

## Screening optimization and production of biosurfactants from *Bacillus* and *Pseudomonas* sp

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### ABSTRACT

Biosurfactants are surface active compounds produced by microorganisms. These molecules reduce surface tension between aqueous solutions and hydrocarbon mixtures. Oil contaminated soil were collected from different automobile shop. *Bacillus* and *Pseudomonas* sp were isolated from the four soil samples and these isolates were screened for biosurfactans activity using petrol, diesel and kerosene source by emulsification capacity, Surface tension measurement, Oil spreading techniques, Drop collapse method and Haemolytic activity. Biosurfactant production by the isolated bacterium using different pH, temperature and concentration of carbon source was studied. Effective production of biosurfactant in both *Bacillus* and *Pseudomonas* sp using mineral salt broth with optimiazed condition.

**Key words:** Biosurfactant, Oil spreading technique, Haemolytic activity, E24 Index.

### INTRODUCTION

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly microbial cell surfaces or excreted extracellularly and contain hydrophobic and hydrophilic moieties that reduce surface tension and interfacial tension between individual molecules at the surface and interface respectively. (Karanth *et al.*, 1999) biosurfactant producing microorganisms were naturally present in oil contaminated soil. Oil contaminated environment contain large amount of hydrocarbons. i.e., aliphatic and aromatic hydrocarbons. Microorganisms exhibit emulsifying activity by producing biosurfactants and utilize the hydrocarbons as substrate often mineralizing them or converting them into harmless products.

Biosurfactants have gained more importance in the fields of enhanced oil recovery,

environmental bioremediation, food processing, and pharmaceuticals owing to their unique properties-higher biodegradability, lower toxicity, and effectiveness at extremes of temperature, pH and salinity. Biosurfactants can be produced by microbial fermentation processes using cheaper agrobased substrates and waste materials. In various industrial processes, they are potentially useful surface-active agents for emulsion polymerization, wetting, foaming, phase dispersion, emulsification and demulsification. (Desai *et al.*, 1997)

Biosurfactants are mainly categorized mainly by their chemical composition and microbial origin. Generally their structures include a hydrophilic moiety consisting of aminoacids or peptides, mono-di or polysaccharides and hydrophobic moiety comprising unsaturated or saturate fatty acids. Accordingly, the major classes of biosurfactants include glycolipids, lipopeptides,

lipoproteins, phospholipid, fatty acids, polymeric biosurfactant and particulate biosurfactants. (Manneerat *et al.*, 2005)

Among the different classes of biosurfactants rhamnolipid and surfactin are best studied. Rhamnolipid is produced by *Pseudomonas aeruginosa*, a gram-negative, motile, non spore forming bacteria. Surfactin is cyclic lipopeptide commonly used as an antibiotic. Surfactin's structure consists of a peptide loop of seven aminoacids(L-asparagine, glycine, two L-leucine, L-valine, and two D-Leucins) and an hydrophobic fatty acid chain thirteen to fifteen carbon long.(Peypoux *et al.*, 1999)

### MATERIAL AND METHODS

The soil samples were collected from polluted sites and isolate the bacteria *Bacillus* and *Pseudomonas* from oil contaminated soil by Gram staining and by various biochemical test. Screening of biosurfactants using the following methods.

#### Screening for biosurfactant activity

The supernatant was subsequently subjected to the preliminary screening methods by using 3 different oil namely petrol, diesel and kerosene.

#### Oil spreading techniques

The 50ml of distilled water was added to a large petri dish (15cm diameter) followed by the addition of 20ml of Oil to the surface of water, 10ml of supernatant of culture broth (Rodrigues *et al.*, 2006)

#### Emulsification capacity(E24) test

E24 of culture samples was determined by adding 2ml of oil to the same amount of culture, mixing with a vortex for a 2min and leaving to stand for 24h. The E24 index is given as percentage of height of emulsified layer(cm) divided by total height of the liquid column(cm) (Sarubbo 2006)

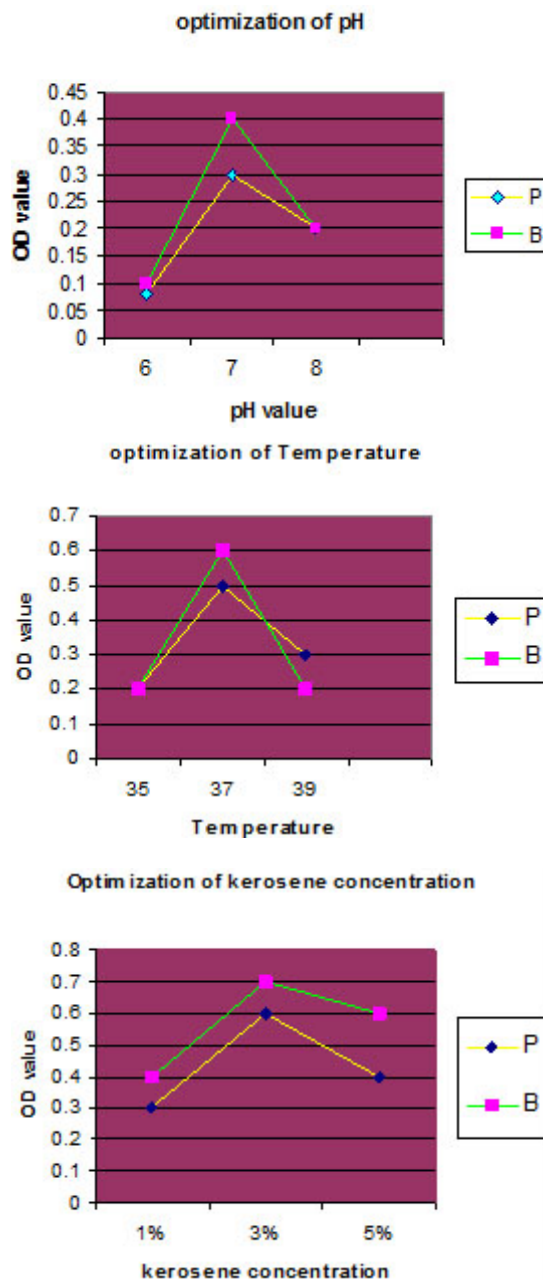
#### Drop collapse method

For drop collapse method 2ml of oil was added to each well of a 96 well containing microtiter plate lid. The lid was equilibrated for 1 h at room temperature. MSM cultures of bacterial isolates was

centrifuged and then 5ml of the drop on the surface of oil was evaluated after 1 min.(Jain *et al.*, 1991)

#### Haemolytic activity

Isolates were screened on blood agar plates containing 5% sheep blood and incubated



**Graph 1**

at 37°C for 48 h. Hemolytic activity was detected as the presence of clear zone around bacterial isolates (Plaza et al., 2006)

#### Surface tension measurement

Surface tension of biosurfactant was measured by drop weight method. A burette is held vertically and liquid drops are allowed to fall from its lower end at a very slow rate about 8 drops per minute. A clean dry beaker is weighed and placed under flow of burette. 50 drops of the liquid are collected and weighed the beaker. Again repeat the same for 3 times. The average mass of one drop is calculated and then surface tension of liquid containing biosurfactant calculated by ( surface tension(T) =  $Mg/3.8r$  )

#### Process optimization and biosurfactant production

The pH ranges from 6,7, and 8, temperature ranges from 35°C, 37°C and 39°C and kerosene concentration from 1%, 3% and 5% for

optimization of biosurfactants production in *Bacillus* and *Pseudomonas sp.* Both the organisms have maximum growth rate observed at pH 7, temperature of 37°C in 0.3% kerosene concentration. Effective biosurfactants production from *Bacillus* and *Pseudomonas sp.* can be achieved using Mineral salt broth.

### RESULTS AND DISCUSSION

Four isolates of *Bacillus* and *Pseudomonas* have highest E24 value was observed in diesel (Priya and usharani 2009). In case our study *Bacillus* and *Pseudomonas* have the ability to emulsifying oils. The highest E24 value was observed in petrol and *Pseudomonas* showed the better E24 value than *Bacillus*. (table -1). In oil spreading techniques *Bacillus* shows higher zone formation of 4.1mm, 3.6mm, 3.2mm in petrol, diesel, and kerosene respectively. Similarly our study focused *Pseudomonas* showed 3.9mm, 3.3mm, 2.8mm in petrol, diesel, and kerosene.

Table 1(a): Emulsification index in petrol

Organisms	Emulsified Layer (cm)	Total Liquid Column (cm)	% of E24
<i>Bacillus subtilis</i>	0.6	2.9	21
<i>Pseudomonas aeruginosa</i>	0.8	2.7	30

Table 1(b): Emulsification index in diesel

Organisms	Emulsified Layer (cm)	Total Liquid Column (cm)	% of E24
<i>Bacillus subtilis</i>	0.3	3.2	9
<i>Pseudomonas aeruginosa</i>	0.2	3.0	17

Table 1(c): Emulsification index in kerosene

Organisms	Emulsified Layer (cm)	Total Liquid Column (cm)	% of E24
<i>Bacillus subtilis</i>	0.3	3.2	9
<i>Pseudomonas aeruginosa</i>	0.4	3.1	13

Surface tension reduction was measured by Kruss Hamburg Nr2215 Tensio meter. Results were compared to medium composition as negative control ( Pavitran *et al.*, 2004) Since our study of surface tension reduction was measured by drop weight method and *Pseudomonas* shows 0.007 surface tension reduction per drop.

Blood agar method is often used for a preliminary screening of microorganisms for the ability to produce biosurfactants on hydrophilic media. (Schulz *et al.* , 1991). From our results both the isolates will cause lysis of the blood cells and exhibit a colorless, transparent ring around the colonies.

Optimization of biosurfactants was carried out by using different pH, temperature and concentration of carbon source ( kerosene) (chart-1)

### CONCLUSION

From the above observation, it was concluded that both bacterial isolates of *bacillus* and *pseudomonas* have the ability to secrete surface active agents it is gain more important in future for industrial and environmental applications.

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