

ANALYSIS OF MICROBIAL CULTURES FOR BIOSURFACTANT PRODUCTION IN MICROBIAL ENHANCED OIL RECOVERY IN BHOGPARA OIL FIELD OF UPPER ASSAM BASIN

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ABSTRACT

The global demand for energy has been steadily rising, driven by population growth, industrialization, and urbanization. This increasing energy demand necessitates the exploration of unconventional methods to maximize oil recovery from reservoirs. Enhanced Oil Recovery (EOR) techniques have emerged as a promising solution to extract more oil from mature and unconventional reservoirs. Among these techniques, Microbial Enhanced Oil Recovery (MEOR) has gained significant attention due to its eco-friendly nature and potential for improving oil recovery. This paper presents a comprehensive study on the application of MEOR using microbial cultures and biosurfactants for enhanced oil recovery.

The mechanism of MEOR involves the introduction of specific microbial cultures into oil reservoirs. These microorganisms produce biosurfactants that reduce the interfacial tension between oil and water, enhancing oil mobilization and recovery. In this study, microbes were revived and grown using suitable cultivation techniques. The cultures of *Pseudomonas*, *Bacillus*, and *Clostridium* were utilized for their known biosurfactant production capabilities.

To assess the effectiveness of biosurfactants, the interfacial tension between oil and water was measured, and contact angle analysis was conducted. The results demonstrated a significant reduction in interfacial tension, indicating the successful production of biosurfactants by the microbial cultures. The contact angle measurements further supported the effectiveness of biosurfactants in altering the wettability of the oil reservoir, promoting improved oil recovery. Finally, core flooding validated the result of higher recovery as it was increased by 11.92% and a dip in residual oil saturation was observed.

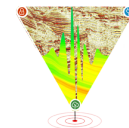
The successful production of biosurfactants by microbial cultures and their impact on interfacial tension and contact angle indicate their potential for enhancing oil recovery. The findings of this research emphasize the significance of MEOR as an environmentally friendly and effective technique, providing a pathway for sustainable oil extraction from reservoirs.

1. INTRODUCTION

Back in the day when the oil production first started there was one main mechanism that made it possible to get the oil up from under the ground, the pressure differential. Higher pressure in the reservoir than in the wellbore pushed the oil into the well and up what we call it as the primary recovery. But as oil is produced, the pressure in the reservoir decreases. This can be mitigated by drilling another well and injecting water or gas, and goes under the term secondary production. Again, this doesn't mean there is no more oil in the reservoir. So, reservoir engineers started devising even more advanced ways to get more. This includes injection of exotic fluids into the reservoir. This is called EOR.

1.1. ABOUT MEOR

Conventional recovery techniques often leave a significant portion of the oil trapped underground. To address this issue, researchers and engineers have been exploring unconventional methods, such as Enhanced Oil Recovery (EOR), to extract a greater amount of oil from reservoirs. Among these techniques, Microbial Enhanced Oil Recovery (MEOR) has emerged as a promising and sustainable approach, harnessing the power of microorganisms and their ability to produce bio-surfactants. MEOR involves the introduction of specific microbial cultures into oil reservoirs to stimulate oil recovery.



1.2. REASON FOR SELECTION OF UPPER ASSAM BASIN

The research emphasizes on recovery of the unswept oil from the pore spaces of the porous media with maximum feasibility to explore the reservoir of Upper Assam basin. The core sample allotted was of Bhogpara oil field. The oil field is the depleted and matured oil field of Upper Assam Basin. The recovery of oil in this field is getting decreased day by day as conventional methods are unable to recover more oil. The demand for using an unconventional method becomes an attractive option for the matured oil fields.

1.3. NOVELTY OF THE RESEARCH

The research conducted presents a comprehensive analysis of different microbial cultures, namely *Pseudomonas*, *Bacillus*, and *Clostridium*, for biosurfactant production in the context of Microbial Enhanced Oil Recovery (MEOR). This study explores the potential of these microbial cultures as alternative sources of biosurfactants for oil recovery, offering a novel perspective in the field. The research goes beyond the typical focus on chemical surfactants and instead investigates the use of biosurfactants produced by microbial cultures. This approach presents a sustainable and eco-friendly alternative for enhancing oil recovery, highlighting the novelty of the research.

Moreover, the study delves into the specific mechanism of biosurfactant production by the microbial cultures and assesses their performance through various analytical techniques. By measuring interfacial tension and contact angle, the research provides a detailed understanding of the effectiveness of the biosurfactants in altering oil-water interactions and facilitating oil recovery.

Additionally, the research addresses the cost-related challenges associated with biosurfactant production in MEOR. While commercial biosurfactants can be expensive, this study proposes a cost-effective solution by synthesizing biosurfactants using the microbial cultures rather than relying on purchased products. This aspect further emphasizes the novelty of the research, as it offers a viable and economical approach for biosurfactant formulation.

2. EXPERIMENTAL ANALYSIS

2.1. MATERIALS USED

1. Lyophilised cultures- *Pseudomonas*, *Bacillus*, and *Clostridium*
2. Eosin Methylene blue broth (EMB), Nutrient broth & cooked meat medium
3. Bushnell Haas Broth

2.2. METHODS EMPLOYED

2.2.1. REVIVAL AND GROWTH OF MICROBES

As per literature study, it was found that *Pseudomonas*, *Bacillus* and *Clostridium* are the microbe species which yield maximum Biosurfactants. So the above three products were ordered from MTCC Chandigarh. The products are in Lyophilised form or freeze dried form. Lyophilised cultures are supplied in vacuum sealed ampoules.

Before we make use of the species, we have to prepare the nutrient mixture. Here the nutrient mix is termed as nutrient broth in Biotechnological terms.

- a. Check the number of the culture on the label inside the ampoule.
- b. Make a file cut on the ampoule near the middle of the plug (see Figure: 1).

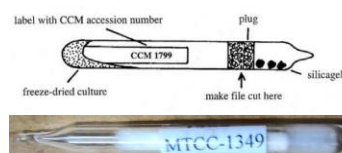


Figure: 1

- c. Disinfect the ampoule with alcohol-dampened gauze or alcohol-dampened cotton wool from just below the plug to the pointed end.
- d. Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule.
- e. Remove the pointed end of the ampoule into disinfectant.
- f. First of all we've to prepare the broth corresponding to the species being ordered.
- g. For *pseudomonas*, *Bacillus* & *Clostridium* the broths used were Eosin Methylene blue broth (EMB), Nutrient broth & cooked meat medium respectively.



Figure: 2

- h. Add about 0.3 ml appropriate broth to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid creating aerosols. Transfer the contents to one or more suitable solid and /or liquid media (Figure: 2).
- i. Incubate the inoculated medium at appropriate conditions for several days.
- j. Autoclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding.

After the initial revival of above mentioned species we will start preparing the nutrients for production of Bio-surfactants for individual species.

2.2.2. PROCEDURE FOR BIO-SURFACTANT PRODUCTION

- a. Grow bacteria in Nutrient broth for 24 hours. (figure: 3)
- b. Bushnell Haas Broth (300 mL) Autoclave.
- c. Cultured Nutrient broth (1mL) + BH broth.
- d. Incubation 37 deg C for 5 days.

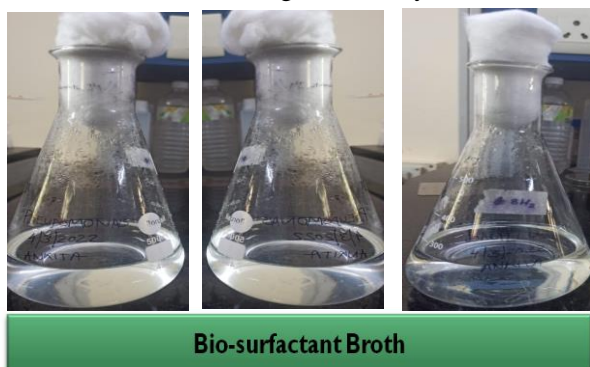


Figure: 3

Through Emulsification Index (EI) test it was found that *Pseudomonas* produced the maximum Bio-surfactants.

2.2.3. CORE ANALYSIS

For using unconventional methods like EOR requires proper nourishment of the core sample by cleaning it thoroughly and a proper characterization of porous media.

Core analysis includes cleaning of the core samples, drying in the oven & measurement of porosity.

Core sample was cleaned in the Soxhlet apparatus shown in figure: 4. Then the core was dried in Humidifier Oven at 37 deg C. Next using saturation method the porosity was found.



Figure: 4

2.2.4. Fluid Formulation

2.2.4.1. Interfacial tension (IFT)

The IFT test was conducted in KRUSS Easy Dyne Tensiometer, which is intended only for measurements of the surface tension of liquids, the interfacial tensions between two liquids and measurements of the density of a liquid. The principle of the measuring method is the attractive forces between molecules (cohesion), for which, a certain work is necessary to change the size of a liquid interface or surface.

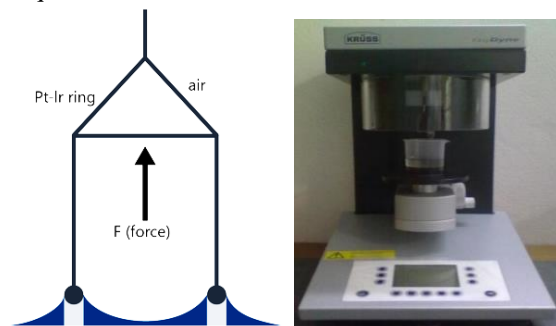
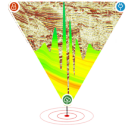


Figure: 5



The Easy Dyne measures the surface or interfacial tension with a measuring probe suspended from a force sensor. This probe is a ring consisting of a material with optimum wetting properties material (platinum iridium) as shown in figure 5.

2.2.4.2. Contact angle measurement

Wettability is the tendency of one fluid to spread onto or adhere to a solid surface in the presence of other immiscible fluids. Wettability alteration is one of the main mechanisms to enhance oil recovery. Figure: 6 shows a schematic of the angle between core disc and the Microbial slug drop. Device used to measure the contact angle is KYOWA Interface Science Co Ltd. (figure: 7)

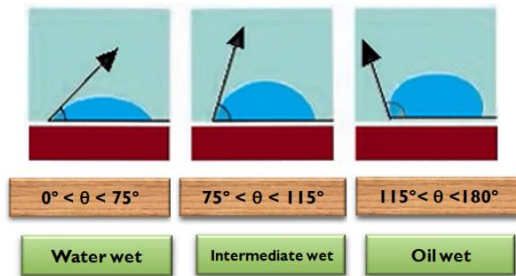


Figure: 6



Figure: 7

2.2.5. Recovery using core flooding experiment

Core flooding experiment was done to calculate the recovery efficiency by secondary brine flooding (3000 ppm) and Microbial slug. Pore volume of the rock sample was calculated by saturation method in

vacuum chamber. The core was placed in the Hassel core holder in core flooding instrument made by D.Cam engineering (Core flooding Instrument).



Figure: 8

2.2.5.1. Calculation involved

$$S_{oi} = \frac{\text{Volume of liquid-line volume}}{\text{Pore volume}}$$

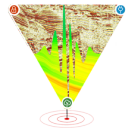
$$\text{Recovery \%} = \frac{S_{oi} - S_{or}}{S_{oi}}$$

3. RESULTS AND DISCUSSION

3.1. Core Analysis:

	Bhogpara
Diameter of the core	37.30 mm
Length	50.25 mm
Dry weight	127.59 g
Bulk volume	$\pi/4 * (D^2) * L$ =54.88 cc
Saturated weight	137.73 g
Porosity	Next we prepared a 4000 ppm Brine solution for saturating both the core samples. As per SOP, 4g NaCl powder was mixed with 1000 mL distilled water. After measuring the saturated weight using the analytical method of saturation we measured the porosity. Density of brine = 1g/cc Since, porosity = P.V/B.V Porosity = (saturated wt-dry wt)/(density)*(B.V)
	18.47%

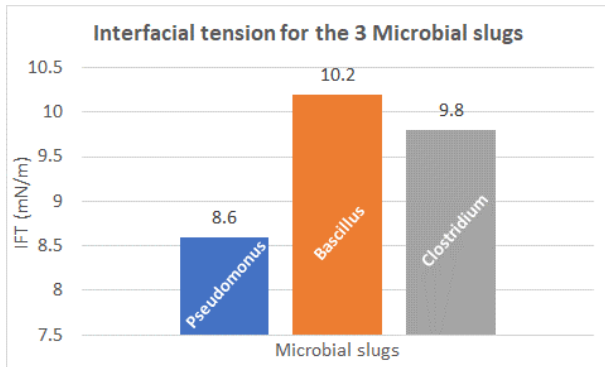
The porosity of the Bhogpara core sample was found to be **18.47%**.



3.2. Fluid Formulation

3.2.1. IFT

The graph: 1 displays Interfacial tension (in mN/m) vs. the microbial slugs being prepared for testing.

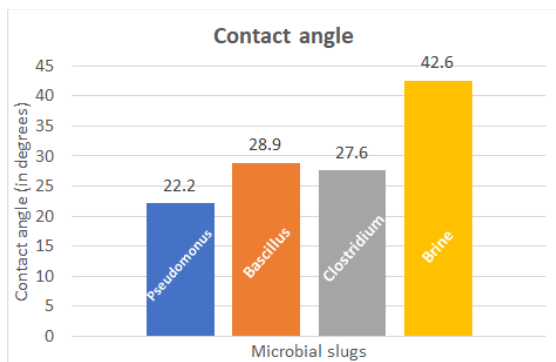


Graph: 1

From the graph it is clear that sample no 1 i.e., (Pseudomonas microbial slug) displays least IFT value w.r.t the other samples are considered.

3.2.2. Contact angle

Graph: 2 displays contact angle (in degrees) vs. the samples being prepared for testing. The graph is used for interpreting the wettability condition for the core sample in use which is of Bhogpara.



Graph: 2

It is observed from the contact angle graph that sample no. 1 (Pseudomonas microbial slug) yields the lowest contact angle value.

Hence, **sample no 1 (Pseudomonas microbial slug) is**

selected as candidate for core flooding experiment.

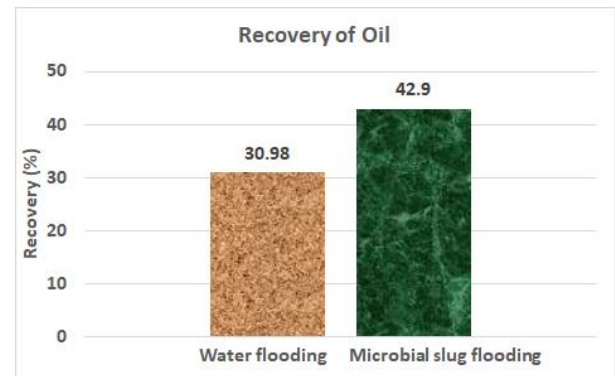
3.3. CORE FLOODING

Core sample	Initial oil saturation (Soi) (fr)	Secondary recovery (%)	Sor
Bhogpara	87.71	30.98	56.73

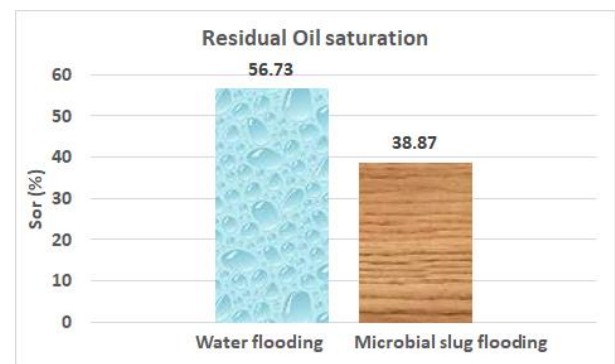
Table: 1a

Core sample	Initial oil saturation (Soi) (fr)	Recovery (%)	Sor	Total ultimate recovery (%)
Bhogpara	56.73	11.92	38.87	42.9

Table: 1b

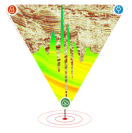


Graph: 3



Graph: 4

Water flooding recovered 30.98% of Oil. While MEOR



slug/fluid extracted an additional recovery of 11.92%.
Total ultimate recovery was found to be 42.9%.

4. Conclusion

The energy crisis faced in the past few years by the decreasing oil prices and the restriction of recovering oil by conventional methods has developed a huge barrier. This barrier is the difficulty to recover oil by unconventional methods because of their high operation cost and the high cost of the materials required to achieve a successful operation. Bhogpara is depleted and matured oil field of Upper Assam Basin. The recovery of oil in this field is getting decreased day by day as conventional methods are unable to recover more oil. The demand for using an unconventional method becomes an attractive option for the matured oil fields. Upper Assam basin is one of the most oil-producing provinces in India. It is a good candidate for CEOR.

First of all the core sample of Bhogpara Oil field was cleaned in Soxhlet apparatus using toluene. Then it was oven dried and finally the porosity was measured using Saturation method which had the usage of vacuum pump. The porosity came out to be 18.47 %. The microbes in the Lyophilised form was revived and then grown. Next proper nutrient was feeded to the microbes for the production of Biosurfactants. As per turbidity check & Emulsification index test it was found that Pseudomonus yielded maximum Biosurfactants which was validated by measuring the contact angle (i.e., wettability) & Interfacial test. Both the contact angle and IFT test proved that Pseudomonus produces the maximum Biosurfactants out of the 3 considered. So pseudomonas was selected as candidate for core flooding.

Core flooding validated the fact of higher recovery as recovery was increased by 11.92 % by making the use of microbial slug. Total ultimate recovery was found to be 42.9 % and a dip in residual oil saturation was observed. The higher recoveries by the microbial slug could be attributed to the reduction of IFT & alteration/reduction of wettability. The results of the experimental findings highlighted the successful implementation of the synergic application of microbial slug for improving recovery efficiency.