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Revolutionizing Soil Health: Enhanced Microbial Bioremediation of BTEX Contaminants

Muskaan Sanawar Husain Mansuri[1], Aishwarya Ajayakumar[2], Dr. Udaybhan Yadav[3*]

[1] PG Student of Department of Microbiology, Thakur Shyamnarayan Degree College, University of Mumbai, Maharashtra, India
[2] Asst. Professor, Department of Microbiology, Thakur Shyamnarayan Degree College, University of Mumbai, Maharashtra, India
[3] Project Guide, Asst. Professor & Head of Department of Microbiology, Thakur Shyamnarayan Degree College, University of Mumbai, Maharashtra, India

Corresponding Author: Dr. Udaybhan Yadav*

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Abstract

BTEX compounds benzene, toluene, ethylbenzene, and xylene are hazardous pollutants from petroleum activities that threaten soil, water, and human health. This study explores an eco-friendly bioremediation strategy using indigenous BTEX-degrading bacteria and biochar derived from Ricinus communis. The isolated bacterial strains showed strong metabolic potential for degrading BTEX. Biochar, prepared via pyrolysis, enhanced microbial activity and BTEX adsorption. Although their combined effect remains validated in the field, this integrated approach offers a cost-effective and sustainable alternative to chemical remediation. Future work may include molecular identification and pilot-scale trials to scale the application of this green remediation method.

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1. INTRODUCTION

BTEX compounds benzene, toluene, ethylbenzene, and xylene—are volatile, toxic hydrocarbons frequently found in soils near fuel stations, industrial zones, and traffic-heavy areas. These pollutants originate primarily from petroleum spills, leakage, and industrial waste, posing severe risks to environmental and public health. Benzene is a known human carcinogen, while the others can lead to neurological, respiratory, and ecological damage. Traditional remediation techniques like soil excavation or chemical oxidation are costly, invasive, and often produce secondary pollution. This has driven the demand for sustainable, eco-friendly alternatives. Bioremediation using microbes capable of degrading BTEX offers a promising solution. However, natural attenuation is often too slow in highly contaminated or stressed environments. Hence, this study emphasizes bioaugmentation the introduction of indigenous BTEX-degrading bacteria isolated from contaminated soil. To enhance this process, biochar derived from *Ricinus communis* (castor plant) was used. Castor biomass is lignocellulose-rich and, when converted to biochar via pyrolysis, yields high porosity and surface area. These properties make it an ideal microbial carrier and BTEX adsorbent. Biochar improves microbial survival, boosts contaminant bioavailability, and stabilizes enzymes involved in degradation. This research investigates the synergistic use of native bacterial strains and *Ricinus communis* biochar to improve BTEX degradation efficiency in contaminated soils. The aim is to develop a scalable, sustainable method suitable for real-world remediation, especially in developing countries like India, where industrial pollution is rapidly rising. The outcomes may offer practical, nature-based solutions that restore soil health and reduce long-term environmental risks.

2. LITERATURE REVIEW

Numerous studies, both national and international, have advanced the field of BTEX bioremediation by investigating microbial diversity, degradation mechanisms, and bioaugmentation strategies.

National Status

Singh [1] emphasized the alarming presence of BTEX compounds in Indian soils, particularly near fuel-handling sites, highlighting the urgent need for eco-friendly remediation strategies. Jain *et al.,* [2] demonstrated the adaptability of native microbes to petroleum hydrocarbons, reinforcing the importance of site-specific solutions. Megharaj *et al.,* [3] showed that indigenous bacteria, under optimized conditions, can degrade hazardous pollutants efficiently. Sinha [4] pioneered vermitechnology in India, combining earthworms and microbial consortia for effective soil remediation. Kakodkar [5] explored microbial consortia for BTEX degradation, finding that mixed cultures outperform individual strains. Kuhad [6] analyzed the enzymatic mechanisms of microbial degradation, underlining the need to select highly efficient strains for bioremediation. Vinothini [7] supported bioaugmentation approaches, while Juwarkar [8] validated the success of indigenous microbes through field trials.

International Status: Atlas [9] laid the foundation for petroleum hydrocarbon biodegradation, demonstrating microbial roles in BTEX removal. Nicholson and Fathepure [10] studied BTEX degradation under hypersaline conditions, expanding bioremediation's applicability to arid regions. Aburto and Peimbert [11] confirmed rapid BTEX degradation using adapted microbial communities. Rittmann [12] highlighted the need for integrated ecological approaches. Woese [13] and Tiedje *et al.,* [14] revolutionized microbial monitoring using molecular tools like 16S rRNA sequencing, improving the tracking of BTEX-degrading species. More recently, Wongbunmak *et al.,* [15] isolated *Bacillus* strains capable of degrading all six BTEX compounds, suggesting their use in *in situ* remediation.

3. METHODOLOGY

Soil samples were collected from multiple BTEX-contaminated sites, including oil spill zones, service stations, and petrol pumps in Kandivali, Mumbai. Sampling was performed at a depth of 10–20 cm using sterile tools as outlined by Mirdamadian [17], and samples were immediately transferred into sterile bags for laboratory analysis. For the enrichment of BTEX-degrading bacteria, 1 g of soil was inoculated into Bushnell-Haas (BH) medium supplemented with a standard BTEX mixture as the sole carbon source, following the bioaugmentation strategy described by Vinothini and Kandasamy [16]. Enrichment cultures underwent serial transfers with gradually increasing BTEX concentrations to select for robust degraders. After enrichment, aliquots were plated onto BH agar containing BTEX. Distinct colonies were visually selected and further purified by repeated streaking on nutrient agar. Pure isolates were screened for BTEX degradation by inoculating them into BH medium with BTEX, followed by incubation under shaking conditions to ensure uniform growth, as outlined in Brock Biology of Microorganisms by Madigan *et al.,* [22]. To assess degradation efficiency, residual BTEX levels were extracted using petroleum ether and quantified by Gas Chromatography – Flame Ionization Detection (GC–FID), as per EPA Method 5035 [19]. Biochemical characterization of promising isolates was performed using classical microbiological tests described by Marge and Schinner [18] and Madigan *et al.,* [22]. In parallel, biochar was synthesized from *Ricinus communis* (castor plant) residues via pyrolysis at 400–700°C, following the method proposed by Lehmann and Joseph [20], and its suitability as a microbial carrier was evaluated based on nanoparticle stabilization insights from Ramanayaka *et al.,* [21]. All experiments were performed in triplicate, and appropriate controls were maintained throughout the study. The methodology was designed to isolate indigenous BTEX-degrading bacteria and evaluate their potential for application in sustainable soil bioremediation, with a focus on synergistic use with biochar.

Figure 1: Soil collection from the oil-contaminated zone

Figure 2: Soil collection from the oil spillage area

Figure 3: Collected soil was inoculated in enrichment media

Figure 4: *Ricinus communis* (castor) plant residues.

4. RESULTS AND DISCUSSION

The degradation efficiency of indigenous bacterial isolates was assessed through controlled microcosm experiments over 21 days. Soil samples artificially spiked with BTEX compounds benzene, toluene, ethylbenzene, and xylene, were inoculated with selected bacterial strains isolated from the original contaminated site. These isolates had previously shown growth in enrichment cultures containing BTEX as the sole carbon source. Quantitative analysis using Gas Chromatography - Flame Ionization Detection (GC-FID) showed a consistent and measurable decline in BTEX concentrations in all inoculated samples. Among the four compounds, benzene the most volatile and toxic was completely degraded by day 21. Toluene and ethylbenzene showed approximately 93% and 91% reduction, respectively, while xylene was reduced by about 90%.

Table 1: CG-FID of BTEX Degradation

COMPOUND	DAY 1	DAY 8	DAY 21
BENZENE	108.7	17.61	0
TOULENE	139.79	51.95	3.24
ETHYL BENZENE	265.4	174.76	20.53
M/P XYLENE	80.76	29.83	3.08
O XYLENE	28.61	10.4	1.34

Biochemical tests were used to assess the metabolic capabilities of the selected isolates. The following results were obtained.

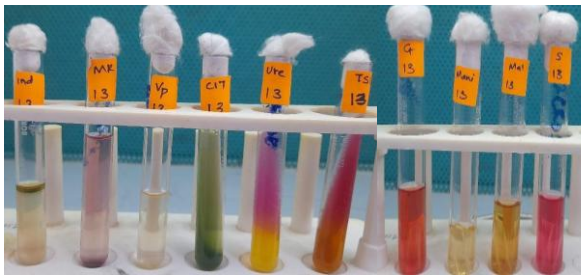

Figure 5: Standard biochemical tests were performed

Table 2: Biochemical tests

Biochemical Test	Result
Indole	Negative
Methyl Red (MR)	Positive
Voges-Proskauer (VP)	Negative
Citrate	Negative
TSI	K/A
Urease	Positive
Mannitol fermentation	Acid + Gas
Maltose fermentation	Acid + Gas
Glucose fermentation	Acid
Sucrose fermentation	Acid

Simultaneously, bacterial growth was monitored using spectrophotometric OD_{600} measurements. A gradual increase in OD values was observed across all test flasks, indicating active microbial proliferation. This growth was particularly prominent between days 7 and 14, suggesting that the isolates had acclimatized and metabolically adapted to use BTEX compounds as energy sources.

Table 3: OD_{600} Microbial Growth

Parameter	OD_{600}(Microbial Growth)
Control	0.00
Benzene	0.44
Toluene	0.38
Ethylbenzene	0.23
Xylene	0.38

These results validate the metabolic potential of native soil bacteria to break down hazardous aromatic hydrocarbons under aerobic conditions. The strong correlation between biomass growth and BTEX degradation further confirms that the isolates were not only surviving but also actively transforming the pollutants into less harmful compounds possibly through enzyme-mediated hydroxylation and ring-cleavage mechanisms known in BTEX degradation pathways.

5. DISCUSSION

The findings of this study reinforce the potential of indigenous microbial strains for effective BTEX biodegradation in contaminated soils. The isolated bacterium, identified through biochemical profiling, showed significant efficiency in degrading BTEX compounds, with complete elimination of benzene within 21 days. The steady increase in OD600 during the incubation period supports active microbial proliferation and utilization of BTEX as the sole carbon source [22].

The observed degradation is consistent with the findings of Vinothini and Kandasamy [16], who demonstrated the effectiveness of native bacterial consortia under hydrocarbon stress. The enzymatic traits observed such as urease and methyl red activity highlight the isolate's metabolic versatility, which is essential for survival in nutrient-limited or chemically harsh conditions [22]. Although the synergistic use of biochar was not tested in this phase, the prepared *Ricinus communis* biochar showed desirable physicochemical properties. Previous studies by Lehmann and Joseph [20] and Ramanayaka *et al.,* [21] have reported biochar's ability to enhance microbial colonization and pollutant adsorption, supporting its future use as a microbial carrier and BTEX adsorbent. Collectively, these results support the integration of bioaugmentation and biochar-based biostimulation as a promising nature-based strategy for remediating petroleum-contaminated soils in an eco-friendly and cost-effective manner.

6. CONCLUSION AND RECOMMENDATION

This study was undertaken to address the persistent environmental threat posed by BTEX compounds benzene, toluene, ethylbenzene, and xylene commonly found in fuel-contaminated sites. Through an integrated bio-based approach, the research demonstrated that indigenous soil bacteria, when carefully isolated and screened, possess significant metabolic versatility to degrade these harmful aromatic hydrocarbons. One of the most promising findings was the successful isolation and biochemical characterization of a potent BTEX-degrading bacterium, which showed excellent degradation capabilities and adaptability to nitrogen-limited and pH-variable environments. The strain exhibited enzymatic traits, such as positive methyl red and urease activity, suggesting robust metabolic flexibility essential for effective bioremediation in real-world conditions.

Furthermore, biochar was successfully synthesized from *Ricinus communis* (castor plant), a fast-growing and widely available agricultural residue. Although its application with bacteria was not yet tested in this study, the prepared biochar showed favorable characteristics such as high surface area and porosity, indicating its potential as both a microbial carrier and adsorbent for BTEX compounds. This work emphasizes the importance of nature-inspired solutions that utilize local resources. It shows that low-cost, scalable, and sustainable remediation techniques are possible without relying on synthetic chemicals or energy-intensive systems. The results support the idea that bioaugmentation combined with biochar-based biostimulation could offer a powerful strategy for urban and rural soil rehabilitation efforts.

7. RECOMMENDATIONS FOR FUTURE RESEARCH

1. Combine biochar and bacterial isolates in controlled and field-scale experiments to test synergistic effects on BTEX degradation rates.

2. Perform molecular identification (e.g., 16S rRNA sequencing) of the bacterial isolates to precisely understand their taxonomy and degradation pathways.
3. Characterize the biochar fully surface area, porosity, chemical composition to optimize its function as a microbial support and BTEX adsorbent.
4. Expand the use of local biomass types for biochar production to make the solution regionally adaptable.
5. Conduct pilot-scale field trials to evaluate practical application and environmental impact in diverse soil conditions.

ABBREVIATIONS

1) **BTEX:** Benzene, Toluene, Ethylbenzene, Xylene
2) **GC–FID:** Gas Chromatography–Flame Ionization Detector
3) **BH:** Bushnell-Haas (medium)
4) **OD_{600}:** Optical Density at 600 nm
5) **ATSDR:** Agency for Toxic Substances and Disease Registry

APPENDIX

Bushnell-Haas (BH) Medium Composition:

Component	Amount/ Liter (g/L)
Magnesium sulfate ($MgSO_4$)	0.2
Calcium chloride ($CaCl_2$)	0.02
Monopotassium phosphate (KH_2PO_4)	1.0
Dipotassium phosphate (K_2HPO_4)	1.0
Ammonium nitrate (NH_4NO_3)	1.0
Ferric chloride ($FeCl_3$)	0.05
Distilled water	1000 mL

pH: Adjust to 7.0

Carbon Source: BH medium does not contain a carbon source by default. For BTEX degradation studies, BTEX compounds are added as the sole carbon source.
Sterilization: Autoclave the medium at 121°C for 15 minutes before use. Add BTEX compounds aseptically after cooling, if required.

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About the Corresponding Author

Dr. Udaybhan Yadav is an Assistant Professor and Head of the Department of Microbiology at Thakur Shyamnarayan Degree College, University of Mumbai, Maharashtra, India. He also serves as a Project Guide and is actively involved in mentoring undergraduate and postgraduate research in microbiology. His academic interests include microbial biotechnology, environmental microbiology, and applied research in microbial remediation.