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Enhanced Bioavailability of Sparsely Soluble PAHs

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Abstract: ***Polyaromatic hydrocarbons (PAHs) are organic pollutants of concern because of the health hazards they promote. In the present work, a bacterial isolate from crude oil was found to promote enhanced bioavailability of two model PAHs namely fluoranthene and pyrene within a span of 24 h which is very short in comparison to previous reports. The bacterium showed scanty growth in presence of these PAHs as the sole source of nutrient and energy. However it showed good growth in peptone glycerol ammonium salts (PGAS) medium supplemented with these PAHs. It also promoted biosurfactant production in the PAH supplemented medium. After initiation of biosurfactant production, addition of test PAHs was done at a concentration of about 10 times their aqueous solubility. Analysis by TLC showed that the PAHs in their entirety were scavenged off thus advocating for the immense bioremediation potential of biosurfactant from the test bacterium.***

Keywords: PAH; biosurfactant; solubility enhancement; bioremediation potential

1. Introduction

Polyaromatic hydrocarbons (PAHs) are organic compounds consisting of fused aromatic rings and their release into the environment can lead to contamination of natural resources. They are of major concern because of their persistence in the environment and also the deleterious roles of being mutagens and carcinogens [1]. Fluoranthene is a PAH found in many combustion products. It is a structural isomer of another PAH, pyrene. They are recalcitrant to biodegradation mainly because of their sparse solubility in water [2].

Bioremediation is the technique of using a bacterium or a microbial consortium for metabolizing the PAHs to inert non-toxic substances. This microbial degradation potential may sometimes get attenuated by the inhibitory concentrations of pollutants [3]. Added to this is the problem of PAH unavailability for microbial degradation. It is often addressed by adding chemical surfactants which definitely increase bioavailability but inhibit microbial growth and biodegradation at concentration above their critical micelle concentrations [4]. Biosurfactants can be a favourable alternative in this regard as they are biodegradable and hence environmentally compatible. These molecules are generated by various genera of microorganisms and are structurally diverse [5]. These amphiphilic molecules can be produced from cheap renewable agro-based substrates thus making the process cost-effective. Biosurfactants form micelles and facilitate hydrocarbon emulsification thus enhancing their availability for microbial uptake and subsequent degradation. The present work is aimed at bioremediation of two sparsely soluble PAHs namely fluoranthene and pyrene. Removal of PAH concentrations several folds higher than their solubility in water was observed.

2. Materials and Methods

2.1 Microorganism and Culture Conditions

A bacterial isolate from crude oil was used in the present study. The culture was maintained on Luria Bertanni (LB) agar plates and LB medium was used for the preparation of the primary inoculum. The inoculum from LB was then transferred to peptone glycerol ammonium salts (PGAS) medium [6] for biosurfactant production and incubated at 37°C with an agitation speed of 200 rpm.

2.2 Biosurfactant Production and Emulsification Studies

The biosurfactant production by the test culture was determined by the drop collapse assay. Briefly 5 µl of the cell free supernatant from the test culture was dropped on a plain surface and the shape of the drop was observed. Same volume of water and a solution of SDS, a well-known chemical surfactant were also added to the surface separately and the resultant shapes of the drops noted.

The property of the biosurfactant to emulsify petrol and diesel was tested by mixing equal volumes of cell free supernatant and either of petrol or diesel and vortexing it at high speed for 5 min. The resulting mixture was incubated at 25 °C for 24 h and then the emulsification index (EI) value was calculated [4] using the formula:

$$EI = \frac{\text{Height of emulsion layer}}{\text{Total height}} \times 100 \quad (1)$$

Emulsification of petrol and diesel with chemical surfactants like SDS and Tween 20 was also observed as a positive control.

2.3 PAH Utilization by the Test Bacterium

Two model PAHs namely fluoranthene and pyrene were used in this study. In one set of experiment, they were used as the sole carbon source for the bacterial growth. In another set of experiment, the bacterium was grown in PGAS medium and after the initiation of biosurfactant production, PAHs were separately added to the media. Both the PAHs were added at a concentration of 10 mg l^{-1}. Samples from the cultures were collected and observed for bacterial growth and the PAH contents.

2.4 Thin Layer Chromatography to Detect PAH Solubilization

To detect the content of PAH remaining in the medium, PAH solubilization assay was performed. Culture supernatant was collected immediately after PAH addition and also after PAH addition to the growth medium and incubation for 24 h. The supernatants collected were extracted with hexane and the absorbance was measured at 250 nm [4]. The percentages of PAHs present in the medium was calculated.

The cell free supernatants collected from the cultures were spotted manually on silica gel plates and allowed to air-dry. The plates were then developed in a solvent system consisting of chloroform and methanol in a ratio of 90:10. The developing jar was saturated with the solvent system for half an hour prior to development. After development the plates were observed under UV lamp to detect the PAHs.

3. Results

3.1 Biosurfactant production by the test culture

The test bacterium was identified to be *Pseudomonas aeruginosa* D2 and its evolutionary position was found to be among related *Pseudomonas* species.

Biosurfactant production by the test bacterial culture was confirmed from the drop collapse assay. This assay showed the relatively flattened appearance of the drop from the bacterial culture with respect to water which is free of any surface active agent and has a beaded appearance while the known surfactant SDS showed also showed a flat surface (Fig. 1).

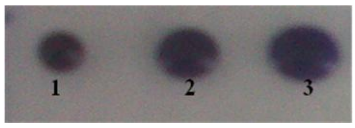

Figure 1: Drop collapse assay indicating biosurfactant production (1- water, 2-test bacterial culture supernatant, 3-SDS solution)

3.2 Emulsification Assay

The supernatant from the test bacterial culture promoted formation of emulsions of hydrocarbons (Fig. 2) due to the presence of biosurfactants. If biosurfactants had been absent, there would have been no emulsification as in the control where the aqueous phase and the hydrocarbon phase remained separated.

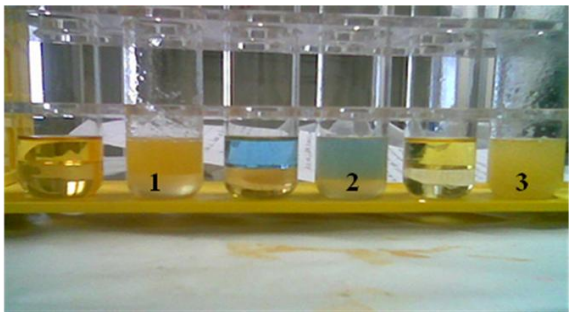

Figure 2: Emulsification of petrol (1), kerosene (2) and diesel (3) by the test culture supernatant

3.3 Bacterial growth in presence of different PAHs

The test bacterium showed scanty growth in presence of both pyrene and fluoranthene as the sole carbon source while the growth was significantly high when the PAHs were added to the PGAS medium, although lower than that in only PGAS medium (Fig. 3).

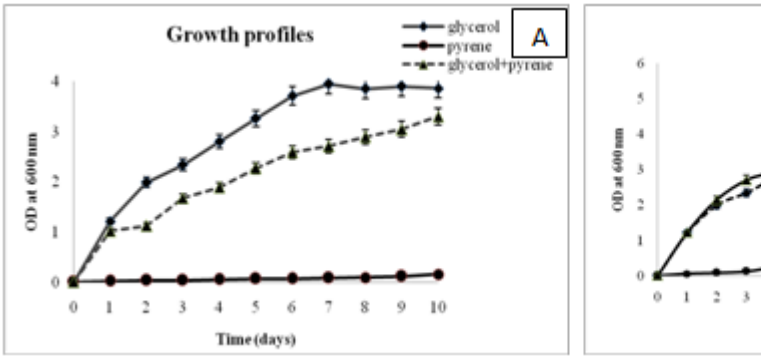

Figure 3. Growth profile of the test bacterium in presence of PAHs alone and in PGAS alone as well as in conjugation with pyrene (A) and fluoranthene (B)

3.4 PAH solulization

PAH solubilization experiments showed that both pyrene and fluoranthene were almost wholly solubilized within 24 h of addition to the bacterial culture (Fig. 4).

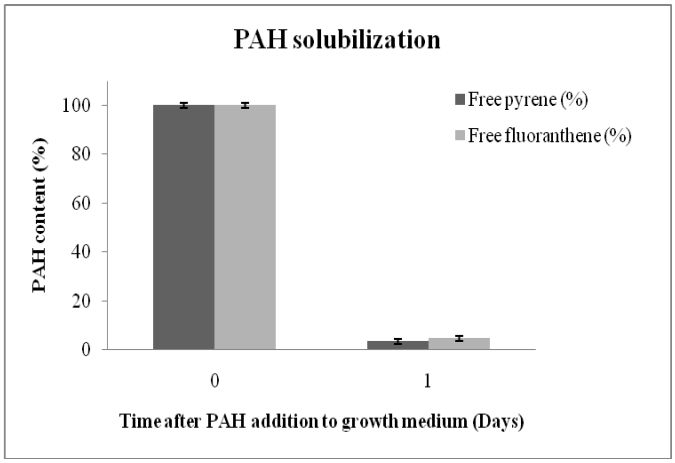

Figure 4: Solubilization of PAHs within 24 h of addition to the growth medium

TLC of the cell free supernatant from the cultures containing PAHs further substantiated this finding. Cell free supernatants from cultures grown in PGAS in conjugation with PAHs, showed no traces of the PAH indicating their complete remediation (Fig. 5). In both cases there were no spots corresponding to the PAHs as was observed in the control.

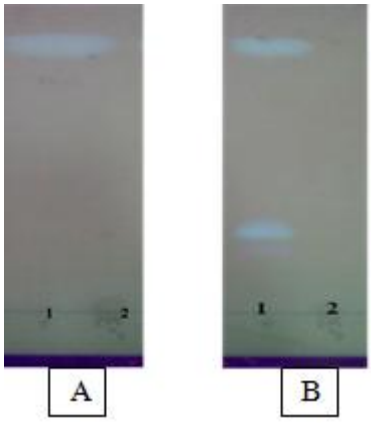

Figure 5: TLC showing complete PAH remediation (1- control PAH and 2- after remediation)

4. Conclusion

The test bacterium was very slow in utilizing pyrene and fluoranthene as sole carbon substrates. In the PGAS medium the biosurfactant production was initiated after around 48 h of culture inoculation. At this time point when the PAHs were added, the whole of the PAH was removed from the medium within 24 h. The solubility of fluoranthene is 0.206 mg l^{-1} while that of pyrene is 0.13 mg l^{-1} [7]. Such sparsely soluble compounds were made soluble by the biosurfactant produced by the test bacterium. The concentration of the PAHs added were 10 mg l^{-1} which was more than five folds to ten folds of their aqueous solubility. The PAH remediation occurred almost immediately with the initiation of biosurfactant production. Earlier reports have shown that around 5 mg l^{-1} of pyrene was degraded within 7 days [8] but in the present work tremendous enhancement in remediation was achieved and that too within a very short span of time. Although the inoculation of the test bacterium to any polluted site might not help as the growth of the bacterium was very feeble upon these substrates but after the biosurfactant production is initiated, the mix of the test bacterium and biosurfactant can be added to any polluted site to promote bioremediation. The efficiency of the test bacterium and its biosurfactant seems to open an avenue of development of a viable method for bioremediation within a short duration of time.

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