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Surfactant mediated recovery of anthracene from contaminated soil

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ABSTRACT

The present study was to evaluate the recovery of anthracene, a polyaromatic hydrocarbon from contaminated soil using chemical surfactant and rhamnolipid biosurfactant produced by *Pseudomonas fluorescens*. Comparative study of biosurfactants to chemical surfactant was carried out. The observation indicates that biosurfactants produced by the bacterial strain was found to be more effective than chemical surfactants. Polycyclic aromatic hydrocarbon (PAH) contamination of the environment represents a serious threat to the health of humans and ecosystems. Bioremediation has shown promise as a potentially effective and low-cost treatment option, but concerns about the slow process rate and bioavailability limitations have hampered more widespread use of this technology. An option to enhance the bioavailability of PAHs is to add surfactants directly to soil in situ or ex situ in bioreactors. Surfactants increase the apparent solubility and desorption rate of the PAH to the aqueous phase.

KEYWORDS

Pseudomonas fluorescens;
Biosurfactant;
Chemical surfactant;
PAH;
Anthracene.

INTRODUCTION

Biosurfactants or microbial surfactants are surface-active agents. They are amphiphilic compounds that consist of a hydrophilic head and a hydrophobic tail. They are produced on living surfaces, mostly microbial cell surfaces or excreted extra cellular. Most of the applications today involve the use of chemically synthesized surfactants. Production of surfactants in the United States and worldwide is estimated at 3.4 -109 kg and 7.109 kg in 2007, respectively. The US surfactant industry shipments were $3.65 billion in 2007.

There are many advantages of biosurfacants if compared to their chemically synthesized counterparts. Their biodegradability, generally low toxicity, biocompatibility and digestibility are their significances. Biosurfactants can be produced from cheap raw materials, which are available in large quantities; the carbon source may come from hydrocarbons, carbohydrates and/or lipids, which may be used separately or in combination with each other. Depending upon application, biosurfactants can also be produced from industrial wastes and by-products and this is of particular interest for bulk production (*e.g.* for use in petroleum-related technologies).

Biosurfactants can be efficiently used in handling industrial emulsions, control of oil spills, biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil. Biosurfactants, being complex organic molecules with specific functional groups, are often specific in their action[1].

Anthracene greatly affects the whole ecological system. It has got both acute and chronic effects on the ecosystem. Acute ecological effects include the death of animals, birds, fishes and death or low growth in plants. It stunts the root and shoots growth in plants. Acute effects are seen after 2-4 days after the animals or plants are exposed to anthracene. These pose a high threat to aquatic animals and birds[2].

Microorganisms utilize a variety of organic compounds as the source of carbon and energy for their growth. When the carbon source is an insoluble substrate like a hydrocarbon, microorganism facilitates their diffusion into the cells by producing a variety of substances the Biosurfactants[3]. Considering these views the present study was designed to evaluate the recovery of anthracene from contaminated soil.

MATERIALS AND METHOD

Using a sterile spatula oil spilled soil was collected in a sterile glass container from a petrol bunk in and around Ottapalam, kerala. The *Pseudomonas fluorescence* was isolated from the oil-spilled soil collected from petrol bunk. The different colonies observed in serial dilution were streaked on separate Nutrient agar slants, and Pseudomonas selective media plates and incubated at 37°C for 24 hours. After incubation, plates were observed for the growth and the colony morphology was studied. Both morphological and biochemical characterizations were carried out.

Production of biosurfactant

For the production of biosurfactant, *Pseudomonas fluorescence* isolated from oil-spilled soil was grown on Mineral salt agar medium containing diesel oil (78% alkanes, 6.4%aromatics, 15.4% of resin, and asphaltenes) as the sole carbon source[4].

Extraction of biosurfacant

The organism was inoculated and incubated at 30°C for 24 hours. The culture was centrifuged at 10,000 rpm for 15 minutes and the supernatant was extracted three times with hexane. The bottom layer was again centrifuged at 10,000 rpm for 15 minutes and the pellet was obtained. The pellets were vacuum dried and stored at 70°C. It was then freeze dried in a lyophiliser to obtain biosurfactant in powder form for further use[5].

Preparations of different concentrations of surfactants

Crude surfactants (biosurfactant and SDS) were weighed and dissolved in sterile water. The percentage recovery of anthracene was calculated by

$$\frac{\text{Final OD} - \text{Initial OD}}{\text{Final OD}} \times 100$$

Test for emulsifying activity

The emulsification activity of the biosurfactant produced by *Pseudomonas fluorescens* was estimated every 24 hours[6]. The protein and carbohydrate content were estimated following the method of Bradford and Dubois[7] respectively.

Estimation of critical micelle concentration (CMC)

CMC was estimated to find out the particular concentrations at which the surface tension remains constant as the concentration above CMC are proved to be toxic for bacteria. The determination of surfactant concentration in an unknown sample was performed by diluting the sample to several folds and measuring in a surface tensiometre.

Detection of antimicrobial activity

The antimicrobial activity was determined by the disc diffusion method[8]. A suspension of the test microorganism was spread on the Mueller Hinton Agar. Filter paper discs of 6mm diameter which contained 10µl of the biosurfactant were placed on the inoculated plates. The petri dishes were subsequently incubated at 37°C for 24 hours. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm (including the diameter of the disc) from the lower surface of the petri dishes[9] and the control consisted of paper disc soaked with appropriate solvent and evaporated to dryness. All the assays were carried out in triplicates.

Detection of rhamnolipid

A new semi-quantitative agar plate test for the de-

tection of extra cellular rhamnolipid has been developed. The test is specific for anionic biosurfactants and can be applied to other glycolipid-producing microorganisms[10].

RESULTS AND DISCUSSION

The organisms were observed as short curved rods on Nutrient agar and king's medium. *Pseudomonas* growth occurred on king's media. Gram staining indicated that the organism isolated from oil spill soil appeared as pink coloured rods, which indicated that they are gram negative (TABLE 1).

TABLE 1 : Biochemical test

Biochemical test	Indole	Methyl red	Voges proskauer	Simmon's citrate utilization	Catalase	Nitrate reduction
Pseudomonas fluorescens	-Ve	-Ve	-Ve	+Ve	+Ve	+Ve

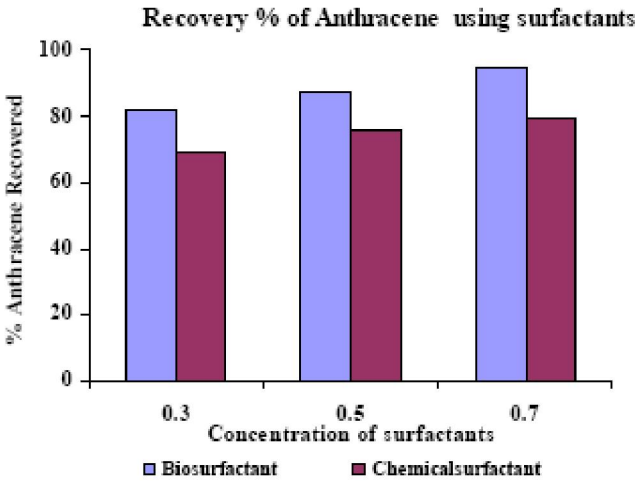
Surfactant production by *Pseudomonas fluorescens*

The amount of surfactant produced by *Pseudomonas fluorescens* cultured in mineral salt agar medium with diesel oil as substrate was 0.3 mg/L at 24 hours and it increased to 3.8 g/L at 120 hours. The above result showed that biosurfactant production by *P.fluorescens* is induced by hydrophobic substrates such as diesel oil. Similar observations were reported for cell-associated biosurfactants produced by psychrotroph *Rhodococcus sp*. strain 215 during growth on n-alkanes[11] and rhamnolipid produced by *P. Aeruginosa* G11 on glycerol[12]. Lin[13] also reported that chemically induced *Bacillus licheniformis* mutant KGL 11 produced lipopeptide biosurfactants up to a concentration of 391 mg/L, which is twelve times more than the parent strain with a surface tension of 26.5 dyn/cm.

Recovery of anthracene

The surfactants at two concentrations namely 1) at CMC and 2) above CMC were used. The recovery of anthracene in the presence of crude biosurfactants was 81.8% - 94.7 % and in the presence of chemical surfactant it was 69.2 – 79.3 % (Graph 1). The above-mentioned results showed that biosurfactant was more effective in the recovery process. Noordman and Janssen[14] reported that rhamnolipid biosurfactant produced by *P. aeruginosa* strain enhanced fast uptake of aliphatic hydrocarbon at the rate of 73% with 0.2 mg of rhamnolipid/mL.

Emulsifying activity



Graph 1 : Recovery of anthracene using biosurfactant

Emulsifying activity was found estimated by growing culture on mineral salt broth with hexane and it was found to possess high emulsification property.

Detection of antimicrobial activity and detection of rhamnolipid

The zone of clearance was observed around the filter paper disc spotted with biosurfactant on Mueller Hinton agar plates swabbed with *Bacillus* culture. This shows that the biosurfactant possess (0.9cm) antibacterial activity (Figure 1). Biosurfacants were observed by the formation of dark blue halos around the colonies (Figure 2).

1- Rhamnolipid (biosurfactants)
2- SDS (Chemical surfactants)

Figure 1 : Antimicrobial activity of surfactants

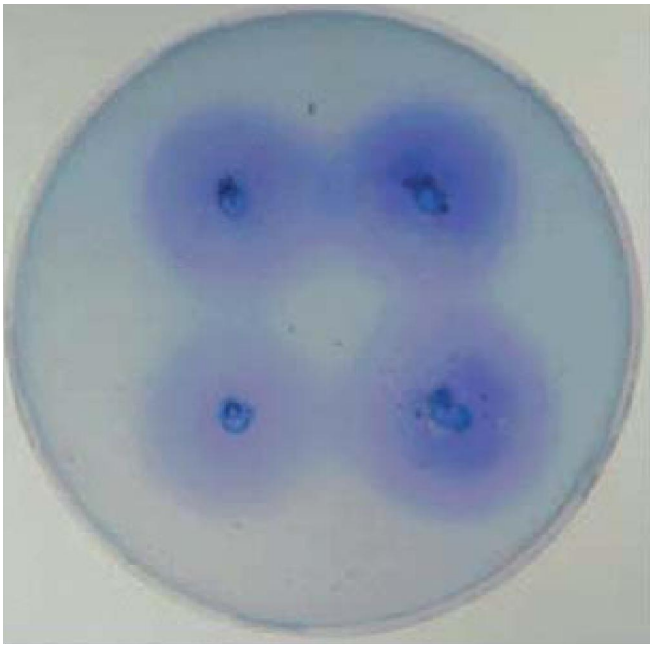


Figure 2 : Detection of Rhamnolipid - Presence of Blue Halos

Comparative study of biosurfactant and chemical surfactant

The percentage recovery of anthracene from soil in the presence of biosurfactant was 81% to 94%. While recovery in the presence of chemical surfactant (SDS) was 69% to 79%. The biosurfactant was found to be more efficient in anthracene recovery from contaminated soil when compared to chemical surfactant. The biosurfactant had a carbohydrate content of 6.12%, lipid content of 60% and protein content of 0.2. Clear bands were obtained by PAGE. The presences of protein bands were visually detected.

Various studies were carried out to estimate the recovery of PAH compounds from contaminated soil, since it has got many side effects on human, animals and plants. It causes mutations in humans and animals whereas stunts the root and shoot growth of plants.

Pseudomonas fluorescence has the ability to produce biosurfactants as its secondary metabolites. Rhamnolipids produced by this strain was proved to be more efficient than any other biosurfactant. These biosurfactant have better surface acting property and minimal side effects when compared to chemical surfactants.

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