Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

# Co-metabolic degradation of benzo(e)pyrene by halophilic bacterial consortium at different saline conditions

**P. Arulazhagan[1], C. Sivaraman[1], S. Adish Kumar[2], M. Aslam[1] and J. Rajesh Banu[2]***

[1]Center of Excellence in Environmental Studies, King Abdulaziz University, Jeddah-21589, Saudi Arabia
[2]Department of Civil Engineering, Regional Centre of Anna University, Tirunelveli-627 007, India
*Corresponding Authors Email : rajeshces@gmail.com

**Publication Info**

*Paper received:*
29 April 2013

*Revised received:*
22 August 2013

*Re-revised received:*
30 September 2013

*Accepted:*
30 October 2013

**Abstract**

Polyaromatic hydrocarbons (PAHs) with high molecular weight (more than three benzene rings) were difficult to degrade in saline environment. The present study details about the bacterial consortium enriched from industrial sludge from salt manufacturing company, Tuticorin, Tamilnadu (India), which was capable of degrading 1, 4 dioxane (Emerging micropollutant) and also phenanthrene as sole carbon source under saline condition. The halophilic bacterial consortium was able to degrade low molecular weight (LMW) phenanthrene, but unable to degrade high molecular weight (HMW) benzo(e)pyrene. To overcome this problem, phenanthrene was added as co-substrate along with benzo(e)pyrene which enhanced the biodegradation process by co-metabolism under saline conditions. The consortium potentially degraded 80% and 99% of benzo(e)pyrene in 7 days and phenanthrene in 5 days at 30 g $l^{-1}$ of NaCl concentration. When the saline concentration increased to 60 g $l^{-1}$, degradation of phenanthrene (97% in 8 days) and benzo(e)pyrene (65% in 10 days) was observed. Further increase in saline concentration to 90 g $l^{-1}$ of NaCl showed reduction in the percent degradation of phenanthrene and benzo(e)pyrene leads to 30.3% and 9% respectively in 6 days. Potential bacterial strains, present in PAHs degrading bacterial consortium were identified as *Achromobacter* sp. AYS3 (JQ419751), *Marinobacter* sp. AYS4 (JQ419752) and *Rhodanobacter* sp. AYS5 (JQ419753). The present study details about the effect of salinity on PAHs degradation and vital role of co-metabolism on biodegradation of benzo(e)pyrene with phenanthrene under saline conditions.

**Key words**

Biodegradation, Co-metabolism, Halophilic bacterial consortium, Polycyclic aromatic hydrocarbons (PAHs)



## Introduction

Marine environment poses serious threat due to accidental oil spill and industrial discharge specifically from refineries. The major concern is about polycyclic aromatic hydrocarbons (PAHs) which act as ubiquitous pollutant with potential toxic and carcinogenic effect, persist in the environment for longer period due to their hydrophobic nature (Menzie *et al.*, 1992, Yuan *et al.*, 2000). The genotoxicity of PAHs also increases with their size, up to at least four or five fused benzene rings (Cerniglia, 1992). Release of toxic PAHs by natural and anthropogenic activities into the ecosystem is the main root cause for air, water and soil pollution which leads to deleterious effects on plants, animals and human health. Due to the release, cleanup of these harmful pollutants is essential (Wilson and Jones 1993; Kasai *et al.*, 2002). Hydrocarbons are recalcitrant organic compounds and thus a significant concern exists in isolating microorganisms present in contaminated environments as a means for bioremediation (Haritash and Kaushik, 2009; Tam *et al.*, 2002, Zhao *et al.,* 2009). The fate of hydrocarbons in the environment is associated with both abiotic and biotic processes which include volatilization, photooxidation, chemical oxidation, bioaccumulation and microbial transformation (Danne *et al.*, 2001).

Limited research studies have been done on degradation of PAHs by bacterial consortium under saline condition (Arulazhagan and Vasudevan 2009; McKew *et al.*, 2007;

Dastgheib *et al.,* 2012). Role of surfactant (Li and Bai, 2005) and enzyme activity (Garcia *et al.,* 2005) on PAHs degradation under saline condition has been reported. In our previous work, the role of nutrients in degradation of PAHs by halotolerant bacterial strain and consortium was reported (Arulazhagan and Vasudevan 2011, Arulazhagan *et al.,* 2010). Juhasz *et al.* (1997) reported high molecular weight PAH degradation rates and lag times preceding degradation were influenced by the presence of lower molecular weight PAHs, which was consistent with degradative capabilities of the micro-organism. High molecular weight (HMW) polyaromtic hydrocarbons cannot be efficiently utilized as sole carbon substrate by the microorganisms. Under this condition, phenanthrene is added as co-substrate to increase the bacterial cell count which also helps to utilize high molecular weight benzo(e)pyrene. Transformation of non-growth substrate in the obligate, presence of growth substrate or another transformable compound is termed as co-metabolism (Dalton and Stirling 1982).

Details on co-metabolism in biodegradation of PAHs under saline condition is an open field for research to identify cell adaptation to extreme environment and bacterial strains involved in the process (De Carvalho, 2012). The present study details about the cometabolic degradation of benzo(e)pyrene in the presence of phenanthrene as co-substrate by a halophilic bacterial consortium under saline condition.

## Materials and Methods

**Enrichment of bacterial consortium :** The salt samples were collected from Toovepuram, Cruzpuram, Keela Arasadi, Tharuvaikulam and Vaippar in Tuticorin, Tamil Nadu, India (Fig. 1). The sample collection area was located at latitude of 8.8100° N and longitude of 78.1400° E. The sampling sites were reported to be polluted with oil spills for more than 25 years from the motor pump oil used to pump sea water. This is the first report on bacterial consortium capable of degradation PAHs co-metabolically from Tuticorin saline environment. The bacterial consortium was enriched from salt industries of Tuticorin, with phenanthrene as sole carbon source. Among the LMW, PAHs, phenanthrene acted as best substrate to study the co-metabolism (Supaka *et al*., 2001, Roy *et al*., 2013). PAHs were purchased from Sigma, USA (99% purity), and all other chemicals (Analar grade) were purchased from Merck, India. Carbon-free mineral salt medium (MSM) contained : 2.5 g $NH_4Cl$, 5.46 g $KH_2PO_4$, 4.76 g $Na_2HPO_4$, 0.20 g $MgSO_4$, 30.0 g NaCl, pH-7.4 $\pm$ 0.2 and 1l distilled water. The final pH of medium was adjusted to 7.4 with 0.1 N NaOH, and the medium was sterilized in an autoclave (Technico, India) at 121 ºC for 15 min, prior to addition of PAH substrates. Stock solution of each PAH (500 ppm) was prepared in ethyl acetate and stored.

**PAH clearing zone-spray-plate technique :** PAH degradation by bacterial consortium was analyzed by spray-plate technique using PAH as sole carbon and energy source. Agar was added to the mineral salt medium (NaCl: 30g $l^{-1}$) for plating in Petri dishes. After solidification, ethyl acetate-dissolved phenanthrene (5 ppm) was sprayed on top of the medium. The halophilic bacterial consortium in sterile disc was inoculated on PAH amended medium and incubated at 37º C for 48 hr. After incubation, the clearing zones formed around the colonies indicated degradation of PAH sprayed on the medium (Kiyohara *et al.,* 1982).

**Analysis of PAH degradation :** For degradation study, the bacterial consortium was inoculated in mineral medium containing PAH. Different compositions used in degradation of PAH were (i) medium + PAH + consortium; (ii) medium + PAH and (iii) medium + consortium, where (ii) and (iii) served as control. The bacterial consortium was added to mineral salt medium (MSM) at a concentration of $10^4$ –$10^5$ cfu $ml^{-1}$. The cultures, prepared in duplicate, were incubated at 37 ºC in a shaker at 150 rpm and were extracted at every 24 hr time interval during the course of study. Plate count was done to analyse the growth pattern of bacterial consortium during biodegradation experiments. The samples were extracted twice with ethyl acetate (v/v) after acidification to pH 2.5 with 1 N HCl. The extracts were filtered through anhydrous sodium sulfate and condensed to 1 ml for chromatographical analysis of PAH degradation using Rota-evaporator. The condensed sample was filtered through 0.22 µm syringe filter and was analyzed using Ultra Fast liquid chromatography (UFLC). UFLC analysis was performed with Shimadzu (LC-20AD, Japan) unit equipped with a PAH-specific column (Pinnacle II PAH 4µm, 150x3.2mm, Restek, USA), column oven and UV–VIS detector (SPD-20A, Shimadzu, Japan) connected to LC Solutions software, which was used to process the data. The mobile phase was acetonitrile. Standard solutions of different PAHs (99.9% purity, Sigma, USA) were used as reference. The flow rate of mobile phase was maintained at 1 ml $min^{-1}$ and the column oven temperature was maintained at 40º C. The samples were injected individually, and the utilization rate of PAHs was calculated based on the peak area percent and retention time.

**$CO_2$ evolution test :** The ability of bacterial consortium to mineralize phenanthrene was studied by respirometric experiments in mineral salt medium (MSM). The experimental setup included. i) MSM + Bacterial consortium + phenanthrene served as test bottles and ii) MSM + phenanthrene served as control bottles. Evolution of $CO_2$ through abiotic process such as hydrolysis, oxidation and photolysis could be evaluated from control bottles. Along with $CO_2$ evolution, degradation of phenanthrene present in the medium was analysed in order to correlate percent degradation with $CO_2$ evolution. PAH (5 ppm phenanthrene) dissolved in ethyl acetate was added to sterile saline bottles (100 ml). After evaporation of the solvent, 25 ml of mineral salts medium was added to the bottle. The bottle was completely sealed (airtight) with aluminum stopper. The medium

was kept in an orbital shaker at 150 rpm. Samples were collected at 24 hr time interval and analyzed for $CO_2$ evolution in a gas chromatograph (Shimadzu, Japan). The carbon dioxide content was measured in a Porapak Q column (80/100 mesh, 2 m) with a thermal conductivity detector using an external standard. The carrier gas was helium, and the column temperature was 50ºC. The temperature of the injector and of the detector was 100º C. Samples (250 µl) of headspace gas of culture flask were withdrawn with a gas-tight syringe and injected into gas chromatograph for $CO_2$ determination. Samples in saline bottles were extracted and analyzed on a PAH-specific column using UFLC to measure degradation of PAH.

**Co-metabolic degradation of benzo(e)pyrene :** The effect of salinity on co-metabolic degradation of benzo(e)pyrene in the presence of phenathrene was studied. This study was carried out at different saline concentrations (NaCl: 30g $l^{-1}$, 60g $l^{-1}$ and 90g $l^{-1}$) of the mineral salts medium. The concentration of phenanthrene and benzo(e)Pyrene used in the study were 10ppm and 3 ppm respectively. The experimental setup was as follows: PAH + MSM and MSM + bacterial consortium served as controls and PAH + MSM + bacterial consortium were used as test sample. Samples were collected at 24 hr time interval and analyzed for PAH concentration with UFLC as described before. Plate count was done to measure the growth pattern of bacterial consortium during biodegradation experiments.

**Analysis of bacterial consortium :** DNA from bacterial cells was extracted using Qiagen (QIAamp DNA stool Mini kit Cat. No. 51504) DNA isolation kit. Using protocol from the manufacturer, DNA was eluted in 200µl of AE buffer and stored at 4ºC for further use. The purity of extracted genomic DNA was checked on 1% agarose gel with 1Kb ladder. PCR amplifications were conducted with PCR master mix (Invitrogen). Universal eubacterial primers (27F and 1492R) were used (Frank *et al*, 2008). The PCR programme used was with an initial 95°C denaturation step for 5 min, followed by 32 amplification cycles of denaturation for 30 sec at 94°C, 30 sec at 52°C, and 1 min at 72°C and final elongation step at 72°C for 10 min. After successful amplification, 5 µl aliquot of the above PCR products were taken for a second, PCR utilizing 968 F primer with a GC clamp and 1492R primers (Szekely *et al.,* 2009).

Denaturing gradient gel electrophoresis (DGGE) was conducted using CBS Scientific system. 10–15 µl of PCR products were loaded onto 8% (w/v) polyacrylamide gels (40% acrylamide stock solution, 2% bis solution 37.5:1). 30-60% gradient of denaturant (7M urea and 40% formamide v/v as 100% denaturant, (Nikolausz *et al,* 2008, Sivaraman *et al.,* 2011) was used to prepare the gel and the gel was run for 16 hr in 1X TAE buffer at 60 volts and was finally stained with SYBR green. The stained gels were inspected under UV transillumination and pictures were taken using a digital camera. The central, 1mm portions of strong DGGE bands were excised with a sterile razor blade and soaked in 50µl of sterile water overnight. A portion (15µl) was removed and used as template for PCR as described above to confirm the band ability to amplify. Purified DNA was sequenced with an automatic sequencer.

The cyclic sequencing reaction was performed using Big Dye terminatorV3.1 cycle sequencing kit containing Ampli Taq DNA polymerase (Applied Biosystems, P/N: 4337457). The sequencing reaction mixture was prepared by combining 1 µl of BigDyeV3.1, 2 µl of 5X sequencing buffer and 1 µl of 50% Dimethyl sulfoxide (DMSO). To 4 µl of sequencing reaction mixture, 4 pico moles of primer (2 µl) and a sufficient amount of purified PCR product was added. The constituted reaction was denatured at 95 ºC for 5 min. Cycling began with denaturing at 95 ºC for 30 sec, annealing at 52 ºC for 30 sec and extension for 4 min at 60 ºC and the cycle was repeated 30 times in MWG thermocycler. The reaction was purified on a sephadex plate (Edge Biosystems) by centrifugation to remove unbound labeled and unlabeled nucleotides and salts. The purified reaction was loaded on to 96 capillary ABI 3700 automated DNA analyzer and electrophoresis was carried out for 4 hr. The nucleotide sequences were registered in the computer attached to ABI 3700 DNA analyzer. The nucleotide sequences obtained from ABI DNA analyzer were studied by BLAST software available at NCBI website (www.ncbi.nlm.nlm.gov). After editing, the sequence was analysed with BLAST software to identify specific type of bacteria corresponding to the nucleotide sequence. A phylogenetic tree was derived according to maximum identity of the nucleotide sequence of bacterial strain.

## Results and Discussion

The bacterial consortium was enriched on phenanthrene as sole carbon source in mineral salts medium with 30 g $l^{-1}$ of NaCl concentration. When the bacterial consortium was grown on PAH-coated (phenanthrene) mineral agar plates, clearing zones were visualized, indicating the PAH-utilizing ability of the strain (Fig. 2). During biodegradation study, the cell count of the bacterial consortium increased from 4 x $10^4$ cfu $ml^{-1}$ to 9 x $10^9$ cfu $ml^{-1}$. The bacterial consortium degraded 5ppm of phenanthrene completely in 4 days (Fig. 3). Increase in PAH concentration (phenanthrene) reduced the utilization of phenanthrene by the bacterial consortium. When phenanthrene concentration was increased, percent degradation reduced to 85% at 25 ppm and 75.7% at 50 ppm in 5 days (Fig. 3). The consortium was able to degrade phenanthrene without any additional growth substrates. The enriched bacterial consortium was checked for its ability to grow on a HMW PAH (Benzo(e)pyrene) as sole carbon source. The concentration of benzo(e)pyrene used in biodegradation experiments was 3 ppm. The bacterial consortium was able to degrade only 12.5% of benzo(e)pyrene in 4 days. No significant growth of bacterial consortium on benzo(e)pyrene was found which was enumerated through plate count during biodegra dation experiments (Fig. 4). Decrease in benzo(e)pyrene degradation

was due to increase in the number of benzene rings of benzo (e) pyrene when compared with LMW compound phenanthrene (Kanaly and Harayama 2000).

The ratio between amount of carbon dioxide produced and residual hydrocarbons gives a complete picture of hydrocarbon degradation (Penet *et al.,* 2004). In the present study, bacterial consortium degraded phenanthrene up to 99% by converting 98% into $CO_2$. The release of $CO_2$ showed complete degradation of phenanthrene (Fig. 5). Growth of bacterial consortium on mineral salts medium containing phenanthrene as sole carbon source was corroborated by an increase in bacterial cell number and concomitant PAH degradation. No significant PAH degradation was found in sterile controls.

Limited information was available on bacterial biodegradation of benzo(e)pyrene in both environmental samples and pure or mixed cultures. Due to its potential hazards on human health, most studies have focused on the five-ring benzo(e)pyrene molecule (Juhasz *et al.*, 2000). The degradation of benzo(e)pyrene was studied in the presence of phenanthrene at varying salt concentrations (30 g $l^{-1}$, 60 g $l^{-1}$ and 90 g $l^{-1}$). The concentration of phenanthrene and benzo(e)pyrene used in the experiments are 10 ppm and 3 ppm respectively. In the absence of phenanthrene, degradation of benzo(e)pyrene by the bacterial consortium was extremely low. While using phenanthrene as a means for co-metabolism, the bacterial consortium degraded benzo(e)pyrene upto 80% in 7 days. Phenanthrene, being a LMW PAH compound, was degraded faster by the consortium within a span of 5 days during the course of study (Fig. 6a). Phenanthrene accelerated bacterial growth and enhanced biodegradation of benzo(e)pyrene. Degradation of benzo(e)pyrene was slow during initial days of the experiment, whereas benzo(e)pyrene degradation increased after complete degradation of phenanthrene at the end of 5$^{th}$ day. Increase in bacterial cell count during the course of biodegradation experiments indicate utilization of PAH compound by the bacterial consortium as sole carbon source. The bacterial consortium was able to adapt to saline condition and degrade PAHs as sole carbon source. The extent of cell adaptation for PAH degradation under saline condition may be due to the changes in bacterial cell membrane and cell-wall level (De Carvalho, 2012). Increase in salt concentration up to 60 g $l^{-1}$ and 90 g $l^{-1}$ significantly reduced the


**Fig. 1 :** Geographical representation of sampling sites in Tuticorin, Tamil Nadu, India

degradation rates of phenanthrene and benzo(e)pyrene. Sohn *et al.* (2004) reported *Novosphingobium pentaromativorans* sp. nov was able to degrade a mixture of high molecular weight PAHs at optimized 2.5% NaCl concentration. The time taken for degradation of benzo(e)pyrene and phenanthrene at 60g $l^{-1}$ increased to 10 days (Fig. 6b). Degradation of benzo(e)pyrene and phenanthrene at 60g $l^{-1}$ was found to be 97% and 65% respectively. Degradation of phenanthrene at 90 g $l^{-1}$ was 30.5% at the end of seven day study. No significant degradation of benzo(e)pyrene (9%) found at 90 g $l^{-1}$ (Fig. 6c). This can be due to the fact that bacterial consortium was unable to tolerate the high salinities and degrade PAH compounds. This can be corroborated by decrease in bacterial plate count in the biodegradation experiments. DGGE analysis showed the presence of two bacterial strains at 90 g $l^{-1}$ *Achromobacter sp* AYS3 and *Marinobacter* sp. AYS4. *Rhodanobacter sp* AYS5 disappeared from the consortium due to increase in salinity. Yu *et al.* (2005) also reported that, in a mixture of PAHs (phenanthrene, fluorene and pyrene) each at 10 mg $l^{-1}$ in liquid medium, a consortium isolated from marine sediments degraded phenanthrene and fluorene completely in 2 weeks. Complete biodegradation of pyrene took longer time (4 weeks), indicating that enriched bacterial consortium prefered to utilize low molecular weight PAHs. In agreement with the above research, the present study revealed the importance of phenanthrene as co-substrate to accelerate the degradation of benzo(e) pyrene.

From DGGE analysis, DNA bands confirmed that the bacterial consortium consisted of three bacterial strains. The 16S rRNA sequences were deposited in Genbank and accession numbers were obtained. JQ419751 showed 100% similarity with *Achromobacter* sp. and was found closely related to *Achromobacter xylosoxidans* by phylogenetic analysis. Genus, *Achromobacter* is known to occur in oil polluted environment (Farjadfard *et al.*, 2012). Strain with accession number JQ419752 showed 100% similarity with *Marinobacter* sp. which is known to degrade a wide range of nitroaromatic and phenolic compounds. Dastgheib *et al.* (2012) also reported that halophilic bacterial consortium possessing *Halomonas* and *Marinobacter* was

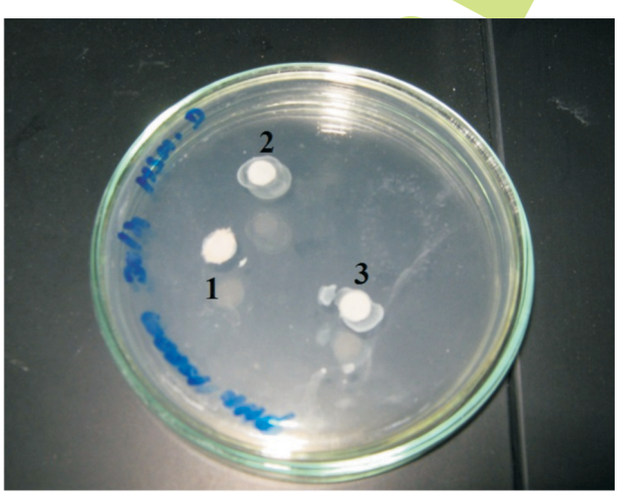


**Fig. 2 :** PAH clearing zone in mineral salt agar medium (1: Control; 2 and 3 : Phenanthrene clearing zone by the halophilic bacterial consortium)

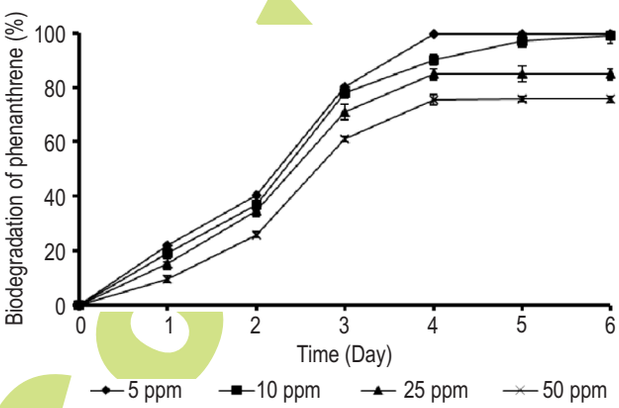


**Fig. 3 :** Biodegradation of phenanthrene at different concentrations by the halophilic bacterial consortium

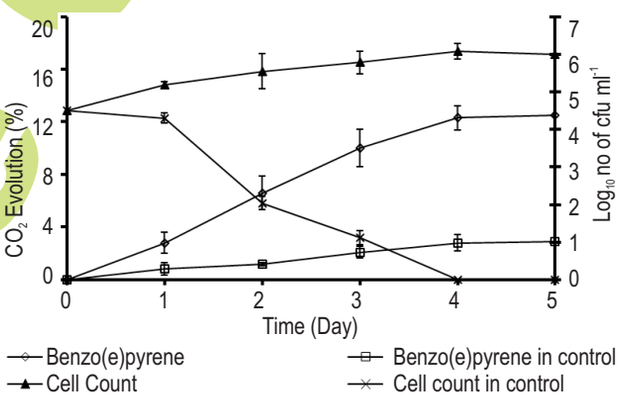


**Fig. 4 :** Biodegradation of benzo(e)pyrene by the halophilic bacterial consortium

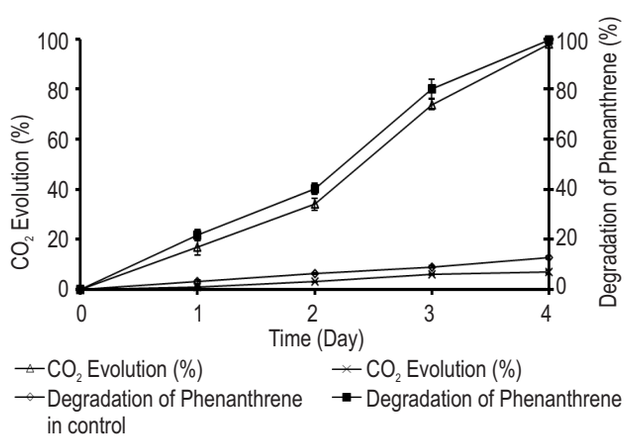


**Fig. 5 :** $CO_2$ evolution during degradation of phenanthrene by halophilic bacterial consortium

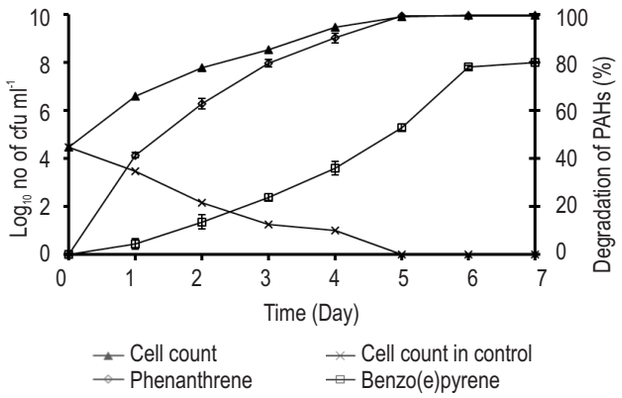


**Fig. 6 (a) :** Cometabolic study on degradation of benzo(e)pyrene at 30g $l^{-1}$ of NaCl concentration by halophilic bacterial consortium

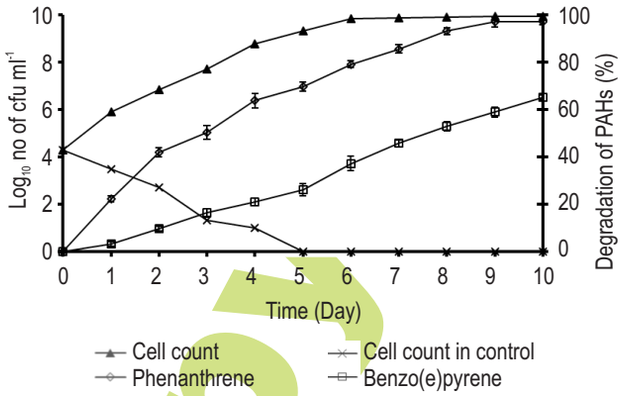


**Fig. 6 (b) :** Cometabolic study on degradation of benzo(e)pyrene at 60g $l^{-1}$ of NaCl concentration by halophilic bacterial consortium

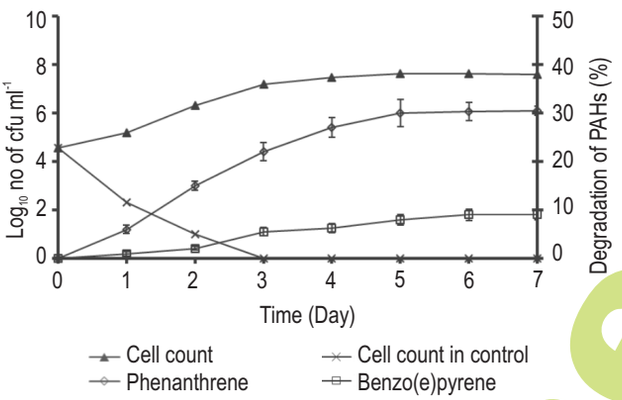


**Fig. 6 (c) :** Cometabolic study on degradation of benzo(e)pyrene at 90g $l^{-1}$ of NaCl concentration by halophilic bacterial consortium

capable of degrading 90% of phenanthrene in 6 days. JQ419753 showed 100% similarity with *Rhodanobacter* sp. which was closely related to *Rhodanobacter thiooxydans* through phylogenetic analysis (Fig. 7). The strain was capable of degrading recalcitrant chlorinated aromatic compounds and a wide variety of aromatic compounds (Kanaly *et al.*, 2002; Nalin *et al.*, 1999, Arulazhagan *et al.*, 2010, Martins and Pexioto 2012, Roy *et al.*, 2013).

The present study reveals that the bacterial consortium, consisting of *Achromobacter* sp. AYS3 (JQ419751), *Marinobacter* sp. AYS4 (JQ419752) and *Rhodanobacter* sp. AYS5 (JQ419753) acted as potential candidate in remediation of HMW PAHs contaminated sites with co-metabolic activity under saline condition. In addition to degradation of petroleum hydrocarbon, the consortium was also able to degrade 1,4

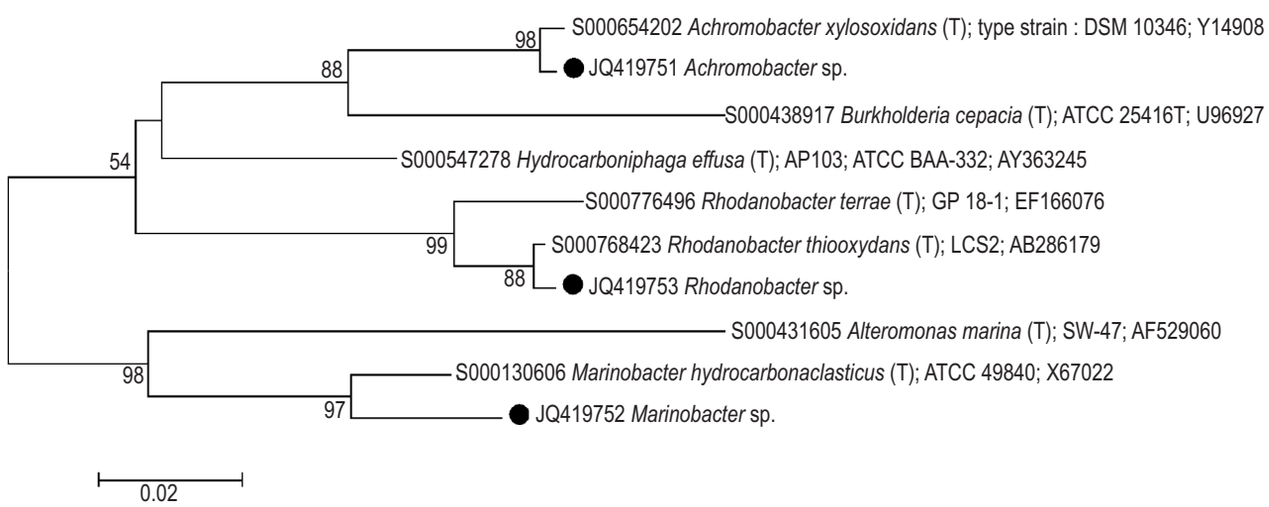


**Fig. 7 :** Phylogenetic tree derived from neighbor joining method of the 16S rRNA gene sequences of *Achromobacter* sp. AYS3 (JQ419751), *Marinobacter* sp. AYS4 (JQ419752) and *Rhodanobacter* sp. AYS5 (JQ419753). Scale bar 0.02 substitution per site

dioxane in industrial wastewater (data not shown). Salinity greatly influences the degradation of benzo(e)pyrene by co-metabolism at higher concentration. From the study, it is concluded that the halophilic bacterial consortium can be employed for treatment of petroleum hydrocarbon contaminated saline wastewater from oil refineries.

## Acknowledgments

The present research work was funded by Tamil Nadu State Council of Science and Technology, Tamil Nadu (India), collaborated with Centre of Excellence in Environmental Studies, King Abdulaziz Unvierstiy, Jeddah, Saudi Arabia.

## References

Arulazhagan, P. and N. Vasudevan: Role of moderately halophilic bacterial consortium in biodegradation of polyaromatic hydrocarbons. *Mar. Poll. Bull.,* **58**, 256-262 (2009).

Arulazhagan, P. and N. Vasudevan: Role of nutrients in the utilization of polycyclic aromatic hydrocarbons by halotolerant bacterial strain. *J. Environ. Sci.,* **23**, 282-287 (2011).

Arulazhagan, P., N. Vasudevan and I.T. Yeom: Biodegradation of polycyclic aromatic hydrocarbon by a halotolerant bacterial consortium isolated from marine environment. *Int. J. Environ. Sci. Tech.,* **7**, 639-652 (2010).

Cerniglia, C.E.: Biodegradation of polycyclic aromatic hydrocarbons. *Biodegradation*, **3**, 351-368, (1992).

Daane, L.L., I. Harjono, G.J. Zylstra and M.M. Häggblom: Isolation and characterization of polycyclic aromatic hydrocarbon-degrading bacteria associated with the rhizosphere of salt marsh plants. *Appl. Environ. Microbiol.,* **67,** 2683-2691 (2001).

Dalton, H., D.I. Stirling and J.R. Quayle: Co-metabolism (and discussion). *Philos. Trans. R. Soc. Lond. B Biol. Sci.,* **297**, 481-496 (1982).

Dastgheib, S.M., M.A. Amoozegar, K. Khajeh, M. Shavandi and A. Ventosa: Biodegradation of polycyclic aromatic hydrocarbons by a halophilic microbial consortium. *Appl. Microbiol. Biotechnol.,* **95**,789-98 (2012).

De Carvalho, C.C.C.R:Adaptation of *Rhodococcus erythropolis* cells for growth and bioremediation under extreme conditions. *Res. Microbiol.,* **163**, 125-136 (2012).

Farjadfard, S., S.M. Borghei, A.H. Hassani, B. Yakhchali, M. Ardjmand and M. Zeinali: Efficient biodegradation of naphthalene by a newly characterized indigenous *Achromobacter* sp. FBHYA2 isolated from Tehran Oil Refinery Complex. *Wat. Sci. Technol.,* **66**, 594–602 (2012).

Frank, J.A., I.R. Claudia, S. Sharma, J.S. Weisbaum, B.A. Wilson and G. J. Olsen: Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl. Environ. Microbiol.,* **74**, 2461–2470 (2008).

García, M.T., A. Ventosa and E. Mellado: Catabolic versatility of aromatic compound-degrading halophilic bacteria. *FEMS Microbiol. Ecol.,* **1**, 97-109 (2005).

Haritash, A.K and C.P. Kaushik: Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. *J. Haz. Mat.,* **169**, 1-15 (2009).

Juhasz, A.L., M.L. Britz and G.A. Stanley: Degradation of fluoranthene, pyrene, benz[a]anthracene and dibenz[a,h]anthracene by *Burkholderia cepacia*. *J. Appl. Microbiol.,* **83**,189-198 (1997).

Juhasz, A.L., G.A. Stanley and M.L Britz: Microbial degradation and detoxification of high molecular weight polycyclic aromatic hydrocarbons by *Stenotrophomonas maltophilia* strain VUN 10,003 *Lett. Appl. Microbiol*., **30**, 396-401 (2000).

Kanaly, R.A., S. Harayama and K. Watanabe: *Rhodanobacter* sp. strain BPC1 in a benzo[*a*]pyrene mineralizing bacterial consortium. *Appl. Environ. Microbiol.,* **68**, 5826-5833 (2002).

Kanaly, R.A and S. Harayama: Biodegradation of high-molecular weight polycyclic aromatic hydrocarbons by bacteria. *J. Bacteriol.,* **182**,2059-2067 (2000).

Kasai, Y, H. Kishira and S. Harayama: Bacteria belonging to the genus *Cycloclasticus* play a primary role in degradation of aromatic hydrocarbons released in a marine environment. *Appl. Environ. Microbiol.,* **68**, 5625-5633(2002).

Kiyohara, H., K.Nagao and K.Yana: Rapid screen for bacteria degrading water-insoluble, solid hydrocarbons on agar plates. *App. Environ. Microbiol.,* **43**,454-457 (1982).

Li, J and R. Bai: Effect of a commercial alcohol ethoxylate surfactant (C11–15E7) on biodegradation of phenanthrene in a saline water medium by *Neptunomonas naphthovorans*. *Biodegradation.,* **16**, 57-65 (2005).

Martin, F.F and R.S. Pexioto: Biodegradation of petroleum hydrocarbons in hypersaline environments. *Braz. J. Microbiol.,* **43**, 865-872 (2012).

McKew, B.A., F.Coulon, A.M. Osborn, K.N. Timmis and T.J. McGenity: Determining the identity and roles of oil-metabolizing marine bacteria from the Thames estuary, UK. *Environ. Microbiol.,* **9**, 165-176 (2007).

Menzie, C.A., B.B. Potocki and J. Santodonato: Exposure to carcinogenic PAH in the environment. *Environ. Sci. Technol.,* **26**,1278-1284 (1992).

Nalin, R., P. Simonet, T.M. Vogel and P. Normand: *Rhodanobacter lindaniclasticus* gen. nov., sp. nov., a lindane-degrading bacterium. *Int. J. Sys. Bacteriol.,* **49**, 19-23 (1999).

Nikolausz, M., U. Kappelmeyer, A. Székely, A. Rusznyák, K. Márialigeti and M. Kästner: Diurnal redox fluctuation and microbial activity in the rhizosphere of wetland plants. *Eur. J. Soil Biol.,* **44**, 324-333 (2008).

Penet, S., R. Marchal, A. Sghir and F. Monot: Biodegradation of hydrocarbon cuts used for diesel oil formulation. *Appl. Microbiol. Biotechnol.,* **66**, 40-47 (2004).

Roy, M., P. Khara, S. Basu and T.K. Dutta: Catabolic versatility of *Sphingobium* sp. strain PNB capable of degrading structurally diverse aromatic compounds. *J. Biorem. Biodeg.,* **4**, 1-6 (2013).

Sivaraman, C., A. Ganguly, M. Nikolausz and S. Mutnuri: Isolation of hydrocarbonoclastic bacteria from bilge oil contaminated waters. *Int. J. Environ. Sci. Technol.,* **8**, 461-470 (2011).

Sohn, J.H., K.K. Kwon, J.H. Kang, H.B. Jung and S.J. Kim: *Novosphingobium pentaromativorans* sp. nov., a high-molecular-mass polycyclic aromatic hydrocarbon-degrading bacterium isolated from estuarine sediment. *Int. J. Syst. Evol. Microbiol.,* **54**, 1483-1487 (2004).

Supaka, N., P. Pinphanichakarna, K. Pattaragulwanita, S. Thaniyavarna, T. Omorib and K. Juntongjin: Isolation and characterization of a phenanthrene degrading *Sphingomonas* sp. strain P2 and its ability to degrade fluoranthene and pyrene via cometabolism. *Sci.Asia.,* **27**, 21-28 (2001).

Székely, A.J., R. Sipos, B. Berta, B. Vajna, C. Hajdú and K. Márialigeti: DGGE and T-RFLP analysis of bacterial succession during mushroom compost production and sequence-aided T-RFLP profile of mature compost. *Microb. Ecol.,* **57**, 522-533 (2009).

Tam, N.F.Y., C.L. Guo, W.Y. Yau and Y.S. Wong: Preliminary study on biodegradation of phenantherene by bacteria isolated from mangrove sediments in Hong Kong. *Mar. Poll. Bull.,* **45**, 316-324 (2002).

Wilson, S.C and K.C. Jones: Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): A review. *Environ. Pollut.,* **81**, 229-49 (1993).

Yu, S.H., L. Ke, Y.S. Wong and N.F.Y. Tam: Degradation of polycyclic aromatic hydrocarbons (PAHS) by a bacterial consortium enriched from mangrove sediments. *Environ. Int.,* **31**, 149-154 (2005).

Yuan, S.Y., S.H. Wein and B.V. Chang: Biodegradation of polycyclic aromatic hydrocarbons by a mixed culture. *Chemosphere,* **41**, 1463-1468 (2000).

Zhao, H.P., Q.S. Wu, L. Wang, X.T. Zhao, H.W. Gao: Degradation of phenanthrene by bacterial strain isolated from soil in oil refinery fields in Shanghai China. *J. Haz. Mat.,* **164**, 863–869 (2009).