Efficiency and Kinetics of Assam Crude Oil Degradation by *Pseudomonas Aeruginosa* AKS1 and *Bacillus* Sp. AKS2

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Research Article

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Abstract

We report kinetics of Assam crude oil degradation by *Pseudomonas aeruginosa* AKS1 and *Bacillus* sp. AKS2, both isolated from Assam refinery sediments. The isolates exhibited appreciable degrees of hydrophobicity, emulsification index and biosurfactant production. Crude oil degradation efficiency of isolates was assessed in (1) liquid medium amended with 1% v/v crude oil and (2) microcosm sediments (125 mg crude oil/ 10 g sand). In liquid culture, the biodegradation rate (k) and half-life ($t_{1/2}$) values were found to be 0.0383 day⁻¹ and 18.09 days for *P. aeruginosa* AKS1, and 0.0204 day⁻¹ and 33.97 days in case of *Bacillus* sp. AKS2. In microcosm sand sediments, the estimated biodegradation rate (k) and half-life ($t_{1/2}$) values were 0.0138 day⁻¹ and 50 days for *P. aeruginosa* AKS1, and 0.0113 day⁻¹ and 61.34 days in case of *Bacillus* sp. AKS2. The level of nutrient treatment in microcosm sand sediment was 125 µg N & 62.5 µg P/g sediment in case of *P. aeruginosa* AKS1 and 375 µg N & 37.5 µg P/g sediment in case of *Bacillus* sp. AKS2. In microcosms without inorganic nutrients, biodegradation rate (k) and half-life ($t_{1/2}$) values were found to be 0.0069 day⁻¹ and 100 days for *P. aeruginosa* AKS1 and for *Bacillus* sp. AKS2, the respective values were found to be 0.0046 day⁻¹ and 150.68 days. Our data provides important information for predictive hydrocarbon degradation in liquid medium and contaminated sediments.

1. Introduction

Hydrocarbon pollution due to natural seeps and anthropogenic activities is a major problem worldwide posing serious threat to local flora and fauna. Developing economy like India generate a large amount of petroleum sludge from crude oil refining activities carried out to meet the mass production of hydrocarbons for use in pharmaceuticals, solvents, fertilizers, pesticides and plastics, besides fuel oil and gasoline. A part of the low molecular weight hydrocarbon fractions from contaminated sediments is removed by evaporation leaving behind relatively recalcitrant high molecular weight fractions. Reclamation of such contaminated sediments is very difficult task and requires intervention of physical, chemical and biological methods. The hydrocarbons are ultimately biodegraded by microbial populations. Bioremediation of hydrocarbon polluted sites using native bacterial population is considered as the most efficient, sustainable, environmentally friendly, and cost-effective approach. Therefore there is a need to identify and test the hydrocarbon degradation potential of native bacteria for bioremediation. The success of bioremediation is largely governed by the survival, adaptability and catabolic activities of the introduced microorganisms. Some of the bacterial strains successfully used for this purpose include *Pseudomonas aeruginosa* (san ai) (Medic et al. 2020), *Comamonas testosteroni* BR60 (Gentry et al. 2001), *Pseudomonas putida* ZWL73 (Niu et al. 2009), *Bacillus* sp. (Kiamarsi et al. 2018) etc. The contaminated sites selectively promote growth of hydrocarbon degrading bacterial populations. Two bacterial genera namely *Pseudomonas* and *Bacillus* have been extensively reported from hydrocarbon contaminated sites (Das and Mukherjee 2007; Kiamarsi et al. 2018; Medic et al. 2020). Several studies have demonstrated active involvement of *Pseudomonas* (Bhattacharya et al. 2003; Mulet et al. 2011) as the most prominent hydrocarbon degrading bacterial genera. Members of *Pseudomonas* are ubiquitous and metabolically highly versatile. They have been shown to degrade many of the crude oil components

(Belhaj et al. 2002) including recalcitrant and toxic polycyclic aromatic hydrocarbons (PAHs) (Bugg et al. 2000; Tian et al. 2002). Several *Pseudomonads* are also known to produce glycolipid type biosurfactants (Rahman et al. 2003; Singh and Tiwary 2016) that facilitate hydrocarbon bioavailability (Ron and Rosenberg 2002). Similarly members of *Bacillus* sp. have been shown to degrade crude oil (Chettri et al. 2016; Parthipan et al. 2017) components including PAHs (Sathishkumar et al. 2008; Dokic et al. 2011). The biodegradation of crude oil results in breakdown of its various components into non-toxic by-products like CO₂ and H₂O. Estimation of residual crude oil and CO₂ production in polluted environment has been used for determining the kinetic parameters of hydrocarbon degradation (Zucchi et al. 2003; Singh et al. 2011; Singh et al. 2014; Chettri and Singh 2019). Such estimations using native hydrocarbonoclastic bacteria (HCB) provide clues for developing predictive hydrocarbon degradation model in natural environment.

In a previous study we have characterized the kinetic parameters of hydrocarbon degradation of *Pseudomonas aeruginosa* AKS1 and *Bacillus* sp. AKS2 isolated from hydrocarbon contaminated sediments collected from Guwahati refinery, India (Chettri et al. 2016). In present study, both isolates have been tested for their ability to produce biosurfactant and characterized for crude oil degradation activity using first-order kinetics.

2. Materials And Methods

2.1. Bacterial growth

Growth of both isolates was assessed in liquid Bushnell-Haas (BH) medium supplemented with 1% (v/v) *n*-hexadecane. The experiments were carried out in 100 ml Erlenmeyer flasks containing 50 ml BH medium inoculated with exponentially growing cells at a final concentration of OD₆₀₀ equal to 0.035. The culture flasks were then incubated at 25 ± 0.5°C in a rotary shaker at 100 rpm under darkness. Growth was assessed as increase in cell density and total protein content (Lowry et al. 1951). Growth in BH medium without *n*-hexadecane served as control.

2.2. Detection of catabolic genes by PCR

Genomic DNA was isolated from BH-crude oil grown cultures using Genomic DNA extraction Kit (Bioline). The conserved fragments of catabolic alkane hydroxylase gene was amplified using the primer pairs and at annealing temperature listed in Supplementary Table 1. A 25 µl PCR reaction mixture contained 50 ng template DNA, 2.5 µl 10X reaction buffer, 1.25 µl of 25 mmol/L MgCl₂, 0.5 µl of 10 mmol/L dNTPs, 0.5 µl of 10 pmol/µl each primer and 1 U Taq DNA polymerase. Final volume was adjusted by adding sterile double distilled water. PCR conditions were as follows: initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 s, primer annealing for 30 s, elongation at 72°C for 30 s, and a final extension step at 72°C for 10 min using an ABI 2720 thermal cycler. The PCR amplicons were then separated on 1.2% agarose gel in 1x TAE buffer and visualized after staining with ethidium bromide (5 µg/ml) and photographed using a Bio-Rad Gel Doc™ XR + imaging system.

2.3. Cell surface hydrophobicity test

Cell hydrophobicity was measured according to Rosenberg (Rosenberg et al. 1980). In brief, cells were washed twice in Phosphate Urea Magnesium (PUM) buffer pH 7.1 (22.2 g K_2HPO_4 , 7.26 g KH_2PO_4 , 1.8 g urea, 0.2 g $MgSO_4 \cdot 7H_2O$ in final volume of 1000 ml sterile distilled water) and suspended in the same to an OD_{550} of 0.5. The cell suspension (1.0 ml) was then mixed and vortexed with heptane (0.2 ml) for 2 min followed by equilibration for 1 hour. The absorbance of the bottom aqueous phase was then measured at 550 nm. Hydrophobicity expressed as percentage adherence to heptane was calculated as follows:

% Hydrophobicity = $(1 - A/A_0) \times 100$ where $A = OD_{550}$ of aqueous phase

$A_0 = OD_{550}$ of initial cell suspension

2.4. Emulsification index

The emulsification activity was determined as described (Berg et al. 1990). To culture supernatant (0.5 ml) same volume of TM buffer (20 mM Tris[tris(hydroxymethyl)-aminomethane]/HCl buffer, pH7.0 and 10 mM $MgSO_4$) and 0.1 ml of kerosene was added. The tube was vortexed for 1 min, held stationary for 3 min, and then visually examined for turbidity of a stable emulsion. Emulsification index was calculated using the formula

$E_{24} = (\text{Height of emulsion formed} / \text{Total height of solution}) \times 100$

2.5. Isolation of biosurfactant

Supernatant of bacterial culture grown for 24 and 48 hours in 0.5% (v/v) *n*-hexadecane amended nutrient broth were collected by centrifugation at 10,000 rpm for 15 min at 4°C. The pH of cell free supernatant was then adjusted to 2.0 using concentrated HCl and kept at 4°C overnight to allow formation of precipitate. The precipitate was collected by centrifugation at 17,000 rpm for 15 min at 4°C, dissolved in water and lyophilized (Heto LyoLab 3000 Lyophilizer, Germany). The recovery of lyophilized biosurfactant sample was expressed as $g \text{ biosurfactant} \cdot g^{-1} \text{ dry cell wt.}$

2.6. Characterization of biosurfactant

The partially purified biosurfactant was characterized based on simple colorimetric assays.

2.6.1. Carbohydrate: The carbohydrate content was measured using Phenol-sulphuric acid method with glucose as standard. In brief, 5 mg of the biosurfactant sample was dissolved in 1 ml of water. One hundred μ l of this suspension was mixed with 200 μ l of phenol reagent (5% v/v water) followed by addition of 1 ml concentrated H_2SO_4 and incubation at room temperature for 10 min. The mixture was vortexed vigorously and allowed to stand for 30 min. Finally absorbance was read at 490 nm against a reagent blank.

2.6.2. Protein: The extracellular protein in partially purified biosurfactant was measured at 750 nm using bovine serum albumin as standard according to Lowry (Lowry et al. 1951).

2.7. Crude oil degradation in liquid BH medium

Degradation of crude oil by individual bacterial isolate was carried out in biometric flasks containing 100 ml BH-medium (Himedia, India) supplemented with 1% Assam crude oil (v/v) as the sole source of carbon and energy. Bacterial cells grown in BH-medium supplemented with glucose (200 mg L⁻¹) were harvested, washed at 10000 rpm for 10 min in BH-medium and then re-suspended in the same. The cell suspension was inoculated at a final OD₆₀₀ equal to 0.035. Non-inoculated flasks were used as abiotic controls. The culture flasks were incubated as described in Sect. 2.1. Crude oil degradation activity of bacteria was assessed in terms of CO₂ evolution (Zibilski 1994). For estimation of total/residual petroleum hydrocarbons (TPH), each sample was extracted in an equal volume of dichloromethane (DCM) at regular interval and separated from aqueous phase. The DCM phase was then dehydrated with anhydrous sodium sulphate and remaining TPH was extracted using a Soxhlet apparatus. Weight of the residual TPH after rota-evaporation was determined gravimetrically.

2.8. Microcosm design and set up

Laboratory microcosm comprising 10 g sterilized sand sediment amended with 125 mg Assam crude oil and supplemented with 125 µg N.g⁻¹ sediment & 62.5 µg P.g⁻¹ sediment for *P. aeruginosa* AKS1, and 375 µg N.g⁻¹ sediment & 37.5 µg P.g⁻¹ sediment for *Bacillus* sp. AKS2 were set up. Sodium nitrate and potassium dihydrogen phosphate were used as sources of inorganic N and P respectively. Microcosms were inoculated with exponentially growing bacterial cells and incubated at a temperature of 25 ± 0.5°C under darkness. Microbial TPH degradation activity in the sediment was assessed as a function of CO₂ evolution as described in Sect. 2.7 (Zibilski 1994), and in terms of residual TPH as described by Márquez-Rocha (Márquez-Rocha et al. 2001). In short, 10 g of microcosm sand sediment was mixed with equal amount (w/w) of anhydrous sodium sulphate and TPH was extracted in DCM using a Soxhlet apparatus. Non-inoculated or microcosms without crude oil were also incubated to account for any abiotic CO₂ production.

Biodegradability of crude oil in both liquid culture and sand microcosms were fitted into first order kinetics as described in Eq. (1) below.

$C_t = C_0 e^{-kt}$ (1) Where, C_0 is the initial TPH content

C_t is the residual TPH content at time t

k is the biodegradation rate constant (day⁻¹)

Biodegradation half-life times ($t_{1/2}$) was calculated by Eq. (2)

$t_{1/2} = \ln 2/k$ (2)

3. Results

3.1. General characteristics of bacterial isolates:

Growth of bacterial isolates determined in *n*-hexadecane supplemented BH-medium showed increase in cell density and protein content which was 20–25 folds more than zero day (Fig. 1). Detection for presence of alkane hydroxylase gene in both strains was done using newly designed primer pairs AlkB-PA-2F/AlkB-PA-2R and AlkB gene-F/AlkB gene-R. While AlkB-PA-2F & 2R produced 200 bp PCR amplicons of *P. aeruginosa* AKS1 only, AlkB gene-F&R generated 190 bp long amplicons of both *P. aeruginosa* AKS1 and *Bacillus* sp. AKS2 (Fig. S1). Application of primer pair as described by (Kloos et al. 2006) did not produce PCR amplicons. These results highlight the diversity of alkane hydroxylase gene in bacterial community. Since both strains showed satisfactory growth in *n*-hexadecane supplemented BH-medium, we determined their hydrophobicity, emulsification index and biosurfactant production activity. The hydrophobicity (% adherence to heptane) was 77.74% and 81.19% and the E₂₄ values were 39.58% and 34.06% for *P. aeruginosa* AKS1 and *Bacillus* sp. AKS2 respectively (Fig. 2A, B). The capacity to produce biosurfactant was analysed in the supernatant of bacterial culture grown in *n*-hexadecane (0.5% v/v) amended nutrient broth. The partially purified biosurfactant was lyophilized and measured gravimetrically. *Pseudomonas aeruginosa* AKS1 produced 0.138 ± 0.063 g biosurfactant.g⁻¹ cell dry wt. after 24 hours and 0.108 ± 0.0057 g biosurfactant.g⁻¹ cell dry wt. after 48 hours of growth. In case of *Bacillus* sp. AKS2, it was 0.246 ± 0.026 g biosurfactant.g⁻¹ cell dry wt. after 24 hours and 0.201 ± 0.051 g biosurfactant.g⁻¹ cell dry wt. after 48 hours. Partial biochemical analysis of the purified biosurfactant revealed the presence of carbohydrate and protein residues. The purified biosurfactant from *P. aeruginosa* AKS1 contained 1.66 ± 0.06 mg carbohydrate plus 1.68 ± 0.5 mg protein.10 mg⁻¹ biosurfactant and the biosurfactant from *Bacillus* sp. AKS2 contained 0.73 ± 0.08 mg carbohydrate plus 1.76 ± 0.85 mg protein.10 mg⁻¹ biosurfactant (Table 1).

Table 1

Production of biosurfactants by *P. aeruginosa* AKS1 and *Bacillus* sp. AKS2 in nutrient broth containing 0.5% (v/v) *n*-hexadecane.

Isolate	After 24 hours		After 48 hours	
	OD ₆₀₀	Biosurfactant recovery (g. g ⁻¹ dry cell wt.)	OD ₆₀₀	Biosurfactant recovery (g. g ⁻¹ dry cell wt.)
<i>P. aeruginosa</i> AKS1	0.8882 ± 0.0374	0.138 ± 0.063	1.443 ± 0.097	0.108 ± 0.0057
<i>Bacillus</i> sp. AKS2	0.9347 ± 0.1138	0.246 ± 0.026	0.963 ± 0.083	0.201 ± 0.051

3.2. Crude oil degradation kinetics

Degradation of crude oil was monitored by measuring the cumulative CO₂ production and changes in TPH concentration with time in liquid BH-medium (Fig. 3), and microcosm sand sediment (Figs. 4 and 5). In liquid microcosm the cumulative CO₂ production was observed to be 455 ± 17.12 µmoles and 200 ± 1.11 µmoles on day 15 after inoculation with *P. aeruginosa* AKS1 and *Bacillus* sp. AKS2 respectively (Fig. 3A, C). Their respective crude oil biodegradation rate constant (*k*) was observed to be 0.0383 day⁻¹ and 0.0204 day⁻¹. The half-life (*t*_{1/2}) for crude oil degradation was found to be 18.09 days for *P. aeruginosa* AKS1 and 33.97 days for *Bacillus* sp. AKS2. The resulting data fitted to First-order kinetics model with R² value more than 0.9 (Fig. 3B, D). Our data suggest high crude oil degradation efficiency for *P. aeruginosa* AKS1 in liquid culture.

Treatment regime of oiled sand microcosm was as follows: (a) nutrient plus bacteria (N + B), (b) nutrient but no bacteria (N only), and (c) bacterial inoculum only (B only). The level of nutrient treatment in sand microcosm was 125 µg N & 62.5 µg P.g⁻¹ sediment for *P. aeruginosa* AKS1 inoculated microcosms, and 375 µg N and 37.5 µg P.g⁻¹ sediment for *Bacillus* sp. AKS2. The application of these concentrations was based on a previous study (Chettri et al. 2016). The N + B microcosm inoculated with *P. aeruginosa* AKS1 produced 256.7 ± 20.2 µmoles CO₂ after 7 days of incubation which was statistically significantly higher when compared to B only (48.75 ± 12.37 µmoles CO₂) or N only controls (27.5 ± 7.1 µmoles CO₂) (one-way ANOVA; P = 1.1E-06). Total CO₂ evolution in N + B microcosm was 306.7 ± 5.2 µmoles 14 days after incubation which was statistically different from N only and B only controls (one-way ANOVA; P = 9.2E-09). Thereafter no significant increase in CO₂ evolution was observed for the total incubation period of 28 day (one-way ANOVA; P = 0.604) (Fig. 4A). Decrease in TPH with time is shown in Fig. 4B. The initial weight of TPH added was determined by taking average of residual hydrocarbons extracted from all three categories of replicate microcosms (100.6 ± 1.20 mg /10 g sand). In N + B microcosm, the residual TPH was found to reduce by 28%, 30%, 34% and 36% after 7, 14, 21 and 28 days respectively. In B only treated microcosms, the respective residual TPH values were found to reduce by 14%, 16%, 16% and 20% of the initial average value. However, in N only control microcosm the degradation of crude oil was found to be 3% on day 7 and 9% on day 28 after incubation. First order kinetic model was used to determine the rate of crude oil degradation in sand sediment microcosms. The biodegradation rate constant (*k*) and half-life (*t*_{1/2}) of degradation for N + B microcosms was 0.0138 day⁻¹ and 50 days respectively. The corresponding values for B only control microcosms were 0.0069 day⁻¹ and 100 days respectively, and for N only control microcosm were 0.003 day⁻¹ and 231 days. Degradation of crude oil in N + B microcosm was significantly higher than in B only microcosm (one way ANOVA; P = 0.014).

The CO₂ evolution and TPH degradation activities analysed in sand sediment microcosm inoculated with *Bacillus* sp. AKS2 is shown in Fig. 5. In this case, total CO₂ evolved reached to 245 ± 11.5 µmoles CO₂ on 7 days and remained nearly same (267.5 ± 31 µmoles CO₂) on day 28 (one way ANOVA; P = 0.316) in N + B microcosm. The CO₂ evolution activities in B only microcosms was 55 ± 7.1 µmoles CO₂ and 118.8 ± 12.4 µmoles CO₂ on days 14 and 28 respectively (Fig. 5A). The degradation of crude oil by *Bacillus* sp. AKS2 with time is shown in Fig. 5B. In N + B microcosm, TPH content was reduced by 15%, 21%, 24% and

29% on 7, 14, 21 and 28 days after incubation respectively. In B only microcosms, reduction in TPH content ranged from 9–14% between day 7 and day 28 while in N only microcosm the reduction was found to be 3% and 11% between day 7 and day 28. The crude oil degradation data thus obtained were fitted into first-order kinetic model to obtain the biodegradation rate constant (k) and half-life ($t_{1/2}$) of degradation. These respective values for N + B microcosms were 0.0113 day^{-1} and 61.34 days, and in case of B only microcosms were 0.0046 day^{-1} and 150.68 days, and in N only treated microcosms were 0.004 day^{-1} and 173.29 days.

4. Discussion

The bacterial isolates namely *Pseudomonas aeruginosa* AKS1 and *Bacillus* sp. AKS2 have been reported previously for hydrocarbon degrading potentials (Chettri et al. 2016). Both isolates showed good growth in liquid BH-media when amended with *n*-hexadecane as sole carbon source (Fig. 1A, B). They also exhibited high percentages of hydrophobicity which determines the initial adhesion of microorganisms to the interface between the NAPL (non-aqueous- phase liquid) and the aqueous phase. The direct contact between a bacterial cell and a target hydrocarbon significantly increase the rate of entry of hydrocarbon into the cell. The cellular metabolism of hydrocarbons subsequently promotes the bacterial growth. Both isolates were found to degrade and utilize crude oil as sole carbon source as evidenced from their CO_2 evolution activity and decrease in quantity of residual TPH. Their ability to metabolize crude oil was clearly reflected by the presence of genes for hydrocarbon degradation (Fig. S1). It is a well-established fact that biosurfactant produced by bacterial strains significantly reduce the surface tension by enhancing emulsification thereby facilitating the bioavailability and degradation of hydrocarbons in contaminated environments (Banat et al. 2000; Rahman et al. 2003; McKew et al. 2007). Both isolates showed biosurfactant production and emulsification activity. The level of emulsification index was very high when compared with values reported in past (Pruthi and Cameotra 1995; Fleck et al. 2000). These attributes of the isolates make them a desirable natural agent for bioremediation of hydrocarbon contaminated environment.

Bioremediation of polluted habitats using bioaugmentation with native isolates and biostimulation with nutrient and surfactant are effective strategy for neutralization of hazardous components in crude oil (McKew et al. 2007; Varjani and Upasani 2019). Kinetic parameters and the optimum range of inorganic nutrients and crude oil concentrations for degradation of Assam crude oil by *Pseudomonas aeruginosa* AKS1 and *Bacillus* sp. AKS2 (Chettri et al. 2016) and their persistence in hydrocarbon amended microcosms (Chettri et al. 2019) has been described previously. However, to the best of our knowledge, there is only one report on kinetics of hydrocarbon degradation of a heavy metal tolerant *Novosphingobium panipatense* P5: ABC strain isolated from crude oil contaminated sediments collected from north east region of India (Chettri et al. 2019) and no such study has been carried out on hydrocarbon degrading *Pseudomonas* and *Bacillus* sp. Availability of kinetic data is highly essential for the development of predictive model of hydrocarbon degradation. Our study reports the TPH degradation kinetics of *P. aeruginosa* AKS1 and *Bacillus* sp. AKS2 over a period of 28 days. The respective TPH

degradation by AKS1 and AKS2 strains were 48.24% and 40.2% of total TPH added in sand sediment microcosms. A long term study (Das and Mukherjee 2007) has reported TPH degradation by *Pseudomonas aeruginosa* M and NM consortium and *B. subtilis* strains. The respective degradation values reported were 75% and 43%.in TPH contaminated soil 120 days after inoculation. These respective values for 60 days incubated microcosms were 42% and 24% only (Das and Mukherjee 2007). In comparison, our data clearly reflect the high efficiency of *P. aeruginosa* AKS1 and *Bacillus* sp. AKS2 in degradation of TPH during a short term incubation period of 28 days. The degradation rate was high in liquid media and in *P. aeruginosa* AKS1 inoculated microcosms. The reason for reduced degradation rate in sand sediment could be due to restricted mobility of both bacteria and crude oil. Amendment of contaminated sediments with inorganic N & P significantly enhanced the estimated biodegradation rates and concomitant reduction of half-life time as compared to no nutrient amended controls. The biodegradation rate of petroleum hydrocarbon in soil sediment has been described using first order kinetic equation. One such study has revealed the range of kinetic constants between 0.041 and 0.0071 day⁻¹ for indigenous bacterium *Stenotrophomonas multophilia* in *ex situ* bioremediation of petroleum contaminated soil (Abbassi and Shquirat 2008). Our data also fitted well in the first order kinetic equation. First order kinetics has also been used to determine the biodegradation rate of lubricating oil, mineral oil, used motor oil, crude oil and diesel oil under various nutrient supplements (Rončević et al. 2005).

Conclusions

The data presented describe the efficiency and kinetics of hydrocarbon degradation activity of *P. aeruginosa* AKS1 and *Bacillus* sp. AKS2 isolates from crude oil contaminated sediments collected from Assam refinery, India. Both isolates showed high level of biosurfactant production and emulsification index. The CO₂ evolution activity of *P. aeruginosa* AKS1 in liquid medium was more than that of *Bacillus* sp. AKS2. But in the sand sediment, both isolates showed almost similar rate of CO₂ evolution activity. The biodegradation efficiency of both isolates was effectively enhanced in response to inorganic N and P nutrients treatments both in liquid medium and microcosm sand sediments. Similar analyses on this line will generate crucial inputs for developing predictive model of hydrocarbon degradation.

Declarations

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Compliance with ethical standards:

Conflict of Interest: The authors declare no conflicts of Interest.

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Figures

Figure 1

Growth of bacterial isolates in presence of n-hexadecane as sole C source (A) *P. aeruginosa* AKS1 (B) *Bacillus* sp. AKS2. Filled circles represent optical density at 600 nm while bold circles indicate protein concentration.

Figure 2

Hydrophobicity and emulsification index of *P. aeruginosa* AKS1 and *Bacillus sp.* AKS2. Hydrophobicity is indicated as % adhesion to heptane.

Figure 3

Kinetics of crude oil degradation with time in liquid BH medium by *P. aeruginosa* AKS1 (A & B) and *Bacillus* sp. AKS2 (C & D). A & C are cumulative CO₂ production and B & D are plot of ln C (TPH).

Figure 4

Kinetics of crude oil degradation in sand microcosms by *P. aeruginosa* AKS1 (A) Rate of CO₂ production with time (B) TPH degradation with time. Filled circles represent (N+B) microcosms, open circles represent (N only) microcosms and gray circles represent (B only) microcosms.

Figure 5

Kinetics of crude oil degradation in sand microcosms by *Bacillus* sp. AKS2 (A) Rate of CO₂ production with time (B) TPH degradation with time. Filled circles represent (N+B) microcosms, open circles represent (N only) microcosms and gray circles represent (B only) microcosms.

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