Isolation and Characterization of Biosurfactant Producing Bacteria for the Application in Enhanced Oil Recovery

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Abstract. In the present study, a biosurfactant producing bacterial strain was isolated, screened and identified. Further, various fermentation conditions (such as pH (5-10), incubation period (24-96h) and incubation temperature (20-60 ^{o}C) were optimized for maximum production of biosurfactant. The produced biosurfactant was characterized by measuring emulsification index, foaming characteristics, rhamnolipid detection, interfacial tension between water and oil and stability against pH and temperature for its potential application in oil recovery process. The additional oil recovery for two different sand, sand1 and sand2, was found to be 49% and 38%, respectively.

1. Introduction

The percentage of oil remaining in a reservoir varies from field to field. However, in a recent study, it was found that 65% of the original oil in place (OOIP) was left behind after primary and secondary-oil recovery processes [1]. In general residual oil can be recovered by three EOR technologies i.e. thermal, miscible and chemical enhanced oil recoveries (CEOR). Among these, CEOR is one of the most common methods of EOR. This method involves the recovery of residual oil by using different types of chemical formulations which are capable enough in reducing mobility ratio and/or increasing capillary number when injected in the reservoir as displacing fluid. Use of chemical surfactant for this purpose is one of the common method [2]. But, chemical surfactants are having many disadvantages in environmental as well as application point of view. They can be hazardous, non biodegradable, toxic to the environment, and costly compounds [3]. Moreover, with these compounds achieving the ultra low interfacial tension (i.e. less than 10^{-3} mN/m), which is one of the basic requirement for EOR, is one of the big challenges in the field of EOR. Because, the interfacial tension (IFT) between water and oil is directly related with the capillary forces and high capillary force resulted into the entrapment of the oil in the porous matrix of the reservoir [4].

From literature it was found that various articles are available on MEOR from 2008 onwards, using biosurfactants produced from various microorganisms (Table 1) but still there is a scope in achieving the desired characteristics of interfacial tension comparatively more nearer to ultralow level (i.e. 10^{-3} mN/m) and improving the MEOR by reducing the capillary forces for channelizing the flow of oil through the porous matrix of the reservoir. For this purpose, an effort has been made to replace chemical surfactant with the eco-friendly bio-surfactant, which is produced ex-situ from an isolated bacterial strain, characterized in terms of interfacial tension and used for the application in microbial enhanced oil recovery.

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Table 1. Literature Review on MEOR

Microorganism for MEOR	Oil Recovery (%)	Reduction in Surface tension (ST)/IFT	Reference
Bacillus subtilis PT2 and *Pseudomonas aeruginosa* SP4	61.62 and 57.07, respectively	ST= 26.4 and 28.3mN/m, respectively	[5]
Bacillus amyloliquefaciens BZ-6	88% from oily sludge	-	[6]
Bacillus subtilis	-	ST=30 mN/m	[7]
Bacillus subtilis B20	9.7	ST=25 mN/m, IFT = 5.02 mN/m	[8]
Bacillus subtilis B30	light oil=26, heavy oil=31	ST=26 mN/m, IFT=4 mN/m	[9]
Pseudomonas sp. SWP- 4	24	ST=22.7 mN/m	[10]
Pseudomonas aeruginosa Pa4, *Escherichia coli*	9.96 and 12, respectively	IFT= 32.39 mN/m and 27.39 mN/m, respectively	[11]
Pseudomonas putida	26.1	IFT=2.5 mN/m	[12]

2. Experimental Procedure

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2.1. Materials and Methods

The sample collection was aimed for isolation of biosurfactant producing organism. For this purpose, the soil sample was collected near automobile garage in Udhana, Surat-Gujarat analyzed for isolation of oil degrading microorganism. The temperature of the soil was around 28 °C and pH 8.0. Bushnell Hass Mineral Salts (BHMS) medium (purchased from Himedia, Mumbai, India) was used for the isolation of hydrocarbon degrading bacteria. The pH was adjusted to 7. The BHMS medium was supplemented with 1% (v/v) used engine oil as the sole carbon source [7]. For MEOR experiment, paraffin liquid light oil (Finar Chemicals) was used asthe oil phase. Sodium chloride (99%) for salinity and Cetyl trimethylammo-nium bromide (CTAB) (99%) for rhamnolipid detection, were purchased from Sigma-Aldrich(India). All other chemicals like HCl and NaOH used were of analytical grade. All

the solutions were prepared in demineralized water. Interfacial tension was estimated between oil (light paraffin oil) phase and water with different concentrations of the produced biosurfactant using a KRUSS-T9Tensiometer (Germany), (Du-Nuoy ring method) under atmospheric pressure.

2.2. Experimental Setup

All fermentation experiments were performed in 500 ml conical flasks. MEOR experiments were performed in 60 ml syringes, packed with two types of sand (calcite (sand 1) and silicate (sand 2)). Four sets were prepared for two different samples among them one served as control for each sand sample. For this test each syringe was packed with sterile sand 40 gram and was packed tightly using syringe plungers. The end nozzle was temporarily sealed. The experiment was carried out at 30°C.
The syringe system was flooded with water at a constant flow rate 1ml/min. Pore volume (PV, ml) was calculated using following equation:

$$\mathbf{PV} = \frac{\textbf{weight of core 100\% saturated with water} - \textbf{weight of dry core}}{\textbf{density of water}}$$

Porosity(%) of the system was measured by dividing the PV by the total volume of packed syringed with sand(20ml). In the Second step, the paraffin oil was injected into the system to replace water in all the syringe, until there was no water coming out from the nozzle in effluent. Original Oil in Place (OOIP) was calculated as the volume of paraffin oil retained in the system. Thus from this Initial Oil saturation (S_{oi}, %) and initial water saturation (S_{wi}, %) were calculated by following formula:

$$\boldsymbol{S_{oi}} = \frac{\boldsymbol{OOIP}}{\boldsymbol{PV}} \times \mathbf{100}$$

$$\boldsymbol{S_{oi}} = \frac{\boldsymbol{PV - OOIP}}{\boldsymbol{PV}} \times \mathbf{100}$$

Following step was to stabilize the system for 24 h and was flooded again to remove out the excess oil until no more paraffin oil was observed in the water effluent. Oil recovered after flooding (S_{orwf},ml) was determined using volumetric flask. Residual oil saturation (S_{or}, %) was calculated as follow:

$$\boldsymbol{S_{or}} = \frac{\boldsymbol{OOIP - S_{orwf}}}{\boldsymbol{OOIP}} \times \mathbf{100}$$

Finally, the residual oil in the system was supplemented with the supernatant containing biosurfactant and for control only BHM broth was utilized. This system was maintained at 30°C for 48 h at shaker with 100rpm. After incubation the system was again flooded to recover the oil. The recovered oil was measured volumetrically and denoted as S_{orbf}(ml).
The Additional oil recovery (MEOR) was calculated as follow:

$$\boldsymbol{MEOR}\ (\%) = \frac{\boldsymbol{S_{orbf}}}{\boldsymbol{OOIP - S_{orwf}}} \times \mathbf{100}$$

2.3. Characterization and Identification of Isolate and Biosurfactant

The isolated microorganisms were identified by standard gram staining, motility test, colony characterization of isolates, biochemical characterization of isolates and Molecular identification by 16s rRNA sequencing and the produced biosurfactant was characterized by performing emulsification index E24 test, Foaming test, rhamnolipid detection and oil displacement test [13].

3. Results and Discussion

3.1. Isolation, Screening and Characterization of Biosurfactant Producing Bacteria

All 14 isolates were screened with oil displacement test for biosurfactant production on used engine oil. Among these isolated 14 organisms, 2 showed oil displacement and formed a clear halo. Maximum oil displacement was seen in one of the isolates with halo diameter of 72 mm (Figure 1(a)) which confirmed its ability of producing biosurfactant and selected for further characterization, optimization and oil recovery experiments.

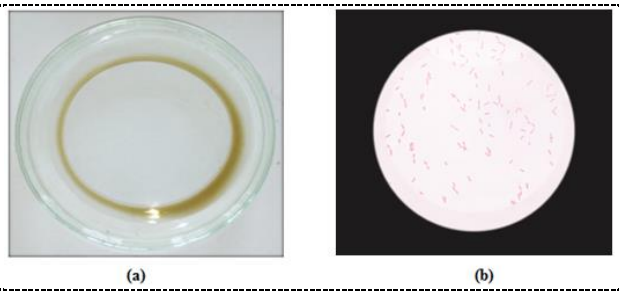

Figure 1 (a) Oil displacemnt zone of halo. **(b)** Microscopic view of Gram stained isolated strain of bacteria.

The isolated strain was found to be gram negative rod shaped and highly motile bacterial strain (Figure 1(b)). Further, molecular identification was done using 16s rRNA sequencing. The obtained sequence was search for its similarity using nucleotide BLAST 2.2.31 (blastn) software. The identification was made evaluating maximum score obtained, coverage, expected Value, and percentage identity. The isolate was confirmed to be Genus-*Pseudomonas* species *aeruginosa* with 99% similarity.

3.2. Effect of Incubation Period, pH and Incubation Temperature on Biomass and Biosurfactant Production

To produce the maximum amount of biosurfactant from the isolated bacteria during the fermentation process, various parameters of the fermentation process needs to be optimized. In this regard, various series of experiments for the fermentation of isolated strain were performed at different conditions of different process parameters like pH (varied from 5-10), temperature (varied from 20 ^{o}C to 50 ℃) and time-period (varied from 24 to 96 h) for production of biosurfactant. For this purpose, the culture was inoculated with the inoculums size of 3.9 $\times 10^{7}$ CFU/ml in BHM broth supplemented with 0.1M Glucose as a sole carbon source and the parameters were optimized by measuring biomass (dry weight) and oil displacement. The results of these experiments are shown in Figure 2. These results indicate that the biosurfactant synthesized from the isolated bacterial strain occurred predominantely during the exponential growth phase, suggesting that the biosurfactant was produced as primary metabolite accompanying cellular biomass formation (growth-associated kinetics) [14]. Based on these experiments, the optimum parameters for the maximum production of biosurfactant were attained by incubating 3.9 $\times 10^{7}$ CFU/ml of the inoculum, upto the incubation period of 96 h at 7.0 pH and 37 ^{o}C.

3.3. Characterization of Crude Biosurfactant for Its Application in Enhanced Oil Recovery

In order to check the applicability of the produced biosurfactant, it is necessary to characterize it in terms of emulsification index (E24), foaming, rhamnolipid detection and interfacial tension at different conditions (pH, salinity, high temperature and pressure). The method of determining these characteristics are described elsewhere [13]. The results of these experiments are shown in Figure 3.

The interfacial tension was measured against paraffin oil. A significant reduction (98% with respect to water) in the interfacial tension (nearer to ultralow) between water and paraffin was observed using the produced biosurfactant, which is desirable for the application enhanced oil recovery. Recently, minimum reduction in interfacial tension using conventional surfactant (CTAB) and ionic liquid surfactant (C_{16}mimBr) achieved were 7.7 mN/m and 3.8 mN/m, respectively [15].

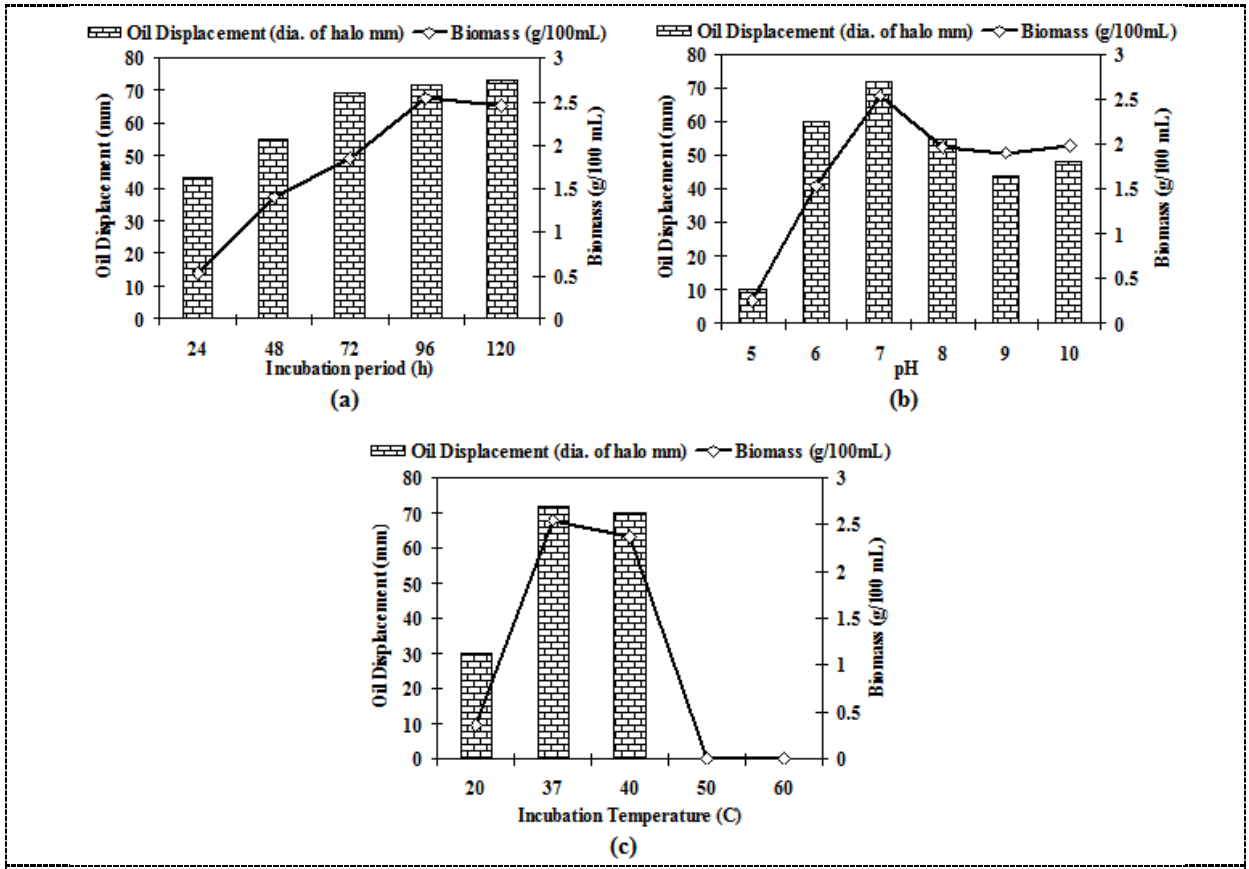

Figure 2. Effect of (a) incubation period with pH 7 and at 37 °C of incubation temperature, (b) pH at 96 h of incubation period and 37 °C of incubation temperature and (c) temperature at pH of 7 and 96 h of incubation period, fermentation and biosurfactant production.

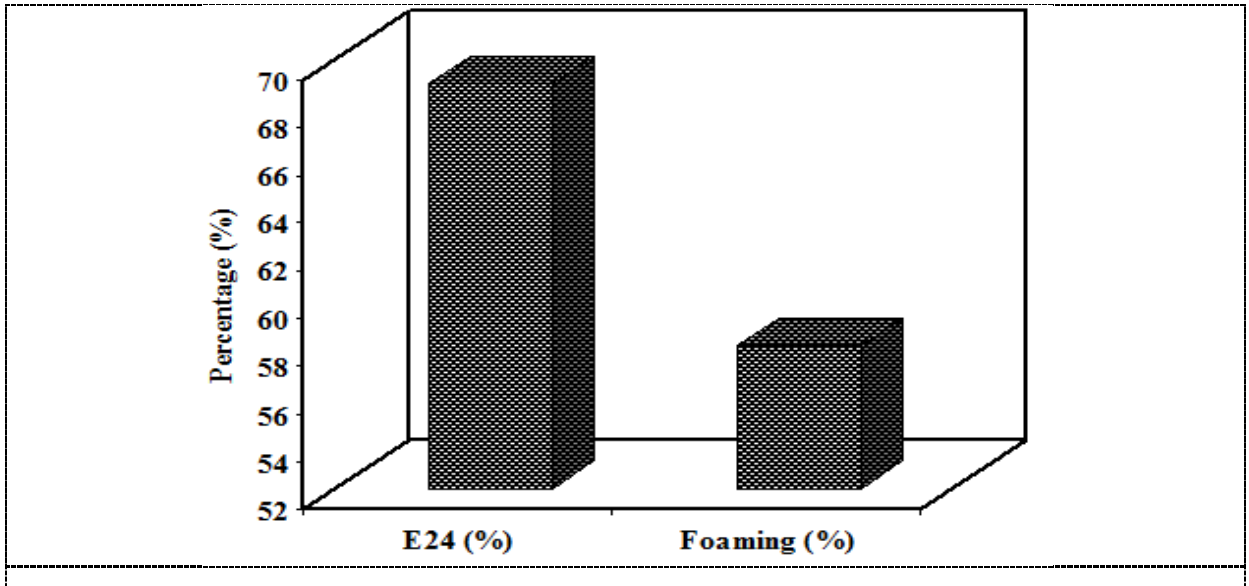

Figure 3. Emulsification index E24 and Foaming detection of the biosurfactant produced from the isolated strain.

3.4. MEOR Column Assay

Biosurfactant produced by the isolated strain was used in this assay and have high potential in decreasing surface tension in varied pH, high temperature and pressure. It shows a significant reduction of interfacial tension between water and light paraffin oil. Thus, it shows good potential for its utilization in MEOR. The additional oil recovery for sand1 and sand2 was measured after nullifying the control volume and was found to be 49% and 38%, respectively. This shows that the biosurfactant produced from isolated strain is highly efficient for MEOR.

4. Conclusions

The isolated strain was biosurfactant producing organism and indentified as Genus-*Pseudomonas* species *aeruginosa.* The isolate showed optimum growth at pH 7.0 and temperature 37℃. The biosurfactant produced by the isolate was detected as rhamnolipid and having significant reduced interfacial tension with good stability at harsh condition of the reservoir. The results show that the produced biosurfactant is highly efficient for MEOR.

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