

Biosurfactant Production by Microorganism for Enhanced Oil Recovery

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Abstract: Biosurfactants are a group of surface active agents which are produced by microorganisms. In this research a bacterial species with the ability of producing biosurfactant was isolated from soil. The bacterium was able to grow on gas oil as the sole source of carbon and energy. Concentrations of gas oil up to about two percent were not detrimental to the bacterial activity. The excess gas oil in the fermentation medium served as an extractant for the biosurfactant. Separation of the organic phase from the aqueous phase and evaporation of the gas oil resulted in a powder of crude biosurfactant. Each liter of the fermentation medium gave about 2.8 grams crude biosurfactant. The critical micelle concentration of the powder was 100 mg/L. The biosurfactant reduced the surface tension of distilled water to 26mN/m. An aqueous solution of the biosurfactant was used to enhance oil recovery in a high permeable and a low permeable laboratory core. The solution enhanced the oil recovery up to 15% in the high permeable core at ambient temperature. This figure was 7.5% in the low permeable core. The biosurfactant retained its activity in enhanced oil recovery when the temperature of the core increased to 80 °C.

Key words: Biosurfactant, Critical Micelle Concentration, Extraction, Microbial Enhanced Oil Recovery.

1 INTRODUCTION

Biosurfactants are a group of surface active molecules that are produced by a variety of microorganisms. Like chemical surfactants, biosurfactants have hydrophobic and hydrophilic moieties. The hydrophilic moiety of a biosurfactant is a carbohydrate (mono, di, or polysaccharides), an amino acid or a peptide, and the hydrophobic moiety is a saturated or unsaturated fatty acid and accordingly biosurfactants are classified as glycolipides, lipoproteins, lipopeptides, polymeric, and particulate biosurfactants [Don, et al, 2003. and Desai, et al, 1997]

Due to environmental concerns about the use of chemical surfactants, there is a growing interest to replace these chemicals by biosurfactants. The potential applications of biosurfactants have been examined in different areas. Biosurfactants have been used in flotation for the removal of metal ions, in bioremediation, in enhanced oil recovery processes, in gene transfer, and in food, pharmaceutical, and cosmetic industries [Christova, et al. 2004, Strappa, 2004, Gregory, et al. 2004] Different microbial species have been used in biosurfactant production. Among the species cited in the literature are *Pseudomonas putida* [Strappa, et al, 2004, Ghadiri, 2003, Syyoun., et al, 2002], *Actinomyces*, *Bacillus subtilis*, *Lactococcus lactis*, *Lactobacillus* strains [Rodrigues, et al, 2006, Rodrigues, et al, 2006.], *Streptococcus thermophilus*, *Bacillus licheniformis*, *Pseudomonas aeruginosa*, *Nocardoides sp.* *Bacillus pumilus*, *Aeromonas sp.* *Serratia sp.* *Rhodococcus* strains, and *Candida ingens* [Rodrigues, et al, 2006].

Both soluble and insoluble carbon substrates have been used for biosurfactant production. Glucose, glycerol, cheese whey, molasses are soluble substrates that have been tested for biosurfactant production. Among insoluble substrates, different kinds of vegetable oils and different hydrocarbons have been used to produce biosurfactants. Insoluble substrates have been reported to be superior in biosurfactant production [Saikr-

shna, et al, 2007., Rodrigues, et al, 2006, Rodrigues, et al, 2006.].

The advantage of bacteria in oil recovery is a well known phenomenon. Bacteria can enhance oil recovery in different ways. They produce gases like carbon dioxide, organic solvents and some surface active agents (biosurfactant). All these can help oil mobilization in reservoirs. Active microorganisms can grow in the reservoirs and block larger pores. This causes the fluid to pass through smaller pores in subsequent flooding.

Three methods have been suggested for microbial enhanced oil recovery. In the first method biosurfactant and/or organic solvents are produced at the surface and the whole fermentation medium is then introduced to the reservoir. In the second method proper microbes are introduced to the reservoir for *in situ* production of gases, solvents, and biosurfactants. In the third method only selected nutrients are injected to the reservoir to stimulate the activity of indigenous bacteria [Rodrigues, et al, 2006]. The disadvantage of injecting the whole fermentation medium to the wells is that the concentration of the useful components like organic solvents and biosurfactants might not be high enough to be effective. Moreover, the fermentation medium contains various constituents like mineral salts and carbohydrates that contaminate the reservoir without contributing to enhanced oil recovery. The second and third methods are applicable only to those reservoirs having compatible environmental conditions (pH, temperature, salinity, nutrient availability) for microbial growth. The drawback for all the three methods is that the efficiency of the methods is not predictable and field trial needs to be done for each specific reservoir [Ghadiri, 2003, Syyoun, 2002, Gregory, 2002, Saikrshna, 2007, Rodrigues, et al, 2006].

Ex situ production and purification of biosurfactants, and application in enhanced oil recovery in known concentrations, is

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an alternative to the existing MEOR methods. Biosurfactants can replace the chemical surfactants. The application of biosurfactants in purified or concentrated forms has not been widely investigated. It is generally believed that using biosurfactants in purified or concentrated forms are costly. In this research we propose a simple method for simultaneous production and separation of a biosurfactant and its application to enhanced oil recovery.

2 MATERIALS AND METHODS

2.1 Mineral medium

A mineral medium with the following composition was used for fermentation:

KH_2PO_4 ; 3.4 gr/lit, K_2HPO_4 ; 4.3 gr/lit, NaNO_3 ; 4 gr/lit, $\text{MgCl}_2 \cdot 7\text{H}_2\text{O}$; 0.2 gr/lit, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 0.04 gr/lit, FeSO_4 ; 0.03 gr/lit, MnCl_2 ; 0.001 gr/lit, NaMoO_4 ; 0.002 gr/lit, CuSO_4 ; 0.0001 gr/lit H_3BO_3 ; 0.0003 g/lit, ZnSO_4 ; 0.0015 gr/lit.

All of the minerals were of analytical grade.

2.2 Carbon source

Gas oil was used as the sole source of carbon and energy for microorganisms. It was obtained from a gas station.

2.3 Isolation of biosurfactant producing microorganisms

Soil samples were collected from different places in the campus. Water was added to each sample and mixed thoroughly. The solid residues were filtered using a filter cloth. One milliliter of each filtrate was added to a flask containing 50mL of

the mineral medium and 1mL gas oil. The flasks were incubated at 30°C. After 3 days the microbial growth was evident in some of the flasks. The flask with the highest turbidity was selected. The microbes from this flask were streaked on nutrient agar and stored at 4 °C for later experiments.

2.4 Biosurfactant production

A 2L jar was used as the bioreactor. The jar had ports for air passage. For each experiment, the jar was filled with 1L mineral medium and 4 mL gas oil. The jar was then inoculated with microorganisms. Air bubbled through the medium using a small air pump. The system was under ambient temperature (25-30 °C). The fermentation lasted for four days and 4 mL gas oil was added to the medium in each day. After fermentation, the whole medium was centrifuged. The microbial pellet was discarded. The organic phase was evaporated to obtain crude biosurfactant as a powder, and the aqueous phase was extracted once more with the half volume of gas oil to obtain any remaining biosurfactant.

2.5 Surface tension measurement

The surface tension of the liquid culture was measured using a digital tensiometer.

2.6 Core experiments

Two laboratory cores with the following characteristics were used to examine the effect of the biosurfactant in enhanced oil recovery.

Table 1: characteristics were used to examine the effect of the biosurfactant in enhanced oil recovery

Core No.	Length (cm)	Diameter (cm)	%Porosity	Permeability(mD)	%Saturated water
1	38	6	20	56	25
2	38	4.5	40	2.5	18

the following composition was used in flooding:

Heavy oil (19 °API) was used for the experiment. Brine with

Table 2: Brine composition was used in flooding

Component	NaCl	KCl	MgCl ₂	KBr	CaCl ₂	NaHCO ₃	Na ₂ SO ₄	NaF
Concentration (gr /lit)	22.53	0.7	4.2	0.031	1.16	0.221	4.1	0.003

The cores were washed with toluene for two days and dried in an oven. After that, the cores were under vacuum for four hours and were then saturated with water and a heavy crude oil successively. The cores were flooded with brine and then with the biosurfactant solution (2 gr/lit).

3 RESULTS AND DISCUSSIONS

3.1 Microorganism

Microscopic observation showed that the isolated microorganism was a bacterium. Soil samples from garden are usually rich in bacteria which many of them are able to grow on hydrocarbons as the sole source of carbon and energy provided that all necessary minerals are present. Preliminary tests

showed that the species was able to reduce the surface tension of the mineral medium from 51 $\text{mN} \cdot \text{m}^{-1}$ to 27 $\text{mN} \cdot \text{m}^{-1}$ indicating that it was a biosurfactant producer. The species were not identified

3.2 Biosurfactant production

Figure (1) shows the fermentation medium after it was centrifuged. Two distinct phases can be seen. The remaining biosurfactant in aqueous phase was extracted with gas oil (Figure 2). The results of three replicate of the experiment have been presented in table 3. Much of the biosurfactant accumulated in the organic phase. The organic phase could be separated from the aqueous phase using a decanter easily. Evaporation of the gas oil resulted in a white powder.

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Addition of excess gas oil was advantageous in extraction of the biosurfactant simultaneous to fermentation. Although application of concentrated biosurfactant has rarely been examined in enhanced oil recovery, recovery of biosurfactants from fermentation media has been done for analytical purposes.



Figure (1): The fermentation medium after it was centrifuged

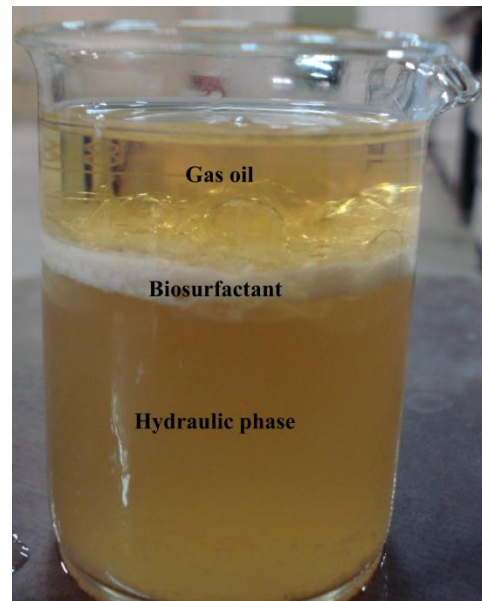


Figure (2): Extraction of the aqueous phase with gas oil
Biosurfactants are usually separated from the fermentation medium by methods such as salting out (saturation of the fermentation medium for precipitation biosurfactant), solvent precipitation (addition of a miscible solvent to the fermentation medium for precipitation of biosurfactant), and solvent extraction. All of the methods require addition of a material to the medium after fermentation. The method proposed here seems to be more economical than existing methods for biosurfactant production.

3.2.1 The effect of concentration of minerals on biosurfactant production

The composition of the mineral medium was adopted from reference [8]. The medium was claimed to be excellent for hydrocarbon degraders. Since the concentrations of some of the medium constituents are rather large, biosurfactant production was examined under lower concentrations.

Table3: Results of biosurfactant production using initial mineral medium in three runs

Run No.	Biosurfactant in organic phase (g)	Biosurfactant in aqueous phase after extraction with gas oil (g)	Total biosurfactant per liter of mineral medium (g)
1	1.95	0.90	2.85
2	1.88	0.84	2.72
3	2.07	0.75	2.82

For this purpose two mineral media were prepared having all components but in concentrations half and one third of those mentioned above, respectively. Biosurfactant production in these media was compared with the initial medium. The results are in Fig.3. Reducing the concentrations causes the biosurfactant production to decrease. The decrease was not much significant when the concentrations of the minerals decreased to half.

3.3 Critical micelle concentration of the biosurfactant

Different concentrations of the biosurfactant in water were prepared. The surface tensions of the solutions were measured and the critical micelle concentration of the biosurfactant was determined. Fig.4 shows the result. The critical micelle concentration of the biosurfactant is rather small (100 mg/L) expressing its potential economical use in enhanced oil recovery.

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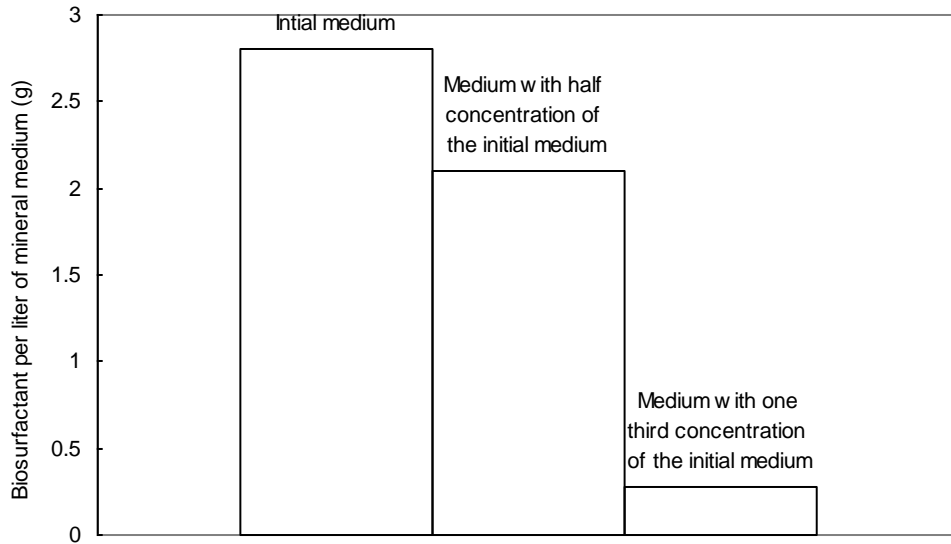


Figure (3): The effect of the minerals concentration on biosurfactant production

3.4 Using biosurfactant in enhanced oil recovery

A $2\text{g}\cdot\text{L}^{-1}$ solution of the biosurfactant in brine was prepared and used in enhanced oil recovery in laboratory cores. The recovery process was performed at temperatures 25°C and 80°C . The results are presented in Figures 5 to 8. In each case the effect of biosurfactant has been compared with a chemical surfactant (Triton x-100). The results indicate that the effect of the biosurfactant in oil recovery is comparable with that of Triton x-100.

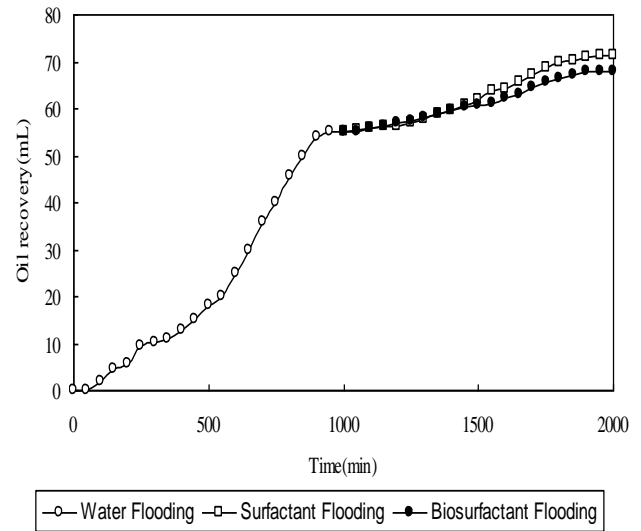


Figure (5): Oil recovery in the high permeable core at 25°C

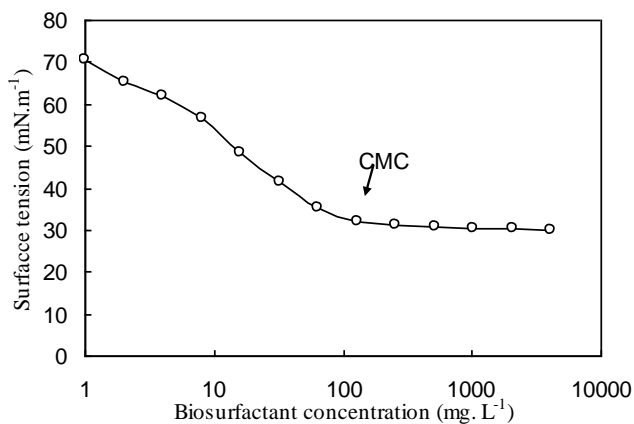


Figure (4): Critical micelle concentration of the biosurfactant

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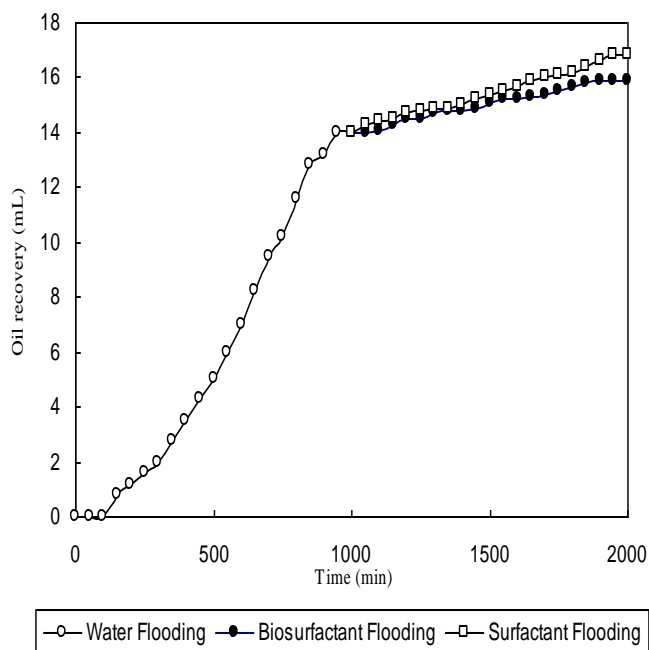


Figure (6): Oil recovery in the low permeable core at 25 °C

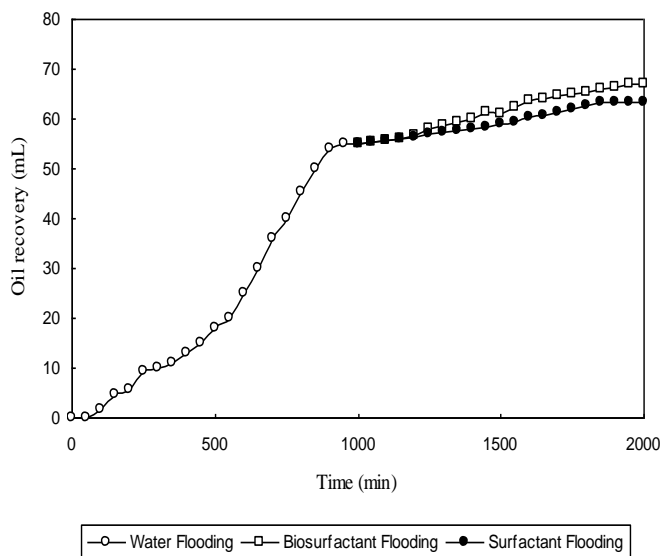


Figure (7): Oil recovery in the high permeable core at 80 °C

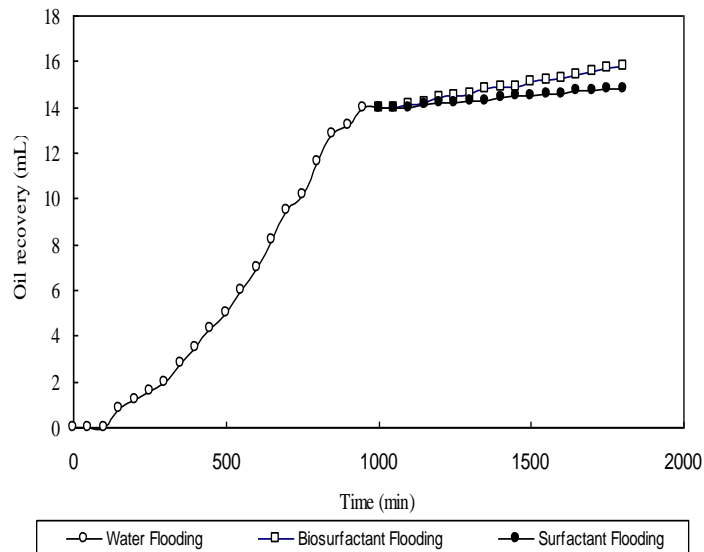


Figure (8): Oil recovery in the low permeable core at 80 °C

4 CONCLUSION

Gas oil is a proper substrate for biosurfactant production. It can serve as both substrate and extracting for the biosurfactant. The biosurfactant obtained here had the critical micelle concentration of 100 mg/L. The biosurfactant was successfully used in enhanced oil recovery in consolidated laboratory cores.

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