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Research Article

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Isolation, purification and production of biosurfactant by microorganism for enhanced oil recovery

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ABSTRACT

Biosurfactants are surface dynamic metabolites created by microorganisms. These are amphiphillic aggravates that are delivered on living surfaces, generally on microbial cell surfaces, or discharged extracellular. The uses of these organic mixes in the field of improved recuperation and bioremediation demonstrated viable. In this project a bacterial animal types with the capacity of delivering biosurfactant was secluded from soil. Division of the natural stage from the fluid stage and vanishing of the gas oil brought about a powder rough biosurfactant. Every liter of maturation medium gave around 2.8 grams rough biosurfactant. The biosurfactant diminished the surface pressure of refined water to 26mN/m. A watery arrangement of the biosurfactant was utilized to upgrade oil recuperation in a high penetrable and a low porous research facility centre. The arrangement improved oil recuperation upto 15% in the high penetrable centre ate encompassing temperature. This figure was 7.5% in the low porous centre expanded to 80^{0}c.

Key words: Biosurfactant, Microorganism, Soil, Oil, Surface activity, Turbidity, Bacterial Culture.

INTRODUCTION

Biosurfactants are class of surface active molecules that are synthesized by microorganisms. Biosurfactants are regarded as chemical compounds. They are produced extracellular as a part of the cell membrane by microorganisms (i.e., by a biological pathway). Biosurfactants are amphiphillic compounds that are produced on living surfaces mostly on microbial surfaces or excreted extracellular[1-5]. They contain both hydrophobic and hydrophilic moieties which partitions preferentially at the interfaces such as liquid/liquid, solid/liquid, and gas/liquid interfaces.

Compared to the surfactants biosurfactants are more effective, selective, stable and environmental friendly. Biosurfactants are ecologically safe and can be applied in process like bioremediation. These biosurfactants play a very important role in living systems and can be regarded as the backbone of the biological membranes which promise the transport and exchange of various important materials.

High molecular weight biosurfactants are produced from a number of different bacteria and comprise lipoproteins, proteins, polysaccharides, lipopolysaccharides, or complexes containing several of these structural types, many of which have yet to be fully characterized [6-8].

This work is conducted because biosurfactants are produced by variety of microorganisms as extracellular compounds. These are superior to the chemical surfactants with respect to surface tension, interfacial activity, temperature and P^{H}. The role of biosurfactants is having a high demand in the field of biotechnology. As biosurfactants are low toxicity in nature they have wide range in industrial applications. Inorder to increase the industrial applications with low cost (i.e., to perform work in economical way) in the fields like food, cosmetic and pharma, Petrochemical, Bioprocess and oil making industries.

EXPERIMENTAL SECTION

PREPARATION OF MINERAL MEDIUM

A mineral medium with the following composition was used for fermentation:
KH_2PO_4:-3.4gr/lit, K_2HPO_4:-4.3gr/lit, $NaNO_3$:-4.0gr/lit, $Mgcl_2.7H_2O$:-0.2gr/lit, $CaCl_2.7H_2O$:-0.04gr/lit, $FeSO_4$:-0.03gr/lit, $Mncl_2$:-0.001gr/lit, $NaMoO_4$:-0.002gr/lit, $CuSO_4$:-0.001gr/lit, H_3BO_3:- 0.003gr/lit, $ZnSO_4$:- 0.0015gr/lit
All the minerals must be of analytical grade.

CARBON SOURCE

Gas oil(palm oil) is used as carbon source and energy for microorganisms.

ISOLATION OF BIOSURFACTANTS

❖Soil samples are collected from different places.
❖ To each sample water was added and mixed thoroughly.
❖The solid particles were filtered using a filter paper or a cloth.
❖To a conical flask 1mL of gas oil and 50mL mineral medium was taken.
❖5mL filtrate was taken and added to the conical flask which contains 50mL mineral medium and 1mL gas oil.
❖The flasks were incubated at 30^{o}c for 72hours.
❖The flasks have evident microbial growth after 3days
❖The flask with highest turbidity was selected.
❖The microbes from that flask is selected and streaked on a agar plate.
❖The plate was stored at 4^{0}c for later experiments.

BIOSURFACTANT PRODUCTION:

❖A 2 liter jar was used as the bioreactor which should have the space for air passagers.
❖For every step of the experiment in production 1Litre of mineral medium and 4Litre of gas oil must be added to the flask.
❖The jar was then inoculated with microorganisms and the air is bubbled through the medium using small air pumps.
❖The system must be maintained at ambient temperature i.e., at 25^{o}c-30^{o}c.
❖The fermentation must be done for 4 days and 4mL gas oil was added each day to the flask during the fermentation.
❖After fermentation the medium should be centrifuged and microbial pellet must be discarded.
❖The organic phase of the medium is 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.

SURFACE TENSION MEASUREMENT

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

CORE EXPERIMENTS

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

RESULTS AND DISCUSSION

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 $mN.m^{-1}$ to 27$mN.m^{-1}$indicating that it was a biosurfactant producer. The species were not identified [9-10].

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. Addition of excess gas oil was advantageous in extraction of the biosurfactant simultaneous to fermentation[11-12]. 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: Fermentation medium after centrifugation

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 as shown in figure 4.

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 [13-15].

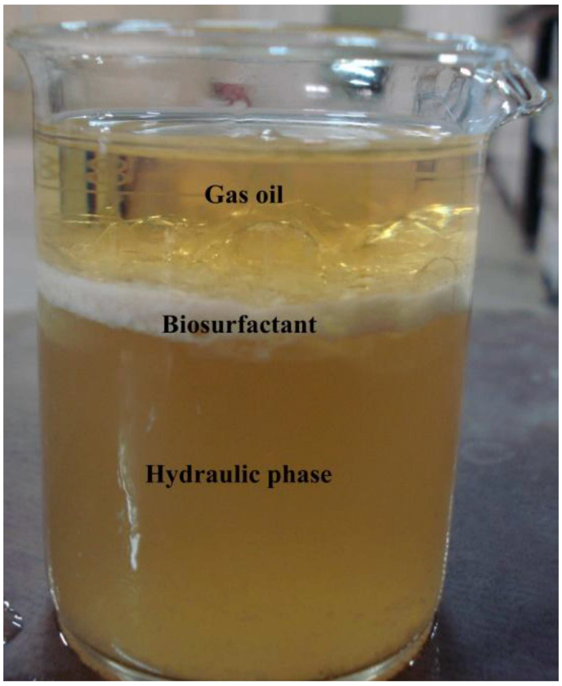

Figure 2: Extraction of the aqueous phase with gas oil

The effect of concentration of minerals on biosurfactant production

The composition of the mineral medium was adopted from reference ®. 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.

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

Core No	Length (cm)	Diameter (cm)	% Porosity	Permeability	% Saturated Water
1	40	8	28	62	35
2	40	6.5	48	4.5	28

The cores were washed with toluene for two days and dried in an oven. After that, the cores were under the 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.

Table 2: The following composition was used for the flooding heavy oil (19^{o} API) was used in the experiment. Brine with Composition was used in flooding

Component	Nacl	Kcl	$Mgcl_2$	KBr	$Cacl_2$	$NaHCO_3$	Na_2SO_4	NaF
Concentration (g/lit)	28.64	0.9	6.8	0.045	2.28	0.435	6.5	0.005

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

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

Using biosurfactant in enhanced oil recovery

A $2g.L^{-1}$ 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.

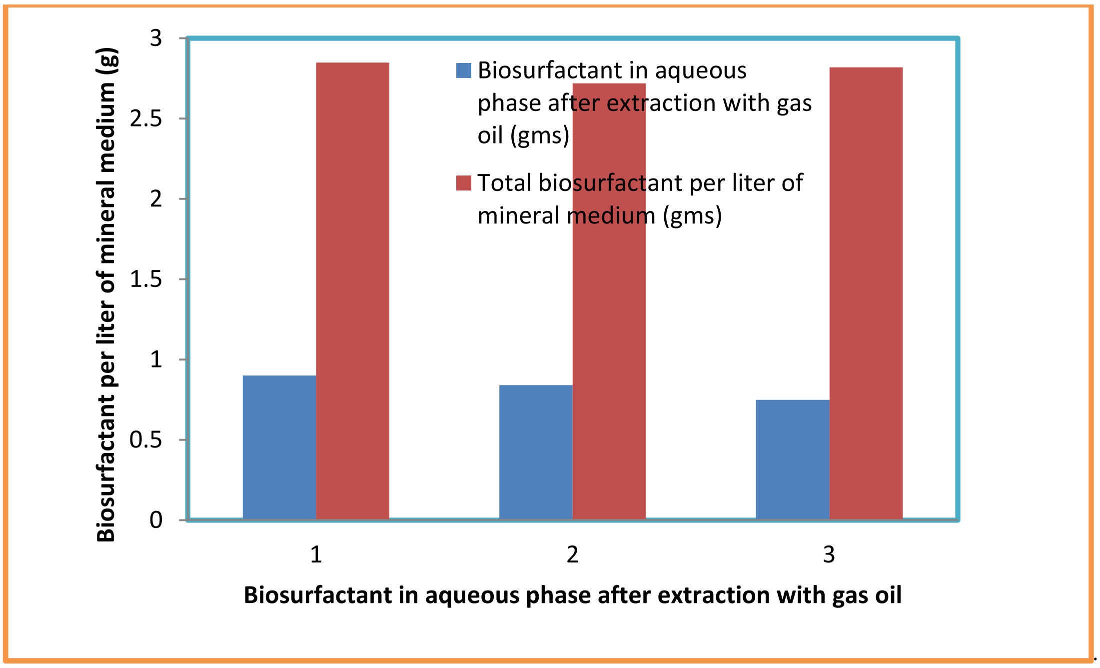

Figure 3: The effect of the minerals concentration on biosurfactant production

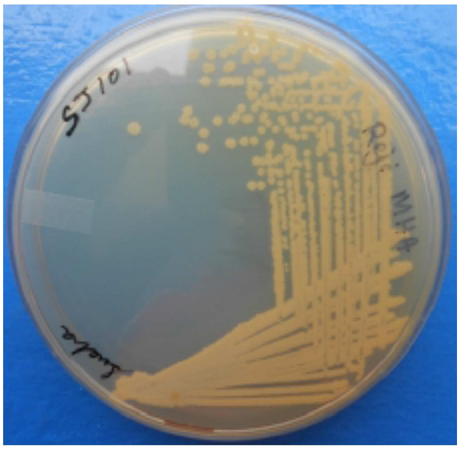

Figure 4: Microorganism developed on petriplate

APPLICATIONS OF BIOSURFACTANTS

Biosurfactants due to their broad range of functional properties and diverse synthetic capabilities they have potential applications in pollution control for enhanced oil revival, removal of heavy metal from contaminated soil and hydrocarbon in aquatic environment. Due to its unique nature low toxicity and they are utilized in replacement of chemically synthesized surfactants in large industrial operations. Now-a-days biosurfactants are playing very vital role due to the above mentioned applications. In addition to these thesebiosurfactants have role in industries like food, medicine industries and in pulp, paper and in ore processing.

BIOSURFACTANT ROLE IN

Potential Food Applications: Biosurfactants can be explored for several food-processing applications.

Used as food formulation ingredients: To control aggregation of fat globules, stabilization of aerated systems, improvement of shelf life period of products that contain starch, modification of rheological properties and in improvement of texture of fat based products. These surfactants are also used in ice cream formulations and rhamnolipids are used to improve the properties of butter cream and frozen confectionery products.

Medical industry: In medicine industry they are used in treatment of succinyltrehalsoe lipid of rhodococcuserthropoles has been reported to inhibit herpes simplex virus and influenza virus. It is also used in treatment of leaves of nicotiana infected with TMV to control potato virus-X-diseases.

Used as Antiadhesive agents: Controlling the adherence of microorganism to food-contact surfaces is an essential step in providing safe and quality products to consumers. These antiadhesive agents are used to control the spoilage of food and transmission of disease and give best product to the consumers.

Used as agents for respiratory failure: A phospholipid protein complex is responsible for the failure of respiration in prematurely born infants. Isolation of genes for protein molecules of this surfactant and cloning in bacteria has made possible its fermentative production for medical applications.

In environmental pollution control: It can be used in handling industrial waste gas pollution, control of oil spill, detoxification of industrial effluents, and bioremediation of contaminated soil.

Economic production: biosurfactants can be produced from industrial wastes and by products
Eg:- use of petroleum related technology

Therapeutic and biomedical applications and antimicrobial activity:
The action of biosurfactants is against algae, fungi, bacteria and viruses. Rhamnolipids repressed the growth of harmful bloom algae and the rhamnolipid mixture obtained from *P.aeruginosa* shows inhibitory activity against the bacteria. *E.Coli* shows excellent antifugal properties against *Aspergillus niger*. The rhamnolipids and sophorolipids were found to be effective antifugalagents plant and seed pathogenic fungi. *Candida antartica*, has shown antimicrobial activity against gram positive bacteria.

Anti-immunodeficiency virus:
Sophorolipid synthesized by *Candida bombicola* has challenged the increased incidence of HIV/AIDS in women aged 15-49 years has identified the urgent need for female-controlled, effective and safe vaginal topical microbicide. The virucidal activity against HIV and sperm-immobilizing activity against human semen are similar to those of nonoxynol.

CONCLUSION

Microscopic observations showed that the isolated microorganism was a bacterium. Soil samples are usually rich in bacteria and provided all necessary minerals are present. Preliminary tests showed the species which were able to reduce the surface tension of the mineral medium indicating that it was a biosurfactant.

The fermentation medium after centrifuged 2 distinct phases can be seen. The remaining biosurfactant in aqueous phase was extracted with gas oil.

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