Effect of biosurfactant on degradation of polyaromatic hydrocarbons in different agricultural soils under different environments

Ashwini Yadav[1], Neera Singh[2], Arti Bhatia[1], Eldho Varghese[3], Kaushik Banerjee[4] and Ashish Khandelwal[1,5,*]

[1]Division of Environmental Sciences, and
[2]Division of Agricultural Chemicals, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India.
[3]Fishery Resources Assessment, Economics and Extension Division (FRAEED), ICAR-Central Marine Fisheries Research Institute, Kochi 682 018, India.
[4]ICAR-National Research Centre for Grapes, Pune 412 307, India.
[5]Marine Biotechnology, Fish Nutrition and Health Division (MBFNHD), ICAR-Central Marine Fisheries Research Institute, Kochi 682 018, India.

A lipopeptide biosurfactant isolated from *Lysinibacillus* sp. MW444883 and characterised using liquid chromatography-high resolution mass spectroscopy. The production of the biosurfactant was optimised under conditions of 27°C and 150 rpm (yield ~2.65 g/l), and Oil dispersion varied from 6 to 8.5 cm. Degradation and half-life of polyaromatic hydrocarbons in inceptisol soils (collected from Delhi and Jharkhand agricultural field) under different environments (28°C and 37°C) varied for naphthalene (20.8%–100%; 1.16–9.86 days), fluorene (5.8–93.8%; 5.6–14.4 days), phenanthrene (6.0%–94.1%; 5.4–22.5 days) and pyrene (5.8%–78.7%; 10.3–27.0 days) respectively. Additionally, dehydrogenase and carbon dioxide varied from 9.6 to 45.1 μg TPF g^{-1} soil h^{-1} and 146.6 to 401.4 mg kg^{-1} d^{-1}, 1 respectively, over 0–21 days.

Keywords: Carbon dioxide release, lipopeptide biosurfactant, oil dispersion, optimisation, PAHs degradation.

SURFACTANTS are extensively utilised in the food, pharmaceutical, agricultural and industrial sectors. However, since they are mostly chemically synthesised, they may lead to environmental and toxicological issues brought by the resistance and the fact that these chemicals are persistent. Surface active agents are molecules that adsorb on the water–surface interface and lower the surface tension of water to improve surface cleaning. Surface tension is lowered as a result of structural configurations of these molecules, which assist in the reduction of cohesive forces between water molecules. Biosurfactants are surfactants that occur spontaneously in microorganisms like yeast and bacteria. According to estimates, the surfactant market would increase at a 4.5%–5% annual rate and surpass USD 58.3 billion by 2024 and USD 81.7 billion by 2030 (ref. 1).

The majority of surfactants sold commercially are made from petroleum derivatives. Biosurfactants are substances of microbiological origin that display surfactant characteristics, such as the ability to emulsify and a decrease in surface tension[2]. Compared to chemical surfactants, biosurfactants are more environmentally friendly, less toxic, more biodegradable, more selective, and more active at high salinity, pH and temperature extremes.

Over the past few decades, as offshore oil exploration and marine traffic have risen, more crude oil spills have entered the marine environment. Over 210 million gallons of oil were spilt into the Gulf of Mexico in 2010 due to the Deepwater Horizon oil leak. Reducing the size of oil droplets by lowering the interfacial tension between the oil and water under wave action is the fundamental idea behind the dispersant used in oil spill response. In this emulsification process, the oil slick must be transformed into distinct droplets that are stable until coalescence by using an efficient dispersant.

According to the United States Environmental Protection Agency (USEPA), 16 polyaromatic hydrocarbons (PAHs) are considered carcinogenic compounds. Among different PAHs, naphthalene, phenanthrene, and anthracene are widely present and considered as signature compounds to detect PAH contamination and are also used as a model substrate for studies on the metabolism of carcinogenic PAHs[3]. Pyrene and phenanthrene are highly associated with industrial processes and are the most widely transported PAHs in the hydrosphere[4]. Global PAH emissions in 2015 were 357 Gg. The lifetime lung cancer deaths caused by exposure to PAH pollution were estimated as 6.9×104. In Korea, PAH concentrations in the paddy soil were reported from 18 to 8,000 ppm. Pyrogenic coal burning and residential biomass combustion were major contributors to the soil contamination in the two cities[5,6]. In India, accidental incidents of crude oil in agricultural soils were reported. Similarly, PAH

*For correspondence. (e-mail: ashish.iasbhu@gmail.com)

incidences due to the burning of fuels and crackers are also reported[7]. Biological remediation can be limited by the bioavailability of soil-bound PAHs due to their low aqueous solubility, high hydrophobicity and strong sorption to soil, which is exacerbated by the long ageing of contaminants in field-contaminated soils[8]. Biosurfactants are surface-active molecules produced by microorganisms and have various industrial applications. These properties include biodegradability, low toxicity, high surface activity, versatility, stability, selectivity and cost-effective[9]. Therefore, under the present study, surface tension reducing substances were isolated, characterised, and their performance for oil spreading and degradation in different types of agricultural soils under different temperatures was evaluated.

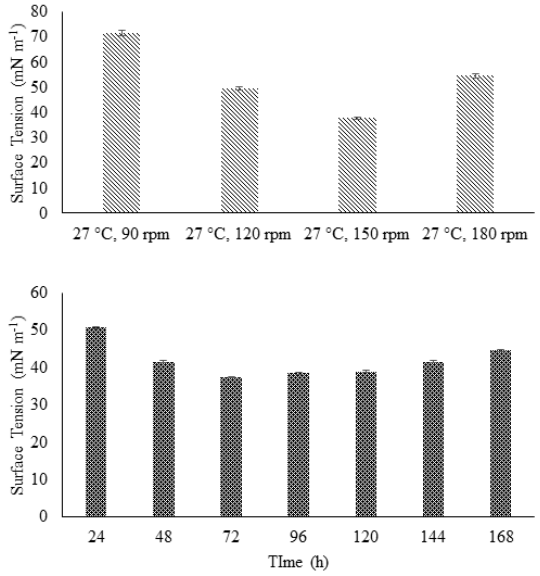

Figure 1. Surface tension of biosurfactant solution (***a***) different rotations at 72 h and 150 rpm and (***b***) at different durations.

Materials and methods

Isolation of biosurfactant and putative characterisation

Lysinibacillus sp. MW444883 (accession number: NAIMCC-B-03918) was cultured at 27°C under different rotations (90, 120, 150 and 180 rpm) for three days. Afterwards, bacteria were cultured for 7 days and surface tension was calculated at different durations (24–168 h) with a one-day interval at 27°C, 150 rpm. For yield purposes, oil displacement and degradation purposes, cell-free supernatants (CFS) obtained after 168 h were centrifuged at 5000 rpm for five minutes. The CFS was lyophilised, and the obtained product is called biosurfactant and putative structural characterisation was performed using liquid chromatography-high resolution mass spectrometry (LC-HRMS).

Surface tension

Surface tension of CFS was calculated using a method described by Veenanadig *et al.*[10]. A dry, clean stalagnometer was fixed to a stand at an angle of 90 degrees. A beaker filled with distilled water was set beneath it. The stalagnometer had a pinchcock attached to the top end of a piece of rubber tubing. Using the rubber tubing, water was drawn into the stalagnometer and the pinchcock was engaged. The water flow was then carefully and gradually adjusted by releasing the pinchcock, forming well-defined spherical drops at a rate of 15–20 drops per minute. After that, the stalagnometer was filled with water again without adjusting the pinchcock until the water level was higher than the upper mark 'A'. After that, the water in the stalagnometer was allowed to fall. We counted and recorded the number of drops that formed between the lower mark, 'B', below the bulb, and the top mark, 'A'. The procedure was repeated two to three times for water, and the average number of drops that formed was recorded. After draining, acetone was used to dry the stalagnometer. After adding the CFS, the experiment was carried out once again. Between markers A and B, the quantity of drops that formed was tallied. The process was conducted two to three times, and the average quantity of drops was determined. Subsequently, the density of the liquid was calculated, and the number of drops was used for the calculation of surface tension (mN m^{-1}) of the liquid solution.

$$\text{Density of liquid} = \frac{\text{Weight of liquid} \times \text{Density of water}}{\text{Weight of water}},$$

$$\text{Surface tension of liquid} = \frac{\text{Weight of liquid} \times \text{Drops of water} \times \text{Surface tension of water}}{\text{Density of water} \times \text{Drops of liquid}},$$

Oil displacement test

For the oil displacement test, crude oil (0.1, 1, 2, 2.5, 3, 4, 5, 6 and 7 ml) was poured into the top of 25 or 30 ml of distilled water in a Petri dish. Biosurfactant (0.1% w/v) was carefully applied in 10 and 20 μl onto the centre of the oil membranes. The diameter was recorded as oil displacement in terms of centimetre[11].

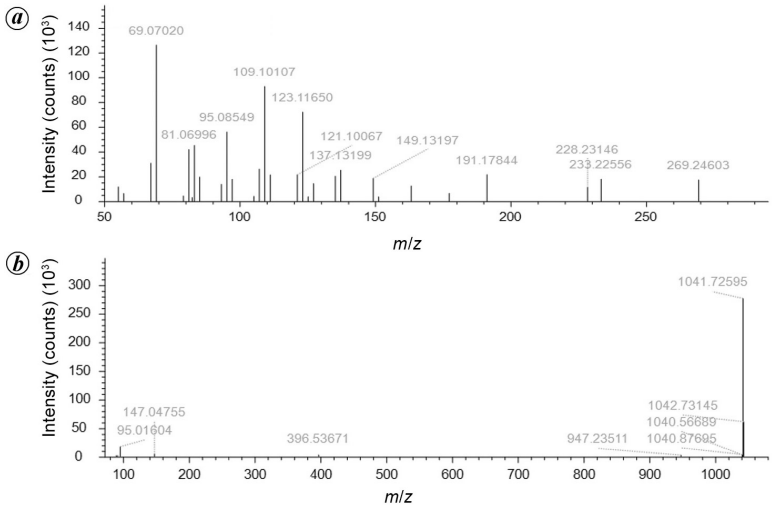

Figure 2. Extracted ion chromatogram of (***a***) palmitelaidic acid methyl ester and (***b***) N∼2∼-acetyl-L-lysyl-L-lysyl-L-alanyl-L-lysyl-L-lysyl-L-alanyl-L-lysyl-L-lysyl-L-alaninamide.

Foaming

After 96 h of incubation of microbial culture, the supernatant (10 ml) was vigorously shaken for two minutes to assess the foaming of the biosurfactant in the culture medium. The foaming was then estimated using the following equation[11].

$$\text{Foaming}\,(\%) = \frac{\text{Height of foaming}}{\text{Total height}} \times 100\,.$$

Figure 3. Foaming effect in biosurfactant solution.

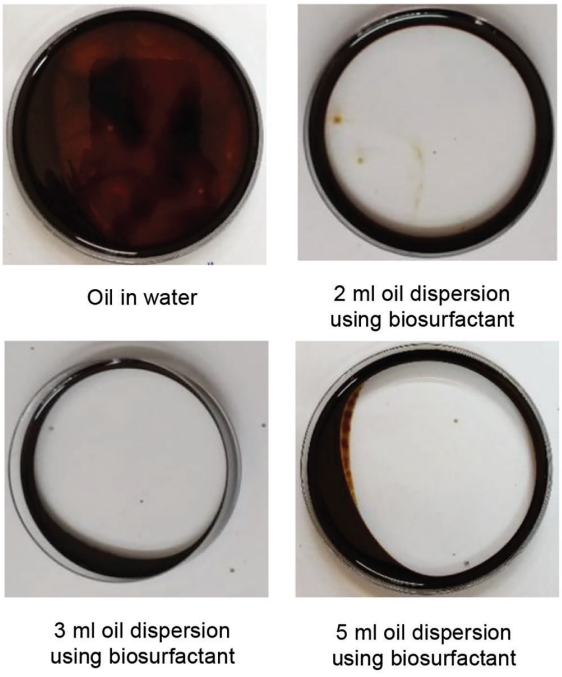

Figure 4. Foil dispersion in the presence of biosurfactant.

Soil collection and characterisation

Two soils were collected from agricultural fields of the ICAR-Indian Agricultural Research Institute, New Delhi (inceptisol, alkaline soil) and the ICAR-Indian Agricultural Research Institute, Jharkhand (inceptisol, acidic soil) for recovery and the degradation study of PAHs. The soils were characterised (Table 1) using standard methodologies[12].

Table 1. Characterisation of soils

Properties	Incepitsol alkaline soil (Delhi soil)	Inceptisol acidic soil (Jharkhand soil)
pH	8.3	6.4
EC (dS m^{-1})	0.5	0.4
Sand (%)	54.4	56
Silt (%)	23.3	22.8
Clay (%)	22.3	21.2
Organic carbon (%)	0.36	036
Available nitrogen (kg ha^{-1})	215	250
Available phosphorus (kg ha^{-1})	25.6	15.2
Available potassium (kg ha^{-1})	185.8	145.0

Table 2. Effect of biosurfactant on oil dispersion

Biosurfactant (μl)	Oil amount on water surface (%)	Oil dispersion (cm)
10	0.33	8.5
	1.66	8.5
	3.33	8.5
	6.66	7.3
	10	6.9
	13.33	6.5
	20	6.1
	40	6
20	0.33	8.5
	1.66	8.5
	3.33	8.5
	6.66	7.5
	10	7.2
	13.33	7
	20	7
	40	6.9

Optimisation of high performance liquid chromatography conditions for analysis of naphthalene, fluorene, phenanthrene and pyrene

Certified reference standards of naphthalene (99%), fluorene (>95%), phenanthrene (>97%) and pyrene (>96%) were purchased from Merck Bangalore, India. The solvents used were purchased locally and were of analytical grade. In the study, a high-performance liquid chromatography (HPLC) method for analysing naphthalene, fluorene, phenanthrene and pyrene was developed using a Shimadzu HPLC apparatus equipped with a degasser and a photodiode array detector linked to a Rheodyne injection system. The analytical conditions comprised an RP-18 (PAH) column measuring 25 cm and 5 μm (Merck) inner diameter and a mobile phase consisting of acetonitrile: water (70 : 30) flowing at a rate of 0.5 ml min^{-1}.

Recovery of PAHs from soil

Based on a prior study[13], a 50 ml conical centrifuge tube (Eppendorf) was filled with five grams of soil from Jharkhand and Delhi. After adding 50 μg of PAH mixture (5 ml 10 mg l^{-1} acetonitrile solution) to the sample, the solvent was allowed to evaporate for two hours. After adding 2.5 ml of distilled water to the fortified soil sample, it was left for 30 min. Then, 5 ml of acetonitrile was added, and the mixture was vortexed for two minutes. After adding 2 g of anhydrous sodium sulphate, vortexing for a minute was performed. Then 0.75 g of sodium chloride was added, and a good shake was given. The tube was centrifuged at 5000 rpm for 5 min after it was vortexed for 1 min. Following centrifugation, 1 ml of the supernatant was transferred to a 2 ml microcentrifuge tube, where it was combined with 150 mg of anhydrous magnesium sulfate and 50 mg of primary secondary ammonia (PSA). The contents underwent a forceful one-minute vortex shaking and a five-minute 5000 rpm centrifugation. After transferring the 0.5 ml of supernatant to a fresh microcentrifuge tube and letting the solvent air dry, the leftovers were diluted to 10 ml of acetonitrile. HPLC was used to examine the samples for PAH fractions.

Effect of biosurfactant on degradation of PAHs in different soils under different environments

The biosurfactant solution was spiked in soil @ 850 μg g^{-1} (as per treatment) and assessed degradation behaviour in inceptisol alkaline soil (Delhi soil) and inceptisol acidic soil (Jharkhand soil) at 27°C and 37°C. Air-dried soil (90 g) in 250 ml sterilised spout-less glass beakers was supplemented with the sterile distilled water/biosurfactant solution to attain field capacity. As per treatments, soil samples were spiked with a mixture of naphthalene, fluorene, phenanthrene and pyrene at 100 μg g^{-1} levels of each PAH. This was done by fortifying 10 g of soil with a 5 ml solution containing 2000 μg ml^{-1} of each PAH and mixing it with 90 g of conditioned soil. As per treatments, study was performed in eight treatments, namely, T1, Delhi soil + PAHs (28^{p}C); T2, Delhi soil + PAHs + BS-850 (28°C); T3, Delhi s+ PAHs (37°C); T4, Delhi soil + PAHs + BS-850 (37°C); T5-Jharkhand soil + PAHs (28°C); T6, Jharkhand soil + PAHs + BS-850 (28°C); T7, Jharkhand soil + PAHs (37°C); T8, Jharkhand soil + PAHs + BS-850 (37°C) and each treatment had three replicates. 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 percentage of degradation, dehydrogenase and CO_2 release patterns were observed for different days.

Dehydrogenase activity

Soil samples collected at different intervals (0, 1, 3, 7, 14 and 21 days) were air dried and then subjected to the addition of triphenyltetrazolium chloride (TTC) and incubated at 27°C after 24 h; a pink colour developed due to the formation of triphenyl formazan (TPF). Changes in pink colour were observed at 685 nm wavelength, and enzymatic activity was recorded.

Carbon dioxide release study

At room temperature, a laboratory experiment was conducted in spout-less glass beakers to evaluate CO_2 release[14]. The measurements of the beaker were 5.5 cm × 5.5 cm × 12 cm (length × width × height). A cork with two sample outlets was used to close the beakers, and the silicone septum sealed off these sampling holes. Silicone grease was used to create an airtight beaker and cork arrangement. CO_2 was sampled at 0, 1, 3, 7, 14 and 21 days using a 50 ml syringe with a hypodermic needle (24 gauge). Internal moisture levels of the beaker were kept at field capacity. Samples of the gas were taken every 0 and 30-minute intervals. Within two hours, the collected samples were evaluated. Using a methaniser and a flame ionisation detector, a gas chromatograph (Shimadzu manufacture) was used to measure the CO_2 concentration[15]. Utilising a GC-computer interface, the peak area was plotted and measured. The calibration was completed. Linde Gases (USA) provided CO_2 standards of 505 and 700 ppmV that were traceable to the National Institute of Standards and Technology (NIST) in Maryland. Samples were taken from every repeated treatment. On sampling days, the average CO_2 emission was measured assuming that it followed a linear trend[14,16].

Statistical analysis and half-life calculation

PAH degradation in Delhi and Jharkhand soils was subjected to two-way ANOVA, and interactions were noted at $P < 0.001$. Further, Tukey's honest significant difference (HSD) test was performed for multiple comparisons among treatment effects and interactions. The degradation data were fitted to the first-order kinetic equation

$$\text{In } C_t/C_0 = -k_t\,,$$

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_{obs} value using the formula

$$t_{1/2} = 0.693/k\,.$$

Results and discussion

Biosurfactant optimisation, putative characterisation and foaming behaviour

During the optimisation step, 10% microbial load (OD600 ~1.0) is added for uniform study. Results showed that minimum surface tension (37.4 mN m$_{-1}$) at 27°C, 150 rpm, 72 h and at different rotations, surface tension varied from 37.4 to 71.5 mN m$_{-1}$ (Figure 1 ***a***). On different days (24–168 h), surface tension varied from 37.4 to 50.7 mN m$_{-1}$ (Figure 1 ***b***). In most of the research studies, minimum surface tension was achieved at 72 h and maintained till 120 h. The yield of biosurfactant was observed as 2.65 g l$_{-1}$ after freeze-drying of supernatant collected from 27$_o$C, 150 rpm, 168 h. The freeze-dried product was used for oil displacement and degradation experiments.

Based on the processing of LC-HRMS data, a number of metabolites were observed. Fatty acid methyl ester, palmi-telaidic acid methyl ester (Figure 2 ***a***) and a series of amino acids, N~2~-acetyl-L-lysyl-L-lysyl-L-alanyl-L-lysyl-L-lysyl-L-alanyl-L-lysyl-L-lysyl-L-alaninamide (Figure 2 ***b***), were also detected. Putative indication showed that the presence of a series of amino acids linked to fatty acid and the combined product might be known as a lipopeptide. The presence of fatty acid and amino acid sequences also confirmed the presence of amino acids in compounds.

Therefore, putative characterisation can be considered as a lipopeptide kind of biosurfactant. Earlier, John *et al.*[17] and Bhardwaj et al.18 also extracted lipopeptide biosurfactant from *Lysinibacillus* sp.

Under different conditions, foaming varied from 40% to 70%. Results showed that biosurfactants can be used as detergents and as an alternative to synthetic surfactants (Figure 3).

Oil displacement

At different amounts of crude oil on water surface (0.33–40%), oil displacement was observed as 6–8.5 cm and 6.9–8.5 cm using 10 and 20 μl biosurfactant (0.1% w/v) respectively (Figure 4 and Table 2). The effect of biosurfactant for oil removal was observed, and the percentage of area reduction was observed as 56.4%–86.6%. Recently, Samadi *et al.*[19] demonstrated the use of rhamnolipid biosurfactant for oil spill removal. Feng *et al.*[20] demonstrated the dispersion effectiveness of lipopeptide biosurfactant as 70.23% at surfactant to oil ratio (SOR) of 1 : 10 at 25°C, pH 7 and 3% salt concentration.

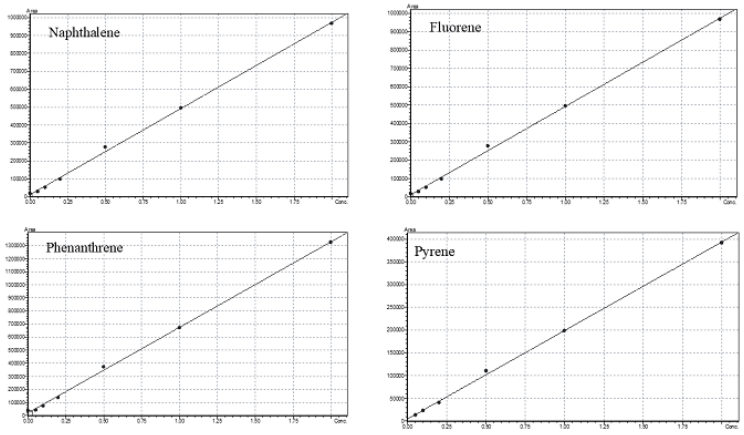

Figure 5. Instrument calibration for naphthalene (220 nm), fluorene (210 nm), phenanthrene (246 nm) and pyrene (246 nm) using high-performance liquid chromatography.

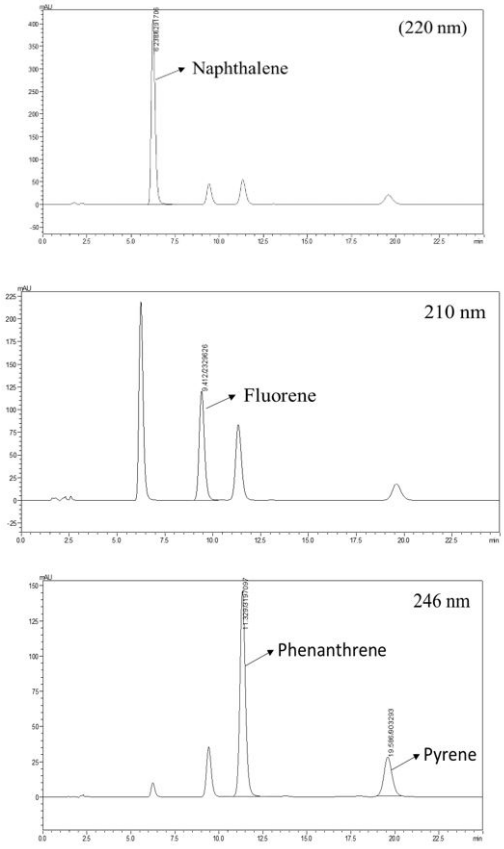

Figure 6. Chromatogram of a mixture of 5 mg l^{-1} naphthalene, fluorine and phenanthrene, anthracene and pyrene at different wave-lengths (246, 210 and 220 nm).

Optimisation of HPLC conditions for analysis of naphthalene, fluorene, phenanthrene and pyrene

Based on the methodology, the HPLC instrument was optimised for analysing naphthalene, fluorene, phenanthrene and pyrene. The analysis wavelengths were 220 nm for naphthalene, 210 nm for fluorene and 246 nm for pyrene and phenanthrene. Figure 5 shows the calibration curve, and Figure 6 shows the chromatograms for each PAH at the wavelength maxima. The limits of detection and quan-

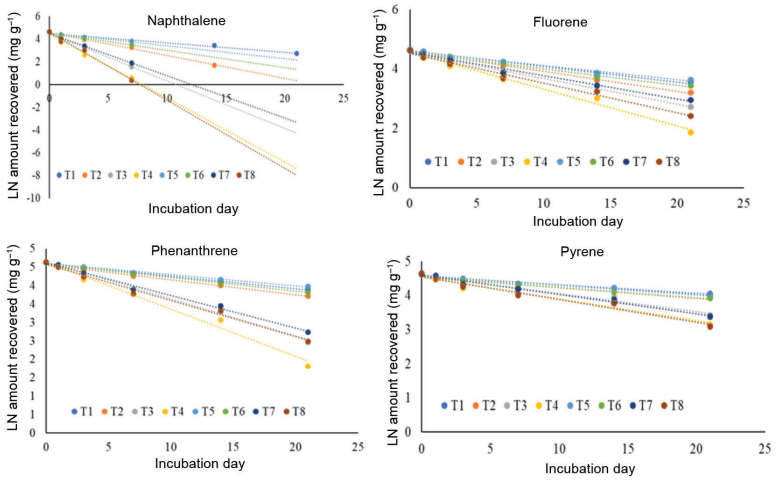

Figure 7. First-order degradation graphs for naphthalene, fluorene, phenanthrene and pyrene under different treat-ments (T1, Delhi soil + PAHs (28°C); T2, Delhi soil + PAHs + BS-850 (28°C); T3, Delhi soil + PAHs (37°C); T4, Delhi soil + PAHs + BS-850 (37°C); T5, Jharkhand soil + PAHs (28°C); T6, Jharkhand soil + PAHs + BS-850 (28°C); T7: Jharkhand soil + PAHs (37°C); T8, Jharkhand soil + PAHs + BS-850 (37°C)).

Table 3. Instrument calibration, limit of detection and limit of quantification for naphthalene, fluorene, phenanthrene and pyrene

Compounds	Equation	R^2	LOD (mg l^{-1})	LOQ (mg l^{-1})
Naphthalene	Y = (1.25316e + 006)X + (5,455.45)	0.9988	0.09	0.27
Fluorene	Y = (481,004)X + (11,588.9)	0.9986	0.09	0.29
Phenanthrene	Y = (653,699)X + (20,799.2)	0.9994	0.086	0.26
Pyrene	Y = (194,077)X + (4,938.11)	0.9991	0.094	0.28

LOD, Limit of detection; LOQ, Limit of quantification.

Table 4. Degradation of naphthalene (100 μg g^{-1}), fluorene(100 μg g^{-1}), phenanthrene (100 μg g^{-1}) and pyrene (100 μg g^{-1}) in different soils at different temperatures in the presence of biosurfactant at different days (n = 3)

	Naphthalene(% degradation)					Fluorene(% degradation)					Phenanthrene (% degradation)					Pyrene (% degradation)				
Treatment	1	3	7	14	21	1	3	7	14	21	1	3	7	14	21	1	3	7	14	21
T1	23.9	38.8	58.0	70.5	85.3	5.8	21.7	33.6	58.5	66.0	6.8	13.3	26.3	40.6	51.8	7.5	15.2	25.5	33.9	43.9
T2	33.2	49.0	74.7	94.7	100.0	9.0	26.8	39.8	64.1	75.9	13.7	19.8	31.4	46.8	60.1	12.8	21.2	31.1	40.6	50.2
T3	53.3	74.4	95.3	100.0	100.0	15.7	30.8	59.1	69.6	85.3	7.5	27.4	52.8	71.5	88.7	8.1	26.9	37.0	49.8	70.3
T4	59.0	86.8	98.3	100.0	100.0	23.6	41.1	62.6	80.5	93.8	12.5	37.3	58.1	79.1	94.1	14.8	34.3	45.5	57.1	77.5
T5	20.8	38.5	56.5	70.0	79.1	6.9	20.1	31.7	53.5	63.4	6.0	11.7	24.9	37.4	48.2	8.0	14.4	28.4	35.4	47.7
T6	28.5	45.6	67.6	85.8	96.3	10.6	27.7	37.3	60.1	69.5	9.5	14.6	26.7	42.6	55.2	11.4	17.4	31.7	41.4	50.5
T7	49.9	70.6	93.4	100.0	100.0	12.9	26.8	52.7	69.4	81.1	7.1	25.2	52.2	69.1	84.9	5.8	26.2	36.2	52.8	71.4
T8	55.6	79.7	98.6	100.0	100.0	21.4	37.3	60.9	75.3	89.2	12.1	32.8	57.6	73.5	88.3	13.3	31.4	46.4	57.9	78.7

T1, Delhi soil + PAHs (28°C); T2, Delhi soil + PAHs + BS-850 (28°C); T3, Delhi soil + PAHs (37°C); T4, Delhi soil + PAHs + BS-850 (37°C); T5, Jharkhand soil + PAHs (28°C); T6, Jharkhand soil + PAHs + BS-850 (28°C); T7, Jharkhand soil + PAHs (37°C); T8, Jharkhand soil + PAHs + BS-850 (37°C).

tification for each of the four PAHs are given in Table 3.

According to the recovery procedure, the percentage of recovered naphthalene (90.1 ± 2.6, 92.5 ± 1.3), fluorene (90.8 ± 2.6, 98.1 ± 3.6), phenanthrene (94.5 ± 1.8, 82.3 ± 0.7) and pyrene (85.4 ± 2.1, 82.4 ± 0.6) were found in both Delhi (inceptisol, alkaline soil) and Jharkhand (inceptisol, acidic soil) respectively.

In-vitro degradation of PAHs in Delhi (inceptisol, alkaline soil) and Jharkhand (inceptisol, acidic soil)

The effect of biosurfactant-mediated degradation was observed in Delhi and Jharkhand PAH-spiked soils. The first-order degradation curve is shown in Figure 7, and the percentage of degradation is shown in Table 4. Results

Table 5. Half-life data for naphthalene, fluorene, phenanthrene and pyrene in different soils at different temperatures in the presence of biosurfactant

	Naphthalene		Fluorene		Phenanthrene		Pyrene	
Treatment	Delhi	Jharkhand	Delhi	Jharkhand	Delhi	Jharkhand	Delhi	Jharkhand
T1	8.30^{b}	9.86^{a}	13.1^{b}	14.4^{a}	20.3^{b}	22.5^{a}	27.0^{a}	24.0^{b}
T2	3.43^{d}	4.70^{c}	10.3^{c}	12.4^{b}	16.9^{d}	18.8^{c}	23.1^{bc}	22.0^{c}
T3	1.66^{e}	1.86^{e}	8.0^{d}	8.8^{d}	6.9^{f}	7.8^{e}	13.0^{d}	12.3^{d}
T4	1.23^{f}	1.16^{f}	5.6^{f}	6.9^{e}	5.4^{g}	7.0^{ef}	10.9^{e}	10.3^{e}
SE	0.084		0.184		0.169		0.251	

T1, Soil + PAHs (28°C); T2, Soil + PAHs (37°C); T3, Soil + PAHs + BS-850 (28°C); T4, Soil + PAHs + BS-850 (37°C).

Table 6. Dehydrogenase activity (μg TPF g^{-1} soil h^{-1}) during degradation of PAHs in Delhi and Jharkhand soils in the presence of biosurfactant at different days under different temperatures.

Treatment	0	1	3	7	14	21
T1	16.5	15.6	18.3	15.2	16.3	14.3
T2	17.3	42.4	18.8	34.5	18.6	16.2
T3	17.8	16.8	20.1	16.1	17.4	15.9
T4	18.4	45.1	21.5	36.4	20.5	18.9
T5	14.1	13.5	15.9	13.5	9.6	12.5
T6	15.2	34.1	14.5	31.5	16.9	13.4
T7	15.9	14.4	16.8	14.5	11.1	14.4
T8	16.8	37.8	16.8	33.1	18.5	16.1

T1, Delhi soil + PAHs (28°C); T2, Delhi soil + PAHs + BS-850 (28°C); T3, Delhi soil + PAHs (37°C); T4, Delhi soil + PAHs + BS-850(37°C); T5, Jharkhand soil + PAHs (28°C); T6, Jharkhand soil + PAHs + BS-850 (28°C); T7, Jharkhand soil + PAHs (37°C); T8,Jharkhand soil + PAHs + BS-850(37°C).

Table 7. Carbon dioxide release (mg kg^{-1} d^{-1}) during degradation of PAHs in Delhi and Jharkhand soil in the presence of biosurfactant at different days under different temperatures

Treatment	0	1	3	7	14	21
T1	238.2	229.5	245.1	211.5	255.2	206.0
T2	264.4	360.6	298.7	316.7	236.8	223.3
T3	259.1	271.4	288.4	288.1	268.1	220.1
T4	289.1	401.4	320.5	345.4	265.4	254.1
T5	211.5	200.9	238.2	146.6	241.9	237.3
T6	274.1	251.0	348.2	360.2	350.6	264.0
T7	225.1	231.4	245.8	220.4	289.4	271.5
T8	305.1	289.4	388.4	391.4	368.4	280.4

T1, Delhi soil + PAHs (28°C); T2, Delhi soil + PAHs + BS-850 (28°C); T3, Delhi soil + PAHs (37°C); T4, Delhi soil + PAHs + BS-850 (37°C); T5, Jharkhand soil + PAHs (28°C); T6, Jharkhand soil + PAHs + BS-850 (28°C); T7, Jharkhand soil + PAHs (37°C); T8, Jharkhand soil + PAHs + BS-850 (37°C).

showed that among different pollutants, naphthalene was readily degraded. Degradation was found to be in the following order: naphthalene > fluorene > phenanthrene > pyrene. An increase in temperature leads to an increase in degradation in both soils. However, degradation was faster in Inceptisol (alkaline soil) as compared to Inceptisol (acidic soil). Per cent degradation of naphthalene (20.8–100), fluorene (5.8–93.8), phenanthrene (6.0–94.1) and pyrene (5.8–78.7) varied over different days, ranging from 1 to 21 days. The naphthalene, fluorene and phenanthrene were degraded faster in T4 as compared to other treatments. During the 7th day, the highest degradation was 98.3% and was at par with T4 (98.6%), followed by T3 (95.3%), T7 (93.4%), T2 (74.7%), T6 (67.6%), T1 (58.0%) and T5 (56.5%). On the 14th day, degradation of fluorene was highest in T4 (80.5%), followed by T8 (75.3%), T3 (69.6%), T7 (69.4%), T2 (64.1%), T6 (60.1%), T1 (58.5%) and T5 (53.5%). During 21st day, phenanthrene was highest degraded in T4 (94.1%) followed by T3 (88.7%), T8 (88.3%), T7 (84.9%), T2 (60.1%), T6 (55.2%), T6 (55.2%) and T1 (51.8%). Pyrene was highly recalcitrant and degraded on the 21st day, and was highest in T8 (78.7%), followed by T4 (77.5%), T7 (71.4%), T3 (70.3%), T6 (50.5%), T2 (50.2%), T5 (47.7%) and T1 (43.9%). However, pyrene degradation seems to be non-significant with respect to temperature. The half-life values were observed in Table 5 and were 1.16 (naphthalene), 5.6 (fluorene), 5.4 (phenanthrene) and 10.3 (pyrene) days at 37°C.

Bioavailability of PAHs is increased due to the addition of phenol in biosurfactant, and a reduction of surface tension is observed. It also causes an increase in the solubilisation of PAHs and helps to enhance degradation in the presence of biosurfactant-based solution using *Stenotrophomonas* sp. N5 (ref. 21).

Rathankumar *et al.*[22] demonstrated that the addition of 1500 mg l^{-1} surface-active compounds enhanced the degradation up to 86%. However, in the absence of surfactant, degradation varied from 54% to 61% on the 21st day. Among different PAHs, 100% degradation was achieved for naphthalene and acenaphthene within 17 days. The use of *Escherichia coli – Pseudomonas putida* consortium enhanced the degradation of phenanthrene from 61.15% to 73.86% within 7 days in 100 ppm spiked soil[23].

Dehydrogenase enzymatic activity and carbon dioxide release

During degradation of PAHs under different environments, dehydrogenase (Table 6) and carbon dioxide release were

observed (Table 7). Across the treatments, the highest dehydrogenase activities over different days were observed at 37°C in both soils. Among different soils, the highest dehydrogenase activities were observed in Delhi soil (18.4–45.1 μg TPF g^{-1} soil h^{-1}) as compared to Jharkhand soil (16.8–37.8 μg TPF g^{-1} soil h^{-1}).

Similarly, CO_2 release was observed to be highest at 37°C in both soils. CO_2 release was observed to be highest in T4 (254.1–401.4 mg kg^{-1} d^{-1}) compared to T8 (280.4–391.4 mg kg^{-1} d^{-1}). In T2 and T4 treatments, the highest activities were recorded between 1 and 7 days, whereas in Jharkhand soil, under T6 and T8, the highest activities were recorded between 1 and 14 days compared to T1 and T4 control treatments.

Conclusion

Surface active lipopeptide biosurfactants were isolated from *Lysinibacillus* sp. and biosurfactant can be used as oil spill removal (due to oil displacement property), alternative to synthetic surfactant (due to foaming property and lower surface tension value) and suitable candidate for degradation of carcinogenic PAHs, namely naphthalene, fluorene, phenanthrene and pyrene and were found to be stable at different temperatures (28°C and 37°C) under different soil types (acidic and alkaline).

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