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Biosurfactant Triggered Degradation of Polyaromatic Hydrocarbons in Agricultural Soil

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Among the various polyaromatic hydrocarbons (PAHs), naphthalene, fluorene, phenanthrene and pyrene are the predominant pollutants commonly detected in agricultural soils, primarily due to transportation emissions, atmospheric deposition and accidental spillages. To facilitate the degradation of these persistent pollutants, a lipopeptide-based biosurfactant was isolated from *Lysinibacillus* sp. MW444883 (Accession No.: NAIMCC-B-03918). The biosurfactant exhibited strong oil dispersion activity, showed no halo formation in the CTAB/ methylene blue agar test, indicating the absence of glycolipid-type surfactants and produced blue and violet colour reactions with bromothymol blue and biuret tests, respectively, confirming its amino-containing (lipopeptide) nature. Its excellent emulsification property, as evidenced by oil fragmentation into fine droplets, enhanced the bioavailability of hydrophobic PAHs, making them more accessible for microbial degradation in soil. Biodegradation studies conducted in Inceptisol alkaline soil (Delhi soil) demonstrated degradation till 21 days and half-life ranging from 1.5-8.3 days for naphthalene, 7.4-14.2 days for fluorene, 6.1-22.6 days for phenanthrene and 12.3-27.2 days for pyrene under different treatment conditions involving oil, biosurfactant, and *Kocuria rosea* in comparison with native microbial activity. These findings highlight the potential of the *Lysinibacillus* derived lipopeptide biosurfactant as an effective bioremediation agent for the degradation of priority pollutant (PAHs) in agricultural soils.

Persistent organic pollutants (POPs) in the environment that pose serious health risks to the general public are polycyclic aromatic hydrocarbons, or PAHs. The United States Environmental Protection Agency (USEPA) has nominated a total of 16 PAHs as “Highlighted pollutants”. Among different PAHs, naphthalene, fluorene, phenanthrene and pyrene are highly associated with industries and widely present in the environments (Liu *et al*, 2019; Mallick *et al*, 2011). The need for crude oil and its byproducts is increasing across a number of industries and as a result, pollution from heavy metals, petroleum hydrocarbons and oil is getting worse. Continuous increasing incidences of oil refineries, emission of pollutant due to biomass burning, coal production, cracker burning have become the major source of pollutant near the surroundings and particularly in the agricultural fields due to deposition of them on soil and transportation to the food produce (Li *et al*, 2022, Kim *et al*, 2019, Gangadhari *et al*, 2021). Such contamination causes ecological harm to aquatic, terrestrial and marine environments. As a result, several methods, such as the use of surfactants and sophisticated technology, have been put forth to remove them from the environment. Biosurfactants are regarded as a sensible choice and environmentally friendly material for remediation technologies.

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PAHs are absorbed through eating, skin contact and breathing. The extremely lipid-soluble characteristics are easily absorbed into human fat tissues from the digestive system and it is anticipated that these features will travel and remain within the human body fat. Some of the negative consequences on people are immunity, tumour formation and reproduction. As a result of the metabolic responses brought on by PAH exposure, people who live in urban neighborhoods run the risk of developing long-term chronic health impacts, such as skin-related disorders and lung cancer (Hishamuddin *et al*, 2023).

A rapidly developing biomolecule for the twenty-first century is microbial biosurfactant. These are amphiphilic substances that are made by microbes and have special qualities that lower surface tension activity (Thundiparambil *et al,* 2024). Biosurfactants, being secondary metabolites, are classified as amphiphilic compounds due to their combination of hydrophilic and hydrophobic components, either generated or connected to the microbial cell. The ability of the biosurfactant to reduce the surface tension or interfacial tension between two immiscible solutions in respect to the cell surface is known as surface active properties. Furthermore, biosurfactants are recognized for their

emulsifying properties, which facilitate the solubility of hydrocarbons in an aqueous solution and vice versa. Biosurfactants are utilized in the industry for the manufacturing of detergents, emulsification, lubrication, foam creation, dispersion and solubilization of various phases because of their special qualities. Biosurfactants are preferred over chemical surfactants due to their high biodegradability, low critical micellization concentration (CMC), biocompatibility, durability in a range of environmental conditions and low toxicity (Ciurko *et al*, 2023). Studies on bioremediation indicate that the addition of biosurfactants increases the hydrocarbons' bioavailability to microbial enzymes for biodegradation and consumption. By solubilizing PAHs in an aqueous solution, biosurfactant might increase the bioavailability of these hydrocarbons to microbes and perhaps speed up the targeted compound's biodegradation. Biosurfactants are amphiphilic, surface-active metabolites produced by several microorganisms. Few researchers have examined the function of biosurfactant fungi in PAH breakdown, despite the fact that numerous fungi have been known to produce them (Gupta *et al,* 2023). Therefore, in present study, biosurfactant was isolated from microbes and its performance for degradation was evaluated for different carcinogenic PAHs in agricultural soil.

MATERIALS AND METHODS

Screening of microbes for biosurfactant production

Previously isolated bacteria from ICAR-IARI, New Delhi, was used for oil dispersion properties (Khandelwal *et al*, 2022). *Lysinibacillus sp.* MW444883*, Kocuria rosea* KY614290, *Arthrobacter sp.* KY614291, *Pseudomonas aeruginosa* KY614293, *Bacillus megaterium* MW444917, *Nocardioides sp.* KY614294, *Pseudomonas sp.* MW444887, *Bacillus amyloliquefaciens* MW532755 and *Staphylococcus sp.* MW44488 were randomly chosen for oil dispersion behaviour of microbes (Figure 1).

Microbes were cultured in nutrient broth for three days and cell free supernatant (CFS) were obtained after centrifugation at 5000 rpm for 5 min. The CFS was tested for oil dispersion behaviour. For this, 30 mL water was added in Petri plate followed by 20 µL crude oil and 10 µL CFS. The observation was recorded for oil dispersion behaviour (Figure 2). Based on observation, two bacteria namely, *Lysinibacillus sp.* and *Bacillus amyloliquefaciens*

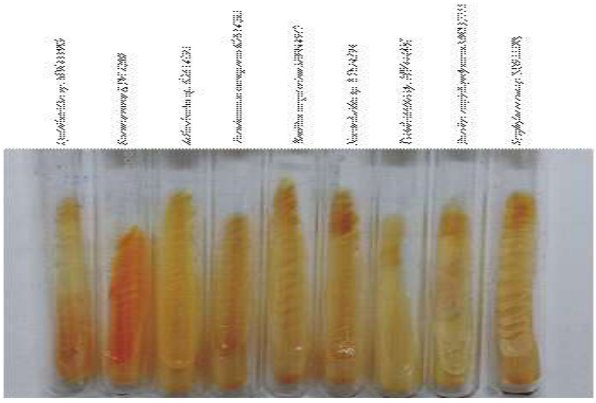

Figure 1. Bacteria used for oil dispersion

were found to be suitable for oil dispersion property. However, consistency and stability for oil dispersion was more in case of *Lysinibacillus* sp. as compared to *Bacillus amyloliquefaciens*. Therefore, *Lysinibacillus* sp. was chosen for further study.

Isolation of biosurfactant and preliminary identification

Lysinibacillus sp. was cultured for different days at different rotation. Cell free supernatant (CFS) was obtained after centrifugation at 5000 rpm for five minutes. The CFS was lyophilized and obtained product is called as crude biosurfactant (BS). The BS was tested using cetyl tri methyl ammonium bromide (CTAB)/methylene blue agar test (Saravanan and Vijayakumar, 2012)), bromothymol blue (BTB) and biuret test (Satake *et al*, 1960; Ong and Wu, 2018).

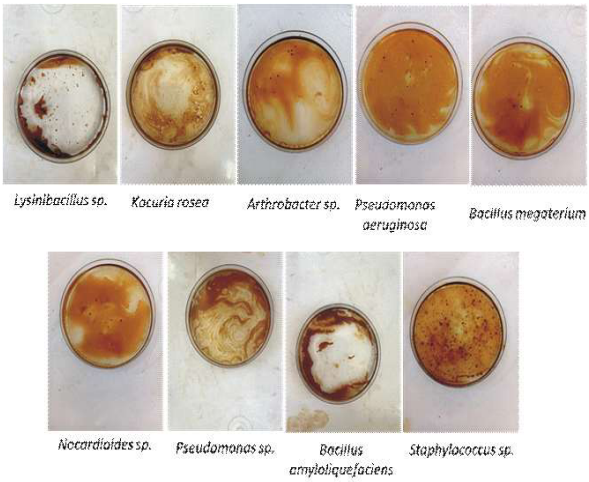

Figure 2. Oil dispersion behaviour using cell free supernatant from different bacteria in *Cetyl trimethyl ammonium bromide (CTAB)/methylene blue agar test*

CTAB agar plates were prepared by dissolving 0.15 g CTAB, 0.005 g methylene blue and agar (12 g) to 1 L distilled water. The pH was adjusted to 7 and autoclaved. After autoclave, the solution was poured into Petri plates and allowed to dry under laminar flow in aseptic conditions. After drying ~6.5 mm two holes were developed and wells were poured with CFS in such a way that it should not come out from well. Afterwards, it was incubated in BOD for 2 d at 37ºC and diameter of halo zone measured.

In bromothymol blue (BTB) test, BTB was dissolved into phosphate-buffered saline (0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4 and 1 M NaCl) and pH of solution was adjusted to 7.2. The qualitative response was observed by changes in colour in control (without biosurfactant) and with biosurfactant.

In biuret test, CFS (cell free supernatant) (2 mL) was heated at 70ºC followed by mixing with 1 M NaOH solution. Drops of copper sulfate (1%) were slowly added and change in colour was observed. The qualitative response was observed with the variation in colour in control (without biosurfactant) and test solution (with biosurfactant) (Feignier *et al*, 1995; Jamal *et al*, 2012).

Monitoring by the optical microscope

Using a Leica DM750 microscope, the steps of water separation and oil droplet fragmentation were seen after adding 10 µL of biosurfactant to 20 µL of crude oil. A 20-times magnification camera mounted in the microscope was used to take the pictures and oil droplet fragmentation pattern were captured (Abdulraheim, 2018).

Soil Collection and Characterization

Inceptisol alkaline soil was collected from agricultural fields of ICAR-Indian Agricultural Research Institute, New Delhi for recovery and degradation study. Each soil profile was sampled to a depth of 15 cm. The soil was air dried at room temperature in the shade, pulverized with a mortar and pestle and sieved through a 2 mm sieve and placed in plastic bags. The soils were characterized using normal analytical processes, and Table 1 shows the physic-chemical properties. Using a Control Dynamics pH meter (APX 175 E/C) equipped with a calomel glass electrode, the pH of soil sample was determined. The Walkley and Black method was used to determine the soil's organic carbon content. Bouyouco's hydrometer method was used to determine the soil texture (sand, silt and clay).

Table 1. Characterization of soil

Properties	
pH	8.3
EC (dS m^{-1})	0.5
Sand (%)	54.4
Silt (%)	23.3
Clay (%)	22.3
Organic Carbon (%)	0.36
Available Nitrogen (kg ha^{-1})	215
Available Phosphorus (kg ha^{-1})	25.6
Available Potassium (kg ha^{-1})	185.8

A conductivity meter was used to determine the soil sample's electrical conductivity (EC). The other criteria were available potassium, available phosphorus and available nitrogen (Day, 1965).

Recovery of PAHs from soil

A 50 mL conical centrifuge tube (Eppendorf) was used to hold 5 g of soil. A mixture of polyaromatic hydrocarbons (PAHs) was introduced by adding 5 mL of a 10 mg L^{-1} acetonitrile solution (equivalent to 50 µg of total PAHs) to the soil, and the solvent was allowed to evaporate for 2 h. Subsequently, 2.5 mL of distilled water was added to the fortified soil sample and left to equilibrate for 30 min. Extraction was initiated by adding 5 mL of acetonitrile, followed by vortexing for 2 min. To remove residual moisture, 2 g of anhydrous sodium sulfate was added and the mixture was vortexed for 1 min. Next, 0.75 g of sodium chloride was introduced and the sample was thoroughly shaken. The mixture was then centrifuged at 5000 rpm for 5 min. After centrifugation, 1 mL of the supernatant was transferred to a 2 mL micro-centrifuge tube containing 150 mg of anhydrous magnesium sulfate and 50 mg of primary secondary amine (PSA) sorbent for cleanup. The tube was vortexed vigorously for 1 min and centrifuged again at 5000 rpm for 5 min. Finally, 0.5 mL of the cleaned supernatant was transferred to a fresh micro-centrifuge tube, the solvent was evaporated to dryness and the residue was reconstituted in 10 mL of acetonitrile. The prepared samples were analyzed for polyaromatic hydrocarbon fractions using high-performance liquid chromatography (HPLC).

Degradation in soil under laboratory condition

Air dried soil (90 g) in 250 mL sterilized conical flask was supplemented with the sterile distilled water/biosurfactant solution to attain field capacity. As per treatments, soil

samples were spiked with mixture of naphthalene, fluorene, phenanthrene and pyrene at 100 µg g^{-1} levels of each PAH. This was done by fortifying 10 g soil with 5 mL solution containing 2000 µg mL^{-1} of each PAH and mixing it with 90 g conditioned soil. In another treatment, crude oil was added at 250 µg g^{-1} to assess the effect. Study was performed with 8 treatments and 3 replicates. In one treatment, *Kocuria rosea* was taken along biosurfactant treatment (@ 850 µg g^{-1}) to evaluate effect of these amendments on PAHs degradation. After inoculation samples were incubated at 28±1°C. At regular intervals (0, 3, 7, 14, 21 days) samples (5 g) were aseptically removed from each treatment for extraction of PAH and analysis using HPLC. The details of treatments are given in Table 2.

Table 2. Treatment details

Treatment	Description
T1	Soil + PAHs
T2	Soil + PAHs + BS (@ 425 µg g^{-1})
T3	Soil + PAHs + BS (@ 850 µg g^{-1})
T4	Soil + PAHs + BS (@ 850 µg g^{-1}) + *Kocuria rosea*
T5	Soil + Oil + PAHs
T6	Soil + Oil + PAHs + BS (@ 425 µg g^{-1})
T7	Soil + Oil + PAHs + BS (@ 850 µg g^{-1})
T8	Soil + Oil + PAHs + BS (@ 850 µg g^{-1}) + *Kocuria rosea*

Statistical Analysis

PAH degradation data was subjected to one-way ANOVA and interactions were noted at $p<0.001$. Further, Tukey's HSD test was performed for multiple comparison among treatments effects and interactions.

The degradation data was fitted to the first order kinetic equation: $\ln (C_t/C_0 = -kt$; where, C_0 is the apparent initial concentration (µg g^{-1}), C_t is the concentration (µg g^{-1}) after a lapse of time t (days), and k is the degradation rate constant. The half-life ($t_{1/2}$) values were calculated from the k value using following formula: $t_{1/2} = 0.693/k$

RESULTS AND DISCUSSION

Identification of functional group/bond in biosurfactant

Incubation of CTAB/methylene blue agar plate at 37ºC for 48 h indicates the absence of blue colour halo zone. It shows the absence of rhamnolipid type of biosurfactant (Figure 3a). Similarly, presence of blue colour in bromothymol blue test indicates complexation reaction between amino and bromothymol blue. Changes in colour from green to blue indicates the presence of amino group in biosurfactant. (Figure 3b) In another biuret test, formation of violet colour indicates the presence of peptide bonds. (Figure 3c).

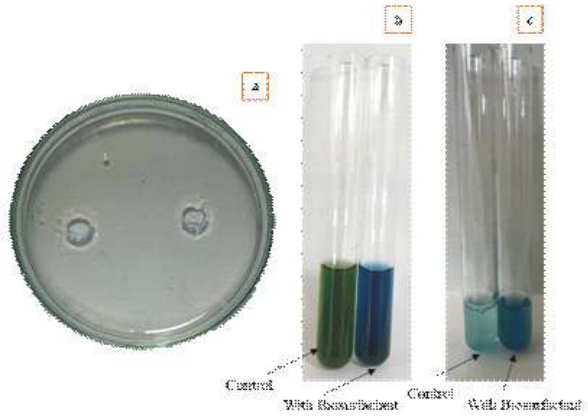

Figure 3. Identification of functional groups/bonds using a) Cetyl trimethyl ammonium bromide (CTAB)/methylene blue agar test, b) Bromothymol blue (BTB) test and c) Biuret test

Preliminary identification revealed the presence of amino and peptide bond in the biosurfactant. Ong and Wu (2018) demonstrated the complexation reaction of amino group with bromothymol blue and variation in colour demonstrated due to changes in pH of solution. In our case also, formation of blue colour from green indicated the changes in pH and complexation reaction between amino group and bromothymol blue. Therefore, putative characterization can be considered as lipopeptide kind of biosurfactant. Earlier, John *et al* (2021) and Bhardwaj *et al* (2016) also extracted lipopeptide biosurfactant from *Lysinibacillus* sp.

Monitoring of water separation and oil droplet fragmentation

The biosurfactant breaks down the oil into small droplets. The droplet accumulates and flocculate and rupturing of droplets took place (Figure 4).

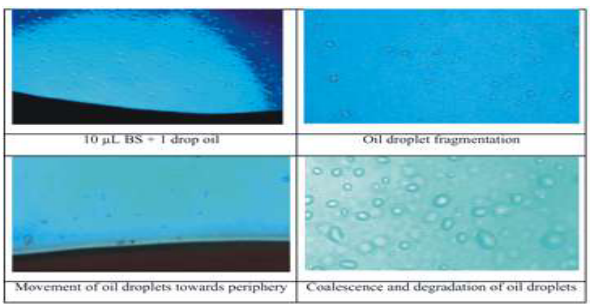

Figure 4. Oil droplet fragmentation and movements towards

Oil droplets start to move towards periphery and due to coalescence, degradation of oil particle into small droplet occurred (Figure 5). The observed oil droplet fragmentation upon biosurfactant addition indicates a substantial reduction in interfacial tension, promoting dispersion stability in the oil–water system. The biosurfactant molecules are likely adsorbed at the interface, reducing cohesive forces and facilitating droplet breakup through steric and electrostatic stabilization (Desai and Banat, 1997). This fragmentation increased the surface area available for microbial and enzymatic interactions, thereby enhancing hydrocarbon biodegradation efficiency (Mukherjee *et al*, 2006). The formation of smaller, uniformly distributed droplets further confirms the strong emulsifying potential of the biosurfactant. Similar fragmentation effects have been reported for rhamnolipids and lipopeptides in hydrocarbon degradation systems (Pacwa-Piociniczak *et al*, 2011). Overall, the findings highlight that biosurfactant-mediated droplet fragmentation plays a crucial role in improving hydrocarbon bioavailability and degradation kinetics.

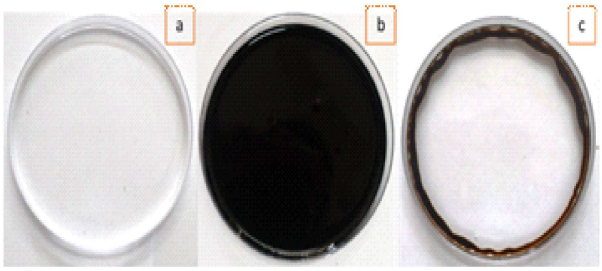

Figure 5. Effect of biosurfactant addition on oil dispersion a) distilled water, b) 100% crude oil and c) biosurfactant added crude oil on water surface

Recovery of PAHs from soil

The calibration curve for naphthalene, fluorene, phenanthrene and pyrene was developed using HPLC. Based on the methodology, HPLC instrument were optimized for analyzing naphthalene, fluorene, phenanthrene, and pyrene. The analysis wavelengths were 220 nm for naphthalene, 210 nm for fluorene, and 246 nm for pyrene and phenanthrene. Based on recovery

Table 3. Degradation of PAHs in the absences and presence of oil and *Kocuria rosea* in Delhi soil

PAH_s	Days	Degradation (%)							
		T1	T2	T3	T4	T5	T6	T7	T8
Naphthalene	1	23.9^{e}	48.0^{d}	53.3^{c}	52.3^{c}	25.1^{e}	48.6^{d}	57.4^{a}	55.5^{b}
	3	38.8^{f}	68.6^{d}	74.4^{b}	72.6^{c}	40.5^{e}	69.2^{d}	76.5^{a}	75.4^{ab}
	7	57.9^{f}	89.4^{d}	95.2^{ab}	94.2^{cb}	59.4^{f}	86.6^{e}	96.6^{a}	93.1^{c}
	14	70.5^{d}	98.6^{b}	100.0^{a}	100.0^{a}	72.3^{c}	99.0^{ab}	100.0^{a}	100.0^{a}
	21	85.2^{c}	100.0^{a}	100.0^{a}	100.0^{a}	87.8^{b}	100.0^{a}	100.0^{a}	100.0^{a}
Fluorene	1	5.7^{d}	9.7^{bc}	15.7^{a}	12.0^{b}	5.1^{d}	8.4^{c}	16.9^{a}	11.4^{b}
	3	21.7^{c}	26.2^{b}	30.7^{a}	25.4^{b}	20.4^{c}	22.3^{c}	32.4^{a}	24.5^{b}
	7	33.6^{e}	49.8^{cd}	59.0^{b}	50.4^{c}	31.7^{e}	47.5^{d}	62.2^{a}	48.8^{cd}
	14	58.5^{e}	65.7^{c}	69.6^{b}	68.4^{b}	54.5^{f}	63.4^{d}	71.4^{a}	66.4^{c}
	21	66.0^{e}	80.8^{c}	85.3^{b}	82.1^{c}	63.5^{f}	78.5^{d}	87.5^{a}	82.1^{c}
Phenanthrene	1	6.8^{cb}	5.4^{cd}	7.5^{ab}	6.6^{cb}	7.3^{ab}	4.0^{d}	8.4^{a}	7.2^{ab}
	3	13.3^{e}	25.4^{c}	27.4^{b}	25.8^{bc}	14.4^{e}	21.1^{d}	29.4^{a}	26.4^{bc}
	7	26.3^{f}	50.1^{c}	52.7^{ab}	51.8^{b}	28.2^{e}	45.6^{d}	54.2^{a}	52.7^{b}
	14	40.5^{g}	68.2^{d}	71.4^{c}	74.2^{ab}	43.2^{f}	63.1^{e}	73.2^{cb}	75.1^{a}
	21	51.7^{f}	84.8^{c}	88.7^{b}	89.5^{b}	54.5^{e}	78.3^{d}	91.5^{a}	91.5^{a}
Pyrene	1	7.5^{cab}	5.7^{c}	8.1^{ab}	6.5^{cb}	6.1^{cb}	6.6^{cb}	9.3^{a}	6.4^{cb}
	3	15.1^{d}	24.1^{c}	26.9^{ab}	25.1^{cb}	13.1^{d}	24.9^{cb}	29.0^{a}	27.4^{a}
	7	25.5^{e}	32.0^{cd}	36.9^{b}	33.1^{c}	22.5^{f}	30.5^{d}	39.2^{a}	35.2^{b}
	14	33.9^{d}	48.0^{cb}	49.7^{ab}	49.0^{ab}	31.6^{e}	46.8^{c}	50.5^{a}	50.4^{a}
	21	43.8^{e}	64.2^{c}	70.3^{a}	66.1^{b}	43.4^{e}	61.3^{d}	71.5^{a}	67.9^{b}

T1: Soil+PAHs; T2: Soil+PAHs+BS (@ 425 µg g^{-1}); T3: Soil+PAHs+BS (@ 850 µg g^{-1}); T4: Soil+PAHs+BS (@ 850 µg g^{-1})+*Kocuria rosea*; T5: Soil+Oil+PAHs; T6: Soil+Oil+PAHs+BS (@ 425 µg g^{-1}); T7:Soil+Oil+PAHs+BS (@ 850 µg g^{-1}); T8: Soil+Oil+PAHs+BS (@ 850 µg g^{-1})+*Kocuria rosea*

experiment, the recovery from soil was naphthalene 90.1±2.6%, fluorene 90.8±2.6%, phenanthrene 94.5±1.8% and pyrene 85.4±2.1%.

Screening of biosurfactant for PAHs degradation in Delhi soil under laboratory condition

Effect of biosurfactant on degradation of PAHs in the presence and absence of oil/native and inoculated microbes were investigated and degradation data is presented in Table 3. Results showed that PAHs degradation for naphthalene, phenanthrene was almost at par for different days in treatment T3 and T4. However, for fluorene and pyrene, degradation was significant little higher in case of T3 as compared to T4. It shows that added biosurfactant @ 850 µg g^{-1} can be better substitute of in native contaminated soil as compared to inoculation of *Kocuria rosea (*microbe) from outside. Compared to T1, degradation was higher in biosurfactant/added microbes in the soil. During 21th day, degradation of naphthalene (85.2-100%), fluorene (66.0-87.5%), phenanthrene (51.7-91.5%) and pyrene (43.4-71.5%) was observed over different treatments T1-T8. As compared to T1, T2, T5, and T6; more than 50% degradation was observed rapidly in the presence of biosurfactant (850 µg g^{-1}) and addition of *Kocuria rosea*. Under different treatments as compared to T1 and T5; half-life of naphthalene (1.5-2.3 d), fluorene (7.4-9.6 d), phenanthrene (6.1-9.5 d) and pyrene (12.3-16.3 d) was significantly lower. Addition of biosurfactant with/without oil as compared to *Kocuria rosea* and native microbial population helped to enhance the degradation of pollutants in contaminated soil. Baharuddin *et al* (2020) developed an ionic liquid based dispersant and effectiveness of formulated ILs was found between 70.75-94.71%.

In another investigation, biosurfactant isolated from *Candida bombicola* URM 3718 was formulated with potassium sorbate and stored till 120 days and tested for surface tension and oil dispersion property (Freitas *et al*, 2016). Rongsayamanont *et al* (2016) developed the lipopeptide-sodium dihexyl sulfosuccinate based micro-emulsion based formulation for developing oil dispersant. Corexit dispersants were formulated with one anionic surfactant, bis-(2-ethylhexyl) sulfosuccinate (DOSS) and three non-ionic surfactants, including sorbitan monooleate (Span 80), sorbitan monooleate polyethoxylate (Tween 80) and sorbitan trioleate polyethoxylate (Tween 85) and used for oil spill removal (Place *et al*, 2016).

CONCLUSION

Lipopeptide based biosurfactant isolated from *Lysinibacillus* sp. and it was found to be effective for oil dispersion and found to be better alternative of adding in the soil as compared to inoculation of microbes for

Table 4. Degradation kinetic parameters for PAHs in the absences and presence of oil and *Kocuria rosea* in Delhi (Inceptisol, alkaline) soil

Treatments	Naphthalene		Fluorene		Phenanthrene		Pyrene	
	Equation (R^2)	$t_{1/2}$	Equation (R^2)	$t_{1/2}$	Equation (R^2)	$t_{1/2}$	Equation (R^2)	$t_{1/2}$
T1	y=-0.0832x+4.4929 (0.975)	8.3	y=-0.0529x+4.6093 (0.979)	13.1	y=-0.0307x+4.6038 (0.991)	22.6	y=-0.0288x+4.5856 (0.966)	24.1
T2	y=-0.2961x+4.4434 (0.993)	2.3	y=-0.0764x+4.5815 (0.992)	9.1	y=-0.0758x+4.632 (0.994)	9.1	y=-0.0469x+4.6003 (0.983)	14.8
T3	y=-0.4166x+4.4919 (0.987)	1.7	y=-0.0865x+4.5517 (0.977)	8.0	y=-0.0882x+4.6157 (0.993)	7.9	y=-0.0564x+4.5955 (0.983)	12.3
T4	y=-0.388x+4.4845 (0.986)	1.8	y=-0.0803x+4.5784 (0.995)	8.6	y=-0.1058x+4.6563 (0.996)	6.6	y=-0.0484x+4.5851 (0.981)	14.3
T5	Y=-0.0912X+4.4887 (0.976)	7.6	y=-0.0488x+4.6081 (0.986)	14.2	y=-0.0368x+4.6014 (0.993)	18.8	y=-0.0255x+4.5895 (0.983)	27.2
T6	y=-0.2858x+4.4456 (0.989)	2.4	Y=-0.0719X+4.5958 (0.992)	9.6	y=-0.0726x+4.6158 (0.994)	9.5	y=-0.0426x+4.5753 (0.976)	16.3
T7	y=-0.4614x+4.4846 (0.986)	1.5	y=-0.0931x+4.549 (0.973)	7.4	y=-0.1123x+4.6554 (0.983)	6.2	y=-0.542x+4.564 (0.964)	12.8
T8	y=-0.359x+4.4017 (0.970)	1.9	y=-0.0795x+4.6013 (0.995)	8.7	y=-0.1145x+4.6723 (0.991)	6.1	y=-0.0506x+4.5823 (0.975)	13.7

T1: Soil+PAHs; T2: Soil+PAHs+BS (@ 425 µg g^{-1}); T3: Soil+PAHs+BS (@ 850 µg g^{-1}); T4: Soil+PAHs+BS (@ 850 µg g^{-1})+*Kocuria rosea*; T5: Soil+Oil+PAHs; T6: Soil+Oil+PAHs+BS (@ 425 µg g^{-1}); T7:Soil+Oil+PAHs+BS (@ 850 µg g^{-1}); T8: Soil+Oil+PAHs+BS (@ 850 µg g^{-1})+*Kocuria rosea*

enhancing degradation of harmful carcinogenic pollutant naphthalene, fluorene, phenanthrene and pyrene. Biosurfactant can be considered as an eco-friendly approach for degradation of harmful pollutant in the soil.

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