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Bio-Surfactant Production and Its Application in Microbial EOR

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***Abstract*—**There are various sources of energies available worldwide and among them, crude oil plays a vital role. Oil recovery is achieved using conventional primary and secondary recovery methods. In-order to recover the remaining residual oil, technologies like Enhanced Oil Recovery (EOR) are utilized which is also known as tertiary recovery. Among EOR, Microbial enhanced oil recovery (MEOR) is a technique which enables the improvement of oil recovery by injection of bio-surfactant produced by microorganisms. Bio-surfactant can retrieve unrecoverable oil from the cap rock which is held by high capillary force. Bio-surfactant is a surface active agent which can reduce the interfacial tension and reduce viscosity of oil and thereby oil can be recovered to the surface as the mobility of the oil is increased. Research in this area has shown promising results besides the method is echo-friendly and cost effective compared with other EOR techniques. In our research, on laboratory scale we produced bio-surfactant using the strain *Pseudomonas putida* (MTCC 2467) and injected into designed simple sand packed column which resembles actual petroleum reservoir. The experiment was conducted in order to determine the efficiency of produced bio-surfactant in oil recovery. The column was made of plastic material with 10 cm in length. The diameter was 2.5 cm. The column was packed with fine sand material. Sand was saturated with brine initially followed by oil saturation. Water flooding followed by bio-surfactant injection was done to determine the amount of oil recovered. Further, the injection of bio-surfactant volume was varied and checked how effectively oil recovery can be achieved. A comparative study was also done by injecting Triton X 100 which is one of the chemical surfactant. Since, bio-surfactant reduced surface and interfacial tension oil can be easily recovered from the porous sand packed column.

***Keywords*—**Bio-surfactant, Bacteria, Interfacial tension, Sand column.

I. Introduction

CRUDE oil plays a vital role in our day to day life. The contribution of energy is about 70% comparatively with other sources of energy. Oil recovery refers to the process by which crude oil is extracted from the cap rock of petroleum reservoir. Oil recovery is categorized into three phase namely primary, secondary and tertiary recovery. In primary recovery method crude oil is extracted to the surface with help of pressure present in the reservoir. The recovery obtained using the method is about 10–15%. Once the pressure gets declined no more oil can be recovered to the surface. At this stage secondary recovery method helps to recover oil. In this method, artificially water is pumped at high pressure to build up pressure. Due to pressure difference oil is moved from high pressure to low pressure zone. In water drive method around 20% of oil is recovered after primary recovery technique [1]. More amount of oil remained trapped in the cap rocks which are held by high interfacial tension. Around 50% of OOIP can be recovered using tertiary recovery method. Injection of different agents like Heat, Polymers, Chemical surfactants and Microbial surfactants to recover crude oil from trapped zone is known as enhanced oil recovery methods. Additionally 20–30% of oil is recovered after secondary recovery. There are many approaches to improve oil recovery rate. The role of microbes and its metabolites helps to recover oil by producing bio-surfactant. This technique is known as Microbial EOR [2]. Bio-surfactants are microbial compounds which are amphiphilic in nature [3]. Bio-surfactants are surface active compounds produced by variety of bacteria [4]. Due to their potential applications in various fields like agriculture, food processing and petroleum industries. Properties of bio-surfactant include surface tension reduction, promoting foaming agents, stable and environment friendly [5]. Bio-surfactants are found to be biodegradable in nature and it is found to be effective at extreme conditions of pH and temperature [6]. In current scenario, interest in bio-surfactants has increased globally to expand the present range of microbial surfactants. These bio-surfactants have potential use in oil industries such as cleaning oil sludge, mobilizing heavy crude oil and managing oil spillage [7]. *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Bacillus licheniformis* are some of the well known bacteria which produce bio-surfactant [8]. Increasing demand for petroleum over recent years and to meet the gap, application of bio-surfactant in oil recovery plays a major role in petroleum industries. However, stability of bio-surfactant at extreme pH and temperature conditions is necessary for enhanced oil recovery. Among the available sources of energy, crude oil plays a critical part in providing major supply to the world. Crude oil is generally recovered using primary recovery, where 10–15% of oil is extracted from Original oil in place (OOIP). The next stage of oil recovery is secondary recovery by water flooding where high pressure water is flushed into the reservoir to recover trapped oil from the pores. 15% of oil is recovered using this method. 65% of more oil is trapped in the reservoir. Enhanced oil recovery methods are the significant method to recover the residual oil. Microbial enhanced oil recovery process is one of

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the EOR techniques where the microorganisms produce bio-surfactant which intern used to retrieve unrecoverable oil. MEOR is cost effective, bio-degradable and eco-friendly method when compared to other EOR techniques. Mechanisms like gas production (to increase reservoir pressure), solvent production (reduce viscosity), bio-surfactant production (alter the oil/water and oil/rock interactions) are generally found to be effective in releasing the trapped residual oil.

II. Materials and Methods

A. Micro-Organism

Pseudomonas putida MTCC 2467 was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India for this study. The culture was maintained in nutrient agar plates with the following composition (g/L): beef extract, 1.0; peptone, 5.0; yeast extract, 2.0; NaCl, 5.0; agar, 15.0; pH 7.0 ± 0.2, storage temperature −2°C to −8°C.

B. Media and Cultivation Conditions

Nutrient broth with the following composition (g/L) was used for inoculum preparation. Beef extract, 1.0; yeast extract, 2.0; peptone, 5.0; NaCl, 5.0. *Pseudomonas putida* (MTCC 2467) grown in Nutrient broth for 8 – 10 h at 30 °C (A_{600nm} 0.7 – 0.9) and 2% (v/v) of the inoculum was used for production of biosurfactant using mineral salt medium with the following composition (g/L) KNO_3, 0.3; Na_2HPO_4, 0.2; KH_2PO_4, 0.014; NaCl, 0.001; $MgSO_4$, 0.06; $CaCl_2$, 0.004; $FeSO_4$, 0.002; 0.1 ml of trace element solution containing (g/L) $ZnSO_4.7H_2O$, 2.32; H_3BO_3, 0.56; $CuSO_4.5H_2O$, 1.0; $MnSO_4.4H_2O$, 1.78; $Na_2MoO_4.2H_2O$, 0.39; $CoCl_2.6H_2O$, 0.42; EDTA, 0.5; $NiCl_2.6H_2O$, 0.004; KI, 0.66; K_2SO_4, 3.0.

C. Effect of Different Parameters on Growth and Bio-Surfactant Production

To identify the best carbon source namely sucrose, glucose and starch were tested to evaluate best carbon source for bio-surfactant production using the strain *Pseudomonas putida*. 100 ml of production medium with 2% (w/v) of above mentioned carbon sources were grown separately at 40°C and 200 rpm for 5 days. Samples were collected for every 12 h and analyzed for bio-surfactant production, growth and other parameters. To identify the best nitrogen source different nitrogen sources such as ammonium sulphate, ammonium nitrate and urea with 0.3% (w/v) were added to the medium containing 2% (w/v) sucrose as carbon source. Fermentation was carried out for 5 days at 40°C and 200 rpm for 5 days. Samples were collected for every 12 h and analyzed for bio-surfactant production, growth and other parameters. To investigate the effect of initial pH on bio-surfactant production, the initial pH of the production medium was adjusted to 5.0, 6.0, 7.0 and 8.0 using 3 M HCl and 3M NaOH and bio-surfactant production experiments were carried out.

D. Surface and Interfacial Tension Measurement

Surface tension measurement of the cell free broth was determined by K6 Tensiometer (Kruss GmbH, Hamburg, Germany), using plate method. 10 ml of sample was placed in the glass container. Measurements were carried out by automatic controller which smoothly pulls down the plate such that gets contacted with the liquid placed. The force acting on the rectangular plate with known length were measured and converted into surface tension digitally. For interfacial tension measurement equal amount of oil is added to the sample and similar procedure is followed.

E. Sand Pack Column Experiment

A simple sand packed column was designed to determine the efficiency of produced bio-surfactant in oil recovery rate. The column was made of plastic material with 10 cm in length. The diameter was 2.5 cm. The column was packed with fine sand material. Sand was saturated with brine initially followed by oil saturation to replicate petroleum reservoir condition. The sand packed column was flooded again with brine until no more oil received at the effluent. 0.5 PV (Pore Volume) of *Pseudomonas putida* MTCC 2467 (OD = 0.23) in mineral salt medium was injected into the column. The column was flooded with water (Secondary flooding) followed by Triton X 100 and Tween separately (Chemical flooding). Produced bio-surfactant was flooded finally to check the recovery rate using various flooding methods. The effluent collected from the outlet of the column gives the amount of oil recovered and determined using standard methods.

A sand packed oil saturated column is considered to be a convenient bench-scale technique to evaluate oil recovery as it simulates the oil recovery operations of oil reservoirs. The oil entrenched column is first subjected to water flooding operation of the secondary phase of oil recovery. At the end of water flooding, the residual oil is believed to be in the form of discontinuous oil which is trapped in the pores of reservoir rocks [9]. This process can be considered in similar situation in an oil saturated column. The oil that is trapped in the reservoir rock is due to competition between two forces which are viscous forces that mobilize the oil and capillary forces that trap the oil. The oil displacement efficiency of a recovery process is determined with the ratio of the two forces. Capillary forces arise from the IFT between oil and water phases, which resist externally applied viscous forces and cause the injected water to bypass the resident oil. These forces cause large quantities of oil to be left behind after water flooding. Capillary number is the ratio of viscous to capillary forces. The recovery of additional l oil by an EOR, the capillary number has to be increased to around 10^{-3} to 10^{-2}. This is usually achieved by surfactants, which can decrease the IFT at oil/brine interface from 20–30 mN/m to 10^{-3} mN/m and by polymer solutions, which act as dry fluids for proper mobility control of oil bank and surfactant slug. The reduction in interfacial tension leads to the mobilization of oil ganglia forming an oil bank. This oil bank is propagated to the production wells by the use of polymer solution followed by the drive water [9]. In general, surfactant injection alone

cannot achieve sufficient oil recovery due to several problems like fingering, adsorption, surfactant–oil interactions, etc. Therefore, a most simple and less expensive process called bio-surfactant flooding is used as a tertiary oil recovery method in current chemical enhanced oil recovery practice.

III. Result and Discussion

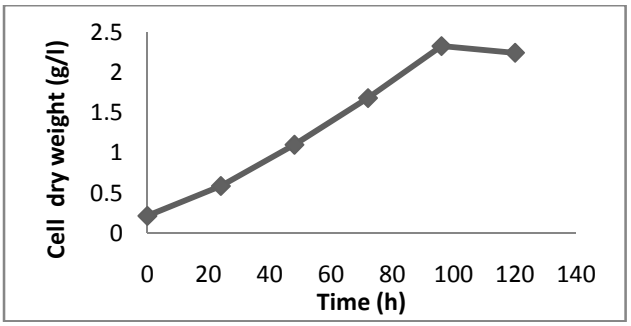


Fig. 1 Cell Growth on Fermentation Time

Biosurfactant are surface active compounds produced by variety of microorganisms belonging to the genus *Bacillus, Pseudomonas and Acinetobacter*. Biosurfactant producing microorganisms grow on both water immiscible hydrocarbons and carbohydrate containing mineral salt medium. Sucrose and Ammonium sulphate was used a carbon and nitrogen sources along with mineral salt for production of bio-surfactnat. The strain *Pseudomonas putida* was able to produce biosurfactant. Fig. 1 gives the profile of cell growth for various time courses. It was found that the maximum cell growth of 2.3 g/l was achieved at 96 hours of fermentation time. Previous studies reported that using *Bacillus subtilis* MTCC1427 in presence of 2% sucrose, 3.3 g/l cell concentration was produced [6].

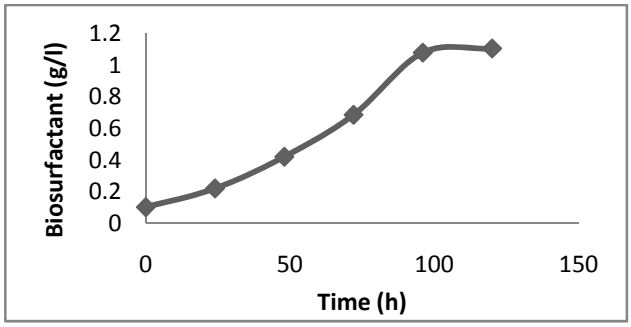


Fig. 2 Bio-Surfactant Concentration for Time Course

Concentration of produced bio-surfactant was shown in Fig. 2. It can be noted that the concentration of bio-surfactant produced depends upon the amount of cell growth. The maximum yield of bio-surfactant was found to be 1.1 g/l. The production of bio-surfactant was considered to be more important since it has the ability to reduce the surface and interfacial tension. The obtained result was compared with previous reports and found that *Bacillus subtilis* MTCC1427 with sucrose and ammonium sulphate gave 1.1 g/l bio-surfactant [6].

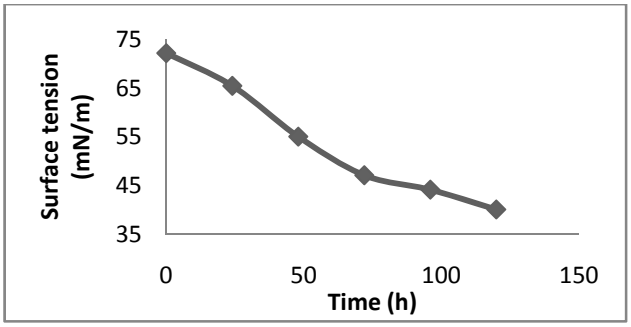


Fig. 3 Surface Tension on Fermentation Time

Fermented culture supernatant was subjected to tensiometer analysis to estimate the efficiency of produced bio-surfactant which intern reduces the surface tension. Initial surface tension was found to be 74 mN/m and as the time proceeds reduction of surface tension was achieved (Fig. 3). Maximum reduction in surface tension was found to be 43.4 mN/m with 120 hours of fermentation time. The obtained results were found to be quite similar to earlier reported data on biosurfactant production by *Bacillus subtilis* MTCC1427 where reduction in surface tension was 34 mN/m [6].

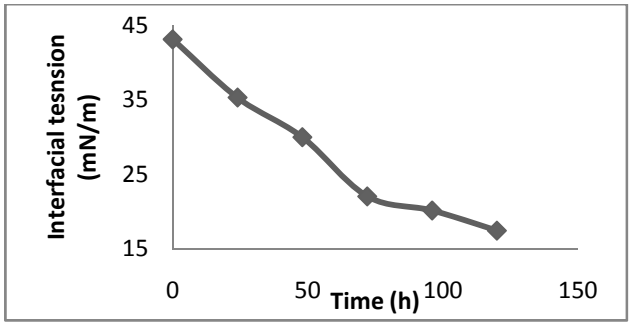


Fig. 4 Profile of Interfacial Tension Reduction *Vs* Time

Interfacial tension reduction was determined by addition of equal volume of crude oil to the bio-surfactant. Fig. 4 gives the profile of interfacial tension reduction for different fermentation time. Interfacial tension was found to be 44 mN/m and as the time precedes reduction of interfacial tension was achieved with the produced bio-surfactant using the strain *Pseudomonas putida*. Maximum reduction was found to be 17.6 mN/m with 120 hours of fermentation time.

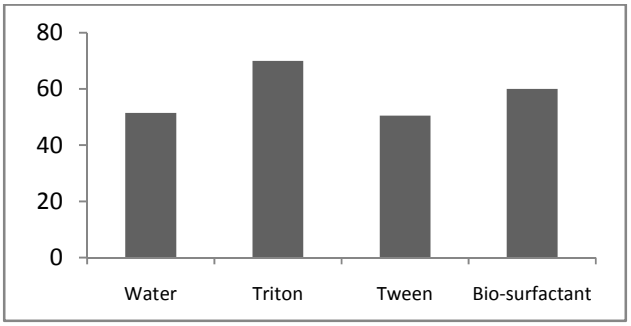


Fig. 5 % Oil Recovered by Non Sequence Pattern with Different Flooding Methods

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Using sand packed column, the efficiency of oil recovery was determined using different flooding methods. All the flooding experiments were carried out separately. Initially crude oil (20 ml) was saturated in the simple sand packed column filled with fine sand. The porosity of the packed material was found to be 26 %. At first stage oil was recovered by injecting 10 ml of water. The amount of oil collected was noted. When no more oil was recovered again another 10 ml of water was injected to recover oil. This was done because as the process of injection was carried out as batch process and the amount of oil collected was calculated. The amount of oil collected by injecting 20 ml of water gives the total amount of oil collected (Fig. 5). This type of water injection and thereby collection of oil is secondary flooding or water flooding.

Similarly, Triton X 100 and Tween (Chemical surfactants) were injected separately to determine the amount of oil that can be recovered. In order to check the efficiency of chemical surfactant and bio-surfactant non sequence method is followed. The amount of oil collected after Triton and Tween flooding was calculated.

MEOR flooding was carried out by injecting pure bio-surfactant produced by the metabolites produced by the strain *Pseudomonas putida* (MTCC 2467). 20 ml of crude oil was injected in to the designed simple sand column. 10 ml bio-surfactant was initially injected and amount of oil collected was calculated. Another 10 ml of oil bio-surfactant was injected and the amount of oil extracted was measured. The amount of oil recovered by injecting 20 ml of bio-surfactant was determined. It was found that using water flooding, around 51.5 % of oil was recovered. Triton X 100 and Tween injection gave a recovery of 70% and 50.5 % respectively. On the other hand the amount of oil recovered using bio-surfactant flooding was found to be 60%.

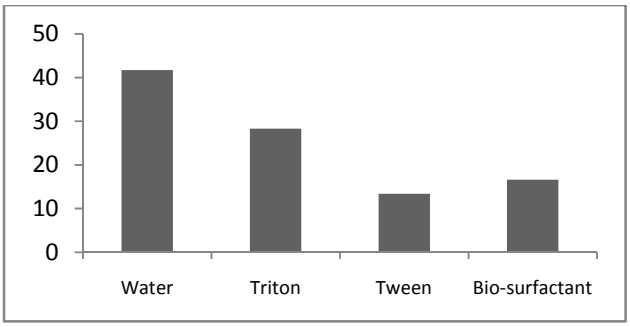


Fig. 6% Oil Recovered by Sequence Pattern of Different Flooding Methods

The flooding experiment was carried out with similar injection process. The only difference here is the injection methods was carried out sequentially. i.e., one crude oil was injected, 20 ml of water injected and the amount of oil collected was determined. To the same column again Triton X 100 followed by Tween and finally bio-surfactant was injected. The amount of oil recovered after each stage of injection was determined. From Fig. 2, it is absorbed that 41.7% of oil was recovered using water flooding. 28.3 and 13.4% of oil was recovered by Triton and Tween injection respectively. Bio-surfactant flooding yielded about 16.6% of oil recovery. The volumetric sweep efficiency is the fraction of the reservoir in total as the injected fluid is effectively in contact. The initial water saturation will have higher effect and considered to be dominant because of wettability adsorbed by crude oil. This is because the water determines which part of the packed material get in contact with the saturated oil. From Fig. 6, the profile shows that, as there is increase in rate of fluid injection there is no vast change in the recovery rate. The reason for this case may be viscous fingering or preferential flow paths which may be associated with the hyperbolic nature of the advective dominant fluid flow. However, considerable amount of oil was recovered post Triton X 100 and Tween flooding. Bio-surfactant which is surface active compounds effectively reduced the capillary force by reducing the interfacial tension. Results on surface tension and interfacial tension reduction were determined using digital tensiometer. The produced bio-surfactant proved to perform positive effects on displacing the crude oil which was considered to be held by higher surface tension [10]. The produced microbes inhibited the negative effects which alters the interaction between the packed material and oil present in the column.

In the present study, bio-surfactant produced by *Pseudomonas putida* was found to be best in increasing the recovery factor. Produced bio-surfactant was found to be stable at reservoir conditions and retained excellent surface activity at various tested temperature, pH and salinity conditions. By non sequence injection methods, the amount of oil recovery was around 60% and with sequence method of injection the recovery rate was around 16%. Production of bio-surfactant with release in gases in turn increases in pressure. Also, the interfacial tension of oil/water and oil/rock was altered with the help of biologically produced surfactant. [11]. Oil recovery rate with bio-surfactant was compared with *Bacillus subtilis.* 23% of oil recovery was reported by [12] with fractured porous media. High permability of micromodel and sand packed column can be suggested that the average pore size of the pores in the column model may be higher in throat size as it is large enough to disperse the bacterial cells to flow free.

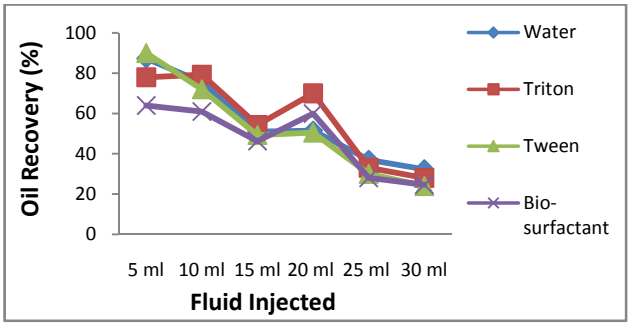


Fig. 7% Oil Recovered by Injecting Varying Volume of Fluids (Fine Sand)

To check the recovery factor, the amount of fluid injected has been varied from 5 ml to 30 ml. This experiment was

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conducted with similar procedure as mentioned in previous experiments. 20 ml of crude oil was saturated. Water flooding was done by injecting 5 ml–30 ml. Similarly the column was saturated with crude oil followed by injecting chemical surfactants (Triton and Tween). Finally bio-surfactant was injected at different volume (5–30 ml). The recovery percentage was shown in Fig. 7. It was found that recovery was higher i.e., 79.3% with triton by injecting 10 ml. Bio-surfactant flooding achieved 60 % of recovery. Water flooding and tween flooding gave higher recovery of 83% and 90% by injecting 5 ml respectively. From the plot it can be noted that recovery was quite higher with 20 ml of triton and bio-surfactant injection. Since, bio-surfactant is more economic than triton; it is injected to increase the recovery rate of oil as it reduces the surface and interfacial tension which normally found to be higher in petroleum reservoir. Also the viscosity of the oil is considerably reduced with the acids and gases generated by microbes during bio-surfactant production. This intern aid in increasing the mobility of oil and thereby the oil is released from the trapped zone in the reservoir. As the injection of water and Tween was increased it was found that the residual oil cannot be accessed by the injecting these fluids. Due to the effect of non dispersion of fluids the recovery of crude oil from the column was not much potential [13]. Potentially, this could be explained in terms of percolation where around 50% oil-wet columns might have enough interconnected oil-wet pores to percolate across the column causing lower residual oil saturation [14]. From the obtained results it is noted that the surface tension and the sand packed in the column may play a vital role by trapping the oil at the exit due to the presence of oil wet surface of the packed column. Thus, the purified lipopeptide biosurfactant produced by the strain *Pseudomonas putida* was found to be best suited for oil recovery applications.

IV. Conclusion

Strain *Pseudomonas putida* has a potential to produce bio-surfactant which reduces surface and interfacial tension effectively. Simple sand packed column were used to evaluate the potential application of bio-surfactant in oil recovery. Triton and bio-surfactant effectively used to increase the recovery rate from the trapped oil zone.

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