Studies on Enhancement of Solubilization and Biodegradation of Polyaromatic Hydrocarbon Using *Acacia Concinna* - A Natural Surfactant

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ABSTRACT

The increased solubilization of PAH (naphthalene and phenanthrene) by Saponin, a derived plant no ionic bio-surfactant, was investigated using biodegradation. The results obtained shows that the dissolving capabilities of Saponin for PAHs were higher than the representative synthetic no ionic surfactant, triton-X 100. The variables which were taken into the study were solution concentration, pH and temperature. The results show that the solubilization has been highly dependent on concentration, pH and temperature of solution. The molar solubilization ratio (MSR) of Saponin in case of naphthalene and phenanthrene was found to be 4-8 times and 5-15 times of those of the triton-X 100 respectively. Also the apparent solubility of naphthalene and phenanthrene decreased to about 40% and 30% respectively with the increase in pH value of solution from 4.0 to 8.0. Saponin is more efficient in increasing PAHs solubilization than synthetic no ionic surfactants which leads to the removal of organic pollutants from contaminated soils.

KEY WORDS: Saponin, Solubilization, Biodegradation and Natural Surfactant.

1. INTRODUCTION

Extensive use, accidental spills, leaks of organic hydrocarbons and improper disposal such as Organic solvents, poly aromatic hydrocarbons and hydrocarbons have resulted in long-term persistent sources of pollution of soil and groundwater, which becomes a major environmental issue because of their adverse effect on human health. One of the main reasons for the long term persistence of water repellant hydrocarbons in polluted environment is their low water solubility, which enhances their absorption to soil particles and restricts their availability to biodegradable microorganisms.

Need for the Study: Conventional bioremediation processes for PAHs has limitations due to the low solubility and strong absorption of PAHs to soil. Bacterial responses to synthetic surfactants have results in no production of any robust. Natural surfactants from plant origin are cheap, abundant, eco-friendly and are easily biodegradable than synthetic surfactants. Vast future research is required to make the usage of natural plant surfactants as a standard tool in biological soil and water prevention measures.

Objectives of The Study: The objectives of this study are,

- To study the effect of *Acacia concinna*-a natural surfactant on the solubilization and biodegradation of naphthalene and phenanthrene.
- To study the effect of operating variables such as the initial concentration of PAHs in individual and mixture of PAHs, initial pH of PAHs, temperature and concentration of surfactant on efficiency of solubilization and biodegradation of PAHs.
- To compare the efficiency of *Acacia concinna on* solubilization and biodegradation of PAHs with that of Synthetic surfactants.

Review of Literature:

- Biodegradation of PAHs is restricted due to their limited bioavailability, which is widely associated with PAH water repellant nature and strong.
- It has been found that the mass transfer rate of PAHs into the aqueous phase is the rate-limiting step in their degradation. The bioavailability of soil contaminants can be increased by stimulating the process that is limiting the rate of biodegradation. However, the most promising way to increase a contaminant's bioavailability is thought to be the addition of surface active agents such as surfactants that stimulate mass transfer rates.
- Microbial surfactants many bacteria, yeasts, and fungi produce extracellular or membrane-related surface active compounds, called Bio-surfactants
- Studies have proved that the use of surfactants result in a several-fold which will results in increasing the solubility of hydrocarbons.
- Studies with synthetic nonionic and an-ionic surfactant additions have showed that they can increase/decrease the biodegradation of soil xenobiotics and a wide range of other hydrocarbons too.
- Nonionic surfactants have also proved to cultivate biodegradation at higher concentrations above their CMC. Indeed wide synthetic surfactants are known to exhibit an inhibitory effect on PAH-degrading microorganisms.
- Anionic and nonionic surfactants are low likely to be absorbed to the soil surface. Cationic surfactants have been used to lower the aquifer permeability by absorption on to the aquifer materials.

- Natural plant derived surfactants mainly surface active agents like saponins without much processing are of main focus in recent years. Saponin glycoside from quillaja bark.
- Another plant based nonionic surfactant derived from Sapindus mukorossi, commonly known as ―Soap nut or ―Reetha in the Indian subcontinent, has been tested to reuse polluted soils. The significant finding from literature review was the solubilization and biodegradation of PAH is increased by using natural surfactant.

2. MATERIALS AND METHODS

This study has been designed for the enhancement of solubilization and biodegradation of PAH by increasing their bioavailability using *Acacia concinna.*
PAHs – Naphthalene and Phenanthrene.
Dry fruit (Acacia concinna or shikakai)
Synthetic Surfactants - TX–100
Surface tensiometer and UV–Vis spectrophotometer

MSM Medium: Biodegradation experiments were conducted at room temperature on an orbital shaker with autoclaved mineral salts medium (MSM), consisting of 435 mg of K_2HPO_4, 668 mg of $Na_2HPO_4 .7H_2O$, 85mg of NH_4Cl, 22.5 mg of $MgSO_4.7H_2O$, 27.5 mg of $CaCl_2$, and 0.25 mg of $FeCl_3.6H_2O$ per liter of deionized water.

Microorganism: The different type of simple to complex carbon compound degrading bacteria used for the study is *Pseudomonas aeruginosa* 2074. Stain is to be maintained in the nutrient medium.

Glass wares: All glass wares like beakers, pipettes, burettes, conical flasks, etc. were required for this study were cleaned with grade1 detergent also they have rinsed several times with water and finally in purified water.

Simulation of PAHs and Surfactant Solution Preparation: Every experiment was done in the borosil tubes of volume 30ml. A stock solution (1g/l) of naphthalene and phenenthrene were prepared by mixing PAHs in pure methanol. Solutions were mixed in rotary shaker at 200 rpm for 48 hours at (20°C). Then solutions were filtered to separate the crystalline portion of the PAHs. Surfactant solution has to be prepared by dissolving 0-2.g/l of TX-100, SDS. The tubes should be put in shaker at 150rpm for 24hrs for complete solubilization.

Extraction of Saponin from *Acacia concinna* pods: After taking off seed from pods, the Peri-Carp was let dry in an oven at 50°c for about two days and then crushed to powder in a mixer. The extracted powdered sample with methanol will give brown syrup. The suspended particles are separated by centrifugation at 8000 rpm for 15 – 20 minutes simultaneously followed by vacuum evaporation and let the extract to dry at 60 – 80°c which would give a pale yellow colored sticky paste.

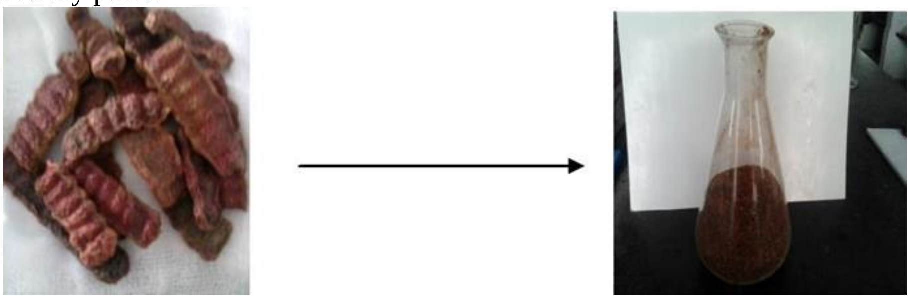


Figure.1. Preparation of Acacia concinna powder

PAHs, in general, are ubiquitous environmental pollutants and are formed from both natural and anthropogenic sources. Because of their low water solubility and strong sorption to soil limits their availability to microorganism. This study has been designed for the enhancement of solubilization and biodegradation of PAH by increasing their bioavailability using *Acacia concinna.*

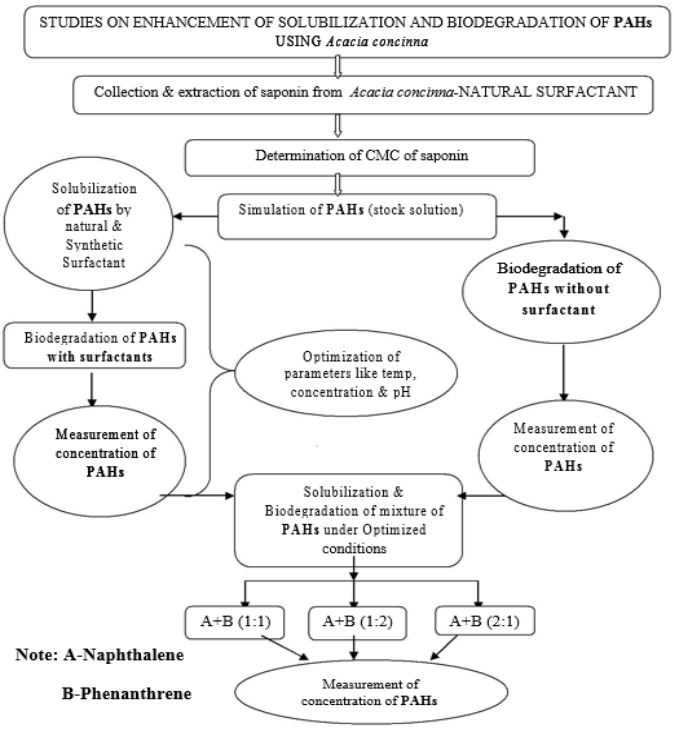
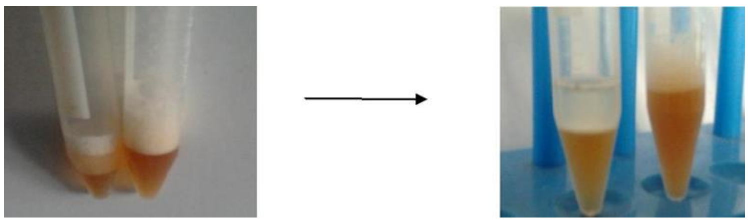


Figure.1. The methodology of the proposed study consists of the above steps

Qualitative Analysis of Saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.



Formation of emulsion **Persistence of emulsion**

Figure.2. Qualitative analysis of saponin

Quantitative Analysis of Saponin: The samples were ground and 10g of each was put into a conical flask and 100 cm^3 of aqueous ethanol were added. Total precipitated saponin obtained was 18.23 g. The scheme is as shown in Figure 3.2.The amount of saponin obtained from acacia concinna was 800mg/g.

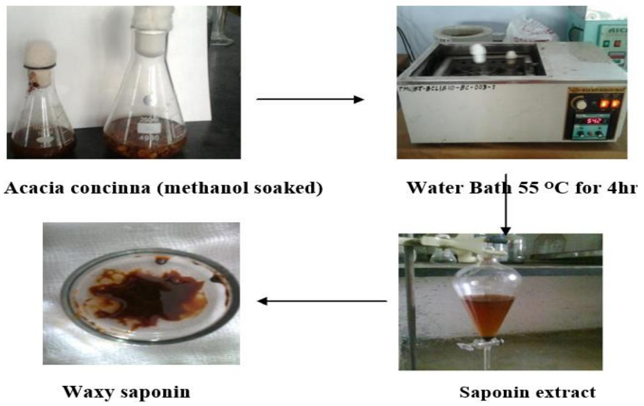
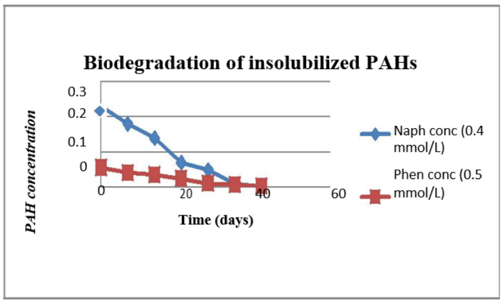


Figure.3. Quanitative analysis of saponin

Biodegradation of PAHs: Stock solutions (1g/L) of naphthalene and phenanthrene were prepared with methanol. The biodegradation experiment was carried out in 250 ml flasks on a rotary shaker (150 rpm). A thin PAH crystal layer was obtained on the bottom of the glass vials when the methanol was allowed to evaporate overnight and 0.5 ml mixed culture was added to each vial containing different concentration of PAHs with 50 ml of MSM medium. Abiotic control vial was prepared without culture. Growth of PAHs degrading culture was measured by spectrometer by measuring the optical density.



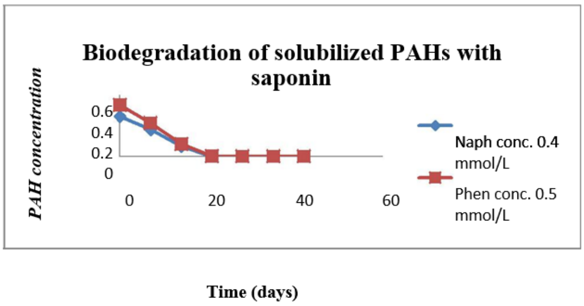


Figure.4. Biodegradation of PAHs with and without surfactant action

Solubilization of PAHs by Surfactants: Solubility test were done in Teflon lined 30 ml borosil glass vials. For solubilization 0.1ml of PAHs (naphthalene and phenanthrene) obtained from the stock will mix with 10 ml of synthetic surfactant and natural surfactant solution. Sample solutions were mixed with magnetic stir at 200 rotations per minute for two days. The concentrations of PAHs were determined by calculating the absorbance of naphthalene at 274 nanometers and phenanthrene at 360 nm wave lengths using an UV–Vis spectrophotometer.

Table.1. Solubilization of naphthalene and phenanthrene using Saponin

Saponin concentration (mg/20 ml)	Optical density values at 274 nm			
	Naphthalene Conc		Phenanthrene Conc	
	5 mg	20 mg	5 mg	20 mg
0	0.022	0.047	0.101	0.400
50	0.138	0. 039	0.0683	0.217
100	0.031	0.022	0.0438	0.0802

3. RESULTS AND DISCUSSION

Based on results and observations the following conclusions can be drawn. The saponin obtained from pods of acacia concinna was 800 mg/g. The saponin showed 97.5% solubility for naphthalene (0.4 mmol/L) and 80% for phenanthrene (0.5 mmol/L), which is comparatively higher than those obtained using triton x-100. Higher MSR and log K_m values (0.836 and 5.0 resp.) was obtained for naphthalene concentration of 1.5 mmol/L, saponin concentration of 1.212 mmol/L, at pH -5, and at temperature 30^O C.

The order of the PAHs biodegradtion reaction was found by fitting the PAHs biodegradtion data (naphthalene concentration - 0.5 millionmol/Litre, phenanthrene -0.5 millionmol/Litre at saponin – 1.212 mmol/L) in the rate equations. The second order rate curve plot showed highest R^2 value of 0.9484 for naphthalene and 0.868 for phenanthrene. Saponin showed higher removal ability as the PAHs was fully utilized within 14 to 21 days for biodegradation, while Triton-X 100 took 42 days for solubilisation and biodegradation.

While solubilizing and degrading mixture of PAHs (Naphthalene: Phenenthrene), effective results are obtained for 1:1 and 1:2 ratios. Higher Phenanthrene concentration in 2:1 mixture ratio, slower the biodegradation phenomena. In the presence of saponin complete biodegradation of PAHs occurred in14 days, whereas it was 42 days in the absence of saponin. Thus the natural surfactant, saponin was found to be effective in enhancing the solubilization and biodegradation of naphthalene and phenanthrene than the synthetic surfactant, triton x-100.

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