas aeruginosa* can decompose the crude oil [6]. In the medium supplemented with 1% crude oil (TAPIS), the emulsification index E24 of Rhamnolipids to TAPIS was maximum value of 42% [6]. The isolated result of Barakat et al., (2017) in Shalateen, Red Sea, Egypt showed that two strain *Bacillus amyloliquefaciens* SH20 and *Bacillus thuringiensis* SH24 can decomposed oil with the highest emulsification index (E24) 57 and 56%, respectively [7]. The fact that biosurfactant can reduce surface tension of both hydrocarbon mixtures and aqueous solutions [8]. Therefore, Joshi et al., (2014) indicated that microbial strain *Pseudomonas stutzeri* isolation from soil samples at petrol pumps and garages in Kalyan also have able produced biosurfactant [8]. The different microbial strains have the biosurfactant-producing capacity and degrading oil differently. The source of initial oil used in many studies is also very diverse, but mainly focus to use DO oil, saraline for lubricating the drill bit or increasing the pressure to push the oil. Thefore, the biosurfactant of *Brevebacterium cerere* strain is the potential compound for drilling mud treatment and oil pollution treatment.

4. CONCLUSION

This study determined that the condition for the highest biosurfactant production of *Brevebacterium cerere* strain is pH = 7.5; temperature 30°C; NaCl concentration is 1%, the carbon source is saraline and DO oil. The analysis result of the remaining oil content showed that the biosurfactant of strain M150 decomposed 82.6% and 74.5% of saraline and DO oil, respectively. The biosurfactant of this strain also decreased the surface tension and viscosity of oil in both experimental and the control sample. Simultaneously, this subtance also affects the biological community in drilling mud samples. These results indicated that the biosurfactants produced by *Brevebacterium celere* strain can be applied in drilling mud treatment and environmental pollution treatment.

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