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Review Article

Analysis and Characterization of Biosurfactant Producing Microbes from Crude Oil Contaminated Soil

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Abstract

Synthetic organophosphate compound are used as agriculture and pesticides and could be chemical agent. The degradation of organophosphate in soil has gained lot of importance due to increase the quantity of its use all over world. The biodegradation of organophosphate was carried out by bacteria, fungi and algae effectively. The biodegradation of organophosphates in flask quoted condition were study by an isolated pseudomonas from the soil under laboratory condition. The biodegradation procedures wereexecuted by the enrichment technique. The biodegradation rate by pseudomonas enhance the rate of biodegradation to 20%- 30% addition of the biosurfactant produce from the pseudomonas enhance the rate of biodegradation to 30% - 45%. The produced biosurfactant were found to be specific to organophosphate.

Keywords: Biosurfactant, crude oil, HPLC, E24, degradation, MSM

Introduction

Environmental pollution due to chemicals,hydrocarbons, solvents and heavy metals are veryharmful to living organisms including human beings and also in developing countries.These pollutant degrading microbes can be classified and appropriately used to eliminate the contaminants to produce value added products. Crude oil, crucial pollutant that involve a low-high molecular weight hydrocarbons (alkanes,asphaltenes , resins and aromatics) in compound **[12].**Biosurfactants are synthesized by microbes that exploit different substrates (oils, hydrocarbons and simple sugars) from polluted environment.Biosurfactants have the capability to slash and interface amongst substanceand lead to dispersed them as emulsions in liquid**[14]**. It is widely believed that oil is not a preferred habitat for microbes because of its potential toxicity and high hydrophobicity **[1]**. However, recent studies have shown that microorganisms including *Acinetobacter, Propionibacterium, Sphingobium, Bacillales, Burkholderia*, and *Brevundimonas* are present in crude oil phase **[18, 19]**. In a recent study, we observed alive microbial cells in crude oil, which could be stimulated to bloom by introduction of other bacteria **[9]**.

Biosurfactantsare broadlyused for numerous purpose (oil recovery, cleaning, food processing industryand crude oil drilling lubricants)**[8, 13]**. Biosurfactants have probable advantage, i.e., eco-friendly, easily degradable, active in any extreme conditions (high salinity/temperature regions)**[5].**In many report the application of biosurfactant producing microbes in the petroleum contaminated environments to eliminate

hydrocarbon and remediate the environment **[6, 11]**.

Material & Method-

Isolation and characterization of biosurfactant-

Petroleum crude oil was obtained from petroleum reservoir for oil field. Biosurfactant microorganisms were isolated by applying enrichment culture technique using minimal salt medium (MSM). The composition of MSMmedium was as described before**[10]**. Biosurfactant microorganisms was used as a sole source of carbon in MSM and incubated at 28 ^{o}C on a rotary shaker at 200 rpm for 7 days. Following the Incubation period, 1mLof culture was transferred to fresh medium and re-incubated for another 7 days. During all cycles of enrichment,salinity was maintained by adding NaCl (30gL-1) in MSM. Following consecutive five cycles of enrichment, 1 mL of culture was diluted and plated on solidified MSM with agar.

Screening for biosurfactant production-

Surfactants are amphipathic molecules that can modify the properties of a liquid medium at a surface by reducing surface tension. The culture growth in 100 ml of mineral media incorporated with 5, 10 and 25 ppm tafgor and nuvan was taken and centrifuged at 10,000 rpm at 4°C for 15 minutes, and the supernatant was separated. This supernatant was challenged with 5, 10 and 25 ppm tafgor and nuvan to check for turbidity, which directly indicated the production of biosurfactant**[7, 17].**

Test for specificity of biosurfactant (Emulsification Index)

For imulsification of biosurfactant 1ml of biosurfactant was added to nine test tubes. Then in first three test tubes, 10 µl of 5, 10 and 25 ppm Methyl Parathion was added, in the next three test tubes, 10 µl of 5, 10 and 25 ppm. Dichlorvos was added, and in last three test tubes, 10 µl of 5, 10 and 25 ppm Chlorpyrifos was added. After a few seconds, the turbidity was found according to degradation of organophosphate**[9]**.

Role of biosurfactant degradation

The biosurfactant produced by the organism is capable of degrading the pesticide that will enhance the degrading rate **[2]**. Three conical flasks (100 ml) were taken for each pesticide. First, with mineral medium and 5, 10 and 25 ppm of concerned organophosphate, is kept as control. The second flask contained the mineral medium incorporated with 5, 10 and 25 ppm of concerned organophosphate, 1ml of culture supernatant, which contained the biosurfactant, and inoculated with isolated organism. These flasks were then incubated in the rotary flask at 200 rpm for 2 weeks. The growth rate of each flask was studied on the third, fifth, seventh, ninth, eleventh, thirteenth and fifteenth day by using a UV spectrophotometer at 660 nm.

Assay of biosurfactant

The assay of the biosurfactant was done in the laboratory conditioned by the following procedure: The culture growth in 100 ml of mineral media incorporated with 5, 10 and 25 ppm of organophosphate (Methyl Parathion, Dichlorvos and Chlorpyrifos), which has an optical density of more than 0.1, was taken and centrifuged at 10,000 rpm at 4°C for 15 minutes. The supernatant was separated and 1 ml of culture supernatant was treated with 10 µl of 5, 10 and 25 ppm organophosphates (Methyl Parathion, Dichlorvos and Chlorpyrifos). It shows turbidity. This indicated the evident production of biosurfactant by the isolated organism to utilisethe carbon from organophosphate**[10]**. The O.D. of the supernatant was checked at 660 nm in UV spectrophotometer with the control.

Physical analysis of biosurfactant

Dry weight of biosurfactant: First a sterile Petri plate was taken to measure the weight. Then 2 ml of pure biosurfactant was poured on the sterile Petri plate. After that, the biosurfactant-poured petri plate was kept inside the hot-air oven for 30 minutes. Then, the dried plate was checked for weight. The dry weight of the biosurfactant was calculated using the below formula.

Formula: Dry weight of biosurfactant=Weight of the plate after drying – Weight of the empty plate

Qualitative analysis of biosurfactant by HPLC

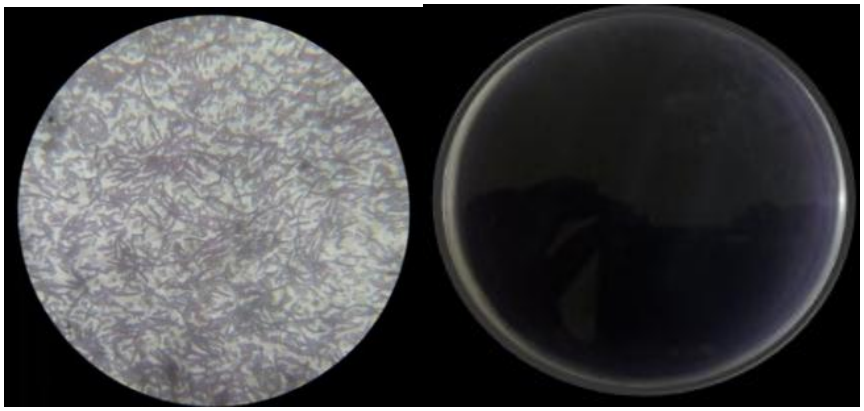
To investigate whether the obtained glycolipid biosurfactant included multiple components or not, it was analyzed by LCMS- 2010 EV (SHIMADZU) system, with a standard of Trehalose 6 6′ dimycolate**[3, 4]**. The mobile phase consisted of 70% methanol and 30% methylene chloride, which was injected into a 20 µl in C18 (25mm×4.6 mm×5 µm) reverse phase column isocratically. The injection flow rate was 0.5 ml min-1 and absorption of the output was detected by detector (SPD-M20A).

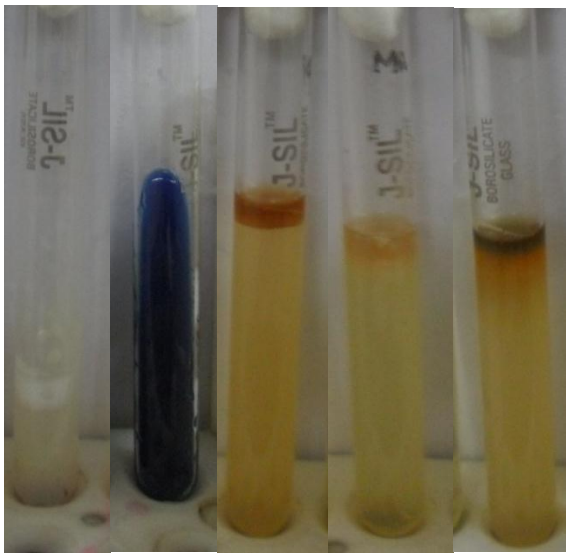
Result & discussion-

Isolation and Characterization of biosurfactant

A biosurfactantproducingbacteriumwasisolated dbyapplyingenrichmentculturetechniqueusingaMSM (as shown in **Figure 1a**).Thebacterialstrainsweredifferentiatedbytheircolony color,Gramstaining,spore-formingand biochemical Test (as shown in **Fig. 1b**).

(a)





(b)

Figure.1: (a)Mineral Salt media plate after 24 hour for growth of desired bacterial colony(b)Gram staining(-ve, rods);Catalse Test (+ve); Citrate UtilizationTest (+ve); Indole Test (-ve); MR Test (-ve); VP Test (-ve)

Screening for biosurfactant production

The biosurfactant production of the strain was confirmed at the end of the repeated sub-culturing and screening methods and identified strain as an excellent biosurfactant producer. In particular, the strain used in this study gave quick positive results for all biosurfactant screening methods.

Test for specificity of biosurfactant (Emulsification Index)

Specifically, drops collapsed within 30 s indicating higher amount of the biosurfactant present in the solution. Emulsification index was recorded as 76% for initial screening as shown in **Table 1**. The present results indicate that biosurfactant produced by strain possess emulsifying activity. In the oil displacement test, a clear zone of 2.4 cm was visualized, followed by addition of surfactant solution in the crude oil layer. These results confirmed the presence of biosurfactant in the cell free culture supernatant as shown in **Fig 2**.

Table 1 : Emulsification index (E24) of biosurfactant against different hydrocarbons

Hydrocarbon source	E24(%)
Glucose	26.07
Olive oil	22.19
Glycerol	25.80
Sucrose	30.21
Kerosene	19.21

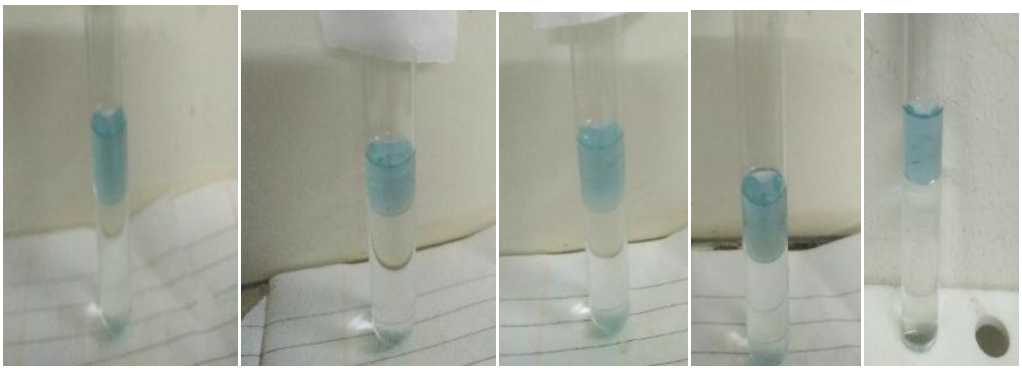


Figure2: EI_{24} values obtained from bio surfactant solution with different carbon source (glucose, olive oil, glycerol, sucrose and kerosene) at emulsification index

Biodegradation of Crude Oil

The obtained O.D of the culture growth on *Tafgor* and *nuvan*is shown in **Table 2** and **Table 3** and the optical density of culture to directly proportional to the culture mass.The turbidity was found according to degradation of organophosphate.

Table 2: OD of culture growing on organophosphate (Tafgor) with olive oil as a carbon sourceS= Sample of olive oil; C= Control; O.D.= Optical Density

Table 3: O.D of culture growing organophosphate (nuvan) with kerosene as a carbon source

Days of Incubation (Days)	O.D. of culture on tafgor of 5ppm at 660mn		O.D. of culture on tafgor of 10ppm at 660nm		O.D. of culture on tafgor 25ppm at 660nm	
	S	C	S	C	S	C
2	0.54	0.00	0.57	0.00	0.62	0.00
4	0.55	0.00	0.59	0.00	0.64	0.00
6	0.59	0.00	0.61	0.00	0.67	0.00
8	0.62	0.00	0.64	0.00	0.70	0.00
10	0.65	0.00	0.67	0.00	0.72	0.00
Average O.D.	0.590	0.00	0.616	0.00	0.670	0.00

Days of Incubation (Days)	O.D. of culture on nuvan of 5ppm at 660mn		O.D. of culture on nuvan of 10ppm at 660nm		O.D. of culture on nuvan 25ppm at 660nm	
	S	C	S	C	S	C
3	0.47	0.00	0.61	0.00	0.43	0.00
5	0.50	0.00	0.63	0.00	0.45	0.00
7	0.52	0.00	0.49	0.00	0.49	0.00
9	0.55	0.00	0.53	0.00	0.51	0.00
11	0.56	0.00	0.51	0.00	0.55	0.00
Average O.D	0.526	0.00	0.554	0.00	0.486	0.00

*S= *Sample of kerosene; C= Control; O.D. = Optical Density*

Physical analysis of biosurfactnt

Dry weight of biosurfactant:

The dry weight of biosurfactant was calculated using the below formula. The result is shown in

Table 4.

Table 4:Dry weight of biosurfactant

Weight of the sterile Plate	**Weight of the plate after drying**	**Dry weight of the Biosurfactant**
11.36	11.40	11.40 – 11.46 = 0.06

Formula: Dry weight of biosurfactant=Weight of the plate after drying – Weight of the empty plate.

Qualitative analysis of biosurfactantby HPLC

The result was shown in **Table 5**. The cellular envelope of *pseudomonas sps*is highly distinctive and harbors a wealth of unique lipids possessing diverse structural and biological properties. However, the ability to conduct global analyses on the full complement of *Psedomonassps.*Lipids has been missing from the repertoire of tools applied to the study of this important pathogen. This methodology is based on efficient chromatographic separation and automated ion identification through accurate mass determination and searching of a newly created database (*Mtb*LipidDB) that contains 2,514 lipid entities. We demonstrate the ability of this methodology to identify changes in lipid content in response to cellular growth phases. This work provides a customizable framework and resource to facilitate future studies on bacterial*sps.*biosynthesis and metabolism.

Table 5: Qualitative analysis of biosurfactant by HPLC

S. No.	**Concentration of the Sample**	**Trehalose 6 6′ dimycolate**
1	**10ppm**	**7.72 mg/ml**
2	**25ppm**	**14.96 mg/ml**

Conclusion

The present investigation the soil sample selected was from area of lucknow (local areas) c-block. *Isolated bacteria (Pseudomonas sps.)*was able to degrade organophosphates (tafgor and nuvan) under laboratory condition by using statistical analysis test. I found out that tafor were finally degraded by *Pseudomonas sps.* When compared with the other nuvan. Biosurfactant produced by this organism were found to be specific to organophosphate and its also plays a major

role in enhancing the degradation process of organophosphates. Physical and biochemical analysis of the biosurfactant was carried out through Dry weight of Biosurfactant and HPLC. Structure identification and quantification analysis of the biosurfactant was carried out by HPLC. The chemical structures of the biosurfactant were identified as glycolipid.

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Conflict of interest: No

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