

# Production of Biosurfactants from Oil Sludge Using Isolated *Pseudomonas*

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## Abstract:

Biosurfactant are amphiphiles produced by microorganism. They are widely used in cleanup process of organic and inorganic contaminants like metals and hydrocarbons. In this study, biosurfactants were produced from local microorganism in laboratory conditions and glass flasks. Contaminated soil or sludge was taken from the sediment tank of Abadan oil Refinery. Biosurfactant synthesis and production was followed by measuring surface tension by tensiometer.

**Key Words:** Biosurfactant, *Pseudomonas*, Amphiphiles, Microorganism.

## 1. Introduction

Biosurfactant are surface active compounds and biological properties produced by variety of microorganisms consist of bacteria, yeast, filamentous fungi [1]. Biosurfactant activities depend on the concentration of the surface-active substances until the critical micelle concentration (CMC) is obtained. Micelle structure has an important role in microemulsion structure [2]. Biosurfactant potential in moving heavy crude oil, transporting petroleum in pipelines, managing oil spills, controlling oil environment pollution, environmental protection and management, and cleaning oil sludge from oil storage facilities in MEOR, bacterial growth produces biosurfactant that reduce surface tension and interfacial between oil and water, lower the capillary forces and cause a decrease in the residual oil saturation in reservoirs [3]. Some of the advantages of biosurfactant over synthetic ones include lower toxicity, biodegradability, and selectivity, specific activity at extreme temperatures, pH and salinity [4]. Biosurfactant can be produced from various substrates, mainly renewable resources such as vegetable oils, industrial waste and from by product. This last feature makes cheap production of biosurfactant possible and allows using waste substrates and reducing their polluting effect at the same time.

In this study isolation, screen and identification of *pseudomonas* bacteria from oil sludge, was capable producing biosurfactant to emulsify crude oil. Biosurfactant were produced from local bacteria and investigated production of biosurfactant at different source carbon.

## 2. Material and method

### 2.1. Medi and Cultivation Conditions

Soil and sludge contaminated samples were taken from the Abadan Oil Refinery, Iran. 10 gr of soil or sludge poured into the 250 ml Erlenmeyer flasks containing 100 ml of phosphate buffered Saline (NaCl: 8g/L ·K<sub>2</sub>HPO<sub>4</sub>: 1.21 g/L ·KH<sub>2</sub>PO<sub>4</sub>: 0.34 g/L) in the end shacked on magnet stirrer for 1 hour. The supernat in the Erlenmeyer flask were used as the main source of isolation bacteria. A mineral salts medium (MSS) used with the following materials with their concentrations and trace element solution was as follows: KHPO<sub>4</sub> 6.3gr/lit, KH<sub>2</sub>PO<sub>4</sub> 1.8 gr/lit, Yeast extract 0.5gr/lit, NH<sub>4</sub>NO<sub>3</sub>0.5gr/lit, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.1 gr/lit, CaCl<sub>2</sub>.H<sub>2</sub>O0.1 gr/lit, FeSO<sub>4</sub>.7H<sub>2</sub>O 0.1 gr/lit, MnSO<sub>4</sub>.H<sub>2</sub>O0.1 gr/lit, H<sub>3</sub>BO<sub>3</sub>0.03 gr/lit, ZnSO<sub>4</sub>.7H<sub>2</sub>O 0.01 gr/lit, CoCl<sub>2</sub>.6H<sub>2</sub>O 0.02gr/lit, CoCl<sub>2</sub>.6H<sub>2</sub>O0.006 gr/lit, CuSO<sub>4</sub>.2H<sub>2</sub>O 0,001 gr/lit. PH of the medium was adjusted to 7.0 ± 0.2.

All of the Erlenmeyer flasks containing 100 ml of MSS were sterilized by the autoclave. In addition of mineral salts, individually following carbon source such as olive oil2%, crude oil2%, glucose2% w/w were added to Erlenmeyer flask. Erlenmeyer flasks were left in incubation of 150 rounds per minute (rpm) rotation in a temperature of 32C° for a week.

## 2.2.Properties of bacteria

**Table 1.** Study the morphology, physiology and biochemistry properties of bacterial

Different Test Of Bacteria		Results
Form and consistency of colony		Small and flat_ Dry consistency
Colony color		Gray
Gram stain		Negative
Gram Gelatinize		Positive
Oxidase production		Positive
TSI Test		Alk/Alk
Nitrate Reduction		Positive
Motility		Positive
O.F Test	Oxidative	Positive
	Fermentative	Negative

## 3. Results and Discussion

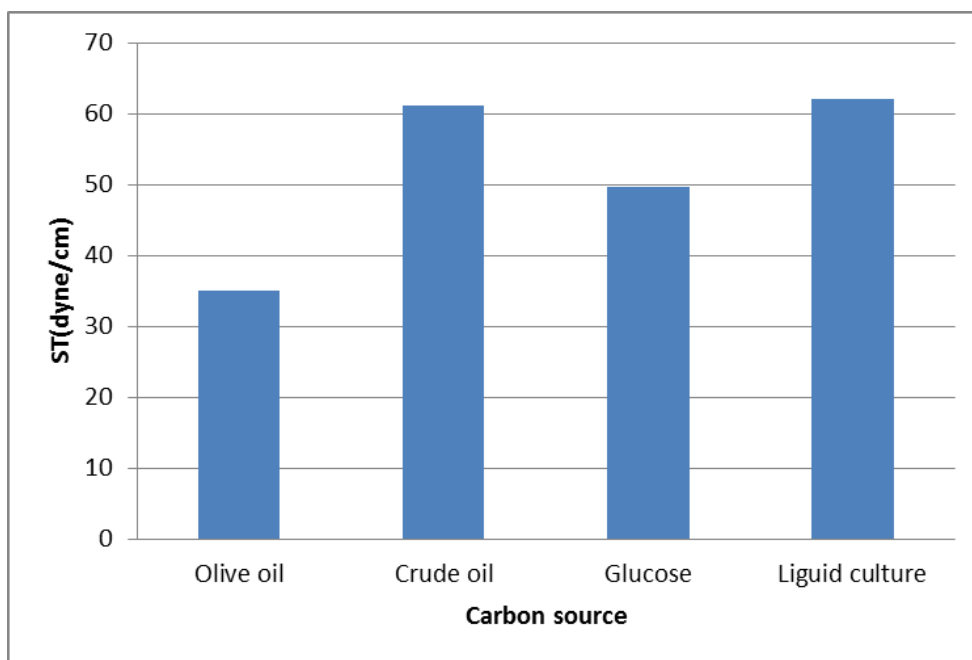
### 3.1.Biosurfactant Production

Bacteria with OD=1 were inoculated into Erlenmeyer flasks. The biosurfactant was a rhamnolipid-type in nature, and had a good foaming, emulsifying, and antimicrobial activities.

The genus *Pseudomonas* is capable of using different substrates, such as glycerol, glucose, mannitol, fructose, n-paraffins and vegetable oils, to produce rhamnolipid-type biosurfactant [6,7]

### 3.2. Effect of Carbon Source

The kinetics of biosurfactant production was followed by measuring surface tension. The surface tension measurement of cell free supernatant was determined in a tensiometer, using the du Nouy ring method. The values reported are the mean of three measurements. All measurements were made on cell-free broth obtained by centrifuging the culture. All experiments were carried out in a temperature of 30°C. Carbon source factor was chosen aiming to obtain higher productivity of the biosurfactant. The carbon sources used were crude oil (2% w/w), olive oil (2%w/w) and glucose (2%w/w). Olive oil was the best carbon source for surfactant synthesis: growth of the on this substrate decreases the surface tension to 35 dyne/cm.



**Figure 1.** Influence of carbon source on the variation of surface tension ST

## 4. Acknowledgements

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## 5. References

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