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Preferential selection of toxic polyaromatic hydrocarbons and their intermediates by *Pseudomonas fragi* driven by catechol dioxygenase-mediated catabolism

Ankita Das, Sourav Debnath, Anand Prakash Maurya, L. Paikhomba Singha, Nandita Das, Piyush Pandey

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Highlights

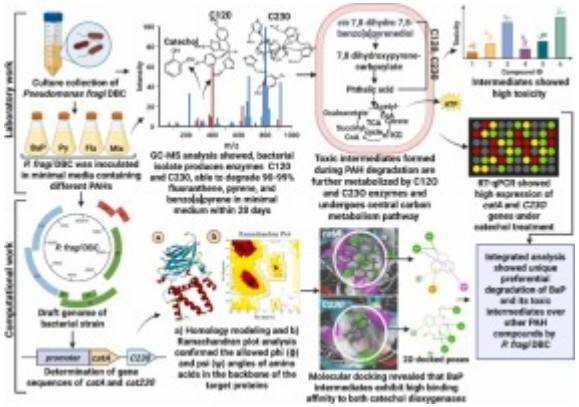
- *Pseudomonas fragi* DBC degrades 90–99 % of high molecular weight PAHs within 28 days.

- Preferential degradation of toxic HMW-PAHs was detected, including benzo(a)pyrene.
- qRT-PCR shows upregulation of dioxygenase genes in response to PAHs, boosting degradation potential.
- Integrative docking, gene-expression, toxicity, GC-MS analyses data confirmed preferential degradation.
- Catechol dioxygenases have higher binding affinity toward benzo(a)pyrene and its intermediates.

Abstract

The extensive use of petroleum crude and its derivatives has caused widespread contamination by polycyclic aromatic hydrocarbons (PAHs), posing serious ecological and health risks. Bioremediation using microbial pathways offers a sustainable solution, yet microbial substrate preference and catabolite repression critically influence PAH degradation efficiency. This study examined the degradation of three high molecular weight PAHs, fluoranthene, pyrene, and benzo(a)pyrene by *Pseudomonas fragi* DBC, focusing on catechol 1,2-dioxygenase and catechol 2,3-dioxygenase. *P. fragi* DBC degraded 90–99% of the PAHs within 28 days, with a marked initial preference for benzo(a)pyrene. Enzyme-substrate analyses revealed higher binding affinity of both dioxygenases for benzo(a)pyrene and its intermediates compared to other PAHs. Toxicity assessments showed that ligands with higher enzyme affinity were more toxic than their degradation products. qRT-PCR analysis indicated increased transcription of both dioxygenase genes in the presence of PAHs, highlighting the strain's enhanced catabolic potential for mineralizing a broad spectrum of PAH pollutants. These findings underscore the crucial role of enzyme-mediated substrate specificity in governing microbial degradation kinetics in complex environmental settings.

Graphical Abstract



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Introduction

Polyaromatic hydrocarbons (PAHs) constitute a group of hazardous organic compounds characterized by multiple fused aromatic rings. These are ubiquitous in the environment and can be found in air, water, soil, and sediment [1]. PAHs have been studied extensively due to their potential health and environmental impacts [2], [3]. PAHs and their intermediates also have the potential to bioaccumulate in the food chain, which increase the chance of human exposure [4]. Due to their long-term persistence, bio-accumulative nature, and harmful effects on ecosystems and human health, regulatory agencies worldwide have established stringent guidelines to monitor and limit PAH concentrations across various environmental matrices. Among the available remediation strategies, microbial bioremediation has emerged as a promising, eco-friendly, and cost-effective approach for PAH degradation [5]. Due to this, bioremediation of PAHs has become a major focus of environmental research over the past few decades with successful applications reported in both terrestrial and marine ecosystems [6], [7]. Some of the reported bacteria in bioremediation studies are *Pseudomonas* spp., *Bacillus* spp., *Mycobacterium* spp., *Rhodococcuss* spp., *Kocuria flava*, *Ochrobactrum anthropic* etc. [3], [8].

The number of aromatic rings in PAHs directly affects their electrochemical stability, persistence, resistance to biodegradation, and carcinogenic index, as these properties becomes more prominent with a greater number of aromatic rings. Conversely, the volatility

of PAHs generally decreases as their molecular weight increases, making it more stable and harder to mineralize [9], [10]. During their degradation, various intermediate compounds are formed, some of which can be more toxic and resistant to further breakdown than the original PAHs [11]. For instance, oxygenated and nitrated PAHs are common degradation byproducts that frequently exhibit higher toxicity than their parent compounds. These intermediates can induce oxidative stress, DNA damage, and other adverse biological effects, which pose risks to both environmental and human health [12], [13]. Moreover, the incomplete degradation of PAHs can lead to the accumulation of stable intermediates that are difficult to mineralize. This persistence highlights the need to monitor both parent PAHs and their transformation products during bioremediation or rhizoremediation efforts.

In natural environments, the presence of multiple carbon sources poses a challenge for bioremediation, as microbes may preferentially utilize more readily available substrates over the target hydrocarbon contaminant, thereby reducing degradation efficiency. The microbes can use simple compounds like glucose, fructose, sucrose and more complex ones like phenolics, aromatics etc. [14]. Mixed PAHs often coexist in the environment along with a complex suite of intermediate compounds formed during degradation generated primarily through microbial processes and abiotic oxidation [15]. These intermediate products accumulate under partial degradation conditions and can be biologically active or toxic. In these situations, the microorganisms prefer to utilize the simple carbon sources first before turning to the more complex ones, resulting in a biphasic growth response due to “catabolite repression” [16]. In contrast, rhizobacteria like *Pseudomonas* sp. exhibit a reverse mechanism called “reverse catabolite repression (rCCR),” where they preferentially utilize high-molecular-weight organic acids and hydrocarbons instead of glucose [17]. In rCCR, the presence of the preferred carbon source enhances the expression of genes involved in the uptake and utilization of alternative carbon sources. For example, the strain *Pseudomonas putida* CSV86 showed a preference for utilizing aromatics first, followed by glucose. Organic acid intermediates generated from aromatic compound metabolism may be responsible for the repression of glucose utilization [18]. However, while glucose-driven catabolite repression is well understood, the potential for additional layers of control like enzyme-level specificity or preferential activity toward PAHs, remains largely unexplored. Degradation of PAHs becomes even more complex when the same dioxygenase enzymes can act on both the parent compound and its intermediate metabolites. In such cases, understanding not only gene expression but also enzyme substrate preference, and consequently the microbial preference for PAHs versus their intermediates, is critical for accurately assessing and optimizing the biodegradation process.

Catechol dioxygenases, including catechol 1,2 dioxygenase (intradiol) and catechol 2,3 dioxygenase (extradiol), cleave catechol and dihydroxylated PAHs intermediates, which aids in their mineralization into central pathways [19]. The dihydroxylated PAHs

intermediates resembles the original PAH structure and molecular weight, as hydroxylation is their primary modification [20]. Intradiol enzymes cleave within the hydroxylated ring, while extradiol enzymes cleave adjacent to it and form a dioxetane intermediate that reacts with oxygen before breaking down into final products [3]. Both enzymes coordinate have an iron cofactor, with intradiol using tyrosine and histidine, and extradiol mainly with histidine and glutamine [21], [22]. Although the sequence similarity of enzymes from different organisms varies, the basic structure and features of the active site in most microorganisms are conserved [23], [24]. These enzymes are produced by various microorganisms, including bacteria, fungi, and actinomycetes, and have been extensively studied for their potential use in PAHs degradation [25]. Catechol 1,2-dioxygenases and catechol 2,3-dioxygenase are encoded by *CatA* and *C23O* respectively and the activity and expression of these genes varies in different microorganisms under varying growth conditions [9], [26], [27], [28]. For example, in *Pseudomonas putida*, *Rhodococcus* sp., and the pyrene-degrading endophyte *Stenotrophomonas maltophilia* PX1, these enzymes are inducible by PAHs and produce intermediates such as 4,5-dihydroxypyrene and salicylate, which are further processed via catechol cleavage by C12O or C23O [29], [30], [31].

In this study, we investigated the catalytic efficiency and substrate affinity of catechol 1,2-dioxygenase (C12O) and catechol 2,3-dioxygenase (C23O) toward various PAHs and their metabolic intermediates during the bioremediation process. Three high molecular weight PAHs (HMW PAH), benzo(a)pyrene (BaP), pyrene (Pyr) and fluoranthene (Fla), were used which has five, four and three benzene rings respectively [32]. The test organism used was *Pseudomonas fragi* DBC, which is known to degrade PAH [26]. Initially, quantitative degradation assays were conducted under different substrate conditions using three representative PAHs: benzo(a)pyrene (BaP), pyrene (Pyr), and fluoranthene (Fla). The catalytic activity of C12O and C23O along with gene expression analysis of *catA* (encoding C12O) and *c23o* (encoding C23O) were assayed in during PAHs degradation. Subsequently, *in silico* toxicity predictions were performed to assess the relative toxicity of the parent PAHs and their intermediate products. The intermediates taken in this study has been given in Fig. 1 and Table 1. Homology modelling of the protein CatA and C23O was carried out using sequences derived from the *P. fragi* DBC genome, followed by molecular docking studies to evaluate the binding interactions between the modelled enzymes and the PAHs and their intermediates. Therefore, the primary objective of this study is to elucidate the relationship between gene expression, enzymatic activity, and the preferential degradation of PAHs and their intermediates. The percentage degradation of PAHs and corresponding enzyme activities along with expression were quantified to assess biodegradation efficiency and enzymatic contribution. Molecular docking revealed substrate binding affinities and specific interactions, which contributes to understanding of enzyme preference and specificity. Thus, this study provides mechanistic insights into the sequential interactions between PAH-degrading enzymes and their substrates, for the understanding of bacterial PAH - catabolic process. The

study provides novel evidence for the enzymatic preferential selection of toxic PAH intermediates, a phenomenon that has not been extensively discussed in the literature.

Unlike most previous studies on *Pseudomonas* spp., which have primarily focused on the degradation of single PAH substrates or general pathway elucidation, the present work uniquely integrates biochemical, molecular, and computational analyses to demonstrate that *P. fragi* DBC can effectively degrade multiple HMW PAHs even under mixed-substrate conditions. Moreover, the study provides the first experimental and *in silico* evidence that this strain preferentially interacts with and transforms toxic PAH and its metabolic intermediates, revealing a hierarchical substrate utilization pattern that enhances detoxification efficiency. This coupling of enzyme specificity, gene regulation, and toxicity prediction establishes a mechanistic framework for understanding microbial preference toward complex PAH mixtures, which represents a significant advancement in the current understanding of PAH biodegradation by *Pseudomonas* spp. and provides a novel perspective for developing targeted bioremediation strategies in multi-contaminant environments.

Section snippets

Chemicals

Analytical grade Ethyl acetate, Hexane and PAHs - Fluoranthene (Fla), Pyrene (Pyr) and Benzo(a)pyrene (BaP) of 99 % purity, were purchased from Sigma-Aldrich. Bushnell-Haas Broth medium (BHB), Nutrient agar (NA), Luria-Bertani agar (LB), glucose, catechol, Tris-base were purchased from Hi-media India having standard composition. …

Microbial strain and culture conditions

The bacterial strains *Pseudomonas fragi* DBC (Accession No. MCC 3580) was collected from culture collection of Soil and Environmental Microbiology laboratory, Dept. of …

Microorganism

P. fragi DBC was selected as test organism for this study, because of its ability to mineralize PAHs, and availability of genome sequence at GenBank (accession number CP021986) [26]. Members of *Pseudomonas* genus had been reported to degrade wide array of pollutants including PAHs, pesticides, heavy metals etc. [21], [52], [53]. The genome of *P. fragi* DBC contains 3993 protein-coding genes, 72 tRNAs, and 25 rRNAs, including genes involved in pathways for PAHs degradation (e.g., *nod*, *nah*, *catA*), …

Conclusion

In this study, the role of specific enzymes in determining substrate preference during the degradation of PAHs by the bacterium *P. fragi* DBC is studied. The focus was on two key enzymes: C12O and C23O, which are integral to the ortho- and meta-cleavage pathways of aromatic compound degradation. Experimental observations revealed that *P. fragi* DBC efficiently degraded HMW PAHs such as BaP, Pyr, and Fla, with notable enzymatic activity from both C12O and C23O during the degradation process. …

Environmental Implications

The study highlights the environmental significance of *Pseudomonas fragi* DBC in efficiently degrading highly toxic, HMW PAHs such as benzo(a)pyrene, pyrene, and fluoranthene through catechol dioxygenase-mediated pathways. By selectively degrading the more toxic polycyclic aromatic hydrocarbons (PAHs) and their intermediates into less harmful derivatives, this bacterium demonstrates a promising and sustainable bioremediation approach for petroleum-contaminated environments. The findings …

CRediT authorship contribution statement

Ankita Das: Writing – original draft, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Sourav Debnath:** Methodology. **Anand Prakash Maurya:** Methodology. **L. Paikhomba Singha:** Methodology. **Nandita Das:** Methodology. **Piyush Pandey:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. …

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Declaration of Competing Interest

The authors declare no computing personal or financial conflicts. …

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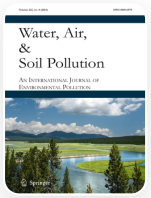
Characterization of Halophilic Bacteria *Vreelandella piezotolerant* DM1 on Biodegradation of Polyaromatic Hydrocarbons and its Pathway

Published: 15 August 2025

Volume 236, article number 807, (2025) Cite this article



Ramanathan Duraimurugan, Krishnan Vignesh, Chenthamarakshan Sahasra, Punniyakotti Parthipan, Jayaraman Narenkumar & Aruliah Rajasekar

Abstract

Polycyclic aromatic hydrocarbons (PAHs) with high molecular weights are significant marine pollutants. This study aimed to determine the ability to degrade PAHs (pyrene and benzo[a]pyrene) by *Vreelandella piezotolerant* DM1 under halophilic conditions. Biodegradation of PAHs was performed in isolate DM1, and the experiment was incubated at 37 °C for 15 days in a hypersaline environment. A bacterial consortium effectively degrades PAHs by secreting alkane hydroxylase (AH) and alcohol dehydrogenase (AD) as key enzymes. At 250 mg/L PAH, the removal efficiencies of TOC and COD were 67–75% and 69–78%, respectively. DM1 was well adapted to saline conditions and effectively metabolised (PAHs). Fourier-transform infrared spectroscopy (FT-IR) confirmed the utilisation of various types of functional groups present in PAHs. Gas chromatography-mass spectroscopy (GC–MS) confirmed that the bacterial strain effectively degraded pyrene (58%) and benzo[a]pyrene (70%) and mixed at 88% within 15 days. The intermediate metabolites, phenanthrene-4,5-dicarboxylic acid, 3,4-dihydroxy phenanthrene, 1-(2-hydroxyphenyl)2-phenylethanone, and phthalic acid, were identified. Based on these metabolites, a possible PAH biodegradation pathway was proposed. Overall, this study elucidated the role of halophilic bacteria in the biodegradation of PAHs and their degradation pathways and found that the strain was suitable for the biodegradation of PAHs in a hypersaline environment.

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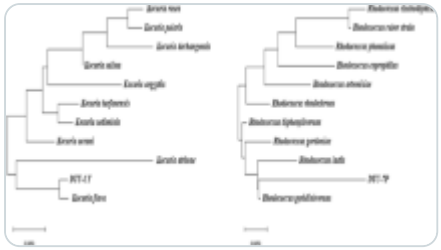

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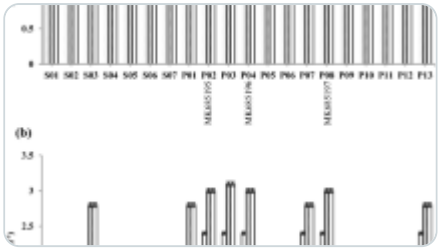

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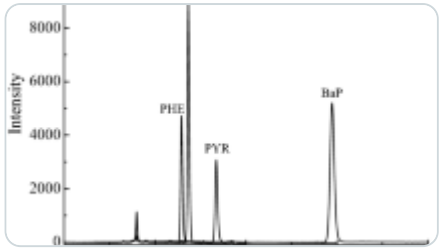

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1 Introduction

Polycyclic aromatic hydrocarbons (PAHs), are typically considered as prior pollutants to environmental issues currently due to their higher hydrophobic nature (Patel et al., 2020). Due to their strong mutagenic and carcinogenic nature, PAHs, common pollutants from human activities, pose a serious threat to the environment and human well-being (Ali et al., 2021; Ofori et al., 2020). Based on the molecular structure of PAHs, it is generally classifying as low and higher molecular weight PAHs (Aziz et al., 2018). PAHs with low molecular weights consist of two to three aromatic rings, while those with high molecular weights have three or more fused aromatic rings (Nene, 2021). PAHs can easily cause mutations and chromosome damage in genetic materials such DNA, RNA and protein molecules due to forming the covalent bonds associated with them (Ifegwu & Anyakora, 2015). Moreover, epoxides are a metabolic intermediate of PAHs which is more lethal over their parent compounds, thus it can easily cause severe cellular effects on the organelles (Elumalai et al., 2024; Maletić et al., 2019). The PAHs with higher molecular weights tend to be more carcinogenic (Wei et al., 2015). Therefore, some higher molecular weight PAHs, such as anthracene, dibenz[ah]anthracene, benzo[a]pyrene, and pyrene, show higher toxicity and carcinogenicity (Bukowska et al., 2022). Pyrene is known to be formed through the incomplete combustion of various types of organic matter, making it ubiquitous in the environment. Among the pollutants, pyrene (four benzene rings), benzo[a]pyrene (five benzene rings) are reported to be more toxic to aquatic vertebrates, and other organisms (Bukowska et al., 2022; Zada et al., 2021). Pyrene and benzo[a]pyrene are representative model compounds owing to their widespread presence in petroleum-contaminated environments, high persistence, and significant toxicity (Ali et al., 2024). Their different

molecular weights and structures facilitate a comparative analysis of microbial degradation across various PAH complexities (Han et al., 2021). Based on clinical symptoms, PAHs cause intoxication, including increased liver weight and reduced renal weight. Removal of PAHs from the environment is a critical and pressing issue for researchers. There are many physicochemical methods available such as precipitation, coagulation, adsorption, ozonation, ion exchange and advanced oxidation to remove PAHs from polluted sites (Coha et al., 2021; Eldos et al. 2022). However, these methods have some disadvantages such as expansive machineries, produce secondary toxic pollutants (Patel et al., 2020). In contrast to this, bioremediation is an effective method in which microorganisms play a crucial role to reduce the pollutants by utilizing it as a carbon source in the environment. However, this method has several limitations (Alori et al., 2022). It depends heavily on specific environmental conditions such as pH, temperature, and nutrient availability. Additionally, the degradation of complex or high molecular weight contaminants is slow. Since microorganisms can decompose or mineralize the toxic organic substances into a simpler or nontoxic end product (Bala et al., 2022).

Compared with other microorganisms, bacterial species have been reported to be efficient candidates for degrading environmental pollutants (Ghosal et al., 2016; Qutob et al., 2022; Xu et al., 2019). However, conventional microbiological treatment processes have failed in high-salt environments (Bacosa et al., 2022; Fathepure, 2014; Huang et al., 2024). Therefore, Castillo-Carvajal et al. (2024) showed that halophilic-resistant microbes are needed for the biodegradation of such environments. Previous studies have limited the biodegradation of pollutants and their biodegradation pathways in hypersaline environments. Consequently, the potential toxicity of contaminants in the accumulation of difficult aqueous systems remains uncharacterized (Castillo-Carvajal et al., 2024). However, recent studies have focused on PAH degradation and its toxicity in halophilic organisms. *Halomonas* sp. can degrade PAHs by cleaving their aromatic rings (Al Farraj et al., 2020; Budiyanto et al., 2018). This haloalkaliphile has also been reported

to be involved in the biodegradation of PAHs, benzoate, phenol, and catechol (Fathepure, 2014). Further characterisation of these degradation processes at high pH and salt concentrations could lead to improved applications of haloalkaliphiles in marine and industrial wastewater treatment processes (Shameer, 2016). PAHs transformation depends upon enzymatic actions. Surface-active biomolecules and enzymes play an important role in PAH degradation (Gupta et al., 2015). More specifically, alkane hydroxylase (AH) and alcohol dehydrogenase (AD), a prior extracellular degradative enzyme that are mainly involved and responsible for the initiation and breakdown of the compounds by bacterial cells during the degradation. that breaks an organic compound's large molecules into smaller molecules (Abbasian et al., 2016). This study aimed to evaluate the efficiency of halophilic bacterial strains in degrading PAHs (pyrene and benzo [a] pyrene) under hypersaline conditions to simulate environments such as saline industrial wastewater or marine-contaminated sites. Biodegradation efficiency (BE) was assessed using gas chromatography-mass spectrometry (GC–MS), while changes in functional groups were analysed using fourier transform infrared spectroscopy (FT-IR). In addition, the activities of key degradative enzymes, aromatic hydroxylase (AH) and aldehyde dehydrogenase (AD), were monitored over time to elucidate the biodegradation mechanisms.

2 Materials and Methods

2.1 Chemicals

The chemicals with high-purity were used in this study such as CHAPS buffer, ferrous sulphate ($FeSO_4$), nicotinamide adenine dinucleotide (NAD), NADH, potassium bromide (KBr), pyrene, benzo[a]pyrene, tris-hydrochloride and halophilic minimal salt medium (HMSM) broth was used for the biodegradation studies. The compositions were ammonium sulfate, monosodium phosphate, calcium chloride heptahydrate, magnesium sulfate heptahydrate, disodium phosphate, ferrous sulphate heptahydrate, and 5% sodium chloride), halophilic broth (Acicase, yeast

extract, protease peptone, trisodium citrate, potassium chloride, magnesium sulfate, sodium chloride and pH7.2 ± 0.2), and LB broth (tryptone, yeast extract, sodium chloride and pH 7.5 ± 0.2) were obtained from Merck, India. The extraction solvents ethyl acetate, dichloromethane, ethanol, and n-hexane were obtained from Himedia (Mumbai, India) with a purity of 99%.

2.2 Bacterial Culture

In this study, the halophilic bacterial strain *Vreelandella piezotolerant* DM1 was isolated from industrial wastewater and was identified by 16S DNA sequencing; further details are reported in our previous study (Duraimurugan et al., 2025). The sequence was deposited under the NCBI GenBank accession number (PP748178). Bacterial cultures were enriched in LB broth containing different concentrations of PAHs (100—400 mg/L) and incubated for 24 h at 37 °C (120 rpm) under halophilic conditions. The resulting optimum concentration was subsequently used for biodegradation studies (250 mg/L). Halophilic mineral salts were used as the media in this study.

2.3 Biodegradation of PAH

Biodegradation was performed in 100 mL of sterilised HMSM broth with pyrene, benzo[a]pyrene (250 mg/L), and mixed PAHs (250 mg/L (pyrene: benzo(a)pyrene = 50:50 w/w)) served as the control system. Fresh bacterial DM1 consortium (10^5 cfu/ml) was inoculated into 100 mL of HMSM liquid medium containing dried PAHs as the carbon source. The system was incubated at 37 °C (150 rpm) under aerobic conditions for 15 days. The obtained PAH stock solutions (50 mg PAHs in 10 ml dichloromethane) were added to separate sterilised Erlenmeyer flasks. Dichloromethane was allowed to evaporate in laminar airflow to obtain dried PAH powders, which were then carefully

transferred to HMSM-containing conical flasks (Muthukumar et al., 2024). The 24 h once the bacterial growth and degradation were analysed.

2.4 Enzyme Analysis

In this study, samples were collected at regular intervals and analysed to quantify alkane hydroxylase (AH) and alcohol dehydrogenase (AD) activity. The AH enzyme activity was determined as reported earlier (Parthipan et al., 2017b). In bacterial supernatant, prepared by centrifuging at 10,000 rpm for 10 min at 4 °C, and added to the reaction mixture of 0.1 mM NADH (nicotinamide adenine dinucleotide hydrogen), 20 mM Tris–HCl buffer (pH 7.4), 0.15% CHAPS buffer (3-[(3-cholamidopropyl)dimethylammonio]propane-1-sulfonate), 10 µL n-hexadecane and 50 µL of culture, brought to a final volume of 1 mL. The enzyme activity was measured spectrophotometrically at 340 nm (Shimadzu UV-1800) (Muthukumar et al., 2022; Pereira et al., 2024).

The AD estimated according to Kong et al., 2017 (Kong et al., 2017). The reaction mixture comprised 1 M Tris–HCl buffer, 4 mM NAD, and 100 µL of 99% pure ethanol. Culture supernatant (50 µL) was obtained after centrifugation at 5000 rpm for 15 min. The activity was determined by measuring 340 nm using a (Shimadzu UV-1800) (Parthipan et al., 2017b).

2.5 Analytical Methods

The residual PAHs were extracted using dichloromethane (DCM) through a separatory funnel and subsequently analysed by gas chromatography-mass spectrometry (GC-MS) (SHIMADZU, QP2010 PLUS) according to previous methods (Parthipan et al., 2017b). PAH concentrations were measured by GC–MS (Agilent, Palo Alto, CA, USA; GC

model 6890, mass selective detector model 5973). The extracted sample was analysed on an Elite GC–MS column (30.0 mm × 0.25 mm × 250 µm) with an initial temperature of 60 °C held for 2 min. The temperature was ramped from 10 °C to 300 °C. Mass spectrometry analysis was performed using electron impact ionisation at 70 eV. Helium served as the carrier gas for the analysis, and the injector temperature was maintained at 280 °C in the splitless mode for 3 min. The PAH and metabolite standards were procured from Sigma-Aldrich (USA). Metabolites produced from hydrocarbon biodegradation were identified using a GC–MS internal library, and biodegradation efficiency (BE) was determined. Chemical oxygen demand (COD) was determined using the open reflux method according to APHA standard 5220 B.45, while total organic carbon (TOC) was measured by direct injection into a Shimadzu TOC-VCPN analyser. The residual PAH were subjected to FTIR spectroscopic analysis to identify their functional components. Following the protocol outlined by PerkinElmer Inc (USA) was employed to analyze the functional groups of the residual PAHs, as per the protocol established (Kapadia et al., 2022). Briefly, the samples were mixed with potassium bromide (KBr) and subjected to hydraulic compression. A GX-FTIR system (PerkinElmer Inc., USA) was used to record the FT-IR spectrum across the wavenumber range of 4000–400 cm^{-1}.

2.6 Statistical Analysis

All data were collected from three replicates (n = 3) and are reported as mean ± standard deviation. Variance were analysed by ANOVA, the Pyrene variance was about p-value < 0.053 and F-1.125, benzo[a]pyrene variance was about p-value < 0.056 and F-1.25 and Mixed PAHs variance was about p-value < 0.093 and F-0.125 respectively.

3 Results and Discussion

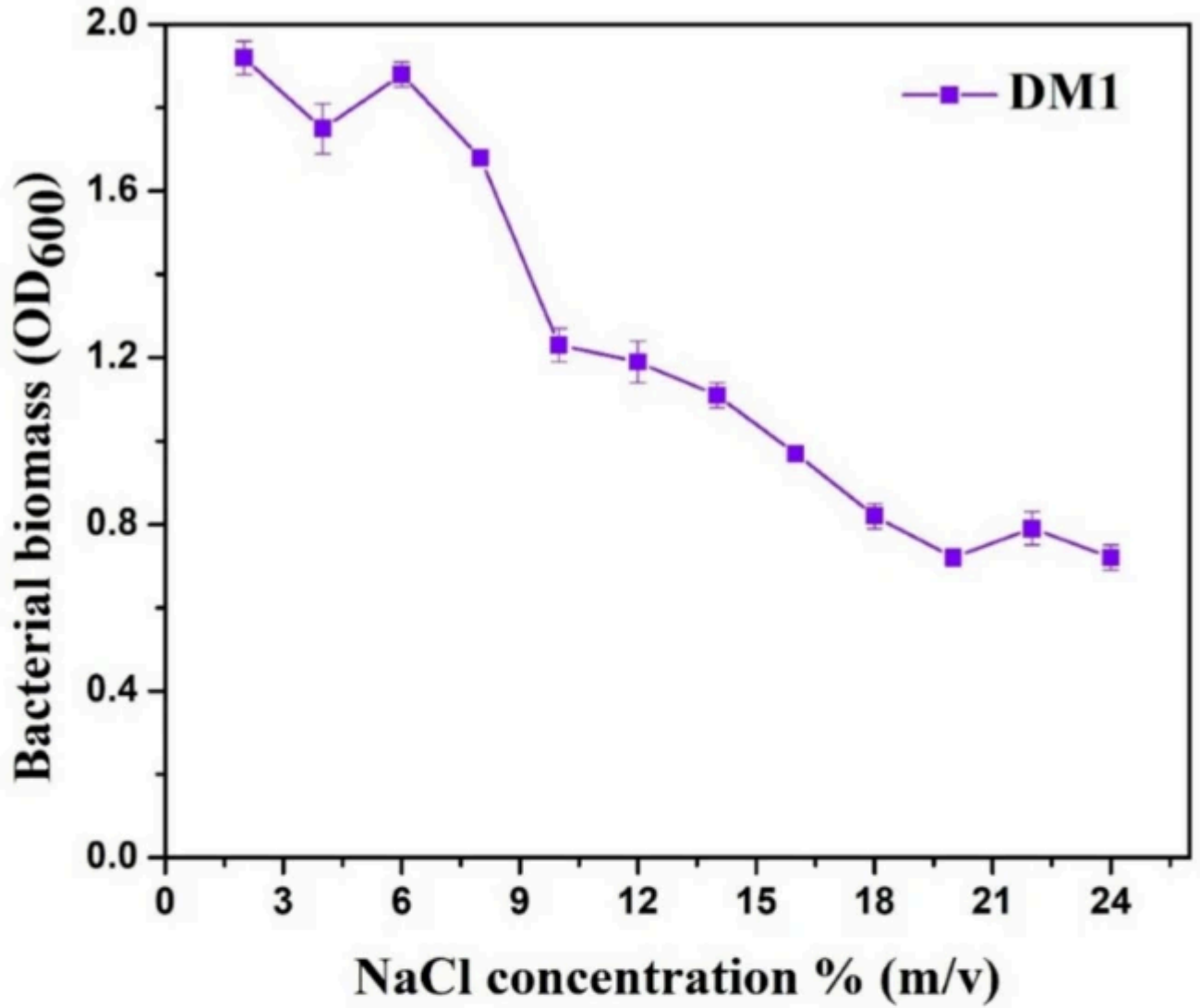
3.1 The Adaptability Threshold of Bacteria in the Salt and PAHs

DM1 was subjected to a simulation test to assess its tolerance to salt and PAHs (Kumawat et al., 2022; Li et al., 2017; Wu et al., 2019). As illustrated in Fig. 1a, the growth curve of the DM1 strain differed when exposed to different concentrations of NaCl (2–25%). This is presumably halophilic or halotolerant in nature. At low to moderate NaCl concentration (2–6%), the strain DM1 showed the optimal growth, with the highest biomass (OD_{600} ≈ 1.92–1.88) was observed. This suggests that DM1 was well-adapted to the saline environment. The slight fluctuation between 2 and 6% may reflect normal biological variability; however, the biomass remained consistent at higher concentrations. However, a sharp decline in biomass was observed beyond 6% NaCl, particularly between 6 to 10%, where the OD_{600} drops from approximately 1.88 to 1.23. This indicated that the onset of osmotic stress begins to inhibit bacterial cell division and metabolic activity. Between 10 and 20% NaCl, a significant decline in bacterial biomass was observed (OD_{600} ~ 1.23 to 0.72). Although DM1 remains viable under high salinity, its metabolic efficiency and replication rate decrease. Beyond 20%, biomass levels was OD_{600} ~ 0.72, it indicating that growth-limited by the salt concentration, where only minimal survival or maintenance of cellular function occurs (Cui et al., 2009). These results confirm that strain DM1 is a halotolerant bacterium with optimal growth under moderate salinity (2–6%) and significant tolerance up to 24% NaCl. This tolerance profile suggests that DM1 has potential applications in the bioremediation of pollutants in saline or hypersaline environments. Subsequently, the bacterial strain DM1 was sub-cultured to characterise its ability to utilise hydrocarbons as a carbon source at an optimum concentration of 300 mg/L PAH (Fig. 1b). *Vreelandella piezotolerant* DM1 exhibited significant biodegradation efficiency and utilisation of PAHs as a carbon source. These halophilic bacteria can be used for the bioremediation of PAH in saline environments (5%). However, biomass production significantly decreased when NaCl concentrations were increased by more than 5%. Despite this, the strain continued to exhibit growth at 5% NaCl (Kapadia et al., 2022; Li et al., 2017). These results indicate that strain DM1 exhibited high tolerance to a wide range of NaCl concentrations, maintaining significant biomass production. The earlier report also supported, the same phenomena and the halophilic species like *Natrialba*

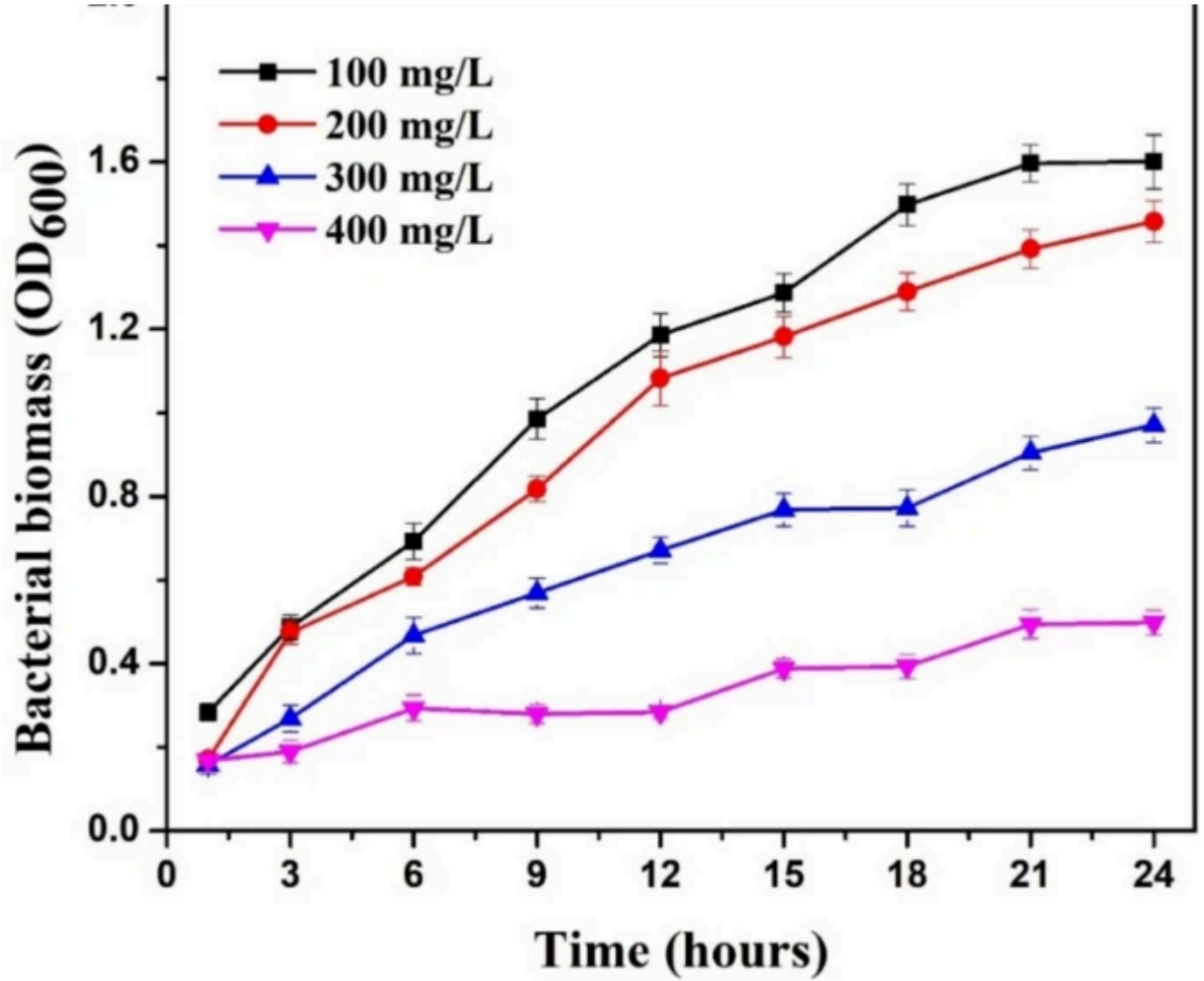
sp. and *Haloarcula* sp. and other halophiles *Marinobacter* sp. and *Arhodomonas* sp. grown in more than 10% salinity (Khemili-Talbi et al., 2015). After exposure to various concentrations of aromatic and aliphatic hydrocarbons.

Fig. 1

a


DM1
Bacterial biomass (OD600)
2.0
1.6
1.2
0.8
0.4
0.0
0
3
6
9
12
15
18
21
24
NaCl concentration % (m/v)

b



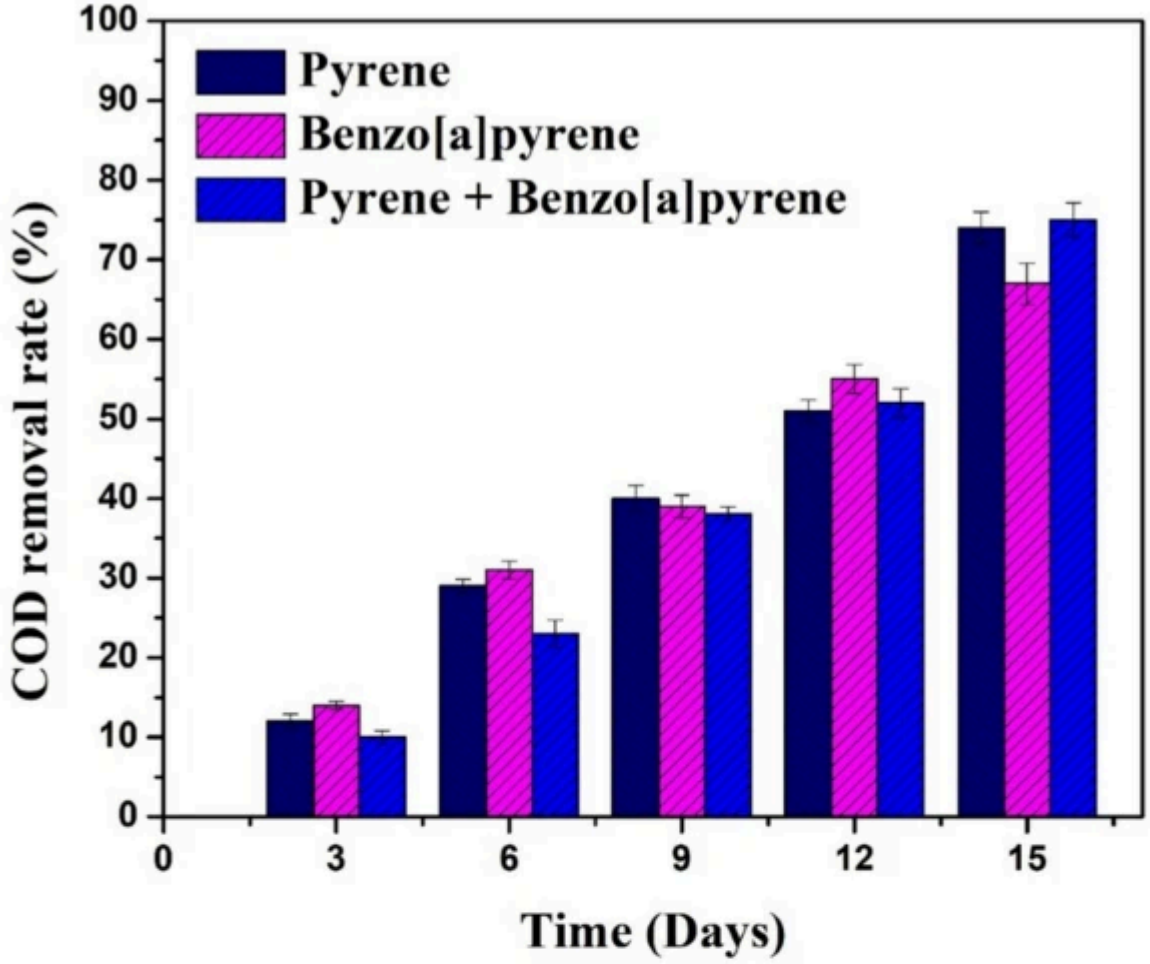
Bacterial Growth efficiency. (**a**) Halophilic bacteria *Vreelandella piezotolerant* DM1 growth at different salt concentrations; (**b**) Halophilic bacteria *Vreelandella piezotolerant* DM1 growth at different concentrations of PAH

3.2 Estimation of Chemical Oxygen Demand and Total Organic Carbon

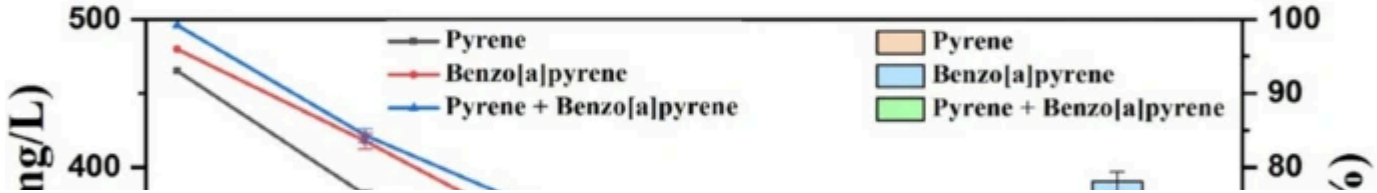
COD and TOC reductions were measured during biodegradation, and the percentage of each degradation system is shown in Fig. 2 (Table S1 and S2). Figure 2a shows that the highest COD reduction was obtained for the mixed PAHs at approximately 75%. The COD reduction levels of PAH obtained for pyrene and benzo[a]pyrene are 74% and 67%, respectively. Higher salt concentrations promoted the growth of non-halophilic bacterial species, which resulted in a lower COD efficiency. In previous studies, the potential bacterium *Vreelandella piezotolerant* DM1for effectively reduced the COD level in a saline environment. In conclusion, COD reduction is possible because the presence of organic compounds in the medium could be utilised by bacterial cells during degradation.

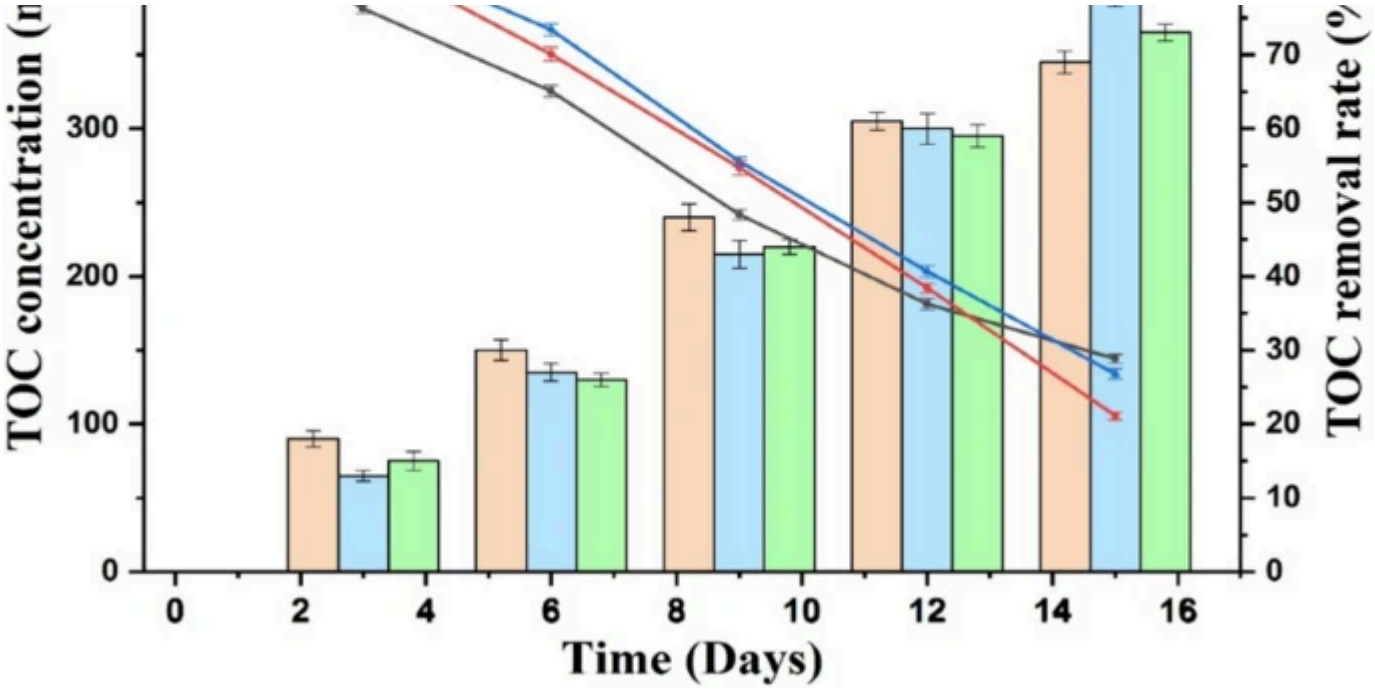
Fig. 2

a


Pyrene
Benzo[a]pyrene
Pyrene + Benzo[a]pyrene
COD removal rate (%)
Time (Days)
0
10
20
30
40
50
60
70
80
90
100
0
3
6
9
12
15

b


Pyrene
Benzo[a]pyrene
Pyrene + Benzo[a]pyrene
Pyrene
Benzo[a]pyrene
Pyrene + Benzo[a]pyrene
500
400
100
90
80



Degradation efficiency of PAHs. (**a**) Chemical oxygen demand removal rate of PAHs; (**b**) Total organic carbon removal rate of PAHs

Figure 2b shows the removal of TOC during PAH biodegradation. TOC exhibited higher percentages of pyrene, benzo[a]pyrene, and mixed PAH solutions of approximately 69%, 78%, and 73%, respectively, within 15 days. This was resolved by the transformation of PAHs into less toxic and the formation of new intermediate compounds during PAHs biodegradation.

3.3 Kinetic Modelling

The linear slope is provided in the supplementary data (Fig. S1). The kinetic investigation of COD confirming the linear increase trends showed slope values of 0.9753 (pyrene), 0.98843 (benzo[a]pyrene), and 0.98277 (pyrene and

benzo[a]pyrene), respectively. The results confirm the high and consistent capacity of the microbial strain for degrading both individual and mixed PAHs. The strong linear correlation ($R^2 > 0.90$) between COD removal and degradation duration highlights the metabolic stability and resilience of the strain under pollutant stress. These findings suggest that the biodegradation process is effective, even with complex contamination. The ability of the strain to perform efficiently under such conditions makes it a strong candidate for application in saline or industrial wastewater treatment systems.

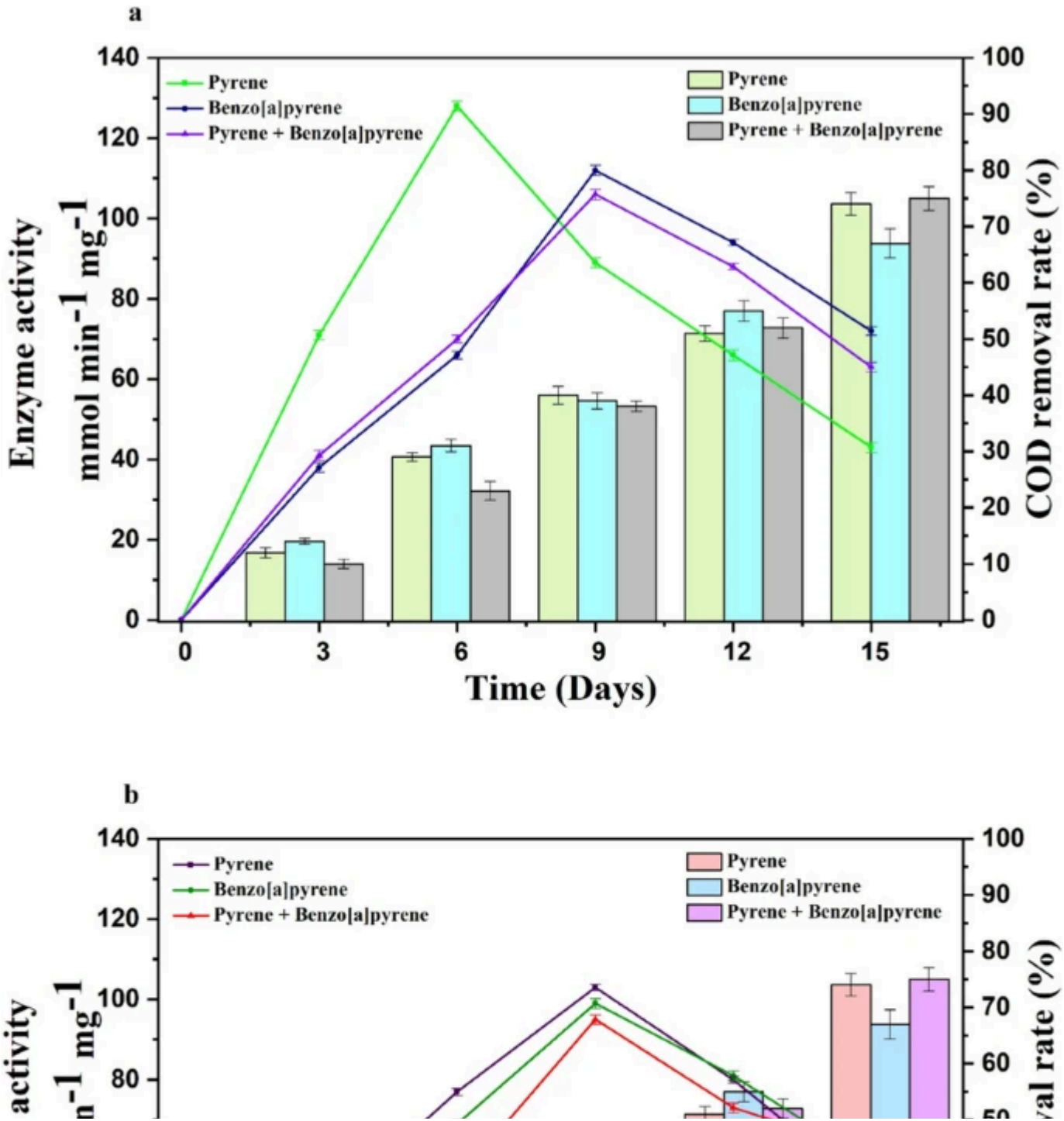
The linear slope is provided in the supplementary data (Fig. S2). The kinetic investigation of TOC, confirming the linear increase trends, showed slope values of 0.98076 (pyrene), 0.99705 (benzo[a]pyrene), and 0.99456 (pyrene and benzo[a]pyrene), respectively. The experimental findings revealed a clear positive correlation between the degradation duration of PAHs and the removal rates of both COD and TOC. This indicated that the biodegradation process was effective and improved consistently over time.

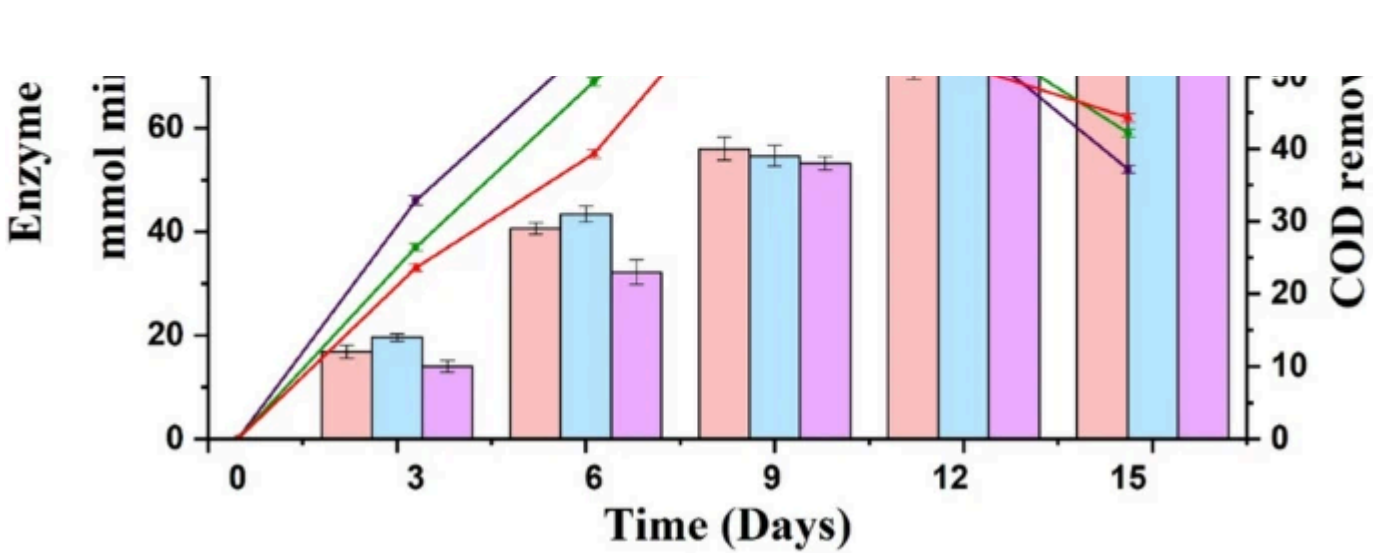
3.4 Enzyme Analysis

The alkane hydroxylase (AH) and alcohol dehydrogenase (AD) enzymes produced were evaluated during biodegradation. AH enzyme activity was induced in strain DM1 on the 3rd, 6th, 9th, 12th and 15th days of hydrocarbon degradation (Fig. 3). AH enzyme activity was higher than that in AD on day 3. The maximum enzyme activity of pyrene was observed on day 6 and then gradually decreased compared with that of AD. The corresponding rates for pyrene, benzo[a]pyrene, and the mixed substrate were 71, 38, and 41 μmol min^{-1}/mg^{-1}, respectively (Fig. 3a, b, and c). After 3rd day, AH enzyme activity gradually increased on the 9th day with respective activities of pyrene 89 μmol min^{-1}/mg^{-1}, benzo[a]pyrene 112 μmol min^{-1}/mg^{-1} and mixed 106 μmol min^{-1}/mg^{-1}. Finally, the selected

bacterial strain produces AH during PAH degradation (Sangkharak et al., 2020). Strain DM1 produced AH during PAH degradation and utilised hydrocarbons as the sole carbon source.

Fig. 3


a
Pyrene
Benzo[a]pyrene
Pyrene + Benzo[a]pyrene
Pyrene
Benzo[a]pyrene
Pyrene + Benzo[a]pyrene
Enzyme activity
mmol min-1 mg-1
COD removal rate (%)
Time (Days)
0
3
6
9
12
15
0
20
40
60
80
100
120
140
0
10
20
30
40
50
60
70
80
90
100
b
Pyrene
Benzo[a]pyrene
Pyrene + Benzo[a]pyrene
Pyrene
Benzo[a]pyrene
Pyrene + Benzo[a]pyrene
80
100
120
140
60
70
80
90
100



Examination of alkane hydroxylase and alcohol dehydrogenase during PAH biodegradation (**a**) Alkane hydroxylase (**b**) Alcohol dehydrogenase

This enzyme catalyzes the breakdown of alkanes to their corresponding alcohols, and it has the first committed step in the metabolic pathway for utilising alkanes as a bacterial source (Zhang et al., 2011). The core function of AH is consistent in both halophilic and non-halophilic bacteria. The enzyme's cofactor requirements may be altered to suit the halophilic environment, such as ions or salts (NADH or $FADH_2$) for their activity. The core mechanism of alkane hydroxylation involves insertion of an oxygen atom into the alkane molecule (Parthipan et al., 2017a). Overall, the bacterial strains exhibited an increase in AH enzyme secretion after the 5th day of incubation. *Pseudomonas and Rhodococcus* species are well known for their characterised enzymatic properties under non-halophilic conditions. AH, an enzyme secreted by diverse hydrocarbon (PAHs)-degrading bacteria, initiates the degradation of long-chain alkanes within PAH and petroleum hydrocarbons. Numerous microorganisms possess specific genes encoding enzymes crucial for degrading complex hydrocarbons in halophilic and non-halophilic conditions (Edbeib et al., 2016;

Fathepure, 2014). An instance of certain hydrocarbon-degrading bacteria is the distribution of aliphatic hydrocarbon-degrading enzymes across three chromosomal loci. Specifically, in non-halophilic strains, the alkane hydroxylase gene is located at a considerable distance from the genes encoding alcohol dehydrogenase and aldehyde dehydrogenase (Abbasian et al., 2016). Genome sequencing has recently shown that moderate halophiles contain four alkane hydroxylation systems (propane monooxygenase, AlkB, CYP, and LadA). Consistent with this, the previously described halophile, Alcanivorax dieselolei B-5, harbours multiple alkane hydroxylases (including two AlkB-, one CYP153-, and one AlmA-type) and exhibits a broad range of alkane-degrading capabilities (Park & Park, 2018).

The AD activity of DM1 during PAHs degradation was shown in Fig. 3. Peak AD activity was observed on the 3rd day in DM1, with pyrene degradation reaching levels of pyrene 46 μmol min^{-1}/mg^{-1} (Fig. 3a), benzo[a]pyrene 37 μmol min^{-1}/mg^{-1} (Fig. 3b), and mixed (pyrene and benzo[a]pyrene) 33 μmol min^{-1}/mg^{-1} (Fig. 3c). AD catalyzes the conversion of alcohols to ketones or aldehydes, which is a critical step in the degradation of organic compounds. A consistent increase in AD activity was observed during the degradation of both PAHs (Gärtner, 2020). DM1 exhibited progressive AD induction from the 3rd day of incubation, which correlated with enhanced PAHs biodegradation.

Microorganisms are crucial for PAHs biodegradation, and enzymes act as catalysts. Alkane hydroxylase, a membrane-bound enzyme that initiates PAHs degradation, is essential for the conversion of aldehydes to carboxylic acids in bacterial metabolism. In this process, both enzymes were maximally produced by strain DM1 on the 15th day before declining, and significantly enhanced overall PAHs degradation.

3.5 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

FT-IR revealed a shift in the organic functional groups of PAHs, indicating the formation of degradation products such as phenol, alcohol monomers, hydrogen-bonded alcohols, carboxylic acids, and aromatic carbon-hydrogen bonds (Marzuki et al., 2021; Marzuki et al., 2023). The FT-IR spectra of PAHs control and biodegradation are presented in Fig. S3. The IR spectrum for the pyrene control absorption band at C-H aliphatic stretch, CH alkanes, C-H bending, C = O stretch, C–O–C, C = C stretch, C–C and CH-OH groups (3044.21 cm^{-1}, 2929.03 cm^{-1}, 2857.05 cm^{-1}, 831.90 cm^{-1}, 1921.26 cm^{-1}, 738.32 cm^{-1}, 712.73 cm^{-1}, 1867.67 cm^{-1}, 1795.68 cm^{-1}, 1746.10 cm^{-1}, 1236.61 cm^{-1}, 1638.12 cm^{-1}, 1587.73 cm^{-1}, 963.87 cm^{-1}, 1434.17 cm^{-1}, 1311.79 cm^{-1}, 1189.42 cm^{-1}, 1093.44 cm^{-1}) were observed. The peak at 3044.21 cm^{-1} indicates C-H stretching vibrations. The range of 1448–1324 cm^{-1} suggests the presence of a ($-CH_3$) alkane group. A peak at 1189.42 cm^{-1} confirms the presence of the (-C-O) carbonyl group. The strong absorption peaks at 963.87, 831.90, and 712.75 cm^{-1} denote C-H aromatic out-of-plane vibrations, and absorption peaks between 400 and 1000 cm^{-1} indicate C-H aromatic in-plane'oop'bonds. The newly shifted peaks at the CH alkanes stretch to 2921.83 cm^{-1}, 2853.85 cm^{-1} and the C = C band 1638.12 cm^{-1} shifted to 1652.52 cm^{-1}. Secondary alcohol stretching was observed as a new peak at 1064.65 cm^{-1} (Majeed et al., 2012). The reduction in peak intensities observed in Figs. S3b and S3e strongly suggests that the bacteria utilised the compounds during the degradation process. Similar results were obtained in Figs. S3 c, d, and e.

In benzo[a]pyrene control system C-H stretching, C = C = C stretching, C = O stretching, C = C stretching, C-H bending, O = H bending, C-O alcohol stretching, C = C bending (3029.81 cm^{-1}, 2921.83 cm^{-1}, 2853.85 cm^{-1}, 1910.86 cm^{-1}, 1759.69 cm^{-1}, 1623.72 cm^{-1}, 1562.14 cm^{-1}, 1596.93 cm^{-1}, 1458.96 cm^{-1}, 1408.57 cm^{-1}, 1350.98 cm^{-1}, 1314.99 cm^{-1}, 1275.80 cm^{-1}, 1229.41 cm^{-1}, 1171.82 cm^{-1}, 1083.04 cm^{-1}, 939.08 cm^{-1}, 877.49 cm^{-1}, 835.10 cm^{-1},

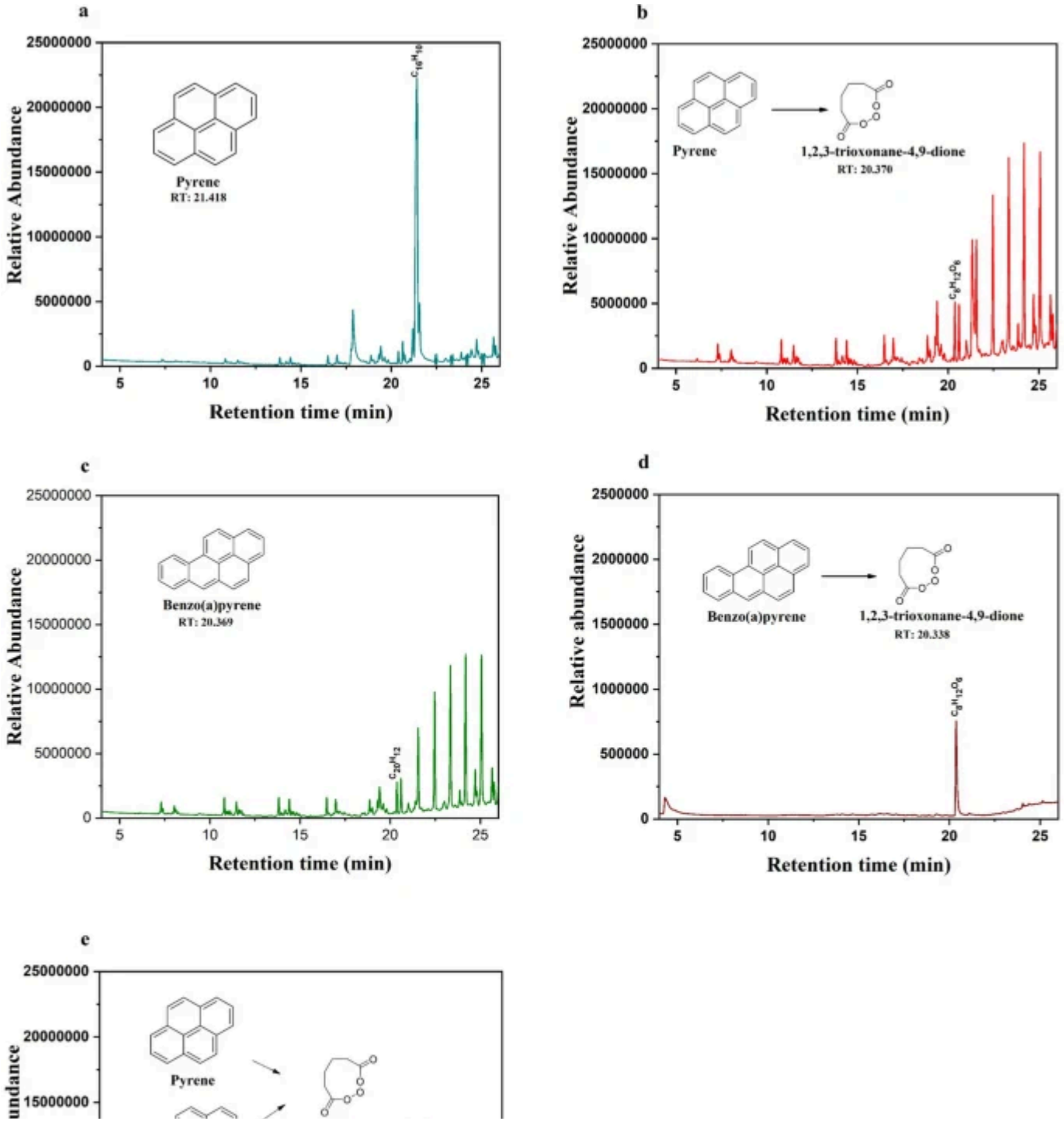
687.13 cm^{-1}, 751.92 cm^{-1}) observed respectively (Nzila & Musa, 2021; Yan et al., 2017). The PAHs of functional groups of C = C = C stretching, O = H bending, C-O alcohol stretching, and C = C bending were significantly reduced during biodegradation. The mixed system (Fig. S3e) is compared to Fig. S3a and c. The peak intensities observed in Fig. S3e are notably lower than those in Fig. S3a and c. This indicates that enhanced compound utilisation can be attributed to bacterial activity in the medium. This finding is consistent with the degradation efficiency obtained through the GC–MS analysis. Overall, the results revealed a significant reduction in peak intensities across Fig. S3b, d, and e compared to the control peaks shown in Fig. S3a and c, indicating substantial compound breakdown. As a result, the functional groups of PAH were drastically decreased by DM1 and utilised as a carbon source.

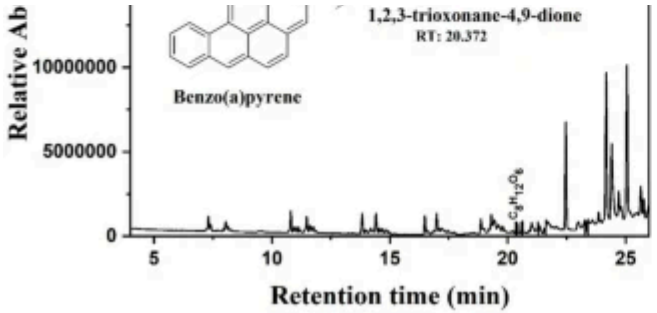
3.6 Gas Chromatography–Mass Spectrometry Analysis

In this study, residual hydrocarbons were extracted after the end of degradation and analysed using GC–MS to evaluate the BE. The resulting bio-derivative compounds were in Table S3.The mass spectral profiles and degradation are shown in Fig. 4 and 5, respectively. DM1 degraded the higher molecular hydrocarbon pyrene by 58%, benzo[a]pyrene by 70%, and mixed PAH by 88% within 15 days. To identify the produced intermediate byproducts during PAHs degradation were analyzed by chromatogram for both control and biodegradation systems (Ladino-Orjuela et al., 2016; Lee et al., 2019; Wang et al., 2018). The control system initially exhibited higher levels of dominant hydrocarbon compounds which were significantly reduced following treatment with biodegradation systems. Based on the above analysis, DM1 metabolises hydrocarbons, possibly via distinct metabolic pathways. These pathways were inferred on the basis of the identified metabolites. As depicted in Fig. 6a and b, the combination of the DM1 bacterial strain produces both enzymes and substantially influences the degradation of PAHs intermediates. In DM1 inoculated systems of biodegradation systems, oxalic acid, 6-ethyloct-3-yl ethyl ester (RT- 10.099), carbonic acid, eicosyl vinyl

ester (RT- 17.434), and cyclohexane (1-methyl ethyl) (RT-19.199) were the primary intermediates. The ability of the bacterial strain to efficiently degrade hydrocarbons at various stages of biodegradation was demonstrated. This was indicated by the efficient bioconversion of the aromatic ring and the addition of hydroxyl and carboxyl functional groups to the intermediate hydrocarbon compounds (Ladino-Orjuela et al., 2016; Lee et al., 2019). The degradation pathway involves the initial deoxygenation of pyrene to form cis-dihydrodiol, followed by dehydrogenation to form dihydroxy pyrene. Subsequent ring cleavages yield intermediates such as phenanthrene-4-carboxylate, cis-3,4-dihydroxyphenanthrene-4-carboxylate and phenanthrene-4,5-dicarboxylic acid (Wang et al., 2018). These intermediates are further oxidized and metabolized through reactions catalysed by dihydrodiol dehydrogenase and ring cleavage dioxygenases, ultimately leading to the formation of phthalic acid and its eventual mineralization through the TCA cycle (Mallick et al., 2011).

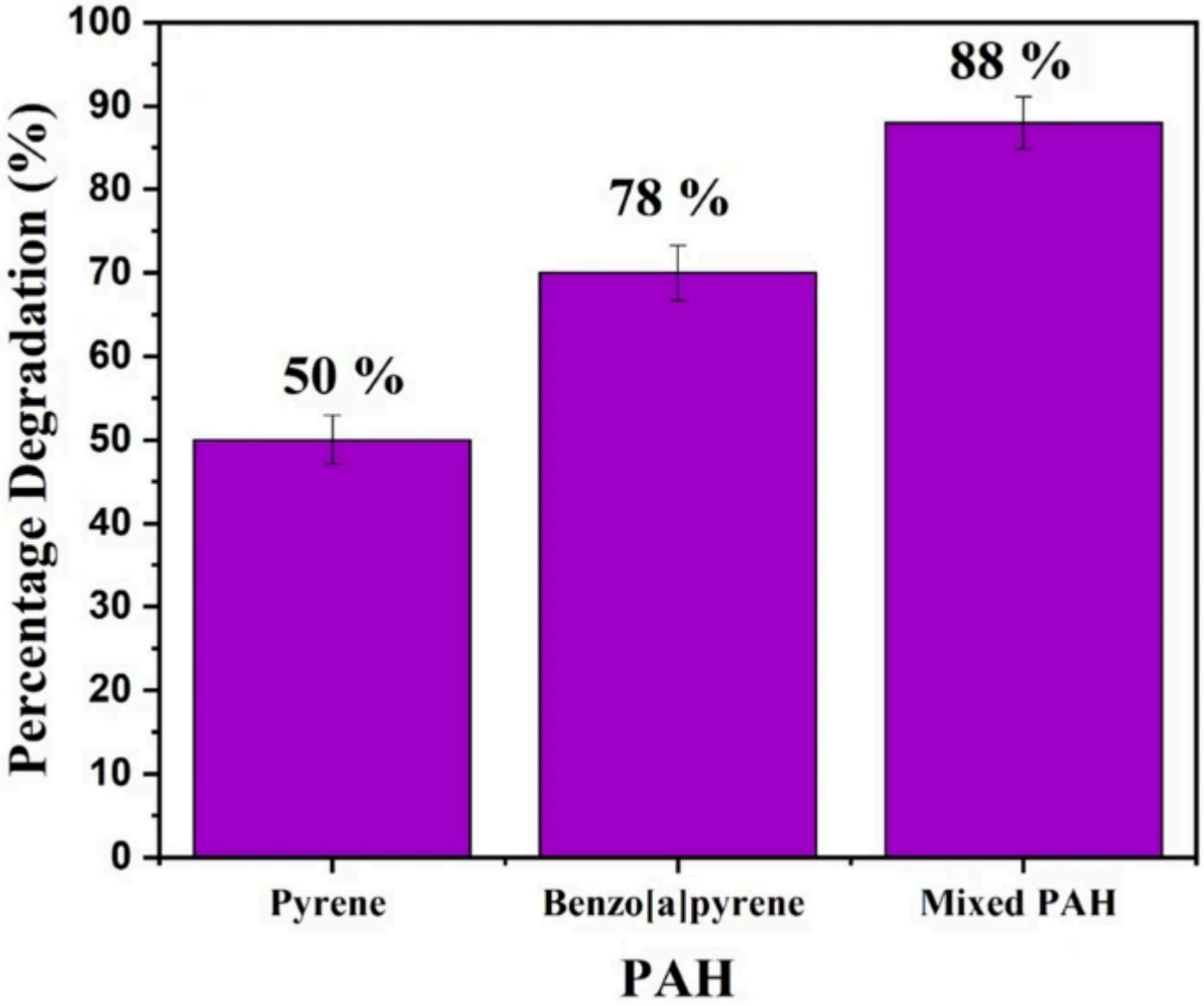
Fig. 4


a
Relative Abundance
Retention time (min)
Pyrene
RT: 21.418
b
Pyrene
1,2,3-trioxonane-4,9-dione
RT: 20.370
c
Benzo(a)pyrene
RT: 20.369
d
Benzo(a)pyrene
1,2,3-trioxonane-4,9-dione
RT: 20.338
e
Pyrene



GC–MS spectra of PAH during biodegradation. (**a**) Pyrene control; (**b**) Pyrene biodegradation; (**c**) Benzo[a]pyrene control; (**d**) Benzo[a]pyrene biodegradation; (**e**) Mixed PAHs (pyrene and benzo[a]pyrene) degradation

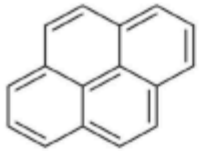
Fig. 5



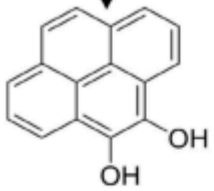
Chromatogram corresponding to the bar graph of PAH degradation

Fig. 6

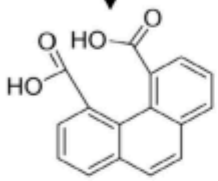
a



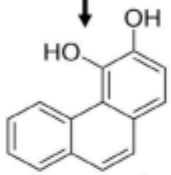
Pyrene



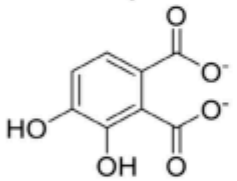
4,5-dihydroxypyrene



Phenanthrene 4,5-dicarboxylic acid

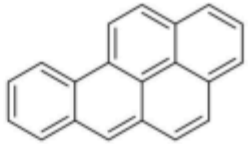


3,4-dihydroxy phenanthrene

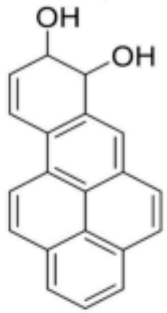


3,4-Dihydroxyphthalate

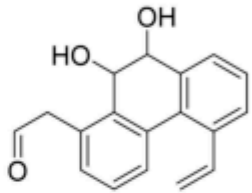
b



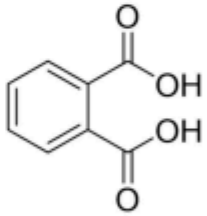
Benzo(a)pyrene



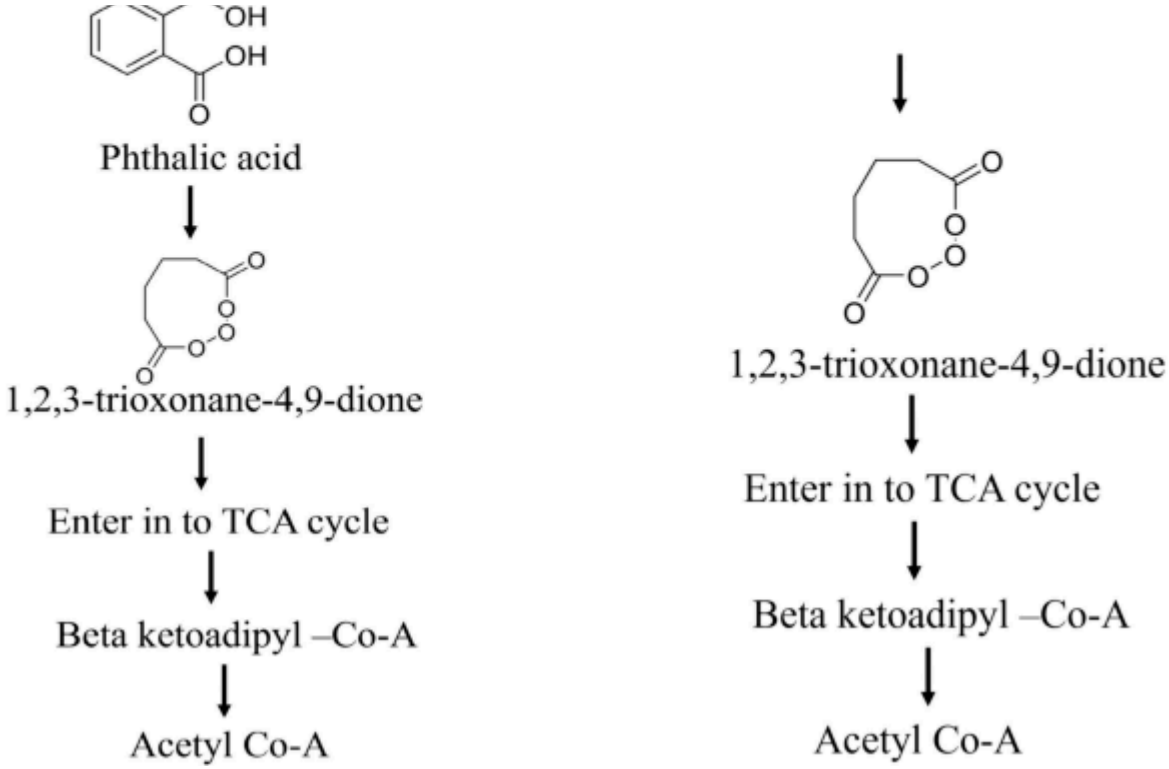
Benzo(a)pyrene-7,8-dihydrodiol



2-(9,10-dihydroxy-5-vinyl-9,10-
ihydrophenanthren-1-yl)acetaldehyde



Phthalic acid



Proposed pathways of PAHs biodegradation. (**a**) Pyrene degradation pathway; (**b**) Benzo[a]pyrene biodegradation pathway

In benzo[a]pyrene degradation, the dioxygenases such as naphthalene dioxygenase or pyrene dioxygenase oxidize for benzo[a]pyrene to benzo[a]pyrene-7,8-dihydrodiol (García de Llasera et al., 2016). Subsequent degradation steps, including ring cleavage and oxidation, produce simpler molecules that are involved in metabolic pathways. The aromatic ring of BaP-7,8-dihydrodiol was cleaved at the 7,8 positions to forming 3,4-dihydrophenanthrene-4-carboxylate. Decarboxylation produces phenanthrene-4-carboxylate, oxidized to 1-(2-hydroxyphenyl)-2-phenylethanone (Nzila & Musa, 2021; Wu et al., 2019). Hydroxylation, an additional reaction, was observed as ring cleavage with oxidation, ultimately leading to the formation of phthalic acid. Furthermore, metabolic intermediate

compounds were completely mineralised through the tricarboxylic acid cycle. The degradation pathways of both pyrene and benzo[a]pyrene converge at phthalic acid and contain a significant intermediate. Phthalic acid then undergoes ring cleavage and oxidation to form 1,2,3-trioxonane-4,9-dione, a crucial step that indicates the breakdown of the aromatic ring structure. This intermediate is subsequently converted to β-ketoadipyl-CoA. CoA-activated compounds are commonly involved in the degradation of protocatechuate and phthalate. As the final degradation intermediate, β-ketoadipyl-CoA is directed into the TCA cycle after its conversion to acetyl-CoA, a vital metabolite for cellular energy production. (Al Farraj et al., 2020; Nzila & Musa, 2021; Radhakrishnan et al., 2023; Wu et al., 2019; Zada et al., 2021). This sequence confirms that the DM1 strain can degrade hydrophobic hydrocarbons with the production of enzymes in a hypersaline environment.

3.7 Proposed Pathway in PAH by the DM1

The proliferation and breakdown of bacteria depend on several factors and conditions. Biodegradation occurs when bacteria break down chemical contaminants, resulting in the loss of their specific chemical composition and properties, such as phenanthrene 4,5-dicarboxylic acid, 3,4-dihydroxy phenanthrene, 1-(2-hydroxyphenyl)2-phenylethanone, and phthalic acid. The bacteria exhibited enhanced metabolic and catabolic activities and efficiently degraded hydrocarbons through the production of specialised extracellular enzymes. These enzymes target specific functional groups within hydrocarbon molecules, breaking them down into smaller hydrocarbon components. This process enables bacteria to absorb energy from hydrocarbons, leading to increased ATP production within their cells (Al Farraj et al., 2020; Wu et al., 2019). During the biodegradation, the bacterial enzymes facilitate the utilization of hydrocarbon and led to the complete mineralisation in an aqueous environment (Radhakrishnan et al., 2023). Extensive research has elucidated the enzymes involved in the degradation pathways and aerobic catabolism of various hydrocarbon compounds by numerous non-halophilic microbes. In subsequent GC–MS studies, different

PAHs were degraded by enzymes, such as dioxygenases or monooxygenases. These enzymes catalyse the addition of oxygen atoms to the alkyl or aromatic region, resulting in the formation of intermediate compounds, including catechols, protocatechuate, and gentisate, which are further degraded through convergent pathways (Elyamine et al., 2021). GC–MS analysis confirmed that the intermediates were cleaved by various ortho- and meta-cleavage dioxygenases, including catechol 2,3-dioxygenase, protocatechuate 3,4-dioxygenases, catechol 1,2-dioxygenase, gentisate 1,2-dioxygenase, and protocatechuate 4,5-dioxygenase enzymes. This process results in the formation of intermediary metabolites like acetyl Co-A, succinyl Co-A, and pyruvate, which subsequently enter the TCA cycle (Al Farraj et al., 2020; Majeed et al., 2012; Nzila & Musa, 2021; Radhakrishnan et al., 2023; Yan et al., 2017; Zada et al., 2021). The proposed metabolic pathway aligned with the detected intermediates during biodegradation. Further, assessed by the comparing the degradation metabolites by the *Halomonas* sp. typically degrade the PAHs via dioxygenation and subsequent ring cleavage. The metabolite profile of DM1 suggested that distinct route involving in the degradation pathway by the monooxygenase-mediated oxidation. This process is similar in the *Marinobacter*, which was known for aliphatic hydrocarbon metaboliser (Khemili-Talbi et al., 2015). However the DM1 exhibit the broader spectrum of biodegradation efficiency of the PAH. Which was due to the enzymatic degradation (Wang et al., 2017). While recent studies suggest that high-salinity microorganisms employ enzymes and degradation pathways akin to non-halophiles for hydrocarbon breakdown, further research is imperative to elucidate the specific steps and intermediates involved in their metabolic processes. However, the natural environment factors such as lower concentrations of contaminant with the presence of competing microorganisms, and fluctuations in salinity and nutrient availability can significantly influence by the biodegradation efficiency.

4 Conclusion

This study demonstrates the potential of DM1 to degrade the high molecular weight PAHs under halophilic conditions. Important degradative enzymes, alkane hydroxylase and alcohol dehydrogenase, were facilitated the biodegradation process. The significant reduction in COD (67–75%) and TOC (69–78%) was observed in the DM1 halophilic biodegradation. FT-IR and GC-MS analysis confirmed the efficient degradation of pyrene (58%), benzo[a]pyrene (70%), and mixed hydrocarbons (88%) in halophilic conditions. The strain DM1 employed the phthalic acid pathway for hydrocarbon metabolite degradation. These findings suggest the strain's remarkable ability to degrade PAHs while tolerating extreme salinity, making it a promising candidate for environmentally sustainable PAHs treatment in saline environments. The degradation of PAHs contaminated water and soil environmental in situ application is in progress.

Data Availability

Data will be made available on request.

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Author information

Authors and Affiliations

Environmental Molecular Microbiology Research Laboratory, Department of Biotechnology, Thiruvalluvar University, Serkkadu, Vellore, Tamil Nadu, 632115, India

Ramanathan Duraimurugan, Krishnan Vignesh, Chenthamarakshan Sahasra & Aruliah Rajasekar

Department of Biotechnology, Faculty of Science and Humanities, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu, Tamil Nadu, 603203, India
Punniyakotti Parthipan

Department of Biochemistry, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, 602105, India
Jayaraman Narenkumar

Contributions

Ramanathan Duraimurugan: Experimental work, Field collection, Writing—original draft. **Krishnan Vignesh:** Experimental work, Field collection. **Chenthamarakshan Sahasra:** Experimental work, Field collection. **Punniyakotti Parthipan:** Writing—review & editing. **Jayaraman Narenkumar:** Writing—review & editing. **Aruliah Rajasekar:** Project administration, Supervision, Validation, Writing—review & editing.

Corresponding author

Correspondence to Aruliah Rajasekar.

Ethics declarations

Ethics Approval

The authors have observed all the ethical issues throughout the experiment.

Competing Interests

Dr. Aruliah Rajasekar serves as an Associate Editor for Water, Air, & Soil Pollution. Appropriate measures were taken to ensure that the editorial process for this manuscript has handled independently to maintain impartiality.

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