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Biodegradation of phenanthrene as a model hydrocarbon: Power display of a super-hydrophobic halotolerant enriched culture derived from a saline-sodic soil

Ahmad Ali Pourbabaee[a,*], Malek Hossein Shahriari[b], Hamidreza Garousin[a]

[a] Biotechnology Lab, Department of Soil Science, University College of Agriculture and Natural Resources, University of Tehran, Iran
[b] Department of Soil Pollution and Chemistry, Persian Gulf University, Bushehr, Iran

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ABSTRACT

In this study, after evaluating the degradation activity of enriched cultures from four crude oil-contaminated soils in mineral salt medium, the most efficient ones were selected for further studies. The chemical analysis of cell-free extract containing phenanthrene by HPLC suggested the superior enriched culture was able to degrade 87.66% of phenanthrene at the concentration of 40 mg L-1 within 10 days. This experiment was done under optimal conditions (37 °C, 10% salinity, and pH around 7 to 7.5). The 16S rRNA sequencing of isolates from this superior enriched culture indicated the highest similarity to *Acidovorax delafieldii* (Q-SH3), *Bacillus hwajinpoensis* (Q-SH12), and *Bacillus rhizosphaerae* (Q-SH14). After biodegradation of phenanthrene in liquid medium, the extracts were analyzed to measure barley and alfalfa germination. Results showed a lower level of toxicity to the seeds, hence this enriched culture could be used for bioremediation of saline environments contaminated by phenanthrene and other similar compounds.

1. Introduction

Environmental contamination by anthropomorphic activities is rather an undeniable part of humans' lifestyle in the industrialized world [1]. Petroleum hydrocarbon, among those, polycyclic aromatic hydrocarbons (PAHs), are major environmental pollutants, notorious for their persistence in environment, and above all, the carcinogenic nature of them [2]. Phenanthrene is a tricyclic compound of polycyclic aromatic hydrocarbons present in most contaminated areas with petroleum hydrocarbons.

Many reports have pointed out the biodegradation of polycyclic aromatic hydrocarbons by microorganisms. In the research, Reddy et al. [3] conveyed that 93.92% of the phenanthrene concentration was degraded by the bacterial strain *Brevibacillus* sp. PDM-3 after 6 days. Based on their notion, the degradation rate increased gradually up to 36 h, and subsequently, a drastic increment took place at the end of 144 h. Moreover, Tiwari et al. [4] concluded that three important polycyclic aromatic compounds considering the degradation priority by Stenotrophomonas sp. strain IITR87, including phenanthrene, pyrene, and benzo[*a*]pyrene, were degraded 15 days after incubation as much as >99, 98 and <50%, respectively.

In general, any remedial approach relys on offsetting the restricting elements of remediation speed. Probably, salinity stress is an utmost importance non-biological factor affecting plant growth and soil microorganisms activity in arid and semi-arid regions [5,6]. Sardinha et al. [7] reported that salinity can be noxious to microbiological processes by limiting the bioavailability of water and affecting the cellular physiology and metabolic processes. Bioremediation of saline environments contaminated with oil hydrocarbons is conditional to the ability of natural microflora to withstand the salinity levels of the environment. Salinity has been reported by many researchers as one of the most prominent factors in the degradation of polycyclic aromatic hydrocarbons [8,9]. When salinity remained high enough, hydrocarbon-degrading microorganisms lost their access to hydrocarbons. Therefore, it was expected that the degradation of crude oil derivatives would be negatively correlated to high salinity.

This study was conducted to isolate and identify the vigorous halotolerant enriched culture which can play a key role in degradation of phenanthrene in petroleum-contaminated saline environments. These promising approaches appear to be helpful especially in environments suffering from petroleum hydrocarbons contamination.

* Corresponding author.
E-mail address: pourbabaei@ut.ac.ir (A.A. Pourbabaee).

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2. Materials and methods

2.1. Sampling and culture medium

Sampling was carried out in four petroleum-contaminated saline and sodic soils (ranging from 0 to 30 cm depth) in oil and gas exploitation area of Sarajeh, Qom, central Iran. Important soil parameters were measured by standard methods (Table 1) [10]. In this study, the modified 6SW-Vit medium McGenity and Gramain [11] containing three solutions, was used for biodegradation assays. Solution A contained (g 800 ml^{-1}) 82.85 NaCl, 24.1 $MgCl_2.6H_2O$, 2.53 KCl, 2.18 Na_2SO_4, 20 ml Tris-HCl (1 M;pH 7.6), 0.5 yeast extract. Solution B contained (ml 100 ml^{-1}) 0.6 H_3BO_3 (400 mM), 0.7 $SrCl_2$ (400 mM), 0.7 NaF (70 mM), 1 NH_4Cl (500 mM), 1 KH_2PO_4 (100 mM), 2 trace element solution SL-10, and solution C contained (ml 100 ml^{-1}) 10 $CaCl_2.2H_2O$ (1 M). First, the solutions autoclaved separately and thereafter mixed when they were cooled down. Trace element solution SL-10 contained (per L) 10 ml HCl (25%; 7.7 M), 1.5 g $FeCl_2.4H_2O$, 70 mg $ZnCl_2$, 100 mg $MnCl_2.4H_2O$, 6 mg H_3BO_3, 190 mg $CoCl_2.6H_2O$, 2 mg $CuCl_2.2H_2O$, 24 mg $NiCl_2.6H_2O$, 36 mg $Na_2MoO_4.2H_2O$. Final pH was adjusted to the range of 7-7.5.

2.2. Enrichment of phenanthrene-degrading halotolerant bacteria

To enrich halotolerant bacteria capable of degrading phenanthrene, 10 g of each crude oil-contaminated soil samples was added into a 500 ml flask containing 100 ml of modified 6SW-Vit medium with 80 mg L^{-1} phenanthrene as the sole carbon source. Samples were shaken for seven days at 150 rpm and 37 °C. To complete the enrichment process, 10 ml of the enriched culture medium was transferred into 90 ml of a fresh salting medium, and this procedure was repeated for 8 times. The final product was used as the enriched culture to evaluate the phenanthrene biodegradation and other relevant tests.

2.3. Phenanthrene biodegradation assay by enriched cultures

1 ml of each enriched cultures suspension which was obtained from soil enrichment in the presence of phenanthrene (turbidity equal to the 0.5 McFarland standard) inoculated in test tubes containing 10 ml of a mineral-salt medium amended with 40 mg L^{-1} phenanthrene and incubated at 37 °C (optimum temperature) in a rotary shaker incubator (150 rpm) for 10 days. After 10 days, the concentration of residual phenanthrene was measured. Thereafter the calculation of residual phenanthrene in the medium, the enriched culture that was more capable to reduce the concentration of phenanthrene was selected for subsequent experiments. All experiments in this study were carried out in triplicate with control sample.

2.4. Assessing the residual phenanthrene concentration

To prepare the samples for high-performance liquid chromatography (HPLC), the contents of test tubes centrifuged at 9000 rpm for 20 min. The supernatant was removed, then 10 ml of n-hexane was added and vortexed for 5 min to extract phenanthrene. Finally, the dehydrated organic phase with anhydrous sodium sulfate was used to determine the concentration of residual phenanthrene by Agilent 1100 series HPLC system [12].

2.5. The impact of salinity on biodegradation

Considering the higher efficiency of the enriched culture from the soil number 3, it was selected for further investigations. In order to determine the impact of different concentrations of salinity on degradation of phenanthrene, a volume of 5% selected supreme enriched culture grown in a minimal medium containing phenanthrene with the turbidity of 0/5 McFarland was inoculated in the modified 6SW-Vit medium at the concentrations of 0.5, 1, 5, 10, 15, 20, 25, 27 and 30% salts comprising 40 mg L^{-1} phenanthrene. After the period of 10 days, the ability of phenanthrene-degrading enriched culture was tested in different concentrations of salt. The growth of enriched culture bacteria was measured spectrophotometrically at 620 nm wavelength in the modified 6SW-Vit medium containing phenanthrene and various aforementioned concentrations of salt.

2.6. The impact of various phenanthrene concentrations on the amount of biodegradation

To investigate the effect of different concentrations of phenanthrene on biodegradation, 40, 100, 500, 1000, and 2000 mg L^{-1} phenanthrene concentrations as the carbon source in the modified 6SW-Vit medium was used. The total remain of phenanthrene's concentration in culture medium was analyzed at 1, 2, 3, 6, and 10 days.

2.7. Isolation and identification of culturable bacterial isolates

To isolation of grown bacteria, a diluted series was prepared from the final enriched culture media and transferred to the plates containing modified 6SW-Vit-agar medium. After incubating at 37 °C, grown isolates were purified from solid culture media. The ability of phenanthrene degradation by pure isolates were probed using spray-plated and turbidity test of medium containing phenanthrene [13]. Qiagen kit Cat. No. 51,504 was used to extract total genomic DNA of bacterial isolates of enriched culture. The 16S rRNA gene of pure isolates was amplified and then the purified PCR products were sequenced by Macrogen Company in South Korea (ABI system 3730XL).

2.8. Measurements of cell hydrophobicity, surface tension and emulsification index

Bacterial adhesion to hydrocarbons (BATH) test was used to evaluate the hydrophobicity of the enriched culture. [14]. The percentage of the cells moved to the hydrocarbon phase indicates cell-surface hydrophobicity.

Various hydrocarbons such as kerosene, benzene, n-hexane, and toluene were used to determine the emulsifying index (E24). After inoculation of the bacterial enriched culture into the medium

Table 1
Chemical parameters of crude oil-contaminated saline and sodic soils.

Soil samples	EC (dS m^{-1})	pH	SAR $(mmol/l)^{1/2}$	SP (%)	Na^+ (meq L^{-1})	Ca^{+2}	Mg^{+2}	K^+	Cl^-	HCO_3^-	$CO3^{-2}$	SO_4^{-2}
1	123.2	7.83	57.11	39.4	1021.7	515	125	2.76	1740	5.6	0	38.34
2	136.8	7.92	66.18	39.22	1193.2	530	120	3.73	1974	5.4	0	45.39
3	226	7.95	150.67	27.23	3309.8	825	140	24.12	4700	10	1.4	18.39
4	222	7.9	143.93	28.47	3159.9	780	145	24.94	4400	9.6	1.2	16.59

containing phenanthrene, the surface tension of the cell-free culture medium was measured at different times by KRUSS tensiometer device [14].

2.9. Isolation and identification of intermediate metabolites

To isolate intermediate metabolites of the phenanthrene biodegradation pathway, the phenanthrene-containing medium was centrifuged on the day of the seventh after incubation and identification of metabolites was conducted based on [15].

2.10. Toxicity survey of phenanthrene on barley and alfalfa germination

To toxicity survey of phenanthrene, the capability of barley and alfalfa germination in agar medium containing phenanthrene and final degradation product was investigated. Due to the fact that the seeds of plants do not have the ability of germination in high salinity, in this experiment, after the phenanthrene degradation by the bacterial enriched culture in the culture medium (include 5% salt and 1000 mg L^{-1} phenanthrene), the culture medium was diluted 10 times with sterilized distilled water. The agar (1.5%) was then added to the medium and also agar culture medium containing 0.5% salt and 100 mg L^{-1} phenanthrene along with agar culture medium comprising 0.5% salt without phenanthrene supplementation were used. Alfalfa and barley seeds were surface sterilized, washed with sterilized distilled water and transferred to Petri dishes containing agar culture media and maintained at room temperature under visible light. The number of buds per Petri dish was tallied during 10 days.

3. Results and discussion

3.1. Selection of bacterial enriched culture

Numerous studies have shown that petroleum hydrocarbon-degrading bacteria have the ability to form a clear zone on contaminated agar plates [16,17]. The inoculation of bacterial enriched cultures in the modified 6SW-Vit medium containing phenanthrene showed that 4 bacterial enriched cultures had the ability to reveal the turbidity and to form a clear zone (Fig. S1). The results of phenanthrene biodegradation suggested that the highest reduction in concentration of 40 mgL^{-1} phenanthrene in the liquid medium was attributed to the enriched culture from the soil sample 3 (87%) (Fig. 1).

3.2. Effects of disparate salinity concentrations on the amount of phenanthrene biodegradation

The results indicated the halotolerant bacterial enriched culture was able to degrade phenanthrene in the wide range of salinity

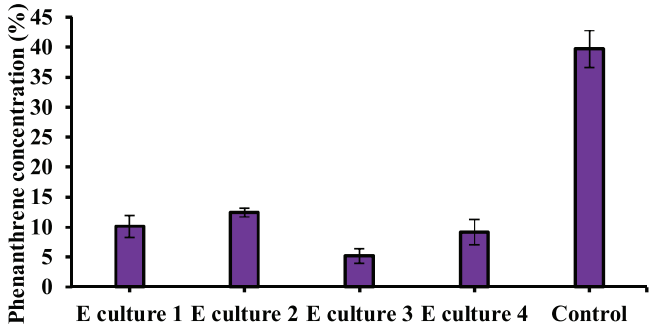

Fig. 1. Residual concentration of phenanthrene with an initial concentration of 40 mgL^{-1} in the medium after 10 days.

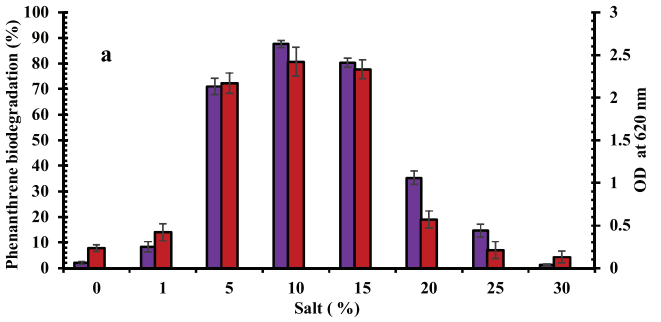

Fig. 2. Phenanthrene degradation (purple columns) with an initial concentration of 40 mgL^{-1} and growth rate of bacteria at 620 nm (dark red columns) in the modified 6SW-Vit medium containing different percentages of salt after 10 days. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(Fig. 2). The highest percentage of phenanthrene degradation was observed at 10% salt concentration (87%). By adding or depleting the salinity, the phenanthrene degradation was reduced. Nonetheless, the halotolerant bacterial enriched culture revealed to be able to degrade more than 70% of phenanthrene at 5, and 15% of salt concentrations within 10 days. Furthermore, higher salinity (more than 20%) imposed a great impact on biodegradation, as it turned out that at the concentration of 25% salt, less than 10% of phenanthrene was degraded. The amount of phenanthrene degradation in concentrations of 0.5 and 30% salt was negligible. In many studies, the concentration of natural NaCl is considered as salt percentage Ventosa et al. [18], but in this study, a mixture of salts which was found in saline soils was used, and the reason was that the usage of a number of salts in the medium was closer to the soils natural conditions. Consequently, the phenanthrene degradation was consistent with the highest growth rate of the bacteria.

More to the point, numerous researchers have reported the salinity as the major issue in degradation of polycyclic aromatic hydrocarbons [8,9]. In an investigation by Zhao et al. [19] the highest degradation of phenanthrene happens in 5% salinity, and meanwhile, phenanthrene biodegradation reduces when salinity either increased or decreased. While in this study, phenanthrene was degraded by the halotolerant bacterial enriched culture at a higher salt concentration (10% salt). This can be explained by the fact that bacterial environmental conditions have long been exposed to high salinity. Although, Arulazhagan and Vasudevan [2] pointed out that in salinity of 3% sodium chloride strain *Ochrobactrum* sp. VA1 was efficient to degrade 92% phenanthrene with an initial concentration of 3 mg L^{-1} over four days, with increasing salinity up to 6% the phenanthrene degradation was dwindled by the same strain.

3.3. Effects of sundry phenanthrene concentrations on the amount of biodegradation

The impact of different concentrations of phenanthrene (40, 100, 500, 1000 and 2000 mgL^{-1}) on biological degradation revealed that the highest percentage of phenanthrene degradation was recorded 87 and 85%, at the concentrations of 40 and 100 mgL^{-1}, respectively after 10 days of incubation (Fig. 3). This bacterial enriched culture also showed an ability to degrade 72, 31 and 13% of phenanthrene, at the concentrations of 500, 1000 and 2000 mgL^{-1} respectively in 10 days. It is clear that, the higher the concentration of petroleum hydrocarbons, the greater the restriction on the transport of nutrients and oxygen, resulting in reduced biodegradation [20]. The previous study showed when rising the phenanthrene concentration from 20 to 50 and 100 mg L^{-1}, combined with 3 percent salinity and microbial

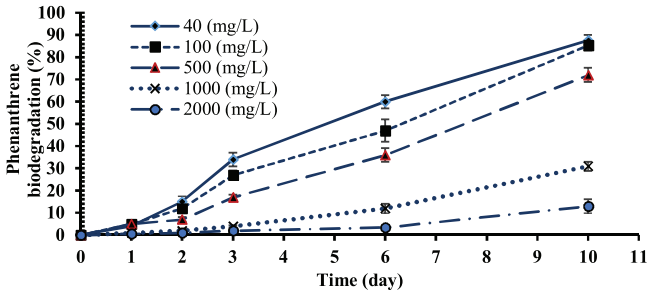

Fig. 3. Phenanthrene biodegradation kinetics by bacterial enriched culture at various concentrations at 37 °C and 10% salt.

population, the phenanthrene biodegradation decreased to 6 and 21%, respectively [21].

3.4. Identification of halotolerant bacterial isolates

The results of 16S rRNA gene sequencing showed that isolate Q-SH12 (Firmicutes, gram-positive, rod-shaped, catalase-positive, the range of growth at different concentrations of NaCl was 0–20%, and the range of temperatures for growth was 20-45 °C)(GenBank accession number KC164254) had the highest similarity (99.05%) to *Bacillus hwajinpoensis* strain SW-72^T with accession number AF541966, and isolate Q-SH3 (b-Proteobacteria, gram-negative, rod-shaped, catalase-positive, the range of growth at different concentrations of NaCl was 0–30%, and the range of temperatures for growth was 20-45 °C)(GenBank accession number KC178692) had the most closely similarity (99.16%) to *Acidovorax delafieldii* strain ATCC 17505^T with accession number AF078764, and lastly, isolate Q-SH14 (Firmicutes, gram-positive, rod-shaped, catalase-positive, the range of growth at different concentrations of NaCl was 0–15%, and the range of temperatures for growth was 20-45 °C)(GenBank accession number KC178693) had the most closely related (98.67%) to *Bacillus rhizosphaerae* strain SC-N012^T with accession number FJ233848.

3.5. Cell surface hydrophobicity, surface tension variations, and emulsifying index

The assay of changes in cell hydrophobicity over time showed that the maximum cell surface hydrophobicity was noticed on the day eighth of the experiment, which 78% of the cells were transferred to the organic phase and it was related to hexane solvent, (Fig. 4).

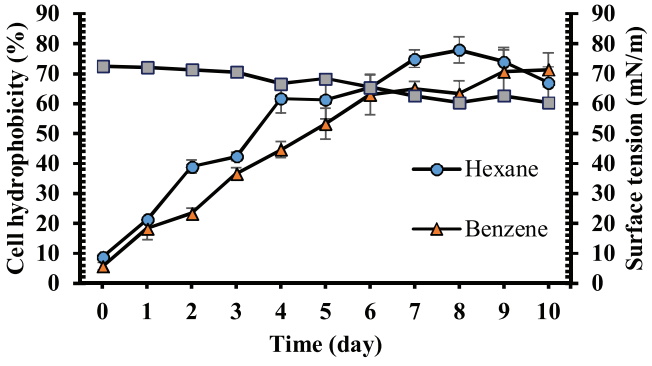

Fig. 4. Changes in the hydrophobicity percentage and surface tension variations of bacterial enriched culture in the modified 6SW-Vit medium amended with phenanthrene over time.

Studies have shown that biosurfactants are not only assisting the formation of emulsions, but also play a vital role in hydrophobicity of the cell surface to improve the surface tension towards the substrate and facilitate their bioavailability and functional activities [14,22]. Gesheva et al. [23] also noted the hyper-hydrophobicity of the cell surface as a predominant process involved in degradation of phenanthrene and the growth of *Rhodococcus fascians*. More to the point, the higher the amount of biosurfactants, the more effective the microbial cells orient towards the hydrophobic substrates. Consequently, this improves the bioavailability of substrates and finally leads to better phenanthrene biodegradation [14].

[11] stated that the cell surface hydrophilicity of the halophilic microorganisms prevents the degradation of hydrophobic compounds. Contrary to this hypothesis, our results suggested a high degree of hydrophobicity at the cell surfaces, which simplified the intimate interaction of cells with hydrocarbon contaminants and could be considered as the main process in the phenanthrene degradation.

The surface tension measurements of the phenanthrene-containing medium showed that the highest surface tension achieved at the beginning of the analysis as much as 72.5 mN m^{-1} (Fig. 4). This elevation in surface tension can be attributed to the salinity of the medium containing 10% salt. The high concentration of minerals in solution increased the surface tension of the liquid [24].

Alterations and slight reduction in surface tension indicated that during phenanthrene degradation the activity of enriched bacteria in the culture medium didn't affect significantly the reduction of surface tension. Previously, some researchers have announced a noteworthy reduction in surface tension as a result of the activity of some hydrocarbon-degrading bacteria. Adding surfactants can decrease the surface tension to less than 30 mN m^{-1} [24]. Moreover, the reduction in surface tension of the culture medium to 26.7 mN m^{-1} by the thermo-halotolerant *Bacillus subtilis* strain has been reported [25]. On the other hand, Dastgheib et al. [26] stated that a halotolerant *Alcanivorax* sp. strain didn't meaningfully decrease the surface tension, despite the ability to degrade hydrocarbons, which was consistent with the results of this study.

Assessing the emulsification index (E24), using kerosene, benzene, n-hexane, and toluene hydrocarbons, showed that the enriched bacteria in the modified 6SW-Vit medium supplemented with phenanthrene didn't have the ability to produce biosurfactants as emulsifying agents which corresponded positively with the results of an insignificant reduction in surface tension. Due to the cell surface high hydrophobicity, direct contact between the cell and hydrocarbon contaminants can be contemplated as the dominant process in the phenanthrene degradation.

3.6. Identification of metabolites as a result of phenanthrene biodegradation

The consequences of the GC–MS analysis displayed that the intermediate metabolites including catechol, phthalic anhydride, naphthol, and naphthalene were detectable (Fig. S2). The degradation of phenanthrene and the metabolites formation during bacterial degradation have been reported by many researchers [15,27].

Presence of phenanthrene-dihydrodiol, dihydroxy phenanthrene, 1-hydroxy-2-naphthoic acid, and phthalic acid metabolites by halophilic microbial consortium are the products of biodegradation [28]. However, due to the high concentration of phthalic anhydride compared to other metabolites, it seems that the phthalic anhydride pathway is the main degradation pathway by the halotolerant bacterial enriched culture.

3.7. The poisonous impact of phenanthrene on barley and alfalfa germination

The impact of phenanthrene with the preliminary concentration of 100 mg L^{-1} on germination of barley and alfalfa seeds were analyzed in agar medium without and after biodegradation (within 10 days). The results delineated that the given concentration reduced the percentage of germination in alfalfa and barley seeds, yet it appeared to be more detrimental and significant in alfalfa's seed germination, indicating the higher level of susceptibility of alfalfa to phenanthrene toxicity (Fig. 5).

The percentage of barely seeds germination exposed to the culture medium containing degraded-phenanthrene and non-degraded treatments was 58 and 45%, respectively. It seemed that the phenanthrene concentration and existing metabolites in the culture medium were still a bit toxic to alfalfa seeds even after biodegradation.

A number of researchers have argued that polycyclic aromatic hydrocarbons can limit the plant's growth by creating toxicity [29,30]. This has been described as a limiting factor in the percentage of germination and plant biomass, so that at the concentration of 1 mM phenanthrene, the germination percentage of *Arabidopsis thaliana* reduced about 50% in the agar-phenanthrene medium [31].

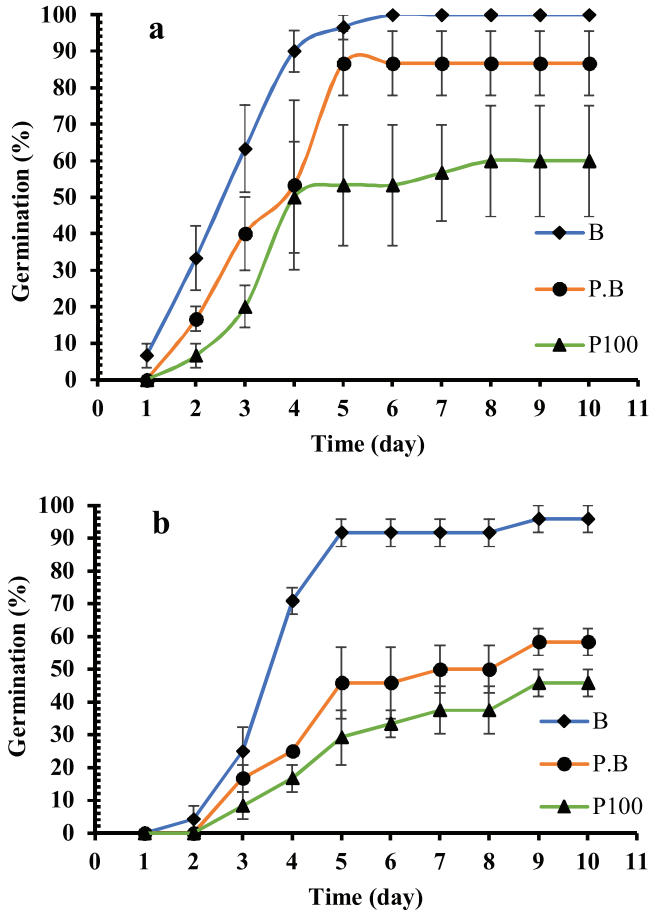

Fig. 5. Germination percentage of barley (a) and alfalfa (b) seeds. B: Control without phenanthrene, P.B: Phenanthrene after biodegradation and P100: culture medium containing 100 mg L^{-1} phenanthrene.

4. Conclusions

In our investigations, this enriched culture was capable to degrade phenanthrene in a wide range of salinity and the phenanthrene concentration. The results revealed that direct contact with hydrocarbon contaminants could be the dominant process in the phenanthrene degradation, despite the inability of the bacterial enriched culture to produce emulsifier surfactants due to the high hydrophobicity of the bacterial cell surface. The produced-metabolites after biodegradation showed that the compounds including catechol, phthalic anhydride, naphthol, and naphthalene were detectable. At the end of the research, the toxicity of phenanthrene to barley and alfalfa germination reduced because of the phenanthrene degradation by this potent enriched culture.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2019.e00388.

References

[1] S.J. Varjani, Microbial degradation of petroleum hydrocarbons, Bioresour. Technol. 223 (2017) 277–286.
[2] P. Arulazhagan, N. Vasudevan, Biodegradation of polycyclic aromatic hydrocarbons by a halotolerant bacterial strain Ochrobactrum sp. VA1, Mar. Pollut. Bull. 62 (2011) 388–394.
[3] M.S. Reddy, B. Naresh, T. Leela, M. Prashanthi, N.C. Madhusudhan, G. Dhanasri, P. Devi, Biodegradation of phenanthrene with biosurfactant production by a new strain of Brevibacillus sp, Bioresour. Technol. 101 (2010) 7980–7983.
[4] B. Tiwari, N. Manickam, S. Kumari, A. Tiwari, Biodegradation and dissolution of polyaromatic hydrocarbons by Stenotrophomonas sp, Bioresour. Technol. 216 (2016) 1102–1105.
[5] M. Kaushal, S.P. Wani, Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands, Ann. Microbiol. 66 (2016) 35–42.
[6] X. Liu, H. Meng, Y. Wang, Y. Katayama, J.-D. Gu, Water is a critical factor in evaluating and assessing microbial colonization and destruction of Angkor sandstone monuments, Int. Biodeterior. Biodegrad. 133 (2018) 9–16.
[7] M. Sardinha, T. Müller, H. Schmeisky, R.G. Joergensen, Microbial performance in soils along a salinity gradient under acidic conditions, Appl. Soil Ecol. 23 (2003) 237–244.
[8] J. Chen, M. Wong, Y.S. Wong, N.F. Tam, Multi-factors on biodegradation kinetics of polycyclic aromatic hydrocarbons (PAHs) by Sphingomonas sp. a bacterial strain isolated from mangrove sediment, Mar. Pollut. Bull. 57 (2008) 695–702.
[9] N. Tam, C. Guo, W. Yau, Y. Wong, Preliminary study on biodegradation of phenanthrene by bacteria isolated from mangrove sediments in Hong Kong, Mar. Pollut. Bull. 45 (2002) 316–324.
[10] D.L. Sparks, Madison, Wisconsin, USA, Methods of Soil Analysis Part 3. Chemical Methods, (1996) .
[11] T.J. Mcgenity, A. Gramain, Halophilic hydrocarbon degraders, in: K.N. Timmis (Ed.), Handbook of Hydrocarbon and Lipid Microbiology, Springer-Verlag, Berlin, 2010.
[12] I. Kelley, C.E. Cerniglia, The metabolism of fluoranthene by a species ofMycobacterium, J. Ind. Microbiol. 7 (1991) 19–26.
[13] M. Kumar, V. León, A.D.S. Materano, O.A. Ilzins, L. Luis, Biosurfactant production and hydrocarbon-degradation by halotolerant and thermotolerant Pseudomonas sp, World J. Microbiol. Biotechnol. 24 (2008) 1047–1057.
[14] M. Rosenberg, D. Gutnick, E. Rosenberg, Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity, FEMS Microbiol. Lett. 9 (1980) 29–33.

[15] Y.-S. Keum, J.-S. Seo, Y. Hu, Q.X. Li, Degradation pathways of phenanthrene by Sinorhizobium sp. C4, Appl. Microbiol. Biotechnol. 71 (2006) 935–941.
[16] H.W. Zhou, T.G. Luan, F. Zou, N.F.Y. Tam, Different bacterial groups for biodegradation of three-and four-ring PAHs isolated from a Hong Kong mangrove sediment, J. Hazard. Mater. 152 (2008) 1179–1185.
[17] H.-P. Zhao, L. Wang, J.-R. Ren, Z. Li, M. Li, H.-W. Gao, Isolation and characterization of phenanthrene-degrading strains Sphingomonas sp. ZP1 and Tistrella sp. ZP5, J. Hazard. Mater. 152 (2008) 1293–1300.
[18] A. Ventosa, J.J. Nieto, A. Oren, Biology of moderately halophilic aerobic bacteria, Microbiol. Mol. Biol. Rev. 62 (1998) 504–544.
[19] B. Zhao, H. Wang, X. Mao, R. Li, Biodegradation of phenanthrene by a halophilic bacterial consortium under aerobic conditions, Curr. Microbiol. 58 (2009) 205–210.
[20] J.G. Leahy, R.R. Colwell, Microbial degradation of hydrocarbons in the environment, Microbiol. Mol. Biol. Rev. 54 (1990) 305–315.
[21] P. Arulazhagan, N. Vasudevan, Role of a moderately halophilic bacterial consortium in the biodegradation of polyaromatic hydrocarbons, Mar. Pollut. Bull. 58 (2009) 256–262.
[22] C. Liao, X. Liu, Prevent the degradation of algicidal ability in Scenedesmus-lysing bacteria using optimized cryopreservation, Environ. Sci. Pollut. Res. - Int. 23 (2016) 5925–5930.
[23] V. Gesheva, E. Stackebrandt, E. Vasileva-Tonkova, Biosurfactant Production by Halotolerant Rhodococcusfascians from Casey Station, Wilkes Land, Antarctica, Curr. Microbiol. 61 (2010) 112–117.
[24] D.G. Cooper, Biosurfactants, Microbiol. Sci. 3 (1986) 145–149.
[25] H. Ghojavand, F. Vahabzadeh, M. Mehranian, M. Radmehr, K.A. Shahraki, F. Zolfagharian, M. Emadi, E. Roayaei, Isolation of thermotolerant, halotolerant, facultative biosurfactant-producing bacteria, Appl. Microbiol. Biotechnol. 80 (2008) 1073–1085.
[26] S.M.M. Dastgheib, M.A. Amoozegar, K. Khajeh, A. Ventosa, A halotolerant Alcanivorax sp. Strain with potential application in saline soil remediation, Appl. Microbiol. Biotechnol. 90 (2011) 305–312.
[27] M. Wu, M. Nie, X. Wang, J. Su, W. Cao, Analysis of phenanthrene biodegradation by using FTIR, UV and GC–MS, Spectrochim. Acta A. Mol. Biomol. Spectrosc. 75 (2010) 1047–1050.
[28] P. Arulazhagan, N. Vasudevan, I.T. Yeom, Biodegradation of polycyclic aromatic hydrocarbon by a halotolerant bacterial consortium isolated from marine environment, Int. J. Environ. Sci. Technol. 7 (2010) 639–652.
[29] E.J. Joner, A. Johansen, A.P. Loibner, M.A. Dela Cruz, O.H. Szolar, J.-M. Portal, C. Leyval, Rhizosphere effects on microbial community structure and dissipation and toxicity of polycyclic aromatic hydrocarbons (PAHs) in spiked soil, Environ. Sci. Technol. 35 (2001) 2773–2777.
[30] Y.F. Song, P. Gong, Q.X. Zhou, T.H. Sun, Phytotoxicity assessment of phenanthrene, pyrene and their mixtures by a soil-based seedling emergence test, J. Environ. Sci. China (China) 17 (2005) 580–583.
[31] H. Liu, D. Weisman, Y.-B. Ye, B. Cui, Y.-H. Huang, A. Colón-Carmona, Z.-H. Wang, An oxidative stress response to polycyclic aromatic hydrocarbon exposure is rapid and complex in Arabidopsis thaliana, Plant Sci. 176 (2009) 375–382.