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Development of the bacterial consortia for the degradation of benzo[a]pyrene, pyrene from hydrocarbons waste

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KEYWORDS

Benzo[a]pyrene

Pyrene

Consortia

PAHs

Bioremadiation

ABSTRACT

The environment is heavily populated with polycyclic aromatic hydrocarbons (PAHs), which are dangerous to human health. Degradation and cleaning of PAH chemicals from water and soil regions are crucial due to their chemical and biological impacts and persistent nature. In this study, we found that a very efficient bacterial consortium A-LOBP-19A+LOP-9 (99.62%) for benzo[a]pyrene up to 1000ppm and B-LOP-9 +GWP-2 (93.8%) for pyrene up to 2000ppm concentration degradation and it was done in MSM medium with isolated bacterial strains and incubated at 37° C for 50 days and 30 days respectively. This consortium consisting of the *Mycobacterium vaanbaalenii* GWP-2 (ON715011), *Staphylococcus aureus* LOP-9(ON715121), and *Stutzerimonas stutzeri* (LOBP-19A) OP389146, and these have capabilities of mentioned PAHs. The HPLC analysis suggested that both benzo[a]pyrene and pyrene degraded through peaks by both consortia. Degraded metabolites were identified by GC-MS and reported the presence of Phthalic acid, Naphthalene, 1,4-benzodicarboxylic acid, Butoxyacetic acid, Benzeneacetic acid and benzo [a]pyrene-1,6-dione. Thus, the study demonstrated efficient bacterial community enhancement for PAHs (benzo[a]pyrene, pyrene) decomposition, and these can be further explored for the cleanup of hydrocarbons pollution.

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1 Introduction

Leakage of petroleum products during oil exploration, disposal, and transportation from drilling sites to refineries harms surrounding agricultural areas and aquatic bodies (Johnston et al. 2019). Accidental and intentional leakage, as well as natural environmental pollution, have presented a significant risk to flora and fauna by introducing harmful compounds into the food chain, including the combinations of various hydrocarbons, metals, and so on (Militon et al. 2010); complex chemical composition, petrol product can have several adverse impacts (Aguilera et al. 2010). Toxicity depends on the secretion of petrol products, dose, route, and organisms, and it can cause rapid death (Kumari and Chandra 2022; Donald et al. 2023). Some oil products have the potential for bio-accumulation within sensitive sea species and transfer to the next phase of the food chain via trophic transfer (Perhar and Arhonditsis 2014). Furthermore, because of the higher expense of secure and appropriate dumping, this problem is exacerbated by improper disposal methods (Rahman et al. 2009). As a result of harmful petroleum contamination, developing a bioremediation technique is critical to disinfecting harmed areas. Because exogenously functional microorganisms typically refuse to carry out the desired stage in an unknown environment, the achievement of biodegradation technology mainly depends on the degrading abilities of various species of microorganisms (Diaz-Ramrez et al. 2003; Venosa and Zhu 2003). Petroleum products showing hydrocarbons became acquainted, demonstrating selection enhancement and hereditary changes (Patowary et al. 2016). Habitual microorganisms can react to pollution containing hydrocarbons in only a brief amount of time and have an advanced biological degradation rate than populations that have never been exposed to such circumstances (Murphy et al. 2021). As a result, the isolation of naturally occurring microorganisms with oil-degrading capacity from an explicitly polluted environment may hold the possibility for the remediation of such contaminated areas. Indeed, such microorganisms are widely regarded as the most efficient hydrocarbon-degrading agents in that environment (Patowary et al. 2016). Although an individual microbe can only process a restricted spectrum of hydrocarbon substrates, the degradation of complex hydrocarbon mixtures generally requires the collaboration of multiple species of bacteria (Das and Chandran 2011). As a result, conglomerations of heterogeneous communities and diverse enzyme capacities are needed to speed up and amplify the value and scope of petrol product degradation (Ozaki et al. 2007). Despite the occurrence of the diverse range of aromatic compounds demeaning microbial organisms, the establishment of particular bacteria on hydrocarbon substrates may be limited by a variety of variables, such as substrate resistance and low solubility of hydrophobic substances in water-based, which limits the potential to biological degradation (Joutey et al. 2013; Zakaria et al. 2021). Some species of the microorganism may release key degrading enzymes and growth factors, whereas some species may be capable of producing biosurfactants, resulting in increased solubilization of polar aromatic compounds for enhanced consumption by bacteria (Patowary et al. 2016).

The study's objective was to develop bacterial consortia from hydrocarbon-contaminated soil from the diesel spilling site in Lucknow, India and create an effective degradation of the inhabitant oil spill region. Three bacterial isolates, namely LOP-9, GWP-2 and LOBP-19A, were selected from two distinct hydrocarbon-contaminated areas, which were chosen for efficient crude oil degradation; this method was described and accepted in the previous study (Kumari et al. 2022; Kumari and Chandra 2023). The consortium, which comprises three bacterial strains, namely *Mycobacterium vaanbaalenii* GWP-2 (ON715011), *Staphylococcus aureus* LOP-9(ON715121), and *Stutzerimonas stutzeri* (LOBP-19A) OP389146 were identified based on their 16s-rRNA sequencing (identified by 16s rRNA sequencing). Consortium A, comprised of LOBP-19A and LOP-9, showed degradation up to 99.62% of benzo[a]pyere after 50 days of incubation, consortia B prepared by LOP-9 and GWP-2 strains with pyrene, which was 93.8% degraded within 30days. FTIR (Fourier transform infrared) and GCMS (Gas chromatography-mass spectrometer) techniques were analyzed, which demonstrates that these consortia eliminated several kinds of petroleum hydrocarbons, including various aliphatic and aromatic hydrocarbons, in comparison to abiotic control.

2 Materials and Methods

2.1 Chemical and reagents

Benzo[a]pyrene (99%) was bought from TCI (Tokyo Chemical Industry) India Pvt. Ltd. Loba Chemie Pvt Ltd provided the HPLC grade 99.8% acetone.

2.2 Sample collection

A diesel-contaminated soil sample was collected from a fuel leaking area near the Charbagh railway station (Lat N 26°49'48.9648" Long E 80°55'26.0436"), Lucknow, Uttar Pradesh, India. Ten grams of soil were collected in autoclaved plastic poly-bag and stored at 4°C until further investigation.

2.3 Preparation of stock solution

In a sterilized individual glass vial, benzo[a]pyrene and pyrene were dissolved in acetone and made a concentration of 1000 ppm for stock production. Before use, the amber vial was airtight, covered in aluminium foil, and kept in the dark at 4°C.

Figure 1 Sample sites A) Hydrocarbons contaminated soil B) Petrochemical plant effluent

2.4 Preparation of medium and inoculum

MSM media was prepared, and fresh bacterial culture with carbon source in individual conical flasks and inoculated with GWP-2, LOP-9 and LOBP -19 A strains, labelled and incubated at 37°C and 130rpm orbital shaker for five days. Bacterial growth should be 1.00 OD by spectrophotometer for biodegradation assay at 600nm OD. Bacterial strains have been isolated as described in the previous study (Kumari et al. 2022; Kumari and Chandra 2023).

2.5 Preparation of bacterial consortia of pyrene and benzo[a]pyrene

Two flasks were prepared to contain 150ml MSM medium, one for benzo[a]pyrene and the second for pyrene degradation by LOBP-19A+LOP-9 and LOP-9+GWP-2 bacterial consortia, respectively. 1000ppm benzo[a]pyrene and 2000 ppm pyrene were included in the prepared medium flask and left for complete evaporation of acetone in the flasks. 1ml LOP-9 and GWP-2 bacterial culture inoculated in pyrene flask, and LOBP-19A and LOP-9 bacterial strains inoculated in benzo[a]pyrene flask. Both flasks were incubated at 37°C, 140 rpm orbital shaker temperature will different for both flasks because benzo[a]pyrene have not easily degrade in a few days, so that benzo[a]pyrene flask was incubated for 50 days and pyrene flask for 30 days. Consortium bacterial growth was checked every 5 days intervals, and the growth curve in OD was observed by spectrophotometer at 600nm. The total benzo[a]pyrene and pyrene degradation analysis were performed with the total standard concentration in HPLC (Aziz et al. 2018; Mandal and Das 2017).

$$\% \text{ of Degradation} = (C_0\text{-}Ce)/C_0 \times 100$$

Where C_0=initial conc. of degradation compound (ppm)

Ce= final conc. of degraded compound (ppm)

2.6 Sample extraction (Liquid-liquid)

The extraction of different compounds in the liquid medium by separating funnel or centrifugation methods is called liquid-liquid extraction. Benzo[a]pyrene and pyrene from bacterial growth culture were extracted (liquid-liquid extraction) with ethyl acetate (1:1), and the organic phase was collected and re-extracted with the same amount of ethyl acetate, and managed organic phase and left for complete evaporation. The evaporated part was diluted in 2ml ethyl acetate and filtered by 0.22µm (Schneider et al. 2021). An aliquot of this sample was prepared containing ethyl acetate solution before HPLC and GCMS analysis.

2.7 HPLC analysis

The distribution of the analysis (sample) throughout a mobile phase (eluent) and a stationary phase (column packing material) is the foundation of the HPLC separation principle. The molecules are retarded while passing through the stationary phase, based on the chemical structure of the molecule. A sample's "on-column" time is analyzed by the precise intermolecular interactions between the sample's molecules and the packing material (Lunn 2005).

Degraded compounds were monitored via HPLC unit (LC-20AP, SHIMADZU, JAPAN) with a C18 column (150 mm × 4.5 µm) with dual λ absorbance detector, and the sample was input by the manual injector. The mobile phase is an isocratic solution flowing at a constant rate of 1.5 mL/min at room temperature. Each analysis continued for a total of 15 minutes. (Nzila et al. 2022).

2.8 Quantitative assay (GCMS analysis)

In the quantitative assay, 50 days old bacterial crude culture (LOBP-19A+LOP-9) and LOP-9+GWP-2 culture incubated for 30 days with the concentration of 1000ppm benzo[a]pyrene and pyrene (2000ppm), respectively, residual were analyzed by GCMS. A negative control was also prepared to compare bacterial strains using GCMS peaks (Minuti et al. 2006; Di Lorenzo et al. 2019). GCMS technique was also carried out as described in a previous study (Kumari et al. 2022).

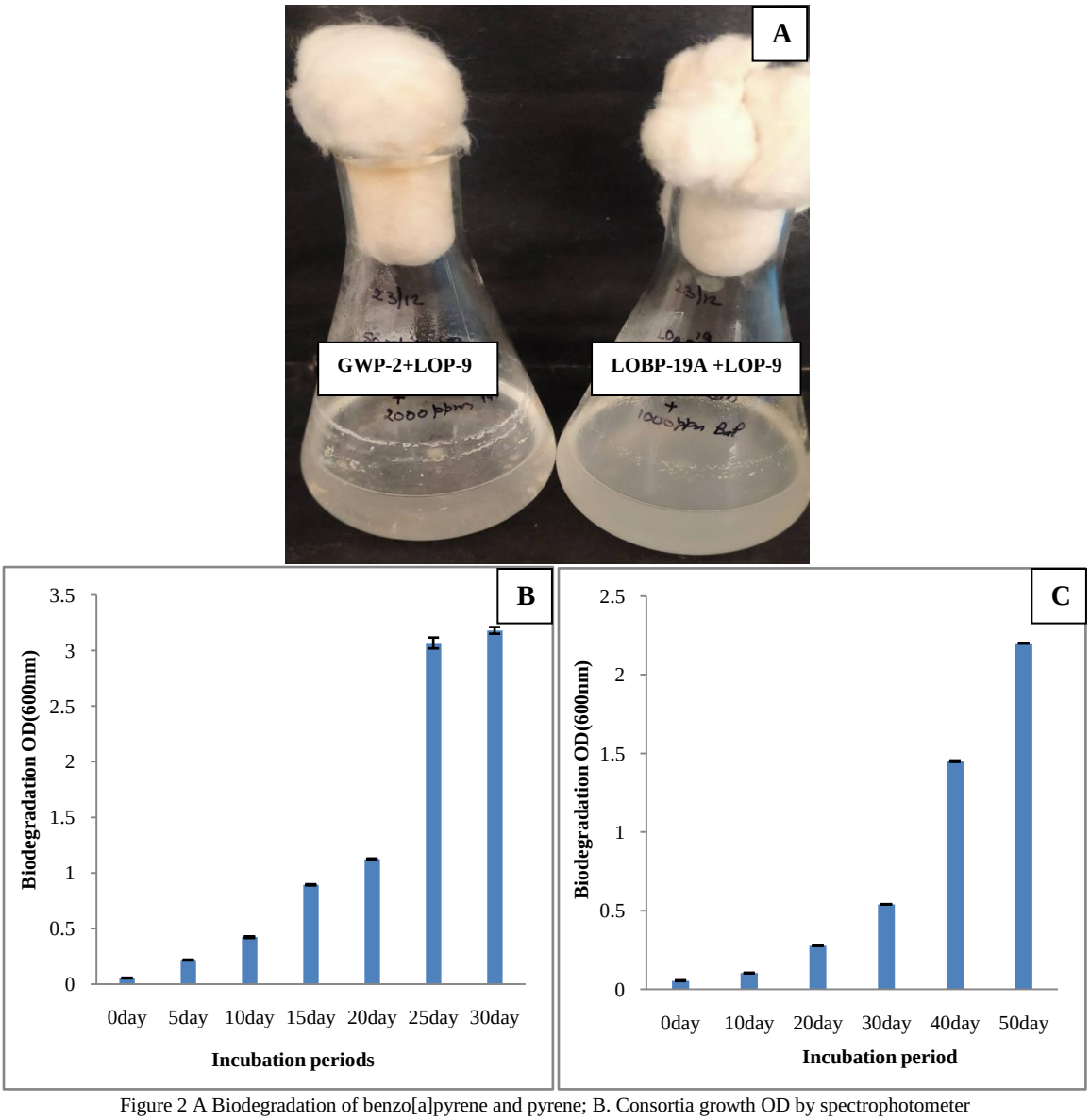


Figure 2 A Biodegradation of benzo[a]pyrene and pyrene; B. Consortia growth OD by spectrophotometer [a. GWP-2+LOP-9; b. LOBP-19A + LOP-9]

Table 1 Percentage of benzo[a]pyrene and pyrene degradation

S.No.	Strains	Degradation (%)
1.	LOP-9	99.3%
2.	GWP-2	97.9%
3.	LOBP-19A	87.5%
4.	LOBP19A+LOP-9(Consortia A) benzo[a]pyrene	99.62%
5.	LOP-9+GWP-2(Consortia B) pyrene	93.8%

2.9 FTIR analysis

Using KBr, IR spectra were collected using a Shimadzu IR affinity-1 FTIR spectrophotometer and the sample was prepared using centrifugal at 10,000 rpm for 10 minutes. The supernatant was preserved via a vacuum dryer. The dried product was extensively combined with KBr after being coarsely powdered. The spectral resolution was 4 cm, and the spectral area was between 3500 and 700 cm^{-1} (Selvi et al. 2014).

2.10 Statistical analysis

In statistical analysis, the T-test was applied to compare the means of benzo[a]pyrene and pyrene degrading bacterial consortia. MS Excel was used to examine the study data and calculate the significance level for each parameter.

3 Results and discussion

3.1 Degradation of benzo[a]pyrene and pyrene by bacterial consortia

Two bacterial consortiums were prepared, in which pyrene was degraded 93.8% in the 30 days and benzo[a]pyrene 99.62% degraded in the 50-day incubation period. The bacterial growth in the biodegradation experiments is shown in Figure 2; the pyrene degradation assay observed that the OD of the LOP-9+GWP-2 consortium was 3.180.03 after 30 days and 2.240.009 after 50 days for the LOP-9+LOBP-19A consortium (optical density checked by spectrophotometer). The removal of benzo[a]pyrene and pyrene was preceded by a prolonged biodegradability lag period, indicating that the lack of benzo[a]pyrene and pyrene removal during the incubation period may be due to poor degradation rates rather than a lack of benzo[a]pyrene and pyrene degradable capacity.

The maximum consortium (*Pseudomonas sp.* ASDP1, *Burkholderia sp.* ASDP2, and *Rhodococcus sp.* ASDP3) rate of growth has been reported to be 0.060/h, both inorganic and organic nutrients, as well as various surfactants, did not affect pyrene degradation (Vaidya et al. 2017). Significantly PAHs degradation has been previously reported increased by mixture of *Selenastrum capricornutum* and *Mycobacterium* sp. strain A1-PYR culture (Ghosal et al. 2016). A previously reported study suggested that consortium degraded 41% of benzo[a]pyrene concentration within 8 days (Aziz et al. 2018).

3.2 HPLC analysis

High-performance liquid chromatography (HPLC) quantification indicated that 99.62% benzo[a]pyrene degraded by LOBP-19A and LOP-9 bacterial consortia, and 93.8% pyrene was degraded by LOP-9 and GWP-2 bacterial consortia as compared with used standard concentration (Figure 3C). Bacterial consortium degradation rate were compared with single bacterial strains degradation of benzo[a]pyrene and pyrene (Table 1).

In previous research, pyrene and benz[a]pyrene degradation have been shown in *M. Luteus*, and BaP is degraded by *Bacillus spp*., including *B. cereus* and *B. vireti* (Nzila et al. 2023; Mohandass et al. 2012). According to Kristanti and Hadibarata (2015), 59% of BaP disappeared in just 20 days. In anaerobic conditions, strain PYR1 has been reported to degrade 94% of pyrene within 15 days and to decompose benzo[a]pyrene after 35 days (Yan et al. 2017). In the current research, degradation of benzo[a]pyrene by the bacterial consortium (LOBP19-A+LOP-9) was given 99.62% degradation, significantly higher than single bacterial strains LOBP-19A. LOP-9 and GWP-2 consortium had a 93.8% degradation rate, which decreased compared to single strain LOP-9 and GWP-2 (described in previous studies).

3.3 GCMS analysis

A qualitative and quantitative examination of their GC traces can illustrate the assessment of benzo[a]pyrene and pyrene degraded residual. The results of this GCMS demonstrated that pyrene and benzo[a]pyrene in a 250-mL flask of MSM broth were degraded into a variety of by-products as a result of the action of the bacterial strains LOBP-19A+LOP-9 and LOP-9+GWP-2. These residual metabolites' GC-MS analysis identified various substances.

These metabolites helped in pyrene degrading metabolic pathways and co-metabolism (Figure 4 D). Metabolites compared with used standard pyrene in the concentration in 2000ppm from 1000ppm standard in acetone concentration. Pyrene showed in GC-MS at 23.15 RT in the chromatogram, and pyrene disappeared in LOP-9 and GWP-2 bacterial consortium after 30 30-day incubation period.

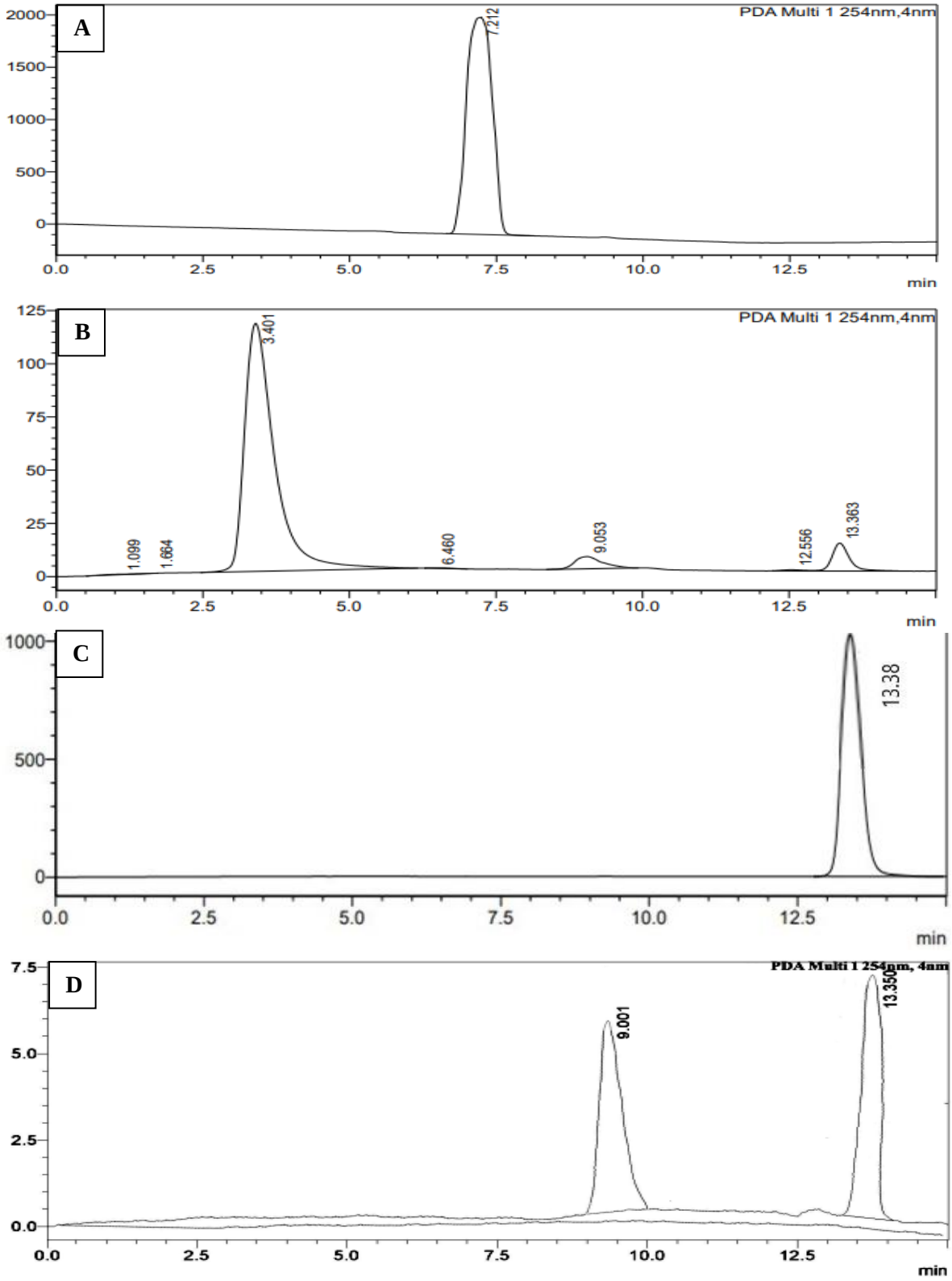
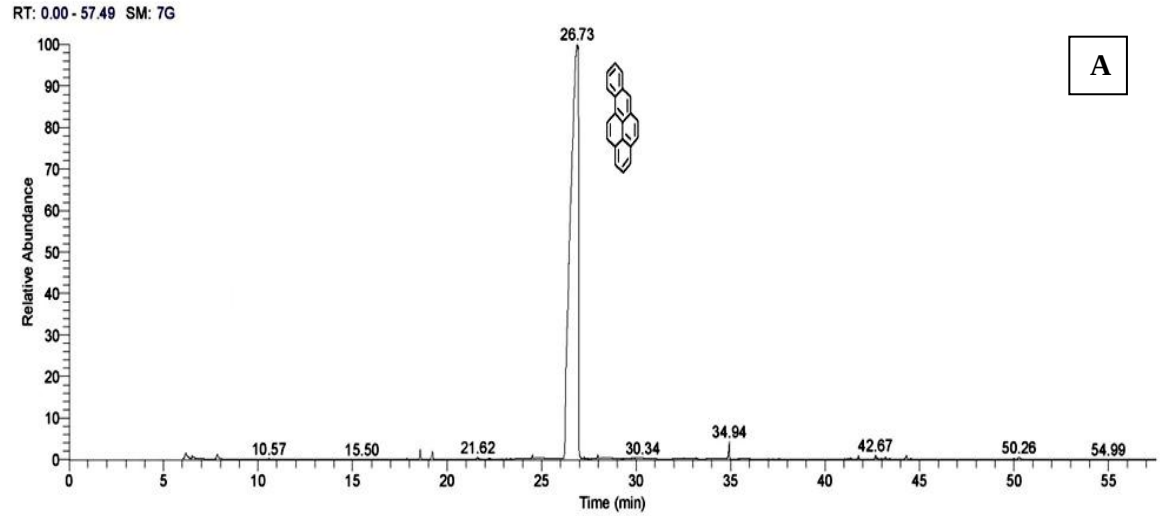
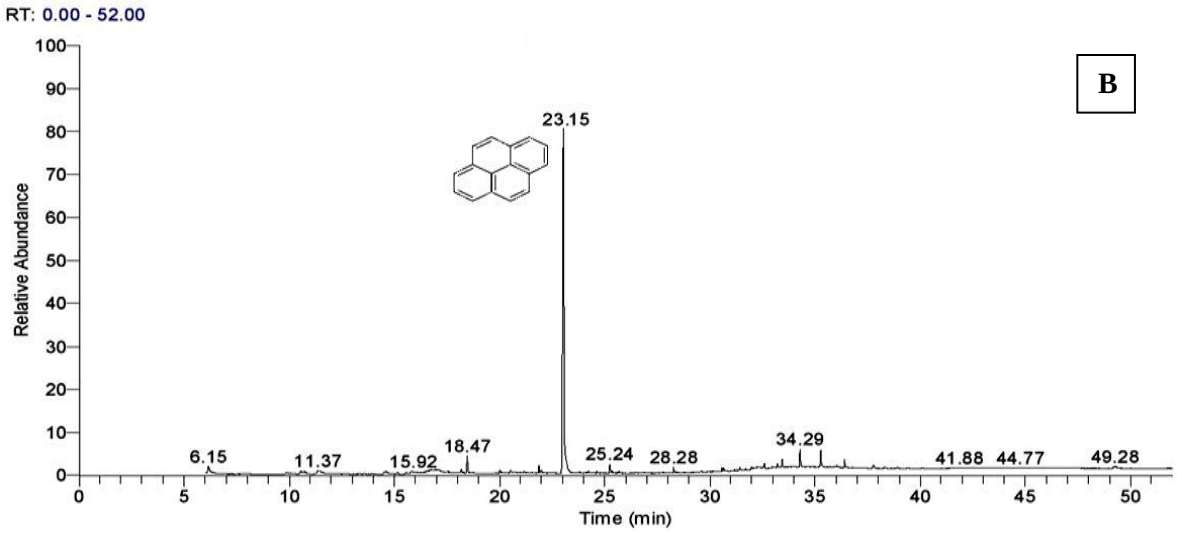
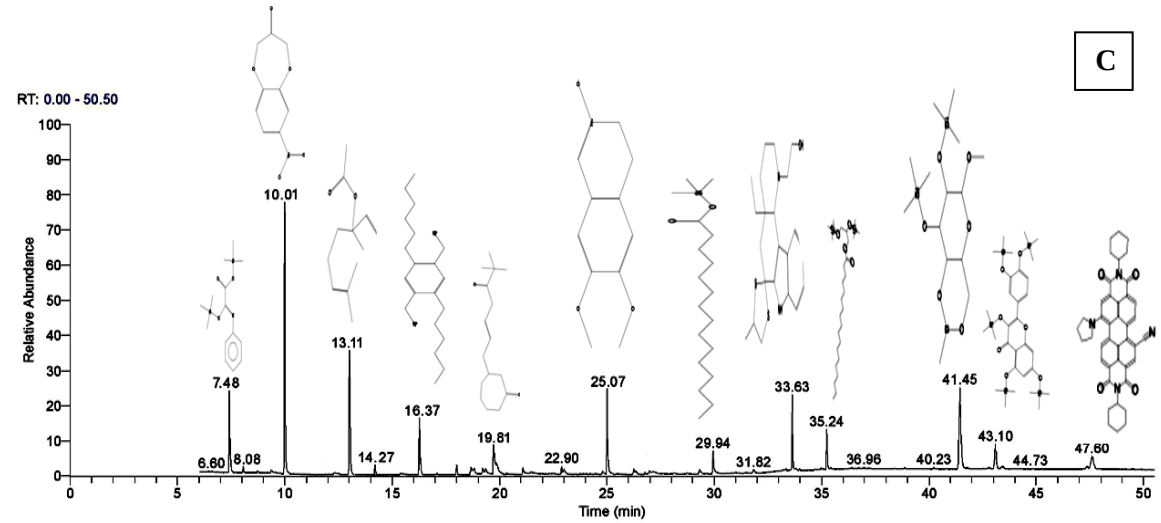


Figure 3 HPLC chromatogram A. Benzo[a]pyrene Std, B. Consortia (LOP-9+LOBP-19A), C. Pyrene Std, and D. Consortia (LOP-9+GWP-2)


RT: 0.00 - 57.49 SM: 7G
A
Relative Abundance
Time (min)
26.73
10.57
15.50
21.62
30.34
34.94
42.67
50.26
54.99


RT: 0.00 - 52.00
B
Relative Abundance
Time (min)
23.15
6.15
11.37
15.92
18.47
25.24
28.28
34.29
41.88
44.77
49.28


RT: 0.00 - 50.50
C
Relative Abundance
Time (min)
10.01
7.48
13.11
16.37
25.07
33.63
41.45
6.80
8.08
14.27
19.81
22.90
29.94
31.82
35.24
36.96
40.23
43.10
44.73
47.60

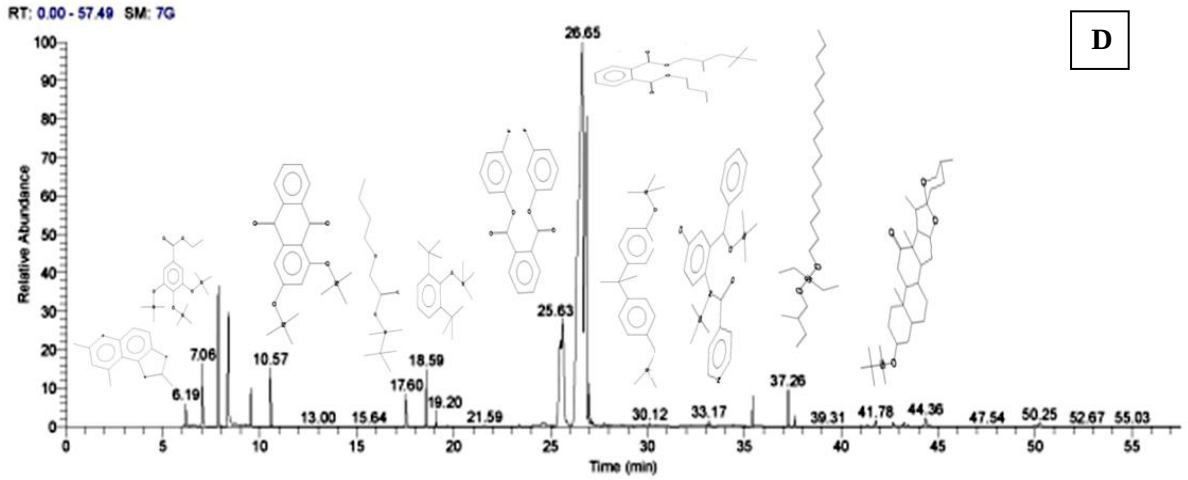


Figure 4 Chromatogram of consortia A. Benzo[a]pyrene std., B. Pyrene std., C. Consortia [LOBP-19A+LOP-9], D. Consortia [LOP-9+GWP-2].

Table 2 Metabolic product of Consortia B (LOP-9+GWP-2)

S. No.	RT	Metabolites (GWP-2+ LOP-9) (after 50 days)	Toxicity
1.	6.19	p-toluenesulfonamide	Irritating to eyes, respiratory system and skin
2.	7.06	3,4,5-trihydroxybenzoic acid ethyl ester	skin and eye irritation
3.	10.57	1,3-dihydroxyanthraquinone	Oral and dermal toxicity
4.	17.60	Butoxyacetic acid	Respiratory, oral and eye irritation
5.	18.59	2,6-bis(tert butyl)phenol	Bronchospasm and pulmonary oedema
6.	19.20	Phthalic acid	Carcinogenic, malformations and reproductive toxicity
7.	19.71	Oxalic acid	Headache, dizziness, nausea and vomiting, convulsions, coma
8.	21.59	Naphthalene	Hemolytic anemia and methemoglobinemia
9.	23.38	Cyclohexane 1,3,5-trimethyl-2-octadecyl	-
10.	24.61	1,4-benzodicarboxylic acid	Irritate the nose, throat and lungs
11.	25.63	Phthalic acid	Carcinogenic, malformations and reproductive toxicity
12.	26.65	Terephthalic acid	Irritate the nose, throat and lungs
13.	27.92	9-octadecenoic acid	-
14.	30.12	Bisphenol	Cytotoxicity, genotoxicity, reproductive toxicity, dioxin-like effects, and neurotoxicity
15.	32.75	9,12-octadecadienoic acid	-
16.	37.26	Silane diethyldecylcloxydodecyloxy	Reproductive and developmental toxicity
17.	41.05	Diethyl(pentafluorobenzyloxy) tetradecycloxy	-

GC-MS chromatogram of the organic compound separated of benzo[a]pyrene degrading metabolites by LOBP-19A and LOP-9 bacterial consortium showed major and minor peaks indicating the occurrence of two bacterial (LOBP-19A+LOP-9) constituents (Figure 4C). The pyrene degrading constituents were characterized by comparing the mass spectra of the constituents with the NIST library. The same compound, oleic acid (cis -9-octadecenoic acid), had been reported in ethanol extract at 10.73 RT (retention time) and methanoic extraction at 13.9 RT by *Staphylococcus aureus* and *Stenotrophomonas maltophilia* in the previous study and present study was at 27.92 RT in ethyl acetate extraction on bacterial viability (Nor et al. 2015). Hexadecanoic acid was found at 23.64 and 27.00 RT in this study, and previous studies have been found at 32.61 and produced by *B. cereus* strain VASB1/TS bacterial constituents (Bayat et al. 2015).

Table 3 Metabolites of consortium (LOP-9+LOBP-19A)

S. No.	RT	Metabolites (LOP-9+LOBP-19A)	Toxicity
1.	6.36	1,2,3,4-tetramethyl-5-(chloromethyl)benzene	Skin and eye irritation
2.	7.46	5,6,7,8-tetrahydro-8,8-dimethyl-2-indolizinecarboxylic acid methyl ester	-
3.	9.05	Butanedioic acid (succinic acid)	*Slight skin irritant and a strong eye irritant*
4.	10.01	Benzo[a]pyrene-1,6-dione	*Carcinogenic, malformations and reproductive toxicity*
5.	11.82	Benzene,[3-chloro-2-propenyl)oxy]-(CAS)	*Irritate the nose, throat and lungs*
6.	13.11	3-acetoxy-3,7-dimethyloocta-1,6-diene	-
7.	14.27	t-butyl 3-(3-methyl-1-butenoxy) propanoate	*Reproductive toxicity*
8.	16.37	2,5-bis(bromomethyl)-1,4-dihexylbenzene	*Severe skin burns and eye damage*
9.	17.30	1-nitro-4-octanol	*Carcinogenicity*
10.	19.81	3-(6,6-dimethyl-5-oxohept-2-enyl)-cycloheptanone	-
11.	21.10	Propenoic acid	Acute eye and dermal irritation effects
12.	22.90	1-heptacosanol	*Oral/Parenteral Toxicity*
13.	23.01	Hexadecane,2-methyl-(CAS)	*CNS depression and gastrointestinal tract irritation*
14.	23.64	Hexadecanoic acid, dimethyl ester	*Low acute toxicities*
15.	25.07	6,7-dimethoxy-3,4-dihydroisoquinoline-N-oxide	Specific *target organ toxicity*
16.	27.00	Hexadecanoic acid	Thrombotic activity
17.	29.94	Tridecanoic acid, trimethyl ester	-
18.	31.82	1,2-dimethylpropyl trifluoroacetate	*Oral/Parenteral Toxicity*
19.	33.63	1,2-dibenzoylbenzo[e]indolizine	
20.	34.09	Benzeneacetic acid	*Specific target organ toxicity*
21.	35.24	2,3-bis [trimethylsilyl] oxypropyl stearate	*Acute toxicity, inhalation*
22.	41.45	Dithioerythritol	*Respiratory tract irritation*
23.	43.10	3,5,7-tri(trimethylsiloxy)-2-[3,4-di(trimethylsiloxy)phenyl]-4H-1-benzopyran-4-one	-
24.	47.60	5[4-(acetylthio)butyl]-15butyl-10,20-diphenylporphyrin	*Slight skin and eye irritation*

Note- According to GC-MS NIST library compound

Table 4 Metabolites functional group

S. No.	Wave number (cm^{-1})	Functional group	GWP-2 + LOP-9	LOBP-19A + LOP-9
1.	3000-2000	Aliphatic C-H Stretching (alkenes, alkanes)	2954.27 (cm^{-1})	2973.39(cm^{-1})
2.	1800-1700	Anhydried simple aliphatic(C=O) stretch,ester,ketone	1731.96 (cm^{-1})	1783(cm^{-1})
3.	1500-1400	C-H aromatic compound, aliphatic group	1447.47 (cm^{-1})	1439.12, (cm^{-1})
4.	1400-1200	C-O carboxy esters, ethers, aromatics, alcohol ether	1374.4 (cm^{-1})	1372.8, 1209.37 (cm^{-1})
5.	1100-1000	C-O bond alkanes, hydroxyl group(O-H)	1097.39 (cm^{-1})	1075.47(cm^{-1})
6.	1000-900	C=C bending (alkene)	938.27(cm^{-1})	975.73(cm^{-1})
7.	900-800	C=C and aromatic ring, C-H bending bending (alkene) inorganic band	847.04(cm^{-1})	884(cm^{-1})

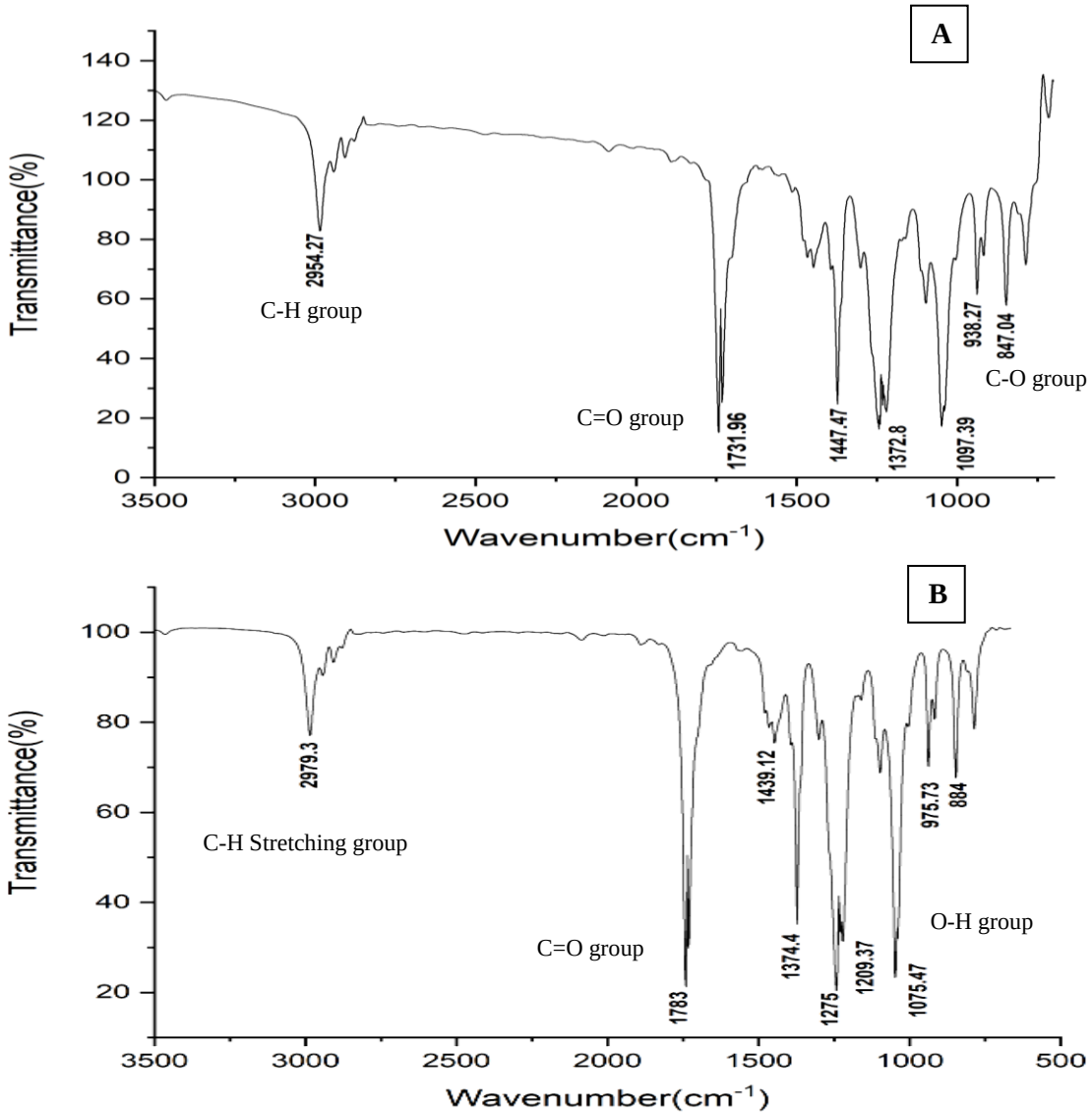


Figure 5 FTIR analysis A. GWP-2+LOP-9 consortia, B. LOBP-19+LOP-9 consortia)

3.4 FTIR analysis

FTIR spectra of pyrene, benzo[a]pyrene degrading metabolites acquired during the degradation phase were compared to demonstrate the change in functional groups between the two compounds and Figure 5 displayed the results. FTIR was used to analyze two bacterial consortium metabolites of degraded benzo[a]pyrene and pyrene. Previous studies reported O-H, C-O, O-H, C-C and C-H vibration in aromatics functional groups (Agrawal and Shahi 2017). In this study, the active group of degrading metabolites (pyrene and benzo[a]pyrene) FTIR graph and Table 4 are represented below (figure 5).

The FTIR library analyzed degrading metabolite's functional groups and bonds. Peaks and wave numbers detailed in the table are 2954.27 (cm^{-1}) in GWP-2+LOP-9 bacterial consortia and 2973.39 (cm^{-1}) produced in LOBP-19A+LOP-9 consortia; these wave numbers were identified as Aliphatic C-H Stretching (alkenes, alkanes) in FTIR library.

Conclusion

The bacterial consortium enriched in MSM and bacterial strains (LOBP-19A, LOP-9 and GWP-2) were used for capable of degrading to different PAHs as pyrene at 2000 ppm and benzo[a]pyrene at 1000 ppm of concentration from 1000ppm standard concentration in acetone. Two consortiums were prepared; one was LOP-9+GWP-2 strains with 2000ppm pyrene, and the second was LOBP-19A+LOP-9 strains with 1000ppm benzo[a]pyrene on environmental conditions. The study proved

that consortium (LOBP-19A and LOP-9) had the better result as compared to single strains, and high benzo[a]pyrene degradation was found (99.62%) where, as LOP-9+GWP-2 consortia had low degradation (93.8%) rate as compared to single bacterial strains pyrene degradation and standard of pyrene (2000ppm) and benzo[a]pyrene (1000ppm). The degradation rate and residual products were identified by HPLC and GCMS, respectively. LOBP-19A+LOP-9 was degraded benzo[a]pyrene, and its degraded metabolites are benzo [a]pyrene-1,6-dione, Butanedioic acid (succinic acid), 2,5-bis(bromomethyl)-1,4-dihexylbenzene, Benzeneacetic acid; besides LOP-9 and GWP-2 bacterial consortium degraded pyrene and degraded metabolites are Naphthalene, 1,4-benzodicarboxylic acid, Terephthalic acid, 9-octadecenoic acid, and these metabolites were degraded with the help of bacterial enzymes secretion and identified by GC-MS. Both aromatic hydrocarbons (pyrene and benzo[a]pyrene) were degraded by bacterial consortium and C-H Stretching (alkenes, alkanes), C-O carboxy esters, ethers, aromatics, alcohol ether and C-O bond alkanes, hydroxyl group (O-H) groups are identified by FTIR analysis which were produced by bacterial consortium degradation. These bacterial consortia may be efficient in the degradation of pyrene and benzo[a]pyrene, and the study may play a significant role in the breakdown of further PAHs. The current study demonstrates the prospective PAHs-degrading bacterial consortium under environmental conditions, which is economical and beneficial to environmental promotion.

Conflict of interest

The authors declare no conflict of interest.

References

Agrawal, N., & Shahi, S. K. (2017). Degradation of polycyclic aromatic hydrocarbon (pyrene) using novel fungal strain *Coriolopsis byrsina* strain APC5. *International Biodeterioration & Biodegradation*, *122*, 69-81.

Aguilera, F., Méndez, J., Pásaro, E., & Laffon, B. (2010). Review on the effects of exposure to spilled oils on human health. *Journal of Applied Toxicology*, *30*(4), 291-301.

Aziz, A., Agamuthu, P., Alaribe, F. O., & Fauziah, S. H. (2018). Biodegradation of benzo [a] pyrene by bacterial consortium isolated from mangrove sediment. *Environmental technology*, *39*(4), 527-535.

Bayat, Z., Hassanshahian, M., & Hesni, M. A. (2015). Enrichment and isolation of crude oil degrading bacteria from some mussels collected from the Persian Gulf. *Marine Pollution Bulletin*, *101*(1), 85-91.

Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology research international*, *2011*, 941810. https://doi.org/10.4061/2011/941810.

Di Lorenzo, R. A., Lobodin, V. V., Cochran, J., Kolic, T., & Besevic, S. (2019). Fast gas chromatography-atmospheric pressure (photo) ionization mass spectrometry of polybrominated diphenylether flame retardants. *Analytica chimica acta*, *1056*, 70-78.

Díaz-Ramírez, I. J., Ramírez-Saad, H., Gutiérrez-Rojas, M., & Favela-Torres, E. (2003). Biodegradation of Maya crude oil fractions by bacterial strains and a defined mixed culture isolated from *Cyperus laxus* rhizosphere soil in a contaminated site. *Canadian Journal of Microbiology*, *49*(12), 755-761.

Donald, C. E., Nakken, C. L., Sørhus, E., Perrichon, P., & Jørgensen, K. B. (2023). Alkyl-phenanthrenes in early life stage fish: differential toxicity in Atlantic haddock (*Melanogrammus aeglefinus*) embryos. *Environmental Science: Processes & Impacts*, *25*(3), 594-608.

Ghosal, D., Ghosh, S., Dutta, T. K., & Ahn, Y. (2016). Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. *Frontiers in microbiology*, *7*, 1369. https://doi.org/10.3389/fmicb.2016.01369.

Johnston, J. E., Lim, E., & Roh, H. (2019). Impact of upstream oil extraction and environmental public health: A review of the evidence. *Science of the Total Environment*, *657*, 187-199.

Joutey, N. T., Bahafid, W., Sayel, H., & El Ghachtouli, N. (2013). Biodegradation: involved microorganisms and genetically engineered microorganisms. *Biodegradation-life of science*, *1*, 289-320.

Kristanti, R. A., & Hadibarata, T. (2015). Biodegradation and identification of transformation products of fluorene by ascomycete fungi. *Water, Air, & Soil Pollution*, *226*, 1-6.

Kumari, B., Chandra, H., & Chandra, R. (2022). Detection of pyrene degrading bacterial strains (LOP-9 *Staphylococcus aureus* and GWP-2 *Mycobacterium vaanbaalenii*) and their metabolic products. *Cleaner Chemical Engineering*, *4*, 100080.

Kumari, B., & Chandra, R. (2022). A Review on Bacterial Degradation of Benzo[a]pyrene and Its Impact on Environmental Health. *Journal of Experimental Biology and Agricultural Sciences*, *10*(6), 1253–1265. https://doi.org/10.18006/2022.10(6).1253.1265

Kumari, B., & Chandra, R. (2023). Benzo [a] pyrene degradation from hydrocarbon-contaminated soil and their degrading

metabolites by Stutzerimonas stutzeri (LOBP-19A). *Waste Management Bulletin*, *1*(3), 115-127.

Lunn, G. (2005). *HPLC methods for recently approved pharmaceuticals*. John Wiley & Sons.

Mandal, S. K., & Das, N. (2017). Biodegradation of benzo [a] pyrene by Rhodotorula sp. NS01 strain isolated from contaminated soil sample. *Research Journal of Pharmacy and Technology*, *10*(6), 1751-1757.

Militon, C., Boucher, D., Vachelard, C., Perchet, G., Barra, V., et al. (2010). Bacterial community changes during bioremediation of aliphatic hydrocarbon-contaminated soil. *FEMS Microbiology Ecology*, *74*(3), 669-681.

Minuti, L., Pellegrino, R. M., & Tesei, I. (2006). Simple extraction method and gas chromatography–mass spectrometry in the selective ion monitoring mode for the determination of phenols in wine. *Journal of Chromatography A*, *1114*(2), 263-268. https://doi.org/10.1016/j.chroma.2006.02.068

Mohandass, R., Rout, P., Jiwal, S., & Sasikala, C. (2012). Biodegradation of benzo [a] pyrene by the mixed culture of *Bacillus cereus* and *Bacillus vireti* isolated from the petrochemical industry. *Journal of Environmental Biology*, *33*(6), 985.

Murphy, S. M., Bautista, M. A., Cramm, M. A., & Hubert, C. R. (2021). Diesel and crude oil biodegradation by cold-adapted microbial communities in the Labrador Sea. *Applied and Environmental Microbiology*, *87*(20), e00800-21.

Nor, N. M., Hadibarata, T., Zubir, M. M. F. A., Lazim, Z. M., Adnan, L. A., & Fulazzaky, M. A. (2015). Mechanism of triphenylmethane Cresol Red degradation by Trichoderma harzianum M06. *Bioprocess and Biosystems Engineering*, *38*, 2167-2175.

Nzila, A., Musa, M. M., Afuecheta, E., Al-Thukair, A., Sankaran, S., Xiang, L., & Li, Q. X. (2023). Benzo [A] Pyrene Biodegradation by Multiple and Individual Mesophilic Bacteria under Axenic Conditions and in Soil Samples. *International Journal of Environmental Research and Public Health*, *20*(3), 1855.

Nzila, A., Musa, M. M., Afuecheta, E., Thukair, A., Sankaran, S., Xiang, L., & Li, Q. X. (2022). Benzo [a] pyrene biodegradation by multiple and individual mesophilic bacteria in axenic conditions and in soil samples. *bioRxiv*, 2022-05.

Ozaki, S., Kishimoto, N., & Fujita, T. (2007). Change in the predominant bacteria in a microbial consortium cultured on media containing aromatic and saturated hydrocarbons as the sole carbon source. *Microbes and Environments*, *22*(2), 128-135.

Patowary, K., Patowary, R., Kalita, M. C., & Deka, S. (2016). Development of an efficient bacterial consortium for the potential remediation of hydrocarbons from contaminated sites. *Frontiers in microbiology*, *7*, 1092.

Perhar, G., & Arhonditsis, G. B. (2014). Aquatic ecosystem dynamics following petroleum hydrocarbon perturbations: A review of the current state of knowledge. *Journal of Great Lakes Research*, *40*, 56-72.

Rahman, P. K., Pasirayi, G., Auger, V., & Ali, Z. (2009). Development of a simple and low cost microbioreactor for high-throughput bioprocessing. *Biotechnology letters*, *31*, 209-214.

Schneider, Y. K., Jørgensen, S. M., Andersen, J. H., & Hansen, E. H. (2021). Qualitative and quantitative comparison of liquid–liquid phase extraction using ethyl acetate and liquid–solid phase extraction using poly-benzyl-resin for natural products. *Applied Sciences*, *11*(21), 10241.

Selvi, A., Salam, J. A., & Das, N. (2014). Biodegradation of cefdinir by a novel yeast strain, *Ustilago* sp. SMN03 isolated from pharmaceutical wastewater. *World Journal of Microbiology and Biotechnology*, *30*, 2839-2850.

Vaidya, S., Jain, K., & Madamwar, D. (2017). Metabolism of pyrene through phthalic acid pathway by enriched bacterial consortium composed of *Pseudomonas*, *Burkholderia*, and *Rhodococcus* (PBR). *3 Biotech*, *7*, 1-15.

Venosa, A. D., & Zhu, X. (2003). Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. *Spill Science & Technology Bulletin*, *8*(2), 163-178.

Yan, Z., Zhang, Y., Wu, H., Yang, M., Zhang, H., Hao, Z., & Jiang, H. (2017). Isolation and characterization of a bacterial strain *Hydrogenophaga* sp. PYR1 for anaerobic pyrene and benzo [a] pyrene biodegradation. *RSC advances*, *7*(74), 46690-46698.

Zakaria, N. N., Gomez-Fuentes, C., Abdul Khalil, K., Convey, P., Roslee, A. F. A., et al. (2021). Statistical optimization of diesel biodegradation at low temperatures by an Antarctic marine bacterial consortium isolated from non-contaminated seawater. *Microorganisms*, *9*(6), 1213.