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A Green Approach for Microbial- Enhanced Bioremediation of BTEX Contaminated Soils

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Abstract

BTEX compounds benzene, toluene, ethylbenzene, and xylene are persistent and toxic petroleum hydrocarbons that pose serious risks to both environmental and human health. This study presents a sustainable microbial- enhanced bioremediation approach focused on the isolation and characterization of indigenous bacterial strains from hydrocarbon-contaminated soils in Mumbai. Strain N04, identified through enrichment in Bushnell-Haas medium with BTEX as the sole carbon source, demonstrated broad-spectrum degradation capacity for benzene, toluene, and xylene (BTX). Its degradation efficiency was confirmed through time-course studies using Gas Chromatography–Flame Ionization Detection (GC–FID), revealing a 98% reduction in BTX over 21 days. Biochemical characterization indicated selective yet diverse metabolic traits, including positive urease activity and carbohydrate fermentation. These findings validate the metabolic adaptability of native microbes in hydrocarbon-stressed environments and reinforce their potential in eco-friendly and cost-effective remediation strategies. This study lays the groundwork for future research involving molecular identification, pathway elucidation, and field-scale implementation of microbial BTEX degradation.

Keywords: BTEX Biodegradation, Indigenous Bacterial Isolates, Microbial Bioremediation, Gas Chromatography (GC–FID), Petroleum-Contaminated Soil

I. INTRODUCTION

BTEX compounds benzene, toluene, ethylbenzene, and xylene are among the most prevalent and hazardous petroleum hydrocarbons released into the environment through spills, leaks, and industrial discharges. Due to their high volatility and solubility, BTEX pollutants can migrate through soil and groundwater, posing critical threats to ecosystems and human health. Benzene, in particular, is classified as a Group 1 human carcinogen, while the other BTEX components are associated with neurological and respiratory toxicity.

Conventional remediation approaches, such as excavation and chemical oxidation, are often expensive, invasive, and prone to generating secondary pollutants. In contrast, microbial bioremediation offers a low-cost, eco-friendly alternative by leveraging the natural metabolic capacity of bacteria to degrade BTEX compounds into less harmful end-products. Indigenous bacterial populations from petroleum-contaminated sites are particularly promising due to their pre-existing adaptation to hydrocarbon stress.

This study focuses on the isolation and characterization of native bacterial strains from fuel-contaminated soils in Mumbai. The aim is to identify organisms capable of efficiently degrading BTEX components under controlled aerobic conditions. Laboratory-scale biodegradation experiments were conducted using Bushnell-Haas medium supplemented with BTEX as the sole carbon and energy source. One isolate, designated as Strain N04, exhibited strong and broad-spectrum degradation of benzene, toluene, and xylene over a 21-day period.

By examining microbial growth patterns and BTX degradation kinetics via Gas Chromatography–Flame Ionization Detection (GC–FID), this study provides valuable insights into the potential of native microbes as sustainable agents for bioremediation. The findings contribute to the ongoing development of nature-based solutions to mitigate petroleum hydrocarbon contamination in urban environments.

II. LITERATURE REVIEW

A. National Status

Singh (2019) emphasized the widespread occurrence of BTEX compounds in industrial effluents and their associated ecological and health impacts. The study recommended green, sustainable technologies to mitigate such pollutants. Rajni Singh and Mary Celin (2010) studied morphological adaptations of bacterial strains under BTEX exposure, highlighting microbial resilience in toxic environments. Mukherjee and Bordoloi (2012) demonstrated that microbial consortia, when supplemented with nutrients under hypoxic conditions, significantly enhance BTX degradation. Haritash and Kaushik (2009) reviewed the role of environmental factors like temperature and pH in influencing microbial biodegradation, stressing the importance of optimized conditions. Mohan et al. (2019) used *Pseudomonas putida* in a bioelectro chemical system to achieve over 90% BTEX degradation, illustrating the potential of microbial systems under controlled parameters. Chattopadhyay et al. (2017) developed a GC×GC–TOFMS technique for improved BTEX detection in complex matrices, which complements analytical approaches such as GC–FID used in the present study.

B. International Status

Nicholson and Fathepure (2004) highlighted the degradation capabilities of *Marinobacter* spp. in saline environments, suggesting their ecological robustness. Wang and Shao (2006) isolated BTEX-degrading *Pseudomonas* and *Acinetobacter* strains with tolerance to variable salinity and temperature, supporting the importance of strain selection. Wang et al. (2008) identified marine *Pseudomonas*, *Rhodococcus*, and *Bacillus* as capable of degrading multiple aromatic hydrocarbons via dioxygenase-mediated pathways. Hendrickx et al. (2006) developed genetic markers such as *tmoA* and *xylM* to track BTEX degradation genes in contaminated soils. Victor et al. (2017) demonstrated the role of biosurfactant-producing bacteria in hydrocarbon breakdown, improving degradation kinetics. Bacosa et al. (2021) confirmed that *Burkholderia* & *Pseudomonas* strains show a preference for degrading aromatic compounds like benzene. Zhang et al. (2021) analyzed microbial pathways in petrochemical-polluted groundwater, identifying *Proteobacteria* dominance and mixed aerobic-anaerobic degradation processes.

III. METHODOLOGY

This study was designed to evaluate the BTEX-degrading potential of indigenous bacteria isolated from petroleum-contaminated soil. All experimental procedures were conducted under aseptic and controlled laboratory conditions in a single primary phase comprising microbial isolation, screening, degradation testing, and biochemical characterization.

A. Sample Collection

Soil samples were collected from hydrocarbon - contaminated sites in Mumbai, specifically garages & petrol stations known for chronic petroleum exposure. Such locations exert selective pressure on microbial communities, promoting the

emergence of hydrocarbon-degrading species (Singh, 2019). Samples were collected from the top 10–15cm of soil using sterile stainless-steel spatulas and transferred to pre-sterilized polyethylene containers. Samples were stored at approximately 4°C during transport to maintain microbial viability (Mukherjee & Bordoloi, 2012).

Fig. 1: Sample collection from a garage.

B. Enrichment of BTEX-Degrading Microorganisms

To enrich BTEX-degrading bacteria, 10 g of soil was inoculated into 100 mL of Bushnell-Haas (BH) broth supplemented with BTEX (benzene, toluene, ethylbenzene, xylene) as the sole carbon and energy source. The flasks were incubated aerobically at 30°C on a rotary shaker at 120 rpm for 7 to 14 days (Wang & Shao, 2006). Microbial growth was assessed through visual turbidity and surface film formation (Al-Yaqout, 2003).

Fig.2: 2.1 – Day 1 of enrichment.
Fig. 2.2 – Day 14 showing turbidity and film.

C. Isolation and Screening of BTEX Degraders

After enrichment, serial dilutions were plated on BH agar supplemented with BTEX. The BTEX mixture was added aseptically post-autoclaving to preserve integrity. Plates were incubated at 30°C for 48–72 hours (Mukherjee & Bordoloi, 2012). Fifteen colonies showing robust growth were isolated, of which six morphologically distinct strains were selected for subculturing and further testing.

D. Gas Chromatography–FID Analysis for BTEX Degradation

To confirm degradation, each isolate was inoculated into separate BH broth flasks containing individual BTX compounds. Abiotic controls without microbes were included. Incubation was carried out at 30°C and 120 rpm for 21 days. Samples were collected on Day 1, 8, 14, and 21 (Hendrickx et al., 2006).

BTEX compounds were extracted using petroleum ether and separated using a funnel. Extracts were passed through anhydrous sodium sulfate to remove residual moisture and stored in sterile vials (Mukherjee & Bordoloi, 2012). Quantification of BTEX degradation was performed using Gas Chromatography equipped with a Flame Ionization Detector (GC–FID), which is highly sensitive for volatile organic compounds (Pascale et al., 2018).

Fig. 3: Rotary shaker incubation.

E. Microbial Characterization

The isolate with the highest degradation activity (Strain N04) was selected for detailed characterization. Morphological traits such as colony size, elevation, margin, color, and surface were recorded on BH and nutrient agar plates. Biochemical profiling included Gram staining, IMViC tests, urease, and sugar fermentation assays (Wang et al., 2008). Strain N04 was Gram-negative and urease-positive. It produced acid and gas from glucose and mannitol but showed no fermentation with maltose. These traits suggest metabolic versatility in hydrocarbon-contaminated environments. Due to time and budget limitations, molecular identification using 16S rRNA sequencing was not performed but is suggested for future work.

Table 1: Colony characters of Strain N04.

Colony characters	Observation Strain N04
Size	~2mm diameter
Shape	Circular
Elevation	Flat
Color	White
Opacity	Opaque
Surface	Wrinkled
Margin	Rough
Consistency	Dry
Growth on BH Agar	Moderate to good
Growth on Nutrient Agar	Consistent

Fig. 4 : Biochemical test

Table 2: Biochemical test results of Strain N04.

Biochemical Test	Result (Strain N04)
Gram Staining	Negative
Indole Test	Negative
Methyl Red (MR) Test	Negative
Voges-Proskauer (VP) Test	Negative
Citrate utilization	Negative
Urease Test	Positive
TSI (Triple Sugar Iron)	K/N, No H_2S

IV. RESULTS

A single isolate, Strain N04, demonstrated the highest degradation potential among the six screened colonies. The strain was tested for its ability to degrade benzene, toluene, and xylene over a 21-day incubation period. Degradation was assessed at regular intervals and expressed as a percentage reduction in compound concentration, as determined by GC–FID analysis.

Table 3. BTX Degradation by Strain N04 over 21 Days

Compound	Day 1%	Day 8%	Day 14%	Day 21%
Benzene	0	67.43	89.92	100
Toluene	0	58.72	76.20	94.72
Xylene	0	53.91	71.56	92.30

Strain N04 achieved complete degradation of benzene within 21 days, while degradation efficiencies for toluene and xylene exceeded 90%. The higher degradation rate of benzene may be attributed to its simpler structure, making it more readily metabolized than its alkyl-substituted counterparts (Wang et al., 2008; Singh, 2019). These results confirm the isolate's broad-spectrum BTX degradation capacity.

Morphologically, Strain N04 formed 2 mm, circular, flat colonies with a wrinkled surface and rough margins. Biochemically, the strain was Gram-negative, urease-positive, and capable of fermenting glucose and mannitol with acid and gas production. These characteristics suggest robust adaptability and metabolic versatility in hydrocarbon-rich environments (Mukherjee & Bordoloi, 2012).

The findings align with previous reports on indigenous bacterial strains exhibiting enhanced hydrocarbon degradation under optimized conditions, reinforcing the potential of Strain N04 as a candidate for eco-friendly BTEX bioremediation.

V. CONCLUSION AND RECOMMENDATION

The present study highlights the potential of native microbial strains in remediating petroleum-contaminated environments. Strain N04, isolated from hydrocarbon-exposed soil in Mumbai, demonstrated efficient degradation of BTX compounds achieving complete degradation of benzene and over 90% reduction of toluene and xylene within 21 days under aerobic laboratory conditions. These findings suggest that naturally adapted bacteria can play a pivotal role in eco-friendly and cost-effective bioremediation strategies for BTEX pollution.

The biochemical profile of Strain N04 indicated favorable enzymatic activity and metabolic adaptability, further supporting its applicability in hydrocarbon degradation. The observed degradation kinetics align with prior studies that emphasize the competence of indigenous bacteria under optimized environmental conditions (Wang & Shao, 2006; Mukherjee & Bordoloi, 2012).

Recommendations:

i. Future work should include **molecular identification** (e.g., 16S rRNA sequencing) of Strain N04 to understand its taxonomy and genetic traits.
ii. Enzymatic assays and pathway mapping should be conducted to elucidate the metabolic routes involved in BTX degradation.
iii. Pilot-scale or **field-level bioremediation trials** using Strain N04 in real contaminated soils are recommended to validate laboratory findings.
iv. Integration with other sustainable remediation techniques (e.g., nutrient supplementation, oxygenation) may further enhance its degradation performance.

The study provides a strong baseline for the development of indigenous microbe-based remediation systems for BTEX-contaminated environments.

ABBREVIATIONS

BTEX: Benzene, Toluene, Ethylbenzene, Xylene
GC–FID: Gas Chromatography–Flame Ionization Detection
BH – Bushnell-Haas
TSI – Triple Sugar Iron
Na_2SO_4 – Anhydrous Sodium Sulfate

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