Research Article

Biodegradation of Phenanthrene, Anthracene and Pyrene *in vitro* by *Bacillus subtilis* SPC14

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ABSTRACT

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of organic pollutants, containing carbon and hydrogen, composed of two or more condensed benzene rings in linear, angular, and cluster arrangements. Number of benzene rings may vary between 2 to 13. Sometimes sulphur, nitrogen and oxygen atoms may get readily substituted in the benzene ring[1]. These are non-polar, hydrophobic and readily soluble in hydrophobic solvents like fat, oil, ethereal solvents. The aqueous solubility of PAHs decreases with increasing molecular size. The hydrophobicity of PAHs plays key role in high persistence and low bioavailability to microbial attack and retained environment for longer times without degradation[2]. The PAHs namely phenanthrene, anthracene and pyrene are common PAHs pollutants that occur in all contaminated sites.

Keywords: Polycyclic Aromatic Hydrocarbons, PAHs, phenanthrene, anthracene, pyrene.

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of organic pollutants, containing carbon and hydrogen, composed of two or more condensed benzene rings in linear, angular, and cluster arrangements. Number of benzene rings may vary between 2 to 13. Sometimes sulphur, nitrogen and oxygen atoms may get readily substituted in the benzene ring[1]. These are non-polar, hydrophobic and readily soluble in hydrophobic solvents like fat, oil, ethereal solvents. The aqueous solubility of PAHs decreases with increasing molecular size. The hydrophobicity of PAHs plays key role in high persistence and low bioavailability to microbial attack and retained environment for longer times without degradation[2]. The PAHs namely phenanthrene, anthracene and pyrene are common PAHs pollutants that occur in all contaminated sites.

In the pioneer research PAHs degrading bacteria were isolated from an oil contaminated site[3]. Davies and Evans[4] for the first time presented the metabolic pathway and the enzymatic reactions resulting in the mineralization of simple PAH, naphthalene. These results encouraged the scientific community towards the studies on the degradation of PAHs by diversified microbes. Intensified work succeeded and paved the way in the identification of PAHs degrading life forms such as bacteria, fungi, and algae and their utilization[5-9]. Studies that related to the degradation of phenanthrene, anthracene and pyrene has extendable interest around the world and number of articles has been publishing every year.

In the present research we have used a novel bacterial strain of *Bacillus subtilis* SPC14 that was isolated from a PAHs contaminated site[10]. The strain has capability to degrade phenanthrene, anthracene and pyrene *in vitro* conditions. We have studied the effects of different factors on the degradation of phenanthrene, anthracene and pyrene and also recorded the respective degrading quantities of phenanthrene, anthracene and pyrene during a course of time *in vitro* using High Performance Liquid Chromatography (HPLC) methods.

MATERIALS AND METHODS

Effect of phenanthrene, anthracene and pyrene concentrations on the growth of *B. subtilis* SPC14

To determine the effect of phenanthrene, anthracene and pyrene concentrations on *B. subtilis* SPC14 growth, precisely 75 ml of Minimal Salt Medium (MSM) was dispensed into 250 ml flasks and sterilized by autoclaving. The flasks were then divided into six sets of six flasks. Further, 10, 50, 100, 150, 200 and 250 ppm levels of phenanthrene, anthracene and pyrene which were separately dissolved in acetone were followed by inoculation with each isolate. Inoculated flasks were then incubated at 28 C for 3 days with a speed 130 rpm. 5 ml of sample was aseptically collected from each flask and assayed for the level of microbial growth. Growth was recorded in terms of Optical Density (OD) readings at 600 nm using a UV spectrophotometer[11].

Study of growth curve on MSM enriched with phenanthrene, anthracene and pyrene

Bacterial growth curve study on MSM enriched with PAHs was adopted from Toledo[12]. Overnight culture of *B. subtilis* SPC14 in nutrient broth was harvested by centrifugation (10,000 g, 10 min) and re-suspended in

sterile phosphate buffer (150 nM, pH 7) to yield an optical density of 0.6 at 660 nm. In control samples no PAHs was added. Aliquots (0.5 ml) of the cell suspensions were transferred to 500 ml Erlenmeyer flasks containing 100 ml of MSM enriched separately with phenanthrene, anthracene or pyrene (100 ppm). Each time 5 ml of sample was collected at the time intervals of 0, 1, 2, 3, 7, 10 and 13 days and recorded OD values at 600 nm using a spectrophotometer and growth curve was plotted using OD values against time intervals.

Effect of pH on the growth of *B. subtilis* SPC14

Effect of pH (negative logarithm of hydrogen ion) on the growth of bacteria was tested at a wide range of pH from 5 to 10 on MSM broth. The pH of the media was adjusted by NaOH/HCl and incubated under standard growth conditions. Growth of the strain was recorded at 600 nm after 3 days of incubation.

Construction of standard chromatograms for HPLC studies

Method for the preparation of standard chromatograms for phenanthrene, anthracene and pyrene using high performance liquid chromatography (HPLC) was adopted from Boonchan[13]. Phenanthrene, anthracene and pyrene with 99% purity were purchased from Sigma-Aldrich, USA and used throughout the experimental work as test PAHs. Accurately weighed 100 mg of phenanthrene, anthracene and pyrene transferred to a 100 ml volumetric flask and 2-3 ml of acetonitrile was added to dissolve. Solution was diluted with acetonitrile to obtain a volume 1000 mg/L stock solution. The stock solution was further diluted to obtain the concentrations of 25, 50, 100, 200 and 250 ppm and the samples were ran in HPLC. Standard chromatograms for phenanthrene, anthracene and pyrene were plotted separately using a software, 'Origin 6.0' by retrieving the peak area values of each PAHs at respective retention times.

HPLC analysis

High performance liquid chromatography (HPLC) studies were conducted with a reverse phase HPLC (SHIMADZU, model RF-10AXL). The instrument consists of dual pump system and connected with UV detector (SPD-20A). Instrument was equipped with column C 18 (250 mm x 4.6 mm, 5 A particle size) of Phenomenex Co. Mobile phase was consisted of 75% acetonitrile and 25% of de-ionized water. Detector was set at 250 nm and mobile phase was maintained at flow rate of 0.8 ml/min in isocratic mode. 20 µl of sample was injected into HPLC with a HPLC injector (Rheodine injector) that prior filtered with 0.22 µm syringe filters. Data of each peak on HPLC chromatogram was analyzed using chromatography software 'LC Solutions'.

Quantification of phenanthrene, anthracene and pyrene concentrations during their degradation *in vitro*

Degradation of phenanthrene, anthracene and pyrene in a mixture was studied by the method of Moody[14]. 250 ml of MSM was prepared in 1000 ml conical flasks and enriched with phenanthrene, anthracene and pyrene (each 50 ppm) and made 150 ppm. One ml of exponential growth phase culture (approximately 10^7 colony forming units) of *B. subtilis* SPC14 was added separately to MSM flasks and in control flasks no culture was added. The cultures were incubated under standard growth conditions (at 24 C on a rotary shaker at 150 rpm speed) for 13 days in the dark. A 5 ml of aqueous portion was withdrawn from each flask at regular time intervals of 0, 1, 2, 3, 7, 8 and 13 days and extracted with three equal volumes of ethyl acetate after adjusting pH to 2.5 using 1N HCl and the step of extraction was repeated thrice. Each time a pinch of anhydrous disodium sulphate (Na_2SO_4) was added to remove residual water content from the samples. Extract was concentrated using rotary evaporator under reduced pressure at 34 C under vacuum conditions. Finally, the samples were dissolved in 3 ml of acetonitrile (ACN) and preserved in a refrigerator at 4 C for HPLC studies.

RESULTS AND DISCUSSION

RESULTS

Effect of phenanthrene, anthracene and pyrene concentrations on growth of the strain

The growth of *B. subtilis* SPC14 was assayed separately on phenanthrene, anthracene and pyrene separately at different concentrations (10 ppm to 250 ppm) using MSM. The growth of strain was decreased with increasing PAHs concentrations from 10 ppm to 250 ppm (Fig. 1) and its maximum growth of *B. subtilis* SPC14 was observed on MSM with anthracene than other PAHs enrichments such as phenanthrene and pyrene. The results are presented in Table1.

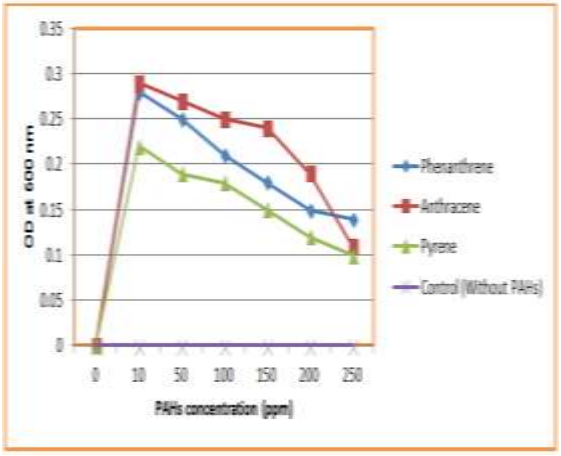

Figure 1: Growth of *B. subtilis* SPC14 on MSM enriched separately with different concentrations of phenanthrene, anthracene and pyrene

Bacterial growth curve of the strain on MSM enriched with phenanthrene, anthracene and pyrene

Study of bacterial growth curve on PAHs enriched media in a course of incubation period has great importance in understanding biodegradation abilities of strain. We have

assayed growth of the strain for 13 days on MSM enriched separately with phenanthrene, anthracene or pyrene. The strain showed gradual increase in growth up to 3rd day. The growth was consistent between 3rd and 7th days and dropped afterwards. The maximum growth of strain was observed on anthracene enrichment over the phenanthrene and pyrene enrichments. The results are presented in Table 2 and Fig 2.

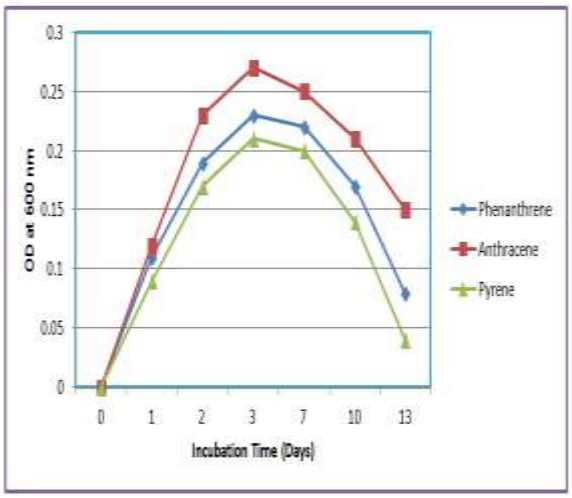

Figure 2: Bacterial growth study of *B. subtilis* SPC14 on MSM

Effect of pH

pH of medium is an important factor that can influences growth of bacteria and thereby degradation of PAHs. In view of this, growth of *B. subtilis* SPC14 was assessed at different pH, acidic to basic on MSM. The strain showed better growth between the pH 6 and 8 and growth was decreased at other pH. The results are presented in Table 3 and Fig. 3.

Degradation of phenanthrene, anthracene or pyrene by *B. subtilis* SPC14 *in vitro*

Preparation of standard chromatograms for PAHs

Standard chromatograms for phenanthrene, anthracene and pyrene were plotted by running HPLC with the known concentrations of 25, 50, 100, 200 and 250 ppm separately. Each PAH formed a peak at constant retention time and peak areas were increased with the increase in concentration. Based on the values of peak areas against known concentrations standard chromatograms for phenanthrene, anthracene and pyrene were plotted separately using the software, 'Origin 6.0'.

The strain recorded good degradation ability for the phenanthrene, anthracene and pyrene *in vitro*. The results are presented in Table 4. Degradation of phenanthrene reached up to 69.51% and its initial concentration (216.32 µg) declined to 65.96 µg in 13 days of incubation. Anthracene was degraded up to 80.37 % as the initial concentration (209.20 µg) was degraded to 41.06 µg in 13 days of incubation. Degradation of pyrene was recorded up to 47.06 %. The initial concentration of pyrene (230.14 µg) was depleted to 121.83 µg (Fig.3 and 4).

Degradation of phenanthrene, anthracene and pyrene by *B. subtilis* SPC14 recorded from first day itself and continued up to 13th day (Fig.3 and 4). In the mixture of PAHs, anthracene was degraded to a maximum extent (80.37%) while pyrene was degraded to minimum percentage (47.06%).

Table 1: Growth of *B. subtilis* SPC14 at different concentrations of phenanthrene, anthracene and pyrene

PAHs enrichment	OD at different concentrations of PAHs (ppm)						
	0	10	50	100	150	200	250
Phenanthrene	0	0.28	0.25	0.21	0.18	0.15	0.14
Anthracene	0	0.29	0.27	0.25	0.24	0.19	0.11
Pyrene	0	0.22	0.19	0.18	0.15	0.12	0.10
Control (Without PAHs)	0	0	0	0	0	0	0

Table 2: Growth of *B. subtilis* SPC14 on MSM enriched separately with phenanthrene, anthracene or pyrene

PAHs enrichment	OD at different time intervals (days)						
	0	1	2	3	7	10	13
Phenanthrene	0	0.11	0.19	0.23	0.22	0.17	0.08
Anthracene	0	0.12	0.23	0.27	0.25	0.21	0.15
Pyrene	0	0.09	0.17	0.21	0.2	0.14	0.04
Control (Without PAHs)	0	0	0	0	0	0	0

Table 3: Growth of *B. subtilis* SPC14 at different pH

pH	4	5	6	7	8	9	10
OD at 600 nm	0.12	0.76	1.54	1.62	1.53	1.06	0.56
Control (Without PAHs)	0	0	0	0	0	0	0

Table 4: Degradation of phenanthrene, anthracene and pyrene by *B. subtilis* SPC14 on MSM enriched with a mixture of PAHs *in vitro* (± represents standard deviation of three replicates)

S. No.	Days	Phenanthrene		Degradation (%)	Anthracene		Degradation (%)	Pyrene		Degradation (%)
		Retention Peak	Conc. (µg/5ml)		Retention Peak	Conc. (µg/5ml)		Retention Peak	Conc. (µg/5ml)	
1	Control (Without Bacteria)	880.750	217±2.07	0	678.142	211±2.03	0	380.241	233±2.08	0
2	0^{th}	880.750	217±2.07	0	678.142	211±2.03	0	380.241	233±2.08	0
3	1^{st} Day	690.162	168±2.01	22.80	371.45	115±2.02	45.72	369.4	225±1.52	3.54
4	2^{nd} Day	606.574	150±1.53	30.73	247.44	75±2.01	64.31	356.21	215±2.08	7.55
5	3^{rd} Day	590.532	143±2.00	34.08	238.09	75±2.08	64.57	336.17	204±1.53	12.33
6	7^{th} Day	505.722	124±1.01	42.91	195.96	61±1.53	71.05	247.177	148±2.00	36.61
7	8^{th} Day	476.326	117±2.00	46.09	179.222	55±2.02	73.81	239.198	146±1.56	37.25
8	13^{th} Day	268.557	66±1.52	69.45	133.104	43±2.00	79.60	201.333	123±2.31	47.10

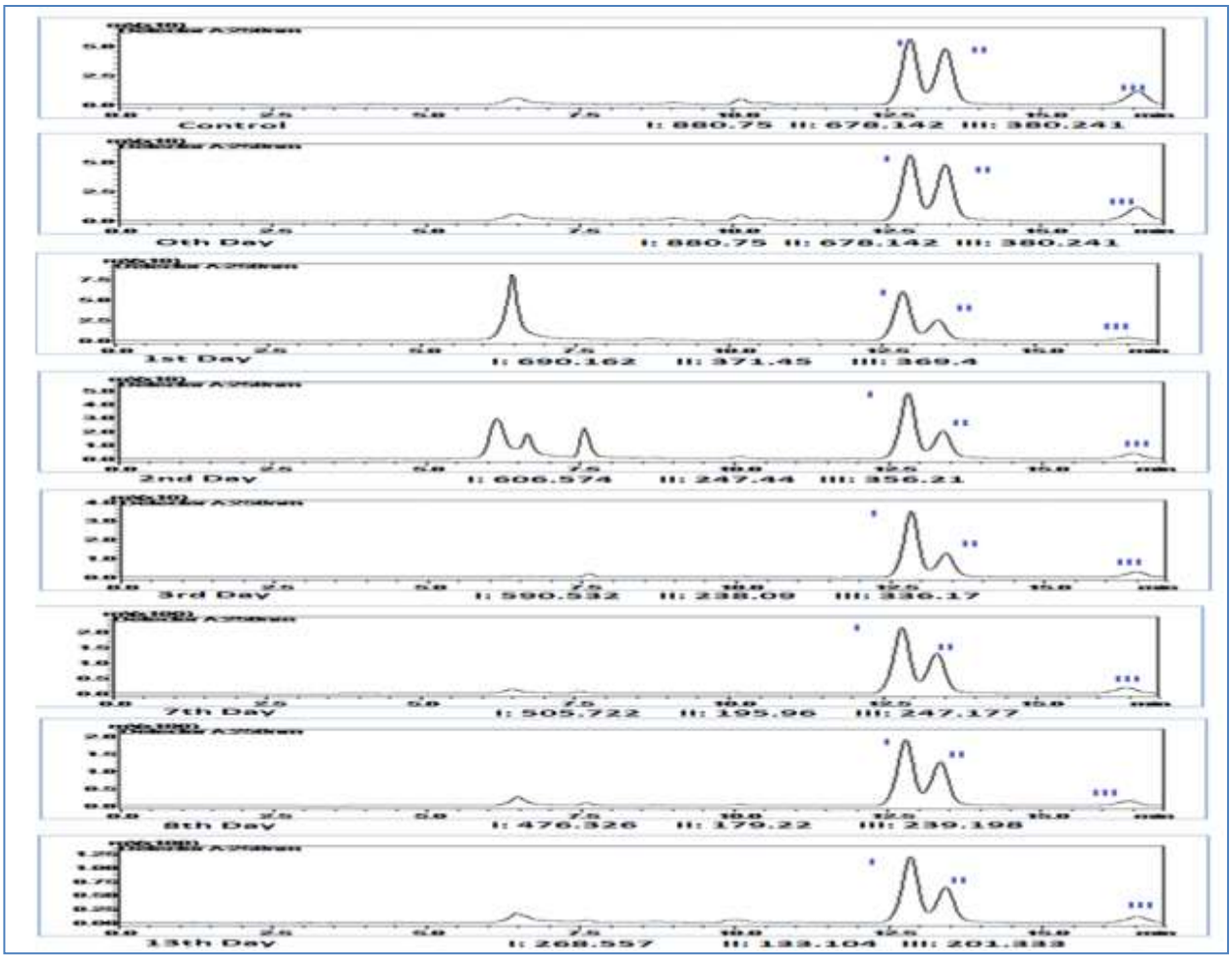

Figure 3: HPLC chromatograms of phenanthrene, anthracene and pyrene recorded during their degradation by *B. subtilis* SPC14 *in vitro* (I= Phenanthrene; II= Anthracene; III= Pyrene)

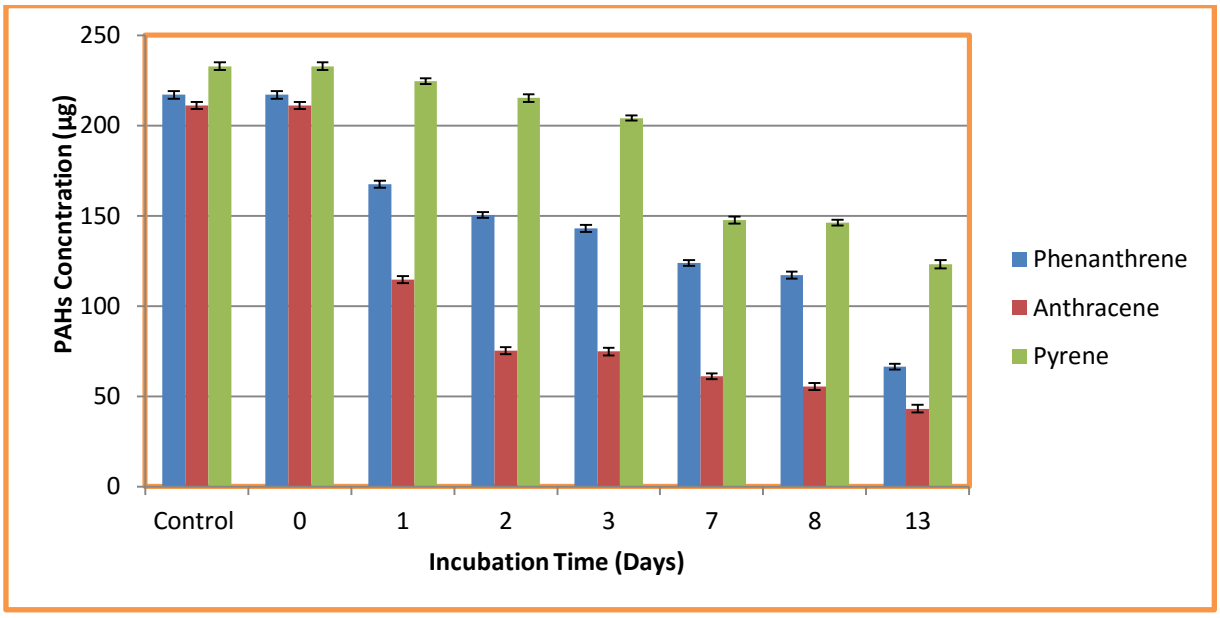

Figure 4: Degradation of phenanthrene, anthracene and pyrene by *B. subtilis* SPC14 in 13 days of incubation (Error bars represent standard deviation of three replacates)

DISCUSSION

Polycyclic aromatic hydrocarbons (PAHs) are one of the important organic pollutants that originate from natural and anthropogenic sources. Now a day pollution of PAHs is has been existing all over the world and soil becoming a big reservoir for PAHs[15]. These pollutants are dangerous to mankind because of their carcinogenic and mutagenic properties. In the natural conditions PAHs degrade slowly and persist for a long time in ecosystems. Hence amelioration of these pollutants becomes necessary and urgent.

Wide array of soil microorganisms have been implicated in the degradation of PAHs. Among the different kinds of microorganisms utilization of bacteria is more adaptable for PAHs. Many bacterial strains have the ability to utilize more than one type of PAHs for their growth and metabolism[16-17]. *Mycobacterium austroafricanum* GTI-23 utilized phenanthrene, fluoranthene and pyrene as sole source of carbon and energy[18].

PAHs as the sources for nutritional and metabolic activities results in their degradation by bacterial strains. Bisht[19] investigated the growth of *Kurthia* sp. SBA4, *Micrococcus variance* SBA8, *Deinococcus radiodurans* SBA6 and *B. circulans* SBA12 on MSM enriched with different concentrations of anthracene and naphthalene. They reported that growth of the bacteria was decreased with the concentration of PAHs. Similarly the growth of *B. subtilis* SPC14 was decreased with increasing concentrations of phenanthrene, anthracene and pyrene and at higher concentrations the decline in growth was rapid. The decrease in bacterial number in many experiments at higher concentrations of PAHs may be due to membrane toxicity of pollutant substrates[20].

In the present study we have studied the growth of *B. subtilis* SPC14 over a period of 13 days at optimum concentrations of phenanthrene, anthracene and pyrene i.e. 100 ppm. Hunter[21] (2005) studied the growth of *B. subtilis* on MSM enriched with PAHs. They reported that the strain, *B. subtilis* reached exponential phase within the first 24 hours, continued growth up to 3 days and bacterial growth was significantly decreased afterwards. Lily[22] reported *B. subtilis* BMT4i grew on MSM enriched with on benzo(a)pyrene and after 12 h of lag phase the strain exponentially increased up to 7th day and declined afterwards. In the present investigation, the strain *B. subtilis* SPC14 showed continuous growth increase from start of the experiment up to 3rd day, became constant between 3 to 7 days and declined afterwards. These results suggest that *B. subtilis* SPC14 can grow better on phenanthrene, anthracene and pyrene enrichments for 7 days and may decline afterwards.

pH of the medium is one of the crucial factor that effects the growth of bacteria in specified media. As several studies suggested that the pH within the range of 7 to 9 supports optimum growth of bacteria and there by degradation of various pollutants[23]. Pathak[24] investigated degradation of naphthalene by *Pseudomonas* sp. HOB1 at different pH and temperature ranges and reported that maximum degradation of naphthalene occurred between pH 7.5-8.5 and temperature 7.5-8.5 and 35-37 C respectively. In the present study, optimum growth of *B. subtilis* SPC14 on MSM enriched with PAHs was observed between pH 6 to 8.

The degradation of phenanthrene, anthracene and pyrene in a mixture by *B. subtilis* SPC14 was studied *in vitro* for 13 days. Phenanthrene, anthracene and pyrene formed different peaks in HPLC chromatograms at respective retention times (RT) based on their respective structures and molecular weights. Sequential appearance of test PAHs on chromatogram is phenanthrene, anthracene and pyrene. The result is in conformity with

the results of Haritash[25]. The retention times observed for phenanthrene, anthracene and pyrene in HPLC chromatograms are 13, 13.5 and 16.5 minutes respectively. We also observed that peak areas of test PAHs are increased with their concentration. Plotted standard chromatograms for each PAH had above the 0.9 correlation coefficient value. Hence, these results are reliable and amenable for determination of unknown concentrations of phenanthrene, anthracene and pyrene.

Daane[26] studied degradation of PAHs individually and as a mixture using naphthalene, fluorene, phenanthrene, pyrene and biphenyl *in vitro* by rhizobacteria for 11 days. Moody (2001) studied degradation of anthracene and phenanthrene by *Mycobacterium* sp. strain PYR-1 for 14 days and reported that *Mycobacterium* sp. degraded anthracene and phenanthrene up to 92 and 90% respectively in 14 days. Zuang[27] reported the species *Pseudomonas, Microbacterium* and *Paracoccus* rapidly degraded PAHs in a mixture like naphthalene, phenanthrene, fluoranthene, chrysene and pyrene within 12 days of incubation. In the present study, degradation of phenanthrene, anthracene and pyrene was observed at all through the incubation period (13 days). However, rapid degradation of test PAHs observed until 7th day and after this time period we noticed low degradation rate. Lily[28] obtained similar results with the degradation of benzo(a)pyrene by *B. subtilis* BMT4i.

As earlier reports suggested degradation of PAHs in a mixture depends on chemical structure and corresponding physiochemical properties of PAHs[29]. Low molecular weight PAHs degrade rapidly than high molecular weight PAHs during biodegradation[30]. Similar results are obtained in the present study. Pyrene a high molecular weight PAH degraded to a low percentage by *B. subtilis* SPC14 i.e. 47.10%. The strain also degraded phenanthrene and anthracene to a greater extent up to 69.45% and 79.60% respectively within 13 days. This may be due to high molecular weight PAHs are more recalcitrant and hard to microbial attack[31].

CONCLUSION

The present study has suggesting that *B. subtilis* SPC14 is very effective in the biodegradation of phenanthrene, anthracene and pyrene for short time implementations when the growth influencing factors such as pH, concentrations of phenanthrene, anthracene and pyrene are in optimum conditions. The study also helps in making procedure for the quantifications of PAHs concentrations during biodegradation.

REFERENCES

1. Wilson SC, Jones KC, Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons PAHs: A review, Environmental Pollution, 813, 1993, 229-249.
2. Heitkamp M.A. and Cerniglia C.E. (1987). 'Effects of chemical structure and exposure on the microbial degradation of polycyclic aromatic hydrocarbons in freshwater and estuarine ecosystems'. *Environ. Tox. Chem*. 6, 546.
3. Cerniglia C.E. and Heitkamp M.A. (1987). 'Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment'. Pages 41-68 in: Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. U. Varanasi, ed. CRC Press, Inc., Boca Raton, FL, U.S.A.
4. Davies J.I. and Evans W.C. (1964). 'Oxidative metabolism of naphthalene by soil Pseudomonads'. *Biochem. J.* 91, 251-261.
5. Leahy J.G. and Colwell R.R. (1990). 'Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev*. 54, 305-315.
6. Sanseverino J., Applegate B.M., King J.M.H. and Sayler G.S. (1993). 'Plasmid mediated mineralization of naphthalene, phenanthrene, and anthracene'. *Appl. Environ. Microbiol.* 59, 1931-1937.
7. Shuttleworth K.L. and Cerniglia C.E. (1996). 'Bacterial degradation of low concentration of phenanthrene and inhibition by naphthalene'. *Microbiol. Ecol.* 31, 305-317.
8. Johnson K. and Jacobsen C.S. (1996). 'Phenotypic and genotypic characterization of phenanthrene-degrading fluorescent *Pseudomonas* biovars'. Applied Environmental Microbiology, 62, 10, 3818-3825.
9. Cerniglia C.E. (1997). 'Fungal metabolism of polycyclic aromatic hydrocarbons; past, present and future applications in bioremediation'. Journal of Industrial Microbiology and Biotechnology, 19, 324-333.
10. Rao MP, Rajithasri AB, Ashokvardhan T, Satyaprasad K, Effective polycyclic aromatic hydrocarbons degrading rhizobacteria with PGP traits from plants growing on fly ash in the premises of thermal power station-Kothagudem, Telangana State, India, International Journal of Science and Research, 2013, 2319-7064 Paper ID: SUB154563.
11. John RC, Essien JP, Akpan SB, Okpokwasili GC, Polycyclic aromatic hydrocarbon-degrading bacteria from aviation fuel spill site at Ibeno, Nigeria, Bulletin Environmental Contamination and Toxicology, 88, 2012, 1014-1019.
12. Toledo FL, Calvo C, Rodelas B, Gonzalez-Lopez J, Selection and identification of bacteria isolated from crude oil with polycyclic aromatic hydrocarbons removal capacities, Systemic Applied Microbiology, 29, 2006, 244-252.
13. Boonchan S, Britz ML, Stanely GA, Biodegradation of and mineralization of high molecular weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures, Applied and Environmental Microbiology. 66, 3, 2000, 1007-1019.
14. Moody JD, Freeman JP, Doerge DR, Cerniglia CE, Degradation of phenanthrene and anthracene by cell suspensions of *Mycobacterium* sp Strain PYR-1, Applied and Environmental Microbiology, 67, 4, 2001, 1476-1483.
15. Wild SR, Jones KC, Polynuclear aromatic hydrocarbons in the United Kingdom environment: A preliminary source inventory and budget, Environmental Pollution, 88, 1995, 91-108.
16. Hunter RD, Ekunwe SI, Dodor DE, Hwang H, Ekunwe L, *Bacillus subtilis* is a potential degrader of pyrene and

benzo(a)pyrene, International Journal of Environmental Research in Public Health, 2, 2, 2005, 267-271.

17. Pizzul L, delPilar Castillo M and Stenstrom J. Effect of rape seed oil on the degradation of polycyclic aromatic hydrocarbons in soil by *Rhodococcus wratislaviensis* Int Biodet Biodegr, 59, 2007, 111-118.
18. Bogan BW, Lahner LM, Sullian WR, Paterek JR, Degradation of straight chain aliphatic and high molecular weight polycyclic aromatic hydrocarbons by a strain of *Mycobacterium austroafricanum*, Journal of Applied Microbiology, 94, 2003, 230-239.
19. Bisht S, Pandey P, Sood A, Sharma S, Bisht NS, Biodegradation of naphthalene and anthracene by chemotactically active rhizobacteria of *Populus deltoids*, Brazilian Journal of Microbiology, 41, 2010, 922-930.
20. Singh S., Canda R., Patel D.K., Rai V. 'Isolation and characterization of novel *Serratia marcescens* (AY927692) for pentachlorophenol degradation from pulp and paper mil waste'. World Journal of Microbiology and Biotechnology, 23, 2007, 1747-1754.
21. Hunter RD, Ekunwe SI, Dodor DE, Hwang H, Ekunwe L, *Bacillus subtilis* is a potential degrader of pyrene and benzo(a)pyrene, International Journal of Environmental Research in Public Health, 2, 2, 2005, 267-271.
22. Lily MK, Bahuguna A, Dangwal K, Garg V, Degradation of Benzo(a)pyrene by a novel strain *Bacillus subtilis* BMT4i MTCC 9447, Brazilian Journal of Microbiology, 40, 2009, 884-892.
23. Frankenberger WT, Camargo FAO, Bento FM, Okeke BC, Chromate reduction by chromium resistant bacteria isolated from soils contaminated with dichromate, Journal of Environmental Quality, 324, 2003, 1228-1233.
24. Pathak H, Kantharia D, Malpani A, Madamwar D, Naphthalene degradation by *Pseudomonas* sp HOB 1: *in vitro* studies and assessment of naphthalene degradation efficiency in stimulated microcosm, Journal of Hazard Mater, 168, 2009, 1456-1473.
25. Haritash AK, Kaushik CP, Biodegradation aspects of polycyclic aromatic hydrocarbons PAHs: A review, Journal of Hazard Mater, 169, 2009, 1-15.
26. Daane LL, Harjono I, Zylstra GJ, Haggblom MM, Isolation and characterization of polycyclic aromatic hydrocarbon degrading bacteria associated with the rhizosphere of salt marsh plants, Applied Environmental Microbiology, 67, 2001, 2683-2691.
27. Zuang H, Kallimanis A, Koukkou AN, Drainas C, Isolation and characterization of novel bacteria degrading polycyclic aromatic hydrocarbons from polluted Greek soils, Applied Microbiology and Biotechnology, 65, 2004, 124-131.
28. Lily MK, Bahuguna A, Dangwal K, Garg V, Degradation of Benzo(a)pyrene by a novel strain *Bacillus subtilis* BMT4i MTCC 9447, Brazilian Journal of Microbiology, 40, 2009, 884-892.
29. Chang BV, Wei SH, Yuan SY, Biodegradation of phenanthrene in soil Journal of Environmental Science and Health, 362, 2001, 177-187.
30. Guo CL, Zhou HW, Wong YS and Tam NFY, Isolation of PAH-degrading bacteria from mangrove sediments and their biodegradation potential, Marine Pollution Bulletin, 51, 2005, 1054-1061.
31. Peng RH, Xiong AS, Xue Y, Fu XY and Gao F, Microbial biodegradation of polyaromatic hydrocarbons, FEMS Microbiology Reviews, 32, 2008, 927-955.

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