

Degradation of Anthracene by Alkaliphilic Bacteria *Bacillus badius*

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Abstract

Alkaliphilic bacterial strain *Bacillus badius* D1 was isolated from Pristine Crater Lake of Lonar, Buldhana, MS, India. It was found that the bacterial strain had a promising potential to degrade aromatic hydrocarbons. The growth conditions of the strain were optimized. The bacterial strain *Bacillus badius* D1 could degrade anthracene at a concentration of 50 mg/100 ml at pH 9.0 with in 60 hrs. Similarly the effect of experimental parameters like pH, temperature, and salinity was also studied on anthracene degradation. The structural determination of the intermediates of anthracene degradation was carried out by spectroscopic analysis like FTIR, GC-MS and ¹HNMR. A tentative pathway of anthracene degradation by *bacillus badius* D1 is also reported.

Keywords: *Bacillus Badius*, anthracene, degradation, Alkaliphilic

1. Introduction

Hydrocarbon contaminations have been the subject of continuous environment and human health concern (Rockne et al., 2000). They occur naturally in coal and crude oil. These compounds have prime importance as energy source and in production of synthetic organic chemicals. Among the hydrocarbons, the monocyclic aromatic compounds benzene, toluene, ethyl benzene and xylene a group called BTEX and the polycyclic aromatic hydrocarbons (PAHs) belongs to the most encountered subsurface contaminant (Holliger et al., 1997).

Polycyclic aromatic hydrocarbons are ubiquitously found in the environment. They are the major cause of concern as anthropogenic pollutants in the environment. These hydrocarbons are fused-ring compounds, which enter in the soil systems and natural water via wastewater effluents from coal and petroleum refineries, accidental spills, leakage of storage tanks or pipe lines. Their persistence in the environment is due to their low water solubility. (Vila et al., 2001; Li et al., 2001) Concern arises because of their recalcitrance and health hazards associated with these compounds. (Vila et al., 2001) Various carcinogenic, mutagenic and genotoxic activities are associated with polycyclic aromatic hydrocarbons (Allen et al., 1999). The carcinogenic effects of these compounds on mammalian cells are the consequences of their metabolic activation to arene oxides which are highly reactive metabolites that covalently binds with DNA. Bacterial and fungal species have the potential to degrade organic pollutants and helps in the mineralization of these compounds. Bacteria have the unique feature in rapidly adapting to toxic nutrient supplies and engaging antagonistic environments. The metabolic diversity and plasticity of bacteria in the face of environmental disaster and limitations provide an immense reservoir of exploitable regularity devices and biochemical activities (Shingler, 2003; Ellis, 2000). Using these abilities, range of naturally occurring as well man made aromatic compounds are discharged through geochemical cycles, urban and industrial discharge are helping the bacteria to develop the potential to degrade them (Timmis, 1999). They normally exhibit specific traits that promote bioavailability, such as adhesion to the pollutants surface (Ortega & Alexander., 1994) production of biosurfactants. (Garcia-Junco et al., 2001) or increased rate of transport by water flow (Lahlou et al., 2000) or by chemotaxis (Ortega et al., 2002). Various strategies are applied by bacteria to degrade aromatic compounds, to overcome the resonance stabilization energy of aromatic ring, which brought about by biotransforming enzymes. Bacteria initiate the attack on these ring compounds by cytochrome P-450 (Cerniglia, 1984; Field et al., 1995). There are many bacterial species known to be able to degrade anthracene under neutral conditions. These species are from the genera

Pseudomonas, *Sphingobium*, *Nocardia*, *Rhodococcus*, and *Mycobacterium* (Takeuchi, 2001; Tongpim and Pickard, 1999). The term "Alkaliphilic" is used for microorganisms that grow optimally or very well at pH values above 9, often between 10 and 12, but cannot grow or grow only slowly at the near-neutral pH value. In our present studies the bacteria strain *Bacillus badius* D1 is isolated from alkaline Crater Lake. Much is known about the degradation of polycyclic aromatic compounds by neutrophiles. However, no data is available about the involvement of alkaliphilic bacterial strain *Bacillus badius* D1 in the biotransformation and degradation of these compounds. Therefore, the attempts have been made to examine the degradation potential of this strain against anthracene.

2. Materials and Methods

2.1 Chemicals

Anthracene (98% Purity) was purchased from sisco research laboratory (SRL). Bacteriological media and solvents were purchased from HI-MEDIA and SRL, Mumbai, India.

2.2 Microorganism and Cultivation

The alkaliphiles bacterial strain *Bacillus badius* D1 was isolated from Pristine Crater Lake of Lonar, Buldhana, M.S, India. This bacterial strain was grown at 37°C in nutrient broth (NB) medium in 500 ml conical flasks. The cultivation media contained the following (in g/100 ml) of the following medium 0.5% Yeast, 0.5% Peptone, 0.5% NaCl, KH₂PO₄ 170 mg, Na₂HPO₄ 980 mg, (NH₄)₂SO₄ 100 mg, MgSO₄ 4.87 mg, MgO 0.1mg, FeSO₄ 0.05 mg, CaCO₃ 0.20 mg, ZnSO₄ 0.08 mg, CuSO₄.5H₂O 0.016 mg, CoSO₄ 0.015 mg, H₃BO₃ 0.006 mg, distilled water 100 ml, drops of 0.1N NaOH to adjusted media to pH-9.0.

2.3 Biodegradation Experiments

Biodegradation was performed by adding 50 mg of anthracene to 100 ml of the 24 hrs grown culture (during the log phase). The conical flasks were placed on a rotary platform incubator shaker at 100 rpm at 37°C for 12, 24, 36, 48, and 60 hours. The culture media of each flask was then centrifuged at 10000 rpm for 15 minutes in cold centrifuge Du Pont Instruments SORVALL RC-5B to separate the bacterial cell mass, and the resulting supernatant was preserved for the extraction of biodegradation products. The supernatant was extracted by dichloromethane (DCM) and then dried over sodium sulphate anhydrous. The solvent was evaporated at 40°C to obtain the residue. The purified residue was subjected to spectroscopic analysis.

2.4 Spectroscopy Analysis

The extracted metabolites were subjected to TLC using hexane and ethylacetate (9:1) as running solvent, and the separated spots were observed under long-UV 365 nm and short-UV 254 nm light. The metabolites were recovered by using silica gel column chromatography and concentrated by rotaevaporation at 40°C. The recovered compounds were subjected to further analysis like Fourier transform infrared spectroscopy (FTIR) analysis by using the KBr pellet technique in the wavelength range of 400-4000cm⁻¹ on the shimadzu-8400 FTIR spectrophotometer. Gas Chromatography Mass (GC-MS) analysis was performed using a GB5 column with 15-20 minutes runtime using helium as carrier gas. The flow rate was 35.7ml/min and temperature between 62-290 °C on a Shimadzu-GC-MS- QP5050. Proton Nuclear Magnetic Resonance (¹HNMR) studies for parent anthracene molecule and for the degraded metabolites were performed in 300 MHz magnetic field using CDCl₃ as a solvent on a Varian Mercury Spectrometer YH 300.

3. Results

3.1 Evaluation of Optimum Condition for Anthracene Degradation

After Sixty hrs of incubation at 37°C and pH 9.0, it was found that complete degradation of anthracene was observed under shaking condition. The degradation pattern was checked after every 12 hr of incubation. Growth and utilization of anthracene are shown in Figure 1. The growth of bacteria was measured at 600_{nm}.

3.2 Identification of Anthracene Degradation Products

GC-MS analysis was carried out to investigate the metabolites formed during the biodegradation of anthracene, eight metabolites were detected in the anthracene degradation pathway as shown in Figure 4. The GC-MS spectra for metabolite I with retention time (Rt) at: 10.992 minutes having a molecular ion at an m/z 210 and characteristic fragment ions at m/z values of 180,152,126 resulting from losses of COH and then either CO-HCO. This metabolite was identified as 1, 2-dihydroxyanthracene. The ¹HNMR analyses are as follows. ¹HNMR { δ 5.911(1H, S, OH D₂O Exchangeable was done), 6.053(1H, S, OH D₂O Exchangeable was done), 7.202-8.319(8H, M Ar-H) FTIR spectra had shown the functional group OH at 3448.84.

Metabolite II showed at Rt 7.442 minutes, with molecular ion at m/z of 241($M^+ - H$) and fragment ions at m/z values of 197, 154 losses of O and $2CO_2$. Metabolite II was identified as (3Z)-4-[3-hydroxy(2-naphthyl)-2-oxobut-3-enoic acid. 1H NMR analysis of the metabolite as $\{\delta 4.984$ (SH, Ar-OH, D₂O Exchangeable was done) 6.990-7.676 (7H, M, Ar H), 10.0 (SH, Ar-COOH), FTIR spectra showed 3419.90 for OH group and 1730.16 for carbonyl group.

Metabolite III was observed at Rt.9.083 minutes and had a molecular ion at m/z of 196 and fragment ions at m/z values of 168, 125 resulting from consecutive losses of CO and this compound was detected as 6,7 benzocoumarin. 1H NMR $\{\delta 6.16$ (1H, S), 6.98-7.42 (7H, M, Ar-H) FTIR spectra have shown the carbonyl group at 1666.55 and C-O group at 1263.42.

Metabolite IV was observed at Rt.10.067 min. The mass spectrum of metabolite IV had a molecular ion at m/z of 224 and fragment ions at m/z values of 209, 181, and 159 that can be attributed to sequential losses of CH_3 and CO. FTIR has shown OH group at 3238.59 and carbonyl group at 1674.27. Metabolite IV was identified as 1-methoxy-2-hydroxyanthracene.

Metabolite V was observed at Rt at 8.725, had a molecular ion at m/z 214 and fragmentation ions at m/z values of 178, 154 representing successive losses of $2H_2O$, $C_2H_4O_2$. This compound was identified as (E)-3-(2-hydroxynaphthalen-3-yl)acrylic acid, the 1H NMR spectra showed the resonances at $\{\delta 5.693$ (1H, S, OH, D₂O Exchangeable was done), 9.68 (1H, S, COOH), 7.215 (1H, D, J=5.8 Hz CH=CH), 7.2388.672 (8H, M, Ar-H).

Metabolite VI had shown Rt at 8.192 minutes and molecular ion at m/z 168 ($M^+ - 2H$). The fragment ions at m/z values of 140, 123, suggesting losses of CO, COOH. This compound was detected as phthalic acid the 1H NMR spectra was shown $\{\delta 10.981$ (2H, d, COOH D₂O Exchangeable is done), 7.961-8.830 (4 H, M, Ar-H)}. FTIR showed the 1660.55 for carbonyl group, Figure 2.

Metabolite VII with Rt at 14.408 minutes and had a molecular ion at 210 and fragment ions at m/z (190, 178, 165) losses H_2O , O_2 and COOH. This metabolite was detected as 9, 10-dihydroxyanthracene. Metabolite VIII (Rt 9.067 minutes) had molecular ion at m/z 208 and fragment ions at m/z value (188, 165) suggesting losses of CO and CO_2 , this compound was identified as 9, 10-anthraquinone.

3.3 Influence of Environmental Parameters on the Degradation of Anthracene

3.3.1 Effect of pH

The effects of pH on the growth of alkaliphilic bacteria as well as biodegradation rate of anthracene is shown in Figure 3A. The 100% degradation was found at pH 9.0 and 95% at pH 10.

3.3.2 Effect of Temperature

Figure 3B shows the effect of temperature on the degradation of anthracene. It was observed that 100% degradation at temperature 35 °C and 95% at 30°C.

3.3.3 Effect of Salinity

The effect of salinity on anthracene degradation has not been widely examined. In this study we observed that the maximum anthracene degradation rate was in 1% of NaCl was 99% by alkaliphilic bacteria *Bacillus badius* D1 strain, Figure 3C.

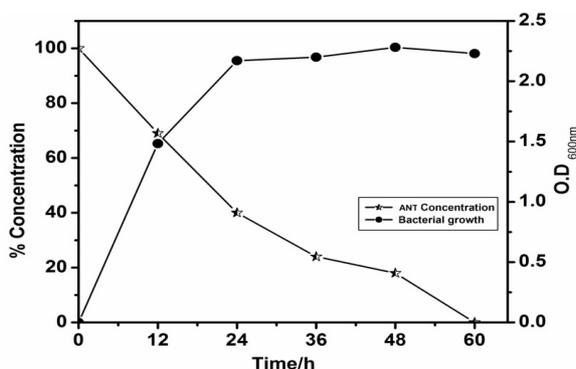


Figure 1. Growth of alkaliphilic bacteria *Bacillus badius* D1 and degradation of anthracene

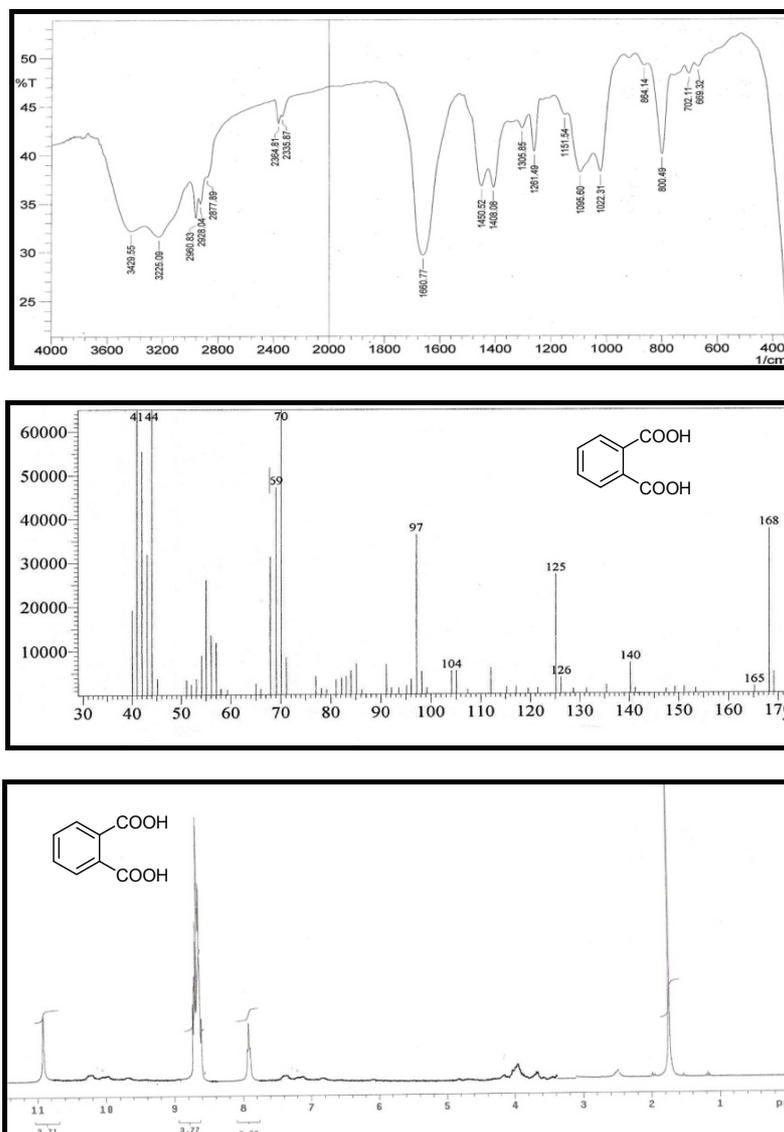
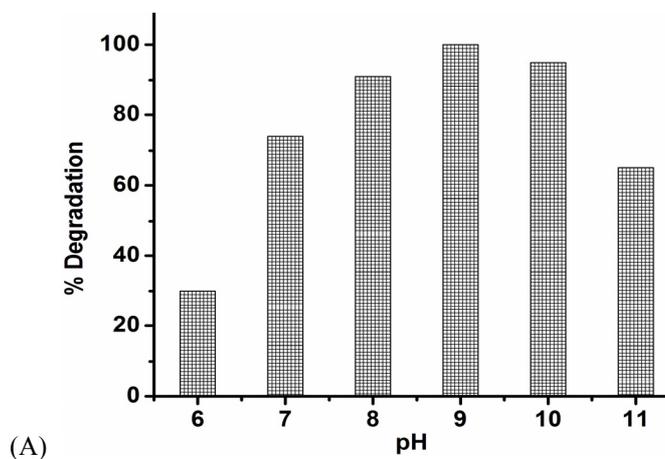


Figure 2. FTIR, GC-MS and ¹H NMR of phthalic acid produced from anthracene degradation by alkaliphilic bacteria *Bacillus badius* after 48 hr of incubation



(A)

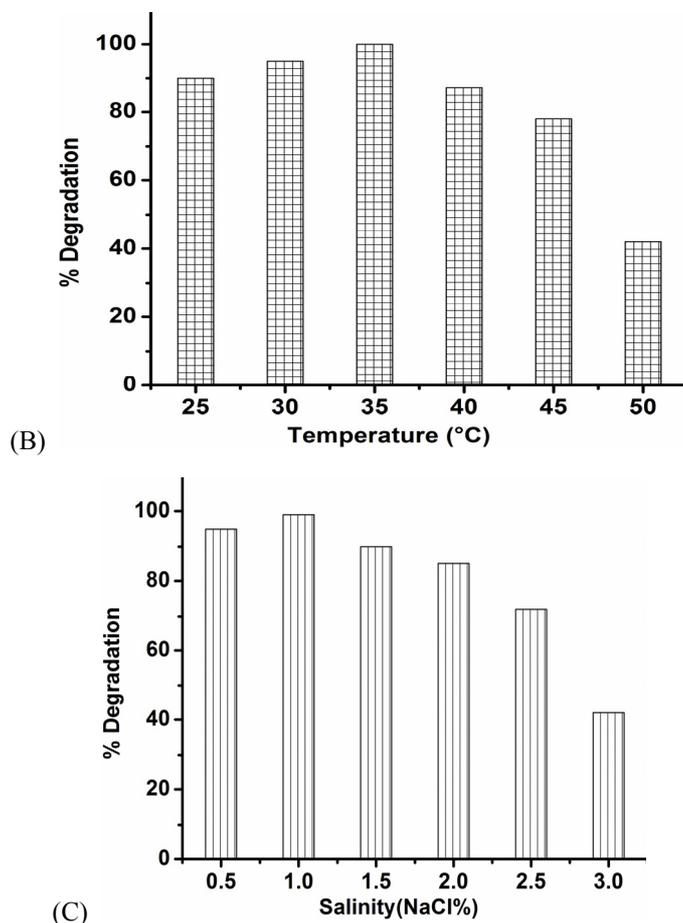


Figure 3. Influence of experimental parameters on anthracene degradation, pH (A), Temperature (B), Salinity (C)

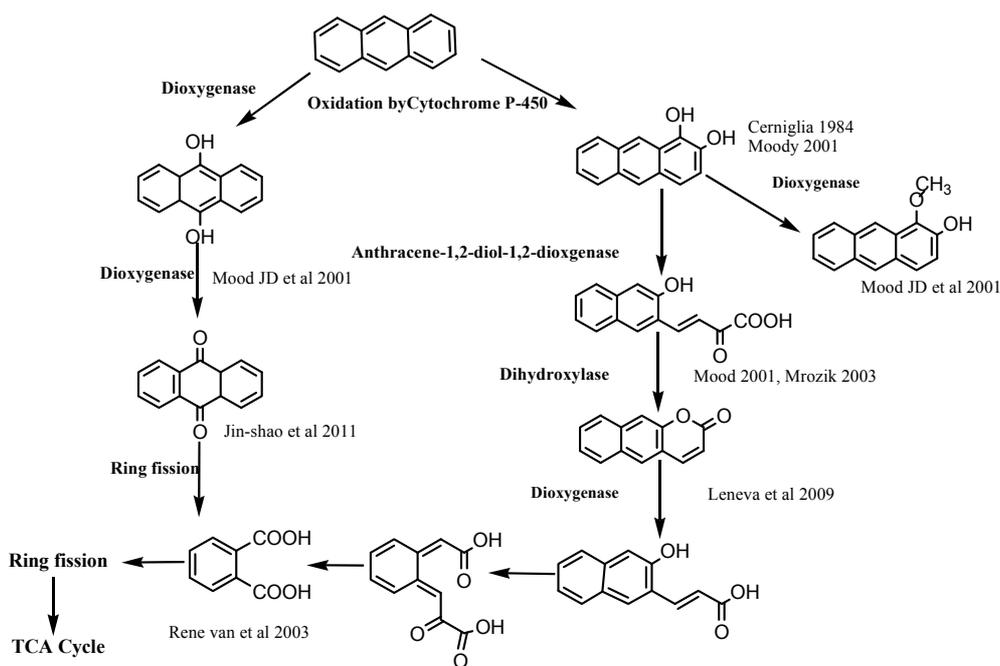


Figure 4. Proposed pathway of anthracene degradation by alkaliphilic bacteria *Bacillus badius* D1

4. Discussion

It is observed that the degradation of anthracene reaches maximal at stationary phase of bacterial growth. In the optimal conditions the observed highest degradation rate could be related with the increasing in cell population under aerobic condition. Information from this and other studies of the degradation of PAHs by microorganisms suggest that both monooxygenase and dioxygenases catalyzes the initial attack on the aromatic ring, it is observed that several dioxygenases are present in alkaliphilic bacteria *Bacillus badius* D1. The identification of *ortho*-ring cleavage intermediates from the degradation of dihydroxylated metabolites of anthracene, phenanthrene, and pyrene involvement of these enzymes (Heitkamp et al., 1988).

The results show that alkaliphilic *Bacillus badius* D1 utilized anthracene as sole carbon and energy source. The isolation and characterization of the major initial oxidation and ring fission products have signified multiple routes of enzymatic attack. The alkaliphilic *Bacillus badius* D1, oxidized anthracene to 1, 2-dihydroxyanthracene via dioxygenase. This data was compared with data published with (Cerniglia, 1984; Moody, 2001). The enzymatic attack in the C-1 and C-2 positions of the anthracene was similar to the naphthalene dioxygenase pathways in *Mycobacterium* sp. strain PYR-1 reported by (Kelley et al., 1990). In the next step, alkaliphilic bacteria oxidize 1, 2-dihydroxyanthracene to the ring fission product (3Z)-4-[3-hydroxy (2-naphthyl)-2-oxobut-3-enoic acid with subsequent conversion to 2-hydroxynaphthoic acid, this data is in agreement with data published by (Moody, 2001; Mrozik, 2003). Further oxidation resulted in the formation of phthalic acid due to the ring fission of (E)-3-(2-hydroxynaphthalen-3-yl) acrylic acid. Phthalic acid is degraded to simple aliphatic compounds via phthalic acid pathway to TCA cycle intermediates. These observations are similar to the result of (Prabhu & Phale, 2003; Rene van et al., 2003).

The accumulation of 1-methoxy -2-hydroxyanthracene provides further evidence for the dioxygenation of anthracene; this has also been shown by (Moody et al., 2001). Methylation of a dihydroxylated PAH intermediate was found earlier in *Mycobacterium* sp. strain PYR-1 with the formation of 8-hydroxy-7-methoxyfluoranthene during the metabolism of fluoranthene (Kelley et al., 1993). The detected of 6, 7-benzocoumarin in culture was formed as *ortho* cleavage of 1, 2-dihydroxyanthracene. 6, 7-benzocoumarin was firstly well-known in the degradation of anthracene by *S.yanoikuyae* BI published by (Kim et al., 1997; Leneva et al., 2009) The detection of these *ortho*-cleavage ring fission products is analogous to evidence in (Annweiler et al., 2000) reports on naphthalene degradation in *Bacillus thermoleovorans*.

During the degradation of anthracene by alkaliphilic bacteria is in the C-9 and C-10 position. The formation of anthrone was detected, which was an expected intermediate and it was followed by the appearance of 9, 10-anthraquinone. (Ceringlia, 1992) has been earlier described anthraquinone as the common oxidation products, this data was in agreement with data published by (Hammel, 1995; Jin-Shao Ye., 2011) who has shown that anthracene inoculated with *Aspergillus fumigates*.

Because, this bacterial strain was isolated from the alkaline environmental, the maximum degradation was observed at alkaline pH 9.00 with optimal temperature at 35°C and NaCl concentration 1%.

(Kiran et al., 2009) has reported that the highest percent of degradation of PAHs was achieved at temperature 30°C with *P.paucimoblis* and *P.putida*. (Rodrigo et al., 2005) has shown the anthracene degradation by *Ps. aeruginosa* isolate 312A had the highest rate of degradation 71% after 48 days at pH 7.0 at 30°C. (Dariush et al., 2009) reported that total PAHs (phenanthrene, anthracene and pyrene) degradation was observed in 1% NaCl (35%).

5. Conclusions

The persistence and toxicity problems associated with PAH compounds in the environment have resulted in too much work and massive efforts, which have been made by researchers in many laboratories. It has concentrated on the ability of a variety of microorganisms to degrade PAHs like anthracene. The pathway of aerobic degradation of PAHs has been established and it is known that the environment contains microbes, which are capable of reducing PAHs concentrations. In this study, it has concluded that the alkaliphilic bacterial strain *Bacillus badius* D1 has a promising potential to degrade anthracene by different ways, either by reduction, oxidation or by induction of some enzymes that degrade these toxic compounds. Also it is concluded that the degradation of anthracene as a model of PAHs degradation under alkaline condition.

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References

- Allen, C., Boyd, D., Hemenstall, F., Larkin, M., & Sharma, N. (1999). Contrasting effects of a non-ionic surfactant on the biotransformation of Polycyclic Aromatic Hydrocarbons to cis-Dihydrodiols by Soil Bacteria. *Appl Environ Microbiol*, 65, 1335-1339.
- Annweiler, E. et al. (2000). Naphthalene degradation and incorporation of naphthalene-derived carbon into biomass of the thermophile *Bacillus thermoleovorans*. *Appl Environ Microbiol*, 66, 518-523. <http://dx.doi.org/10.1128/aem.66.2.518-523.2000>
- Cerniglia, C. E. (1984). Microbial metabolism of polycyclic aromatic hydrocarbons. *Adv Appl Microbiol*, 30, 31. [http://dx.doi.org/10.1016/S0065-2164\(08\)70052-2](http://dx.doi.org/10.1016/S0065-2164(08)70052-2)
- Dariush, M. T., Minoui, S., & Herfatmanesh, A. (2009). Effect of Salinity on Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) of Heavy Crude Oil in Soil. *Bull Environ Contam Toxicol*, 82, 179-184. <http://dx.doi.org/10.1007/s00128-008-9548-9>
- Ellis, L. B. (2000). Environmental biotechnology informatics. *Curr Opin Biotechnol*, 11, 232. [http://dx.doi.org/10.1016/S0958-1669\(00\)00089-6](http://dx.doi.org/10.1016/S0958-1669(00)00089-6)
- Field, J. A., Boelsma, F., Baten, H., & Rulkens, W. H. (1995). Oxidation of anthracene in water/solvent mixtures by the white-rot fungus *Bjerkandera* sp strain BOS55. *Appl Microbiol Biotechnol*, 44, 234. <http://dx.doi.org/10.1007/BF00164508>
- Garcia-Junco, M., De Olmedo, E., & Ortega- Calvo, J. J. (2001). Bioavailability of solid and non-aqueous phase liquid (NAPL)-dissolved phenanthrene to the biosurfactant producing bacterium *Pseudomonas aeruginosa* 19SJ. *Environ. Microbiol*, 3, 561-569. <http://dx.doi.org/10.1046/j.1462-2920.2001.00223>
- Hammel, K. E. (1995). Mechanisms for polycyclic aromatic hydrocarbon by ligninolytic fungi. *Environ Health Perspect*, 103, 41-43.
- Heitkamp, M., Franklin, F., & Cerniglia, C. E. (1988). Microbial metabolism of polycyclic aromatic hydrocarbons: isolation and characterization of a pyrene-degrading bacterium. *Appl Environ Microb.*, 54, 2549-2555.
- Holliger, C. et al. (1997). Contaminated environmental in the subsurface and bioremediation: organic compounds. *FEMS Microbiology Reviews*, 20, 517-523. <http://dx.doi.org/10.1111/j.1574-6976.1997.tb00334x>
- Jin-Shao Ye et al. (2011). Biodegradation of anthracene by *Aspergillus fumigates*. *Journal of Hazardous Materials*, 185, 174-181. <http://dx.doi.org/10.1016/j.jhazmat.2010.09.015>
- Kelley, I. et al. (1990). Identification of metabolites from degradation of naphthalene by a *Mycobacterium* sp. *Biodegradation*, 1, 283-290. <http://dx.doi.org/10.1007/BF00119765>
- Kelley, I., Freeman, J., Evans, F., & Cerniglia, C.E. (1993). Identification of metabolites from the degradation of fluoranthene by *Mycobacterium* sp. strain PYR-1. *Appl Environ Microbiol*, 59, 800-806.
- Kim, E., Zylstra, G. J., Freeman, J. P., Heinze, T. M., Deck, J., & Cerniglia, C. E. (1997). Evidence for the role of 2-hydroxychromene-2-carboxylate isomerase in the degradation of anthracene by *Sphingomonas yanoikuyae* B1. *FEMS Microbiol Lett.*, 153, 479-484. <http://dx.doi.org/10.1111/j.1574-6968.1997.tb12613.x>
- Kiran, BS, et al. (2009). Distribution and biodegradation of polycyclic aromatic hydrocarbons in contaminated sites of hisar (India). *Indian Journal of Experiment Biology*, 47, 210-217.
- Lahlou, M., Harms, H., Springael, D., & Ortega-Calvo, J. J. (2000). Influence of Soil Components on the Transport of Polycyclic Aromatic Hydrocarbon-Degrading Bacteria through Saturated Porous Media. *Environ Sci Technol.*, 34, 3649. <http://dx.doi.org/10.1021/es000021t>
- Leneva, N. A., et al. (2009). Phenanthrene and anthracene degradation by microorganisms of the genus *Rhodococcus*. *Prikl Biokhim Mikrobiol*, 45, 188-94.
- Li, Qing-Shan., Ogawa, J., Schmid, R. D., & Shimizu, S. (2001). Engineering Cytochrome P450 BM-3 for Oxidation of Polycyclic Aromatic Hydrocarbons. *Appl Environ Microbiol*, 67, 5735.
- Moody, J. D. et al. (2001). Degradation of phenanthrene and anthracene by cell suspension of *Mycobacterium* sp. strain PYR-1. *Appl Environ Microbiol*, 67, 1476-1483. <http://dx.doi.org/10.1128/AEM.67.4.1476-1483.2001>
- Mrozik, A. Z., Piotrowska-seget, & Labuzek, S. (2003). Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. *Journal of Environmental Studies*, 12, 15-25.

- Ortega-Calvo, J. J., & Alexander, M. (1994). Roles of bacterial attachment and spontaneous partitioning in the biodegradation of naphthalene initially present in nonaqueous-phase liquids. *Appl Environ Microbiol*, *60*, 2643.
- Ortega-Calvo, J. J., Marchenko, A. I., Borobyou, A. I., & Borovick, R. V. (2002). Chemotaxis in polycyclic aromatic hydrocarbon-degrading bacteria isolated from coal-tar- and oil-polluted rhizospheres. *Microbiol Eco*.
- Prabhu, Y., & Phale, P. S. (2003). Biodegradation of phenanthrene by *Pseudomonas* sp. strain PP2: novel metabolic pathway, role of biosurfactant and cell surface hydrophobicity in hydrocarbon assimilation. *Appl Microbiol Biotechnol.*, *61*, 342-351. <http://dx.doi.org/10.1007/s00253-002-1218-y>
- Rene van., et al. (2003). Degradation of Anthracene by *Mycobacterium* sp. Strain LB501T Proceeds via a Novel Pathway, through o-Phthalic Acid. *Appl Environ Microbiol.*, *69*, 186-190. <http://dx.doi.org/10.1128/AEM.69.1.186-190.2003>
- Rockne, K. J., Chee-Sanford, J. C., Sanford, R. A., Hedlund, B. P., Staley, J. T., & Strand, S. E. (2000). Anaerobic naphthalene degradation by microbial pure cultures under nitrate-reducing conditions. *Appl Environ Microbiol.*, *66*, 1595. <http://dx.doi.org/10.1128/AEM.66.4.1595-1601.2000>
- Rodrigo, J. S. et al. (2005). Anthracene biodegradation by *Pseudomonas* sp. isolated from a petrochemical sludge landfarming site. *International Biodeterioration and biodegradation.*, *56*, 143-150. <http://dx.doi.org/10.1016/j.ibiod.2005.06.005>
- Shingler, V. (2003). Integrated regulation in response to aromatic compounds: from signal sensing to attractive behavior. *Environ Microbiol.*, *12*, 1226-1241. <http://dx.doi.org/10.1111/j.1462-2920.2003.00472.x>
- Takeuchi, M., Hamana, K., & Hiraishi, A. (2001). Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol.*, *51*, 1405-1417. <http://dx.doi.org/content/51/4/1405>
- Timmis, K. N., & Pieper, D. H. (1999). Bacteria designed for bioremediation. *Trends Biotechnol.*, *17*, 200. [http://dx.doi.org/10.1016/S0167-7799\(98\)01295-5](http://dx.doi.org/10.1016/S0167-7799(98)01295-5)
- Tongpim, S., & Pickard, M. A. (1999). Cometabolic oxidation of phenanthrene to phenanthrene trans-9, 10-dihydrodiol by *Mycobacterium* strain S1 growing on anthracene in the presence of phenanthrene. *Can J Microbiol.*, *45*, 369-376.
- Vila, J., López, Z., Sabate, J., Mingullón, C., Solanas, A. M., & Grifoll, M. (2001). Identification of a novel metabolite in the degradation of pyrene by *Mycobacterium* sp. strain AP1: actions of the isolate on two- and three-ring polycyclic aromatic hydrocarbons. *Appl Environ Microbiol*, *67*, 5497. <http://dx.doi.org/10.1128/AEM.67.12.5497-5505.2001>