PRODUCTION SCREENING AND OPTIMIZATION OF BIOSURFACTANTS FROM OIL CONTAMINATED SOIL SAMPLE

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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 Tiruchy railway shed, Dindigul ,Tirunelveli, Indian Oil Corporation (IOC) and Chennai Petroleum Corporation Limited (CPCL) of Tamil Nadu. These isolates were screened for biosurfactans activity using petrol, diesel and engine oil source by Oil spreading techniques, Drop collapse method and Emulsification capacity Biosurfactant production by the isolated bacterium using different pH, temperature ,concentration of carbon source,nitrogen source was studied. Pseudomonas and bacillus sps were isolsted from these contaminated soil and their biochemical characters were studied.

Keyword: Bioremediation, Biosurfactant, Bacillus Sp, Pseudomonas Sp, Oil Polluted Soil.

I. INTRODUCTION

Bio surfactants are amphiphilic compounds produced on living surfaces, mostly microbial cell surfaces or excreted extracellularly and contain hydrophobic and hydrophilic domains that find application in an extremely wide variety of industrial process involving emulsification, foaming, detergency, wetting, dispersing or solublization (Gautam and Tyagi 2006). The enormous market demand for surfactants is currently met by numerous synthetic, mainly petroleum based, chemical surfactants. These compounds are usually toxic to the environment and non biodegradable. Tightening environmental regulations and increasing awareness for the need to protect the ecosystem have efficiently resulted in an increasing interest in biosurfactants as possible alternations to chemical biosurfactants. These molecules could be widely used in cosmetic, pharmaceutical, and food processes as emulsifiers, preservatives, and detergents, and in bioremediation processes. They can be produced from various substrates, mainly renewable resources such as vegetable oils, distillery and dairy wastes. Biosurfactants are categorized mainly by their chemical composition and microbial origin. Generally, their structures include a hydrophilic moiety consisting of amino acids or peptides, mono, di or polysaccharides and hydrophobic moiety comprising unsaturated or saturated fatty acids. Accordingly, the major classes of biosurfactants include glycolipids, lipopeptides, lipoprotein, phospholipids, fatty acids, polymeric biosurfactant and particulate biosurfactants (Mukherjee et al., 2006; Maneerat 2005). Among the different classes of biosurfactants rhamnolipid and surfactin are best studied biosurfactants. Rhamnolipid is one of the type of glycolipids in which one or more molecules of rhamnose are linked to one or more molecules of hydroxydecanoic acid while the OH group of one of the ccids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide,the OH group of the second acid is involved in ester

formation(Karanth *etal* 1999). Surfactin is a cycle lipopeptide commonly used as an antibiotic. In the various course of studies of its properties ,surface was found to exhibit effective characteristics like antibacterial, antiviral, antifungal , antimycoplasma and hemolytic activities (Pooja Singh and Cameotra 2004).

II. MATERIALS AND METHODS

Soil samples were collected from five different regions of hydrocarbon contaminated area. The samples were collected from Tirchy railway shed, Dindugul, Tirunelveli, Indian Oil Corporation (IOC) and Chennai Petroleum Corporation Limited (CPCL) of Tamil Nadu. Finally the soil samples were enriched with (petrol, diesel and engine oil) as substrate. Serial dilution technique using nutrient agar was performed to isolate bacteria from soil samples. Staining techniques and biochemical tests (Koneman, William, Stephen 1988) were done to identify biosurfactant producing organisms.

III. SCREENING OF BIOSURFACTANT ACTIVITY

The following screening assays were carried out for detecting biosurfactant producing organisms. (Youssef *et al* 2004).

3.1 Oil Displacement Method

50ml of distilled water was taken in the petriplate. On this water 20µl of diesel was layered uniformly. Further 10µl of supernatant of culture broth was added at different spots. Occurrence of clear zone was an indication of biosurfactant production. (Rodrigues, Teixenira, Mei 2006).

3.2 Drop Collapse Method

96µl microtitre plates were coated with 2Toil. After coating the plates were left in room temperature for 1 hr. Then drop of supernatant was added and the results were seen less that 1 minute (Jain *et al* 1991).

3.3 Emulsification Index

Emulsifying capacity was evaluated by an emulsification index (E_{24}). This was calculated by adding d and supernatant in (1:1) ratio. Then by vortexing for 2 mts to obtain maximum emulsification (sarubbo 2006). After 24 hrs E_{24} was calculated by the formula.

$$E_{24} - \frac{Height\ of\ emulsion\ formed}{Total\ height\ of\ solution} \times 100$$

IV. OPTIMIZATION PROCESS AND BIOSURFACTANT PRODUCTION

Optimization of bio surfactant production involves changing parameters like pH, Temperature, Carbon source and Nitrogen source were optimized based on one-factor at a time approach.

4. 1EFFECT oF Ph

The effect of pH was studied with various pH namely 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12. The optimum pH for maximum activity was selected by varying the pH of medium and medium from pH 4 to 12 for the culture isolated from the soil.

Fig1: Effect of Different Ph Of Contaminated Soil Sample

4.2 Effect of Temperature

The effect of temperature was studied with various temperatures namely 25ºC, 30ºC, 35ºC, 40ºC, 45ºC and 50ºC. The optimum temperature for maximum activity was selected by varying the temperature of medium (pH-9.0) from 25ºC to 50ºC for the culture isolated from the soil. All other parameters were kept unaltered.

4.3 Effect of Carbon Source

The effect of carbon source was studied with different carbon sources like Glucose, Sucrose, Mannitol, Coconut Oil, Olive Oil, Glycerol, Tween 80 and Palm Oil. The optimum carbon source for maximum e activity was selected by varying the carbon sources of medium (pH-9.0) at 35^{o} C for the culture isolated from the soil. All other parameters were kept unaltered.

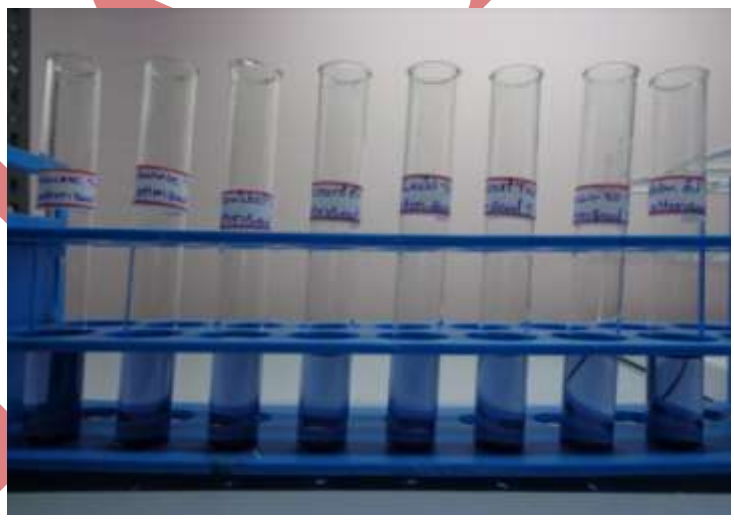

Fig2: Effect of Different Carbon Source Of Contaminated Soil Samples

4.4 Effect of Nitrogen Source

The effect of Nitrogen source was studied with different nitrogen sources like Peptone, Ammonium Nitrate, Potassium Nitrate, Yeast Extract, Tryptone, Beef Extract and Urea .The optimum nitrogen source for maximum activity was selected by varying the nitrogen sources medium (pH-9.0) with mannitol as carbon source medium (pH 8.0) with at 35^{o}C for the culture isolated from soil. All other parameters were kept unaltered.

V. BIOSURFACTANT PRODUCTION

The bacterial strains were inoculated in nutrient broth medium with 0.1% of oil (Petrol, Diesel and Engine oil) and incubated for 48 hours. The 48 hour culture was centrifuged at 5000 rpm for 15mins at 25°C to remove the cells. The supernatant was collected and used as bio-surfactant.

VI. RESULT AND DISCUSSION

Oil contaminated soil samples were collected from five different places namely Tirchy Railway shed, Dindugul, Tirunelveli, Indian Oil Corporation (IOC) and Chennai Petroleum Corporation Limited (CPCL) of Tamil Nadu. Three different bacterial species were isolated from the oil contaminated soil samples and standard biochemical tests were done to identify the organisms (Table 1). The two bacterial species were identified as *Pseudomonas sp., Bacillus sp.*

Table 1: Biochemical Characters Of Isolated Organisms

	Microorganisms	
Biochemical Test	*Pseudomonas sp.*	*Bacillus sp.*
Gram staining	Gram Negative, Rod	Gram Positive, Rod
Catalase	Positive	Positive
Oxidase	Positive	Positive
Indole	Negative	Negative
Methyl Red	Negative	Negative
Voges proskauer	Negative	Positive
Citrate	Positive	Negative
Urease	Negative	Negative
Nitrate Reduction	Negative	Positive

The two different bacterial species isolated from oil contaminated soil were screened for their biosurfactant activity by Drop Collapsing Test, oil spreading technique and emulsification stability test in three different oils namely engine oil, petrol and diesel. In oil spreading test the organisms *Pseudomonas* and *Bacillus* produced clear zone (Table 2) and in the drop collapse test the samples were collapsed. This clearly indicated that the two organisms produced biosurfactant. Similarly the two organisms were able to form stable emulsions for 24 h (Table 3). These emulsification results showed that, biosurfactant produced from a substrate can emulsify different hydrocarbons to a greater extent which confirmed its applicability against different hydrocarbon pollution (Thavasi, Jayalakshmi & Banat, 2010.). Among the three oils the higher E24 value was observed in diesel and *Pseudomonas* sp. showed the highest biosurfactant activity compared to *Bacillus* sp.. The results are shown in (Table 2 and 3).

Table 2: Oil Spreading Test

Micro-organism	Zone formation in various oils tested (diameter in mm)		
	Engine oil	Petrol	Diesel
Bacillus sp.	11	14	22
Pseudomonas sp.	16	28	34

Table 3: Emulsification activity

Micro-organism	E24 value (%)		
	Engine oil	Petrol	Diesel
Bacillus sp.	45	52	66
Pseudomonas sp.	59	64	72

The pH ranges from 4 to 12, temperature ranges from 25°C to 50°C,carbon sources such as Glucose, Sucrose, Mannitol, Coconut Oil, Olive Oil, Glycerol, Tween 80 and Palm Oil and nitrogen sources such as for Peptone, Ammonium Nitrate, Potassium Nitrate, Yeast Extract, Tryptone, Beef Extract and urea in optimization of biosurfactant production in *Pseudomonas* and *Bacillus sp*. Both the organisms have maximum growth rate observed at PH 7, temperature of 37°Cand shown best results in olive oil as carbon source and yeast extract as nitrogen source. Among the two isolates Pseudomonas shows better growth rate than *Bacillus.*

VII. CONCLUSION

These results conclude that two different bacterial species were isolated from oil contaminated soil and their degradation capability was checked individually by various screening methods among which *Pseudomonas* sp. showed highest degradation efficiency than *Bacillus* sp. Thus the above experiment shows that bioremediation can be used effectively to treat oil contaminated soil. By using biological processes, as in the case of bioremediation, usually lowers the costs as compared to chemical treatment processes for various contaminated sites., it was concluded that both bacterial isolates of *pseudomonas* and *bacillus* have the ability to secrete surface active agents it is gain more important in future for industrial and environmental applications.

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