Optimization of Phenanthrene-Degradation by *Dietzia cinnamea* AP for Bioremediation Applications

Zahra Fathi 1, Golamhossein Ebrahimipour 1, Amirarsalan Kavyanifard 2*, Ziba Najmi 1

1 Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran.
2 Department of Science, Payam Noor University (PNU), P.O. Box, 19395-3697, Tehran, Iran.

Received: 22 Apr 2016
Revised : 18 May 2016
Accepted: 2 Jun 2016
Corresponding Author:
Amirarsalan Kavyanifard
Department of Science, Payam Noor University (PNU), Tehran, Iran.
Phone: +98 212 243 1664
E-mail: kavyanifard@gmail.com

Abstract

**Background:** This study focused on isolation and identification of a Phenanthrene (Phe) degrader bacterium and optimization of environmental conditions for Phe degradation.

**Materials and Methods:** Enrichment technique was used for isolation and the most effective isolate; named AP was selected based on its Phe biodegradation abilities. The isolate was identified using morphological and biochemical tests as well as 16S rDNA sequencing. The effects of various factors such as temperature, pH and C/N on bacterial growth and Phe degradation were investigated using protein assay (Bradford) and Gas Chromatography (GC), respectively.

**Results:** The selected isolate was identified as *Dietzia cinnamea* AP. It was able to degrade Phe at pH 6-10 (optimum at 8), temperatures of 25-45 °C (optimum at 35 °C) and NH4Cl concentrations of 0.5-2.5 gL-1 (optimum at 2 g L-1). By optimization of environmental parameters, within 10 days of fermentation, Phe degradation rate increased by more than 1.2 fold (from 60% to 73%).

**Conclusion:** *D. cinnamea* AP was found to be an appropriate candidate for bioremediation applications. To the best of our knowledge, this is the first report of *D. cinnamea* species that can degrade Phe.

**Keywords:** Biodegradation, Phenanthrene, *Dietzia cinnamea* AP, Gas Chromatography

---

**Please cite this article as:** Fathi Z, Ebrahimipour G, Kavyanifard A, Najmi Z. Optimization of Phenanthrene-Degradation by *Dietzia cinnamea* AP for Bioremediation Applications. Res Mol Med. 2016; 4 (3): 22-28

---

**Introduction**

Polycyclic Aromatic Hydrocarbons (PAHs) are organic compounds containing two or more benzene rings. They are hazardous environmental contaminants that have noticeable biological effects, mutagenicity, carcinogenicity and toxicity (1). Phenanthrene (Phe) is a tri-cyclic aromatic hydrocarbon that is found in high concentrations in PAH contaminated surface soils, sediments and waste sites. Due to its potential severe hazards, Phe is of significant environmental concerns (2). Although Phe may undergo volatilization, photolysis, adsorption and chemical degradation but microorganisms play an effective role in its degradation in various ecosystems and microbial biodegradation is the main process in natural bioremediation (3). There are many bacterial species capable of degrading Phe and most of them are isolated from contaminated sediment or soils (4-6). It was found that microorganisms isolated from hydrocarbon contaminated soils are as active as or even more active than those isolated from uncontaminated soils (1). Phe biodegradation varies with the type and number of the microorganisms and the rate of biodegradation depends on pH, temperature, accessibility of nutrients and chemical partitioning in growth medium (7, 8).

Natural biodegradation of hydrocarbons in polluted environments is extremely slow, therefore, for more efficient and rapid elimination of hydrocarbon pollutants, bioaugmentation strategy (adding bioscavengers to the polluted sites) can be applied (9). So, isolation of new bioscavengers and optimization of environmental condition have gained interests for
their better performance. Although many Phe degrading bacteria have been isolated (10-13), more efficient Phe-degraders are always favored in bioremediation of Phe-contaminated sediments and soils (14). The main objective of this paper was to isolate bacterial strains which are able to degrade Phe and optimization of environmental parameters for more efficient removal of this contaminant.

**Materials and methods**

**Sampling**

Hydrocarbon contaminated soil samples were taken from oil exploration sites around Ahvaz, south of Iran; and transferred to laboratory in sterile bottles on ice while aerated every 20 min. The sampling site pH and temperature were 7 and 40 °C, respectively.

**Media and chemicals**

1- A stock solution of Phe was achieved by dissolving 2 g of phe in 100 ml acetone (200 mg L-1). It was stored at 4 °C for further uses. Specific amounts of the stock were used, if necessary, for Phe and acetone solvent was first evaporated at any stage.

2- Mineral Salt Medium (MSM) included 36mM NH4Cl, 1.69mM K2PO4, 0.066mM FeSO4, 16mM MgSO4.7H2O, 1.8mM CaCl2, 0.67mM KCl, 513mM NaCl, along with 1ml of trace element solution. Trace element solution composed of 0.147mM ZnCl2, 1.54mM CoCl2, 0.77mM NiCl, 1.38mM Na2SeO4, 0.24mM Na2MoO4, 0.118mM Na2WO4, 0.054mM NaVO3, and 0.007mM HCl. Final pH of MSM was adjusted at 7.0 using 100mM Tris/HCl (15).

3- MSM agar contained 15g agar in 1L MSM. After distribution of the medium in plates and consolidation, whole surface of plates were coated by spreading a layer of Phe (2 ml of Phe stock). All the materials were supplied from Merck (Darmstadt, Germany); but Tris (Tris hydroxymethylamino methane), CaCl2, arabinose, starch, glucose, and Folin Phenol reagent were purchased from Sigma–Aldrich, Germany; Peptone, SIM and Agar from Difco, MRVP and TSI from Oxid; MgSO4.7H2O from Riedel-de Haën and NH4Cl from Chemlab, Belgium.

**Isolation of Phe-degrading bacteria**

In order to isolate Phe-degrading bacteria, 10 g of samples were mixed with 100 ml aseptic distilled water in 250 ml flasks, shacked at 100 rpm for 10 min and then, kept unshaken to precipitate. For enrichment of Phe degrading bacteria, 10 ml of the supernatant was transferred into the conical flask containing 100 ml MSM and supplemented with 200 mg/L Phe as the sole carbon and energy source (pH7) and finally incubated at 30 °C for two weeks (40 rpm). Phe consumption was monitored by increasing in bacterial biomass (absorbance at 600 nm). Then, subcultures (four times) were performed by transferring 1ml of the enriched culture to the same fresh medium.

At the end of enrichment process, a 10-fold serial diluted concentration of inoculated MSM were made, cultured on MSM agar and incubated at 30 °C. Colonies with clearing zone were purified and tested for Phe degradation ability as follow; equal concentration of any of colonies (an absorbance of 0.3 at 600 nm) were inoculated in MSM and incubated at 30 °C for 10 days (140 rpm). MSM with no bacterial inoculum was used as control. The experiments were conducted in triplicate. The amount of produced protein was considered as a criteria for bacterial growth and Phe degradation (8).

**Protein measurement**

Total produced protein was done as follows: at 48h intervals, 1ml of fermented MSM was centrifuged at 8000 g for 10 min. The harvested bacteria were then suspended in 1ml of Ringer’s solution (5.7mM KCl, 111.2mM NaCl, 2.4mM NaHCO3, and 2.25mM CaCl2), vortexc and centrifuged again (8000 g for 10min). In order to cell lysing, the pellet was re-suspended in 0.5ml of 0.3M NaOH and 1ml of distilled water and then vortexed. The suspension was incubated at 90°C for 1h. The achieved cell lysate was used to the total produced protein measuring that was done by dye-binding assay of Bradford. Bradford protein assay protocol was as follows: for Bradford reagent preparation, 100 mg Coomassie Brilliant Blue G-250 was dissolved in 50 ml 95% ethanol, then after 100 ml, 85% (w/v) phosphoric acid was added. The final volume of mixture was taken to 1 liter using dH2O, when the dye had completely dissolved, and filtered through Whatman 540 paper just before use. Finally, 20μl of the cell lysis was added to 1ml of the diluted reagent and vortexed. Absorbance of the formed blue color was measured at 595nm (Shimadzu, UV-120-02; Japan). The results were checked against the standard curve of a serial dilution series (0.1-1.0 mg/ml) of bovine serum albumin (16).

**Characterization of isolated bacterium**

To identify selected isolate, biochemical and morphological characterization were done according to Bergey’s manual of determinative bacteriology (17). Phylogenetic identification was performed using 16S rDNA analysis. Genomic DNA was extracted using phenol/chloroform/isoamyl alcohol referring (18) method. 16S rDNA gene fragment was amplified by PCR using a set of universal primer including Rd1 (5'-AGGAGGTGATCCAGGC-3') and Fd1 (5'-AGAGTTTGATCCTGCT-3'). The PCR results were checked against the standard curve of a serial dilution series (0.1-1.0 mg/ml) of bovine serum albumin (16).
amplification mixture of 16 μl, contained the bacterial DNA (of about 200 ng), 5 μl of Taq buffer (TAPS, pH 8.8, 3mM MgCl2, 50 mM KCl), 1 μl (3 units) of Taq DNA polymerase, 5 μl of 2 mM dNTP mix and 5 μl of each primer (10 pM/μl). The amplification program was set for 30 cycles; all included a denaturation at 96°C (10 s), an annealing at 50 °C (10 s) and an extension step at 60 °C (4 min); with a final extension step at 72 °C for 5 min. Subsequently, PCR products were electrophoresed on 1% agarose gel and visualized using ethidium bromide. DNA fragments were sequenced using a sequencer (SEQUAB, Germany). Finally, all the sequences were compared with reference sequences in NCBI databases (http://WWW.ncbi.nlm.nih.gov/Genbank) using Blast software. Phylogenetic analysis was done by MEGA software version 4 and genetic relationship was found based on the 16S rDNA gene sequence using neighbor-joining tree with bootstrap value of 1000 replication.

Optimization of Phe degradation
Effects of environmental parameters such as temperature, pH and Carbon/Nitrogen (molar) ratio on Phe degradation and growth of the isolate was investigated. To determine the effect of temperature, log-phase cells (in 5ml MSM) were cultured at a range of temperatures (25 °C to 45 °C by 5 °C intervals) and shaken at 140 rpm. After determination of optimal temperature, in order to determine the effect of pH parameter, the pH of the MSM was adjusted from 6 to10, and temperature was adjusted at 35 °C with shaking at 140 rpm. Finally to determine the effect of C/N ratio, various concentrations of NH4Cl of 0.5 to 2.5 g/L (with 0.5 g/L intervals) were used while other condition was 35 °C, pH8 and shaking at 140 rpm. The experiments were performed in triplicate. Sampling for protein assay was done at two-day intervals.

Extraction and analysis of Phe by GC
A 10-day-old fermented flask (containing 100 ml MSM) under optimal conditions and a control flask (containing 100 ml MSM) were extracted separately by ethyl acetate, and Phe amounts were measured by GC. The concentration of Phe was determined according to Wu et al. (19) 50 ml ethyl acetate were added to the flask and was shaken for 15 min. After forming two phases, Phe-containing organic phase was collected and aqueous phase was re-extracted by 50 ml ethyl acetate. Eventually, the organic phase was dried by Na2SO4 (anhydrous) and the volume was adjusted to 100 ml. Extract samples were analyzed using GC-FID equipped with a HP-5MS fused silica capillary column (60m × 0.25 mm ID × 0.25 lm thicknesses, Agilent Technologies, USA) with the detector and injector temperature of 300 °C and 280 °C, respectively. We used nitrogen as carrier gas. Oven temperature program was as following: 80 °C (for 2 min) to 120 °C at a rate of 10 °C/min and 120 °C to 300 °C at a rate of 4 °C/min and held at 300 °C for15 min. The quantification and identification of chemicals were performed by matching their retention times of standards (20).

Statistical analysis
All experiments were done in triplicate and the values were expressed as means. In order to reveal any significant differences between the parameters and variables we used Kruskal–Wallis one-way analysis of variance, Kolmogorov–Smirnov test, U Mann–Whitney Test, or by one-way ANOVA and the Tukey test (SPSS software, version 19). Results were considered significant if p<0.05.

Results
Isolation and selection of the most efficient Phe-degrading bacterium
Three Phe degrading bacteria, named AP, BP and CP were isolated and purified by aforementioned enrichment and screening techniques. Compared with other isolates, the clear zone formed around AP isolate on MSM agar was the biggest, indicating that the isolate was the most efficient in Phe biodegradation. On the other hand, the amount of protein produced by AP strain was more than that of other isolates in MSM medium (Table 1). Thus, AP isolate was selected as the best Phe degrader strain and other next tests and analyses were done using that.

Table1. Results of screening and selection for most efficient Phe degrader bacterium.

| Isolate | AP | BP | CP |
|---------|----|----|----|
| Produced protein (mg/g Phe) | 29 | 16 | 19 |
| Clear zone diameter (mm) | 5 | 2.3 | 3.1 |

*showed significant difference than the other isolates statistically (using U Mann Whitney test).

Characterization of selected bacterium
For phylogenetic characterization of AP isolate, a fragment of 1442 bp nucleotide of 16S rRNA was amplified and sequenced using universal primers and deposited in NCBI database (GenBank accession number: KR232837). As determined by BLAST analysis, a neighbor joining tree of the isolate was constructed (Figure 1). Results of NCBI blasting revealed that AP isolate clustered with Dietzia cinnamiae strain DSM 44904, with 99% nucleotide identity. AP isolate was also found to be closely related to other strains of D. cinnamiae including
D. cinnamea IMM (NR 042390.1) and D. cinnamea BC07 (KF052080.1) with 98% nucleotide identity.

By comparing the phylogenetic analysis and results achieved from morphological and biochemical tests (Table 2), the isolate was named as Dietzia cinnamea AP.

Table 2. Biochemical and morphological characteristics of isolate AP.

| Cell shape and properties | Gram-positive, short rod and cocccoid with size of 1.0–1.2×1.1–2.4 (in single, double and multiple), non-spore or capsule forming, non-motile |
|---------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Colony Morphology         | convex, smooth-edge, circular, glistening and deep orange                                                                                                                             |
| Positive reactions        | oxidase, urease, catalase, aerobic, nitrification, nitrate reduction and methyl-red                                                                                                         |
| Negative reactions        | denitrification, acid-fast, H2S production, Voges-Proskauer test and indole production                                                                                                    |
| Utilized                  | d-glucose, acetate, ethanol, d-fructose, glycerol, n-hexadecane, n-decane, maltose, d-xylene and starch                                                                                     |
| Not utilized              | d-arabinose, citrate, d-galactose, gelatin, lactose, mannose, raffinose and sucrose                                                                                                          |
| GC content                | 71.6%                                                                                                                                  |
| Tolerated                 | pH (5-10), temperature (15-48 °C) and NaCl (0-9%)                                                                                                                                          |

Optimum conditions for Phe degradation by D. cinnamea AP

Environmental parameters including temperature, pH and NH4Cl concentration were optimized to achieve maximal Phe degradation. As shown in Figure 2A, D. cinnamea AP was able to grow and degrade Phe at temperatures from 25 °C to 45 °C, but with low efficiency at 25 °C and 45 °C. Statistical analysis showed no significant differences between the amounts of protein produced at 35 °C and 40 °C. Since more protein was produced at 35 °C, the temperature considered as optimum for Phe degradation. In the case of pH, it was observed that Phe degradation took place at the range of 6 to 9 by D. cinnamea AP (Figure 2B). Based on statistical analysis, D. cinnamea AP at pH8 showed a significant difference for Phe degradation compared with other values of pH and therefore selected as the optimum pH for Phe degradation. Neither of growth or Phe degradation occurred at acidic pH (pH5). Overall, the isolate preferred alkaline values of pH than acidic values. The selected bacterium grew and degraded Phe in concentration ranges of 1 to 2.5g/L NH4Cl. No significant differences were observed between concentrations of 2 and 2.5g/L NH4Cl, statistically, but these concentrations differed from other concentrations. Since minimum amount of NH4Cl is desired in industrial experiences, therefore the amount of 2g/L NH4Cl was considered as optimum concentration for NH4Cl (Figure 2C). At lower concentrations of NH4Cl, the rates of growth and Phe degradation decreased as NH4Cl concentrations decreased. Figure 2D shows that optimization of environmental parameters resulted in increasing of total produced protein more than 1.5 fold (from 78.24 to 125 µg/200 mg Phe). Since increase in protein

Figure 1. Phylogenic trees according to 16S rRNA gene sequences illustrating the relationships between strain AP and other members of Dietzia genus and Bacillus subtilis strain BCR (DQ993674.1).
produced was a criterion for Phe degradation, it can be concluded that the rate of Phe degradation increased by environmental parameters optimization.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Optimization of Phe degradation by *D. cinnamea* AP. A) Effect of different temperatures on bacterial growth in MSM at pH 7 and 1 g/l NH4Cl. B) Effect of different pH on bacterial growth in MSM at 35 °C and 1 g/l NH4Cl. C) Effect of different NH4Cl concentrations on bacterial growth in MSM at 35 °C and pH 8. D) Effect of optimization on amounts of protein produced by *D. cinnamea* AP.

Before optimization of environmental factors, *D. cinnamea* AP was able to degrade only 60% of Phe at 10-day incubation; but after optimization of environmental factors the value of Phe degradation raised to 73%. These were proved by GC analysis of remained Phe in 10-day-old fermented MSM under optimized (Figure 3).

![Figure 3](https://example.com/figure3.png)

**Figure 3.** GC analysis showing Phe consumption by *D. cinnamea* AP. Phe values in control MSM (left) and in optimized MSM after 10-days of incubation (right).
Discussion
High concentrations of Phe in PAHs contaminated sediments and their potential bioaccumulation and severe hazards, calls for developing a safe procedure for their removal from contaminated sites. Therefore, great attention has been paid to develop effective methods to remediate PAHs contaminated environments in the past decades (14). We successfully isolated three Phe degrader bacterial strains, amongst which one (named AP) was the most efficient and selected for Phe degradation. Based on biochemical morphological and tests as well as 16S rDNA sequencing, our isolate was characterized as Dietzia cinnamonea AP. Some members of Dietzia species have been revealed to degrade aromatic compounds (21) and Phe degrader members in Dietz genus have been reported (22-24). For example, two D. cinnamonea strains were found to have tendency for n-C11 to n-C36 (25, 26) and n-C10 to n-C26 (17) alkanes hydrocarbon biodegradation. Despite the ability of these strains to degrade alkanes hydrocarbon, they were unable to degrade Phe as the sole carbon and energy source; while D. cinnamonea AP could use Phe as the sole carbon and energy source. Based on our current knowledge we can claim that present study is the first report of Phe degradation by D. cinnamonea species.

Since environmental parameters affect various activities of organisms (27, 28) including their ability to degrade and utilize various compounds, additional studies were made to determine optimal conditions for maximum Phe degradation by the bacterium. Total protein produced was considered as a criterion for growth and therefore Phe degradation. Our results showed that D. cinnamonea AP degraded Phe at a wide range of pH (6 to 10), temperature (25-45 °C) and NH4Cl concentration (0.5-2.5 g L-1). Optimization of environmental parameters led to increase in performance of Phe degradation by our isolate. This increase was proven by total protein produced and GC analysis. Precise measurements achieved from GC analysis showed that Phe degradation rate increased more than 1.2 fold (from 60% to 73%) by optimization of environmental parameters. Acceptable Phe degradation rates were achieved by the bacteria under wide ranges of environmental parameters, therefore, it could be suggested as a versatile candidate for bioremediation applications in various media including acidic or alkaline environments at variable temperatures.

To the best of our knowledge this is the first report of Phe degradation by D. cinnamonea species as sole carbon and energy source. According to the results, D. cinnamonea AP could be valuable in bioremediation of Phe-contaminated environments.

Acknowledgments
This work was funded by Shahid Beheshti University of Tehran, Microbiology Laboratory of Industrial Biotechnology.

Authors' contributions
All the authors performed the experiments. EG supervised the study. NZ advised the study KA wrote the manuscript. All authors discussed the data and commented on the manuscript.

Conflict of interest
The authors declare that they have no conflict of interest in this work.

References
1. Al-Awdah H, Sulaiman RH, Mahmoud HM, Radwan S. Alkaliphilic and halophilic hydrocarbon-utilizing bacteria from Kuwaiti coasts of the Arabian Gulf. Appl microb Biootechnol. 2007; 77(1): 183-6. PMID: 17710391
2. Kavvanifard A, Ebrahimipour GH, Ghasempour A. Individually and Synergistic Degradation of Hydrocarbons by Biosurfactant Producing Bacteria. Res Mol Med. 2016; 4 (1): 36-44. doi 10.7508/rmm.2016.01.006
3. Andreoni V, Gianfreda L. Bioremediation and monitoring of aromatic-polluted habitats. Appl Microbiol Biotechnol. 2007; 76(2): 287-308. PMID: 17541581
4. Bihari Z, Szentek A, Szabó Z, Blastyák A, Zombori Z, Balázs M, et al. Functional analysis of long-chain n-alkane degradation by Dietzia spp. FEMS Microbiol. Letters. 2011; 316(2): 100-107. PMID: 21204932
5. Bodtker G, Hvidsten IV, Barth T, Torsvik T. Hydrocarbon degradation by Dietzia sp. A14101 isolated from an oil reservoir model column. Antonie Van Leeuwenhoek 2009; 96(4): 459-469. PMID: 19565350
6. Chang CH, Lee J, Ko BG, Kim SK, Chang JS. Staphylococcus sp. KW-07 contains nahH gene encoding catechol 2,3-dioxygenase for phenanthrene degradation and a test in soil microcosm. Int Biodeterior Biodegrad. 2011; 65(1): 198-203.
7. Zhu X, Ni X, Waigi MG, Liu J, Sun K, Gao Y. Biodegradation of Mixed PAHs by PAH-Degrading Endophytic Bacteria. Int J Environ Res Public Health. 2016; 13: 805. PMID: 27517944
8. Gu H, Lou J, Wang H, Yang Y, Wu L, Wu J, Xu J. Biodegradation, Biosorption of Phenanthrene and Its Trans-Membrane Transport by Massilia sp. WF1 and Phanerochaete chrysosporium. Front Microbiol. 2016; 7: 38. PMID: 26858710
9. Chen J, Wong M, Wong YS, Tam NF. Multi-factors on biodegradation kinetics of polycyclic aromatic hydrocarbons (PAHs) by Sphingomonas sp. a bacterial strain isolated from mangrove sediment. Mar Pollut Bull. 2008; 57(6): 695-702. PMID: 18453800
10. Coulon F, McKew BA, Osborn AM, McGinty TJ, Timmis KN. Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters. Environ Microbiol. 2007; 9(1): 177-186. PMID: 17227422
11. Díaz MP, Boyd KG, Grigson SJ, Burgess JG. Biodegradation
of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M immobilized onto polypropylene fibers. Biotechnol Bioeng. 2002; 79(2):145-153. PMID: 12115430

12. Ebrahimipour GH, Gilavand F, Karkhane M, Kavynifard AA, Teymouri M, Marzban A. Bioemulsification activity assessment of an indigenous strain of halotolerant Planococcus and partial characterization of produced biosurfactants. Int J Environ Sci Technol. 2014. doi 10.1007/s13762-014-0548-5

13. Gharibzahedi SMT, Razavi SH, Mousavi M. Potential applications and emerging trends of species of the genus Dietzia: a review. Ann of Microbiol. 2014; 64(2):421-429.

14. Wang H, Lou J, Gu H, Luo X, Yang L, Wu L, Liu Y, Wu J, Xu J. Efficient biodegradation of phenanthrene by a novel strain Massilia sp. WF1 isolated from a PAH-contaminated soil. Environ Sci Pollut Res Int. 2016; 23(13):13378-88. PMID: 27026540

15. Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST. Bergey's Manual of determinate bacteriology. 1994.

16. Noble JE, Bailey MJA. Quantitation of Protein. Methods Enzymol. 2009; 463:73-95. PMID: 19892168

17. Jiang L, Song M, Luo C, Zhang D, Zhang G. Novel phenanthrene-degrading bacteria identified by DNA-stable isotope probing. PloS One. 2015; 10(6):e0130846. PMID: 26098417

18. Kavynifard A, Ebrahimipour G, Ghasempour A. Optimization of crude oil degradation by Dietzia cinnamomea KAI1 capable of biosurfactant production. J Basic Microbiol. 2015; 55. PMID: 26615815

19. Kucerova R, Fecko P. Biodegradation of PAU PCB and NEL soil samples from the hazardous waste dump in Pozd'átky (Czech Republic) Physicochem. Prob Miner Process. 2006; 40:203-210.

20. Moscoso F, Teijiz I, Sanroman M, Deive F. On the suitability of a bacterial consortium to implement a continuous PAHs biodegradation process in a stirred tank bioreactor. Indust Engin Chem Res. 2012; 51(49):15895-900.

21. Reda A.B. Bacterial bioremediation of polycyclic aromatic hydrocarbons in heavy oil contaminated soil. J Appl Sci Res. 2009 pp. 197-201.

22. Sambrook J, Fritsch E, Maniatis T. Molecular Cloning: A Laboratory Manual 1989. Cold Spring Harbor Laboratory New York. 2001

23. Vinas M, Sabaté J, Espuny MJ, Solanas AM. Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediation of heavily creosote-contaminated soil. Appl Environ Microbiol. 2005; 71(11):7008-7018. PMID: 16269736

24. Wang XB, Chi CQ, Nie Y, Tang YQ, Tan Y, Wu G, Wu XL. Degradation of petroleum hydrocarbons (C6–C40) and crude oil by a novel Dietzia strain. Bioreourc Technol. 2011; 102(17):7755-7761. PMID: 21715162

25. Wu YR, Luo ZH, Vrijmoed L. Biodegradation of anthracene and benz [a] anthracene by two Fusarium solani strains isolated from mangrove sediments. Bioreourc Technol. 2010; 101(24):9666-9672. PMID: 20691587

26. Kavynifard A, Ebrahimipour G, Ghasempour A. Structure Characterization of a Methylated Ester Biosurfactant Produced by a Newly Isolated Dietzia cinnamomea KAI1. Microbiol. 2016; 85(4):430-435. doi 10.1134/S0026261716040111

27. Hassan I, Mohamedelhassan E, Yanful FK, Yuan ZC. A Review Article: Electrokinetic Bioremediation Current Knowledge and New Prospects. Adv Microbiol. 2016; 6:57-72.

28. de la Cueva SC, Rodríguez CH, Cruz NOS, Contreras JAR, Miranda JL. Changes in Bacterial Populations During Bioremediation of Soil Contaminated with Petroleum Hydrocarbons. Water Air Soil Pollut. 2016; 227: 91. doi:10.1007/s11270-016-2789-z