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Optimal Conditions Dramatically Enhance the Degradation Of M-Xylene by Phyllosphere Bacteria, Clearly Outpacing the Breakdown of Other BTEX Compounds.

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Abstract
BTEX compounds comprises of benzene, toluene, ethylbenzene, m-xylene, o-xylene, and p-xylene pose significant environmental pollution risks due to their rising production and use. This study examined optimal conditions for biodegradation by investigating factors like pH, temperature, agitation rates, and nitrogen availability. The results revealed that phyllosphere bacteria could significantly improve the degradation of these toxic hydrocarbons, with Pseudomonas putida HLPSC8 emerging as the most effective strain. Optimal conditions favoured the degradation of m-xylene, achieving the lowest residual hydrocarbon levels with minimal cell biomass generation. These findings point to promising avenues for further research into other bacterial strains for hydrocarbon degradation, which is crucial for environmental management. Understanding the performance of Pseudomonas putida HLPSC8 lays the groundwork for future studies on varying hydrocarbon concentrations and biodegradation strategies..
Keywords: *Benzene, Toluene, Ethylbenzene, m-xylene, o-xylene, p-xylene, gas chromatography, Bioremediation*

1. INTRODUCTION

Hydrocarbons are organic compounds made up of hydrogen and carbon atoms. They are highly combustible and emit carbon dioxide, water and heat when burned. They act as an excellent source of fuel. The usage of hydrocarbons is found mostly in the manufacturing of plastics, polymers, pharmaceuticals and other industries. The increase of carbon dioxide in the atmosphere when energy is produced as a result of the inclusion of oil and natural gas is a major concern with these hydrocarbon compounds. It always acts as a major environmental concern towards global warming. Environmental degradation and climate change are increasingly recognized as the foremost global concerns, fundamentally linked to human well-being and sustainability risks (Wassenius and Crona 2022).

The volatile organic compounds are generally organic, and they exert high vapour pressure at room temperature. Most of these compounds are dangerous to human health.Due to their extensive applications, volatile organic compounds (VOCs) pose direct or indirect exposure to the general population. However, individuals employed in industrial sectors experience prolonged exposure to these compounds, as they are utilized in various chemical processes. Compounds such as xylene and toluene have been demonstrated to produce neurobehavioral effects in rat populations (Armenta-Reséndiz et al., 2019).. The major group of these compounds, termed BTEX, comprises benzene, toluene, ethylbenzene, m-xylene, o-xylene and p-xylene. Benzene is formed as a result of burning fuel such as coal, petrol and wood. The World Health Organisation have stated benzene as a Group 1 carcinogen, which causes leukaemia and impacts red and white blood cells. Toluene, normally known as methylbenzene, is used mostly as a solvent, whereas ethylbenzene is found in the manufacture of styrene compounds. The xylene derivatives were found in the crude oil combustion, production of plastic bottles, and polyester clothing manufacturing industries. All these aromatic compounds are considered as hazardous compounds. Due to the toxic nature, stability, and cumulative effects of BTEX compounds, they can significantly impact both the environment and human health. Therefore, it is crucial to continuously monitor and evaluate the health risks associated with these substances, as this is an important public health issue that needs to be addressed.(Huang B et al.,2014)

The phyllosphere region is generally found at the aerial parts of plants harbours large microbial communities. The large surface areas provide more number of microbiome. The phyllosphere, the external surface of leaves, represents a vital and dynamic habitat teeming with a diverse array of microorganisms. This unique environment plays a crucial role in various ecological processes, providing essential resources and shelter for microbial communities (Vacher et al., 2016). .Actinobacteria, Firmicutes, Bacteroides and Proteobacteria groups of bacteria were found abundantly in these region. The bacteria associated with seed coats that have been identified in the phyllosphere predominantly belong to influential groups, including Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. These microbial communities play a crucial role in plant health and ecosystem dynamics, highlighting their significance in agricultural and ecological research.(Johnston-Monje and Raizada 2011; Rodríguez-Escobar et al. 2018) These epiphyte bacteria's generally found influenced by exposure of light, temperature and presence of plant species. These phyllsophere bacteria generally play a vital role in degradation of organic volatile compounds such as BTEX in a considerable manner. Herby they are useful in maintaining ecological relationship, increasing the yield of crops and removal of harmful substances. But this phyllosphere biodegrading of BTEX compounds is only in the limited edition. Microorganisms can break down hydrocarbons either by individual strains or by groups of different strains, which may belong to the same or different genera.(Boopathy, 2000)

Bioremediation represents a cutting-edge and ecologically sustainable technology that utilizes biological microorganisms to decompose and neutralize pollutants (Ajona and Vasanthi, 2021). This technique plays a crucial role in removing environmental toxins, thereby restoring ecosystems to their natural state and preventing further contamination (Sardrood et al., 2013, Berillo et al., 2021).Bioremediation is a dynamic and evolving field that utilizes the natural abilities of living organisms to clean up contaminated environments (Nazir et al., 2024). By understanding and optimizing the factors that affect microbial degradation, we can improve the efficiency and applicability of bioremediation strategies, ultimately aiding in the restoration and protection of ecosystems (Kalogiouri et al., 2024).Additionally, the process can be enhanced by providing nutrients such as phosphorous, nitrogen, and carbon (Maier et al.2000).

This study investigates the isolation of phyllosphere bacteria from the leaves of Pongamia trees situated near the industrial estate of Puducherry and the Cuddalore SIPCOT area. The isolated bacteria were screened for their potential hydrocarbonoclastic capabilities using both biomass growth and gravimetric methods. Following the screening process, five of the most promising strains were identified through 16S rRNA sequencing. These strains were then evaluated for their tolerance to BTEX compounds and their ability to interact with combined hydrocarbons. The study also examined various factors, including pH, temperature, agitation, and different nitrogen concentrations. The degrading activity was assessed through growth kinetics profiles and gas chromatographic analysis.

2. MATERIALS AND METHODS

2.1 Isolation of Phyllosphere Hydrocarbon Utilising Bacteria

Leaves coated with a hydrocarbon overlayer were collected from Pongamia trees in the industrial areas of Cuddalore (SIPCOT) and Puducherry. Using pre-sterilized tools, the leaves were cut and placed in a sterilized 1-liter container. They were transported at 4°C in an airtight thermocol box and immediately processed to isolate hydrocarbon-utilizing phyllosphere bacteria under aseptic conditions. Selected hydrocarbon spots were sterilely removed, and one gram of the sample was homogenized with pre-sterilized bacteriological saline. This mixture was inoculated into 100 ml of Bushnell Haas broth, supplemented with 1% crude oil as a carbon source, and incubated for 7 days. The bacterial population was then serially diluted and spread-plated on nutrient agar for isolation.Pure cultures were obtained through repeated culturing on fresh nutrient agar plates using the quadrant streaking technique and were confirmed via colony morphology and Gram staining..

2.2 Screening of potential hydrocarbonoclastic bacteria

We tested each type of pure cultured bacteria to see how well they can break down hydrocarbons. First, we prepared the inoculum to a concentration of 108 CFU per milliliter using a 0.5 McFarland turbidity standard. Then, we added 1% of this inoculum to a 250 mL conical flask with 100 mL of Bushnell Haas broth and 10% crude oil. We incubated this mixture at 37°C for 7 days to stimulate microbial growth.After the incubation, we checked the bacteria that can degrade hydrocarbons by extracting the

leftover crude oil with an equal amount of hexane. We measured the remaining oil using gravimetric methods based on the work of Rahul et al. (2013). We also calculated the total dry weight of the cell biomass by centrifuging the cultured broth and drying the cell pellet in a hot air oven at 60°C.

2.3 Molecular Identification of Potential Strains

Potential strains were selected based on cell biomass and crude oil biodegradation percentage. They were then identified using molecular methods, specifically the 16S rRNA gene, for further characterization studies.

DNA isolation procedure: After evaluating the cell biomass and the percentage of crude oil biodegradation activities, we selected potential strains for further in-depth characterization studies. To identify the potential strains, we used the 16S rRNA identification method along with molecular techniques.

2.4 Secondary screening of most potential hydrocarbons utilizing bacterium and identification of potential bacteria

In this study, we tested five types of bacteria that can break down hydrocarbons. Each bacteria strain was checked to see if it could use six different hydrocarbons: benzene, toluene, ethylbenzene, m-xylene, o-xylene, and p-xylene. We prepared each strain to a specific concentration using a standard method. Then, we added 1% of each strain to 100 ml of Bushnell Haas broth in a 250 ml airtight flask to keep the hydrocarbons from evaporating. We also added 0.5% ammonium nitrate for nitrogen.After incubating the mixtures at 37°C for four days, we measured the remaining hydrocarbons by extracting them with hexane. We used a weight measurement method to analyze these hydrocarbons, based on a technique from Luna et al. (2009). We also checked the total dry weight of the bacteria by centrifuging the broth after incubation and drying the cell pellet in an oven at 60°C.We conducted the utilization study with a 1% concentration of each individual BTEX compound. The biomass was calculated using the weight measurement method. Based on these results, we identified the most effective bacteria.

2.5. Tolerance study of potential bacterium

The study focused on how hydrocarbons are used at a concentration of only 1%. To learn more, we conducted a tolerance study using concentrations between 1% and 10% for all BTEX compounds. We based our findings on the cell biomass and the amount of remaining hydrocarbon.

2.6. Biodegradation activity.

The P. putida HLPSC8 strain was optimized to break down various hydrocarbons, such as benzene, toluene, ethylbenzene, m-xylene, o-xylene, and p-xylene. We used a method that changed one factor at a time for testing.To ensure reliable results, we standardized each factor separately. We conducted the tests in a 250 mL airtight conical flask with a 100 mL working volume. The basic conditions included 1% ammonium nitrate, a pH of 7, a temperature of 37°C, and a 120-hour incubation period. The hydrocarbons served as the carbon source at levels that P. putida HLPSC8 could tolerate.We prepared the inoculum from the strain during its exponential growth phase, using the same basic conditions. We adjusted the optical density at 620 nm (OD620nm) of the inoculum to 0.1, following the McFarland turbidity standard of 0.5. This corresponds to a bacterial cell concentration of 1 × 10^8 cfu/mL.We calculated the degradation of hydrocarbons by measuring the remaining amount after extraction with an equal volume of hexane. We estimated this using gravimetric analysis, as described in previous research (Yang et al., 2019). We performed all measurements in triplicate and reported the results as mean ± standard deviation.

2.7. Standardization of various cultural conditions improving biodegradation activity

Standardizing pH, temperature, agitation, and nitrogen sources is vital for enhancing biodegradation. This study examined pH levels from 5 to 9, temperatures between 20°C and 50°C, and agitation speeds from 0 to 400 rpm. Various nitrogen sources, including peptone, yeast extract, malt extract, beef extract, and ammonium nitrate, were also tested at 1% concentration.

2.8 Growth kinetics profile over time in relation to biodegradation activities.

The potential strain was investigated for its biodegradation activities, focusing on the formation of cell biomass at regular 12-hour intervals, from the lag phase (0 hours) to the decline phase (168 hours).

Evaluations were conducted after separating the cell biomass from the cell-free supernatant by centrifugation at 3,000 rpm for 15 minutes. The growth of bacterial cells was estimated by measuring the dry weight of the cell biomass obtained from the centrifuged pellets, which were dried in a hot air oven at 50°C for 30 minutes. The residual hydrocarbons were measured directly from the cell-free supernatant using the gravimetric analysis method described earlier (Yang et al., 2019).

2.9 GC analysis

At the start (0 hours) and the end (168 hours) of each hydrocarbon biodegradation study, we analyzed the cell-free supernatant to check for leftover hydrocarbons using gas chromatography (GC). We used a Thermo Trace GC Ultra connected to a Polaris Q mass spectrometer and a TriPlus auto-sampler. A DB-5 column (0.25 mm × 30 m × 0.22 μm) with helium as the carrier gas conducted the analysis. We set the temperature to go from 50°C to 250°C, increasing at a rate of 10°C per minute. We maintained the initial temperature for 2 minutes and held the final temperature of 250°C for 10 minutes. The gas chromatography flow rate was 1 ml/min, with a total run time of 32 minutes. We obtained mass spectra in scan mode from 0 to 500 m/z using an ion trap with electron ionization (EI+). We also conducted targeted hydrocarbon GC analysis at both 0 and 168 hours of incubation (Hiller et al., 2009) and non-targeted GC analysis. Lastly, we examined fatty acids at the end of the biodegradation experiment. We estimated the proportion of specific hydrocarbon degradation using a particular formula.

$$\% \text{ Hydrocarbon degradation} = \frac{(1 - \text{Peak area of hydrocarbon at 168 hrs})}{\text{Peak area of hydrocarbon at 0 hr}} \times 100$$

3. RESULTS

3.1 Isolation of Phyllosphere hydrocarbon-utilising bacteria

We found 13 types of bacteria that can use hydrocarbons, using 1% crude oil as the carbon source, as shown in Table 1. We identified pure cultures by looking at the colony shape and performing Gram staining. Most of these bacteria were Gram-negative and rod-shaped, as noted in Table 2. We standardised all the pure cultures for testing potential isolates. We studied the ability of these bacteria to use hydrocarbons by measuring their biomass and using gravimetric analysis.

Table 1: Cell biomass concentration and crude oil biodegradation activities of phyllosphere bacteria cultured in Bushnell-Haas broth.

Strains No.	Dry cell biomass (g/L)	Crude oil degradation (%)
PHUB1	0.68	29
PHUB2	1.92	62
PHUB3	0.77	18
PHUB4	0.54	22
PHUB5	1.79	64
PHUB6	0.47	17
PHUB7	2.01	69
PHUB8	1.84	60
PHUB9	0.69	25
PHUB10	0.77	27
PHUB11	1.96	63
PHUB12	0.88	27
PHUB13	0.72	31

*PHUB- Phyllosphere Hydrocarbon Utilising Bacteria

Table 2: Morphological Characteristics by pure culture isolates

Strains No.	Gram Character	Colony Morphology
PHUB2	Positive /rod-shaped	White
PHUB5	Negative/rod-shaped	Creamy white

PHUB7	Negative/rod-shaped	Dull white
PHUB8	Negative/rod-shaped	White
PHUB11	Negative/rod-shaped	Dull white

3.2 Screening of potential hydrocarbonoclastic bacteria

In a comprehensive study involving 13 different isolates, five exceptional strains—PHUB2, PHUB5, PHUB7, PHUB8, and PHUB11—emerged as standout performers, showcasing impressive biomass production that ranged from 1.79 g/L to 2.01 g/L. These remarkable isolates revealed a robust capacity for hydrocarbon degradation, thriving in an environment enriched with 10% crude oil. This was evidenced by the substantial total dry weight of their biomass, reflecting their efficiency in utilizing the hydrocarbons. The degradation rates for crude oil were particularly noteworthy, with PHUB2 achieving 62%, PHUB5 reaching 64%, PHUB7 excelling at 69%, PHUB8 showing 60%, and PHUB11 at 63%. These figures underline the significant potential of these strains for bioremediation efforts. For more detailed insights, please refer to Table 1.

3.3 Molecular identification of potential strains

In this study, all five potential strains were selected for further detailed characterisation utilising 16S rRNA sequencing, which facilitated the identification of specific microorganisms. The isolates were classified as follows: PHUB2 was designated as Bacillus altitudinis (HLPSC2), PHUB5 as Serratia plymuthica (HLPSC5), PHUB7 as Achromobacter xylosoxidans (HLPSC7), PHUB8 as Pseudomonas putida (HLPSC8), and PHUB11 as Proteus vulgaris (HLPSC11).

3.4 Secondary screening of most potential hydrocarbons utilising a bacterium

A study evaluated the utilisation of five potential strains, each exposed individually to 1% of all BTEX compounds. Using a gravimetric method to measure total cell biomass, it was determined that Pseudomonas putida HLPSC8 exhibited cell biomass levels ranging from 4.1 to 4.7 g/L. Additionally, the residual hydrocarbon concentration for this strain varied from 22% to 27% (see Fig. 1). As a result, Pseudomonas putida HLPSC8 is considered a promising strain and has been selected for further tolerance studies.

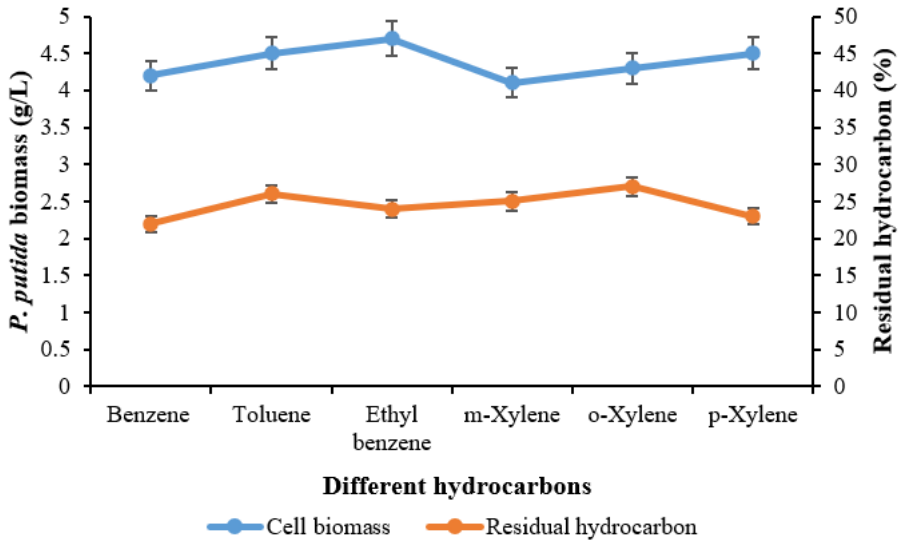

Fig.1 Utilisation of different hydrocarbons using Pseudomonas putida HLPSC8

3.5 Tolerance study of potential bacterium

The potential Pseudomonas putida HLPSC8 strain is used, and a tolerance study is done with BTEX compounds concentration ranging from 1% to 10% see Fig. 2 (a)- (f).

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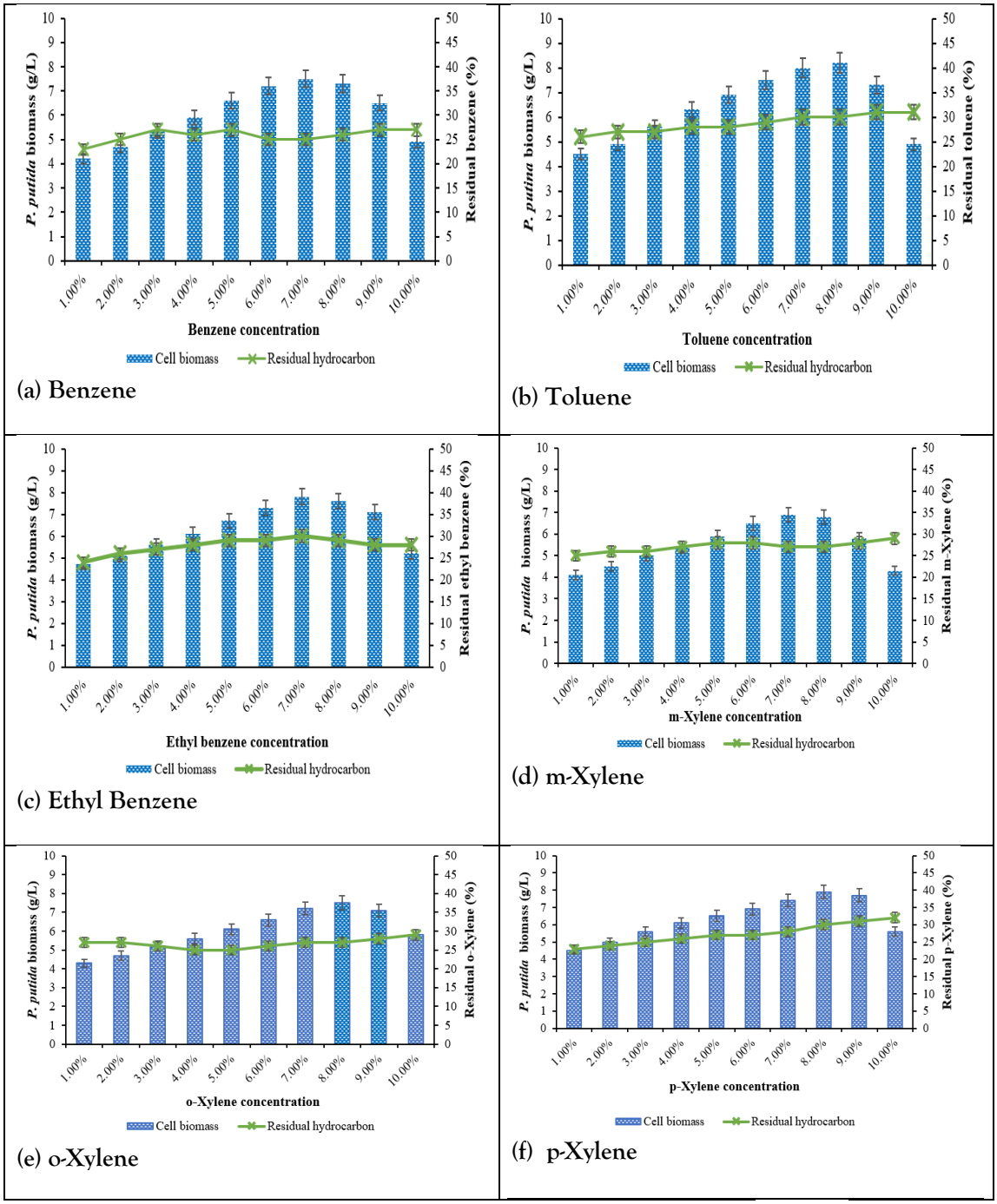

Fig.2 Tolerance of different hydrocarbons (BTEX) by Pseudomonas putida HLPSC8

In the analysis of benzene (Figure 2a), cell biomass increased significantly, peaking at 7%. In contrast, toluene, ethylbenzene, m-xylene, o-xylene, and p-xylene showed slightly higher growth rates of 8% (Figures 2b-f).Residual hydrocarbon concentrations were as follows: benzene at 25%, toluene at 30%, ethylbenzene at 29%, m-xylene and o-xylene at 27%, and p-xylene at 30%. These results illustrate the differing efficiencies of biomass growth in response to various BTEX compounds.

3.6 Biodegradation activity.

The biodegradation activity of BTEX compounds was analysed under various cultural conditions. These conditions included pH levels ranging from 5 to 9, temperatures from 20 to 50 °C, agitation speeds from 0 to 400 rpm, and different nitrogen sources such as peptone, yeast extract, malt extract, beef extract, skim milk, ammonium nitrate, sodium nitrate, and potassium nitrate. By comparing cell biomass and residual hydrocarbon concentration using gravimetric methods, it was determined that the optimal conditions for biodegradation were a pH of 7, a temperature of 35 °C, an agitation speed of 200 rpm, and the use of ammonium nitrate as the nitrogen source. These optimal conditions significantly enhanced the biodegradation of all BTEX compounds.

Table 3: Influence of culture factors on Pseudomonas putida HLPSC8 biodegradation activity.

At Optimum pH 7	Cell biomass g/L	Residual Hydrocarbon %
Benzene	7.6	25
Toluene	8.2	30
Ethyl benzene	7.9	21
m-xylene	**8.3**	**19**
o-xylene	8	20
p-xylene	7.7	24
At Optimum Temperatur-35		
Benzene	7.7	23
Toluene	8.4	26
Ethyl benzene	8.1	18
m-xylene	**8.5**	**16**
o-xylene	8.2	18
p-xylene	8.3	22
At OptimumAgitation-200 rpm		
Benzene	8.3	18
Toluene	8.7	21
Ethyl benzene	8.8	15
m-xylene	**8.9**	**12**
o-xylene	8.7	15
p-xylene	8.6	17
At Optimum Nitrogen source 1% Ammonium nitrate		
Benzene	8.6	17
Toluene	8.6	17
Ethyl benzene	8.8	15
m-xylene	**8.9**	**12**
o-xylene	8.8	15
p-xylene	8.7	17

3.7 Growth Kinetics profile as a function of time on biodegradation activities

The growth of biodegradation activities every 12 hours, from 0 to 168 hours, for the degradation of benzene,ethylbenzene , m-xylene ,o-xylene and p-xylene were found (Table 4).

Table 4: Growth kinetics profile of P. putida HLPSC8 at 168hrs with reference to the biodegradation of BTEX compounds.

GC profile At 168 hrs	Cell biomass g/L	Residual Hydrocarbon %
Benzene	0.9	9
Toluene	0.7	8
Ethyl benzene	0.8	6.8
m-xylene	**0.7**	**3.7**
o-xylene	0.7	4.1
p-xylene	1.2	5.4

3.8 GC Analysis

We conducted gas chromatography (GC) analysis on the biodegradation broth at two points: 0 hours and 168 hours. The goal was to measure and analyze the amounts of m-xylene degraded.At 0 hours, we found the peak level at 108978351 and after 168 hours 4032199 was found as shown in Table 5.The retention times were also recorded during these two hours as 17.849 and 17.893, as shown in Figure 3.

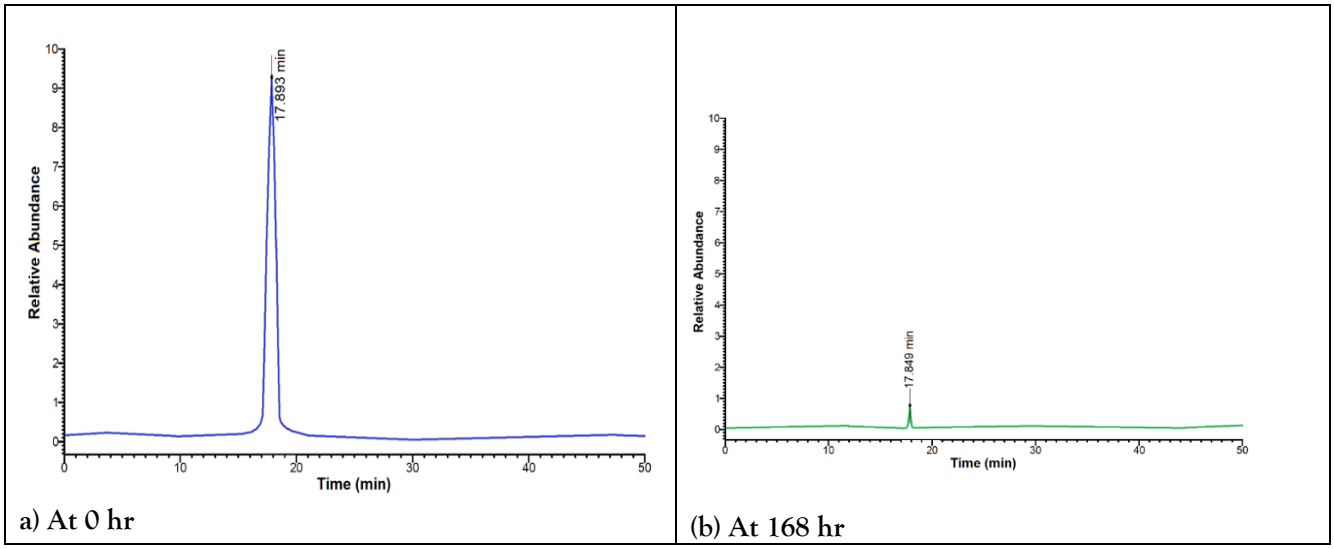

Fig.3 : GC analysis of the biodegradation broth after 0 and 168hrs targeting the quantitative analysis of total m-xylene

Pseudomonas putida HLPSC8 was used to assess the biodegradation of m-xylene compounds. After 168 hours, the percentages of biodegradation for m-xylene found as 96.3% .We also analyzed the gas chromatography (GC) profiles of the biodegradation broth after 168 hours to find the total volatile compounds. The results showed that m-xylene had 18 identifiable compounds.

Table 5: Represents the percentage of m-xylene biodegradation using P. putida HLPSC8 at the end of the experiment (decline phase) after 168 hrs.

Fig no.	Analysis period	Chemical name	Retention time (min.)	Molecular formula and weight	Peak Area
4(a)	0 hrs	m-Xylene	17.849	C_8H_{10}, 106.1	108978351
4(b)	168 hrs	m-Xylene	17.893	C_8H_{10}, 106.1	4032199
Percentage biodegradation of m-Xylene					96.3%

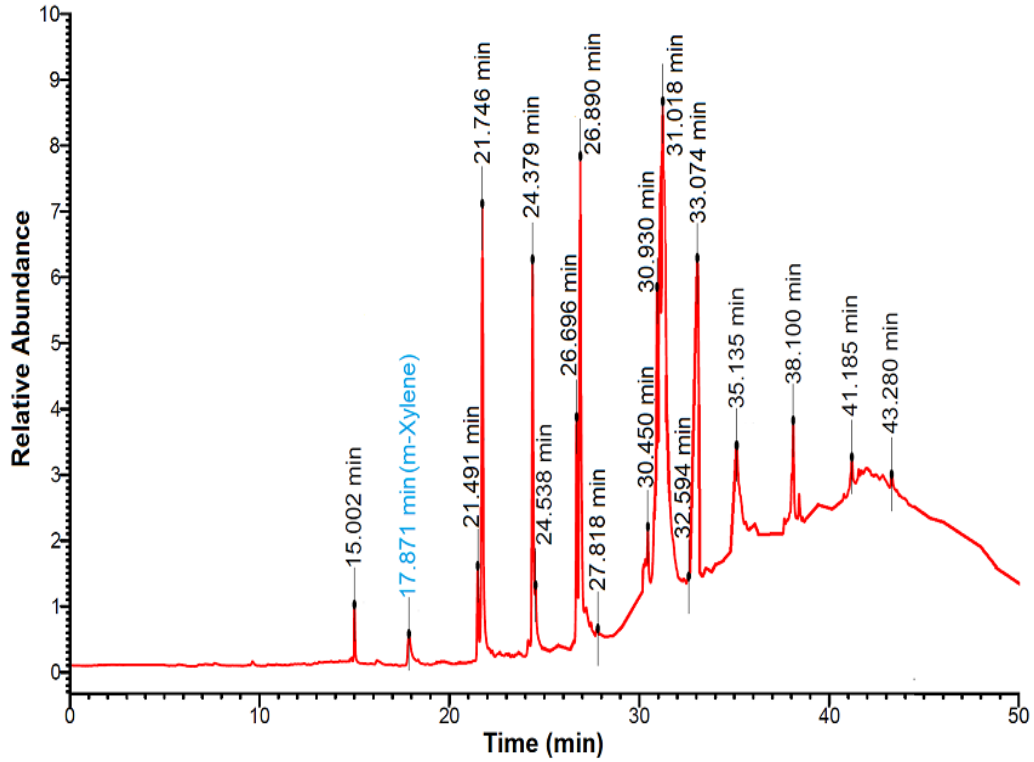

Fig. 4: GC analysis of the cell-free supernatant after 168hrs of m-xylene biodegradation broth

Table 6: GC profile revealing the total volatile compounds in the cell-free supernatant of the broth after 168hrs of m-xylene biodegradation

S. No	Retention time (min)	Chemical name	Molecular weight	Molecular formula	% Area
1	15.002	2-Furanmethanol	98.1	C_5H_6O	1.7
2	17.871	m-Xylene	106.1	C_8H_{10}	1.2
3	21.491	Propanoic acid, 2-hydroxy-2-methyl-	104.1	$C_4H_8O_3$	3.5
4	21.746	N-Methylpyrrole-2-carboxylic acid	125.1	$C_6H_7NO_2$	9.1
5	24.379	Phenol, 2,6-dimethoxy-	154.1	$C_8H_{10}O_3$	7.5
6	24.538	Hydroxylamine, O-decyl-	173.1	$C_{10}H_{23}NO$	3.3
7	26.696	Decane, 2,6,8-trimethyl-	184.3	$C_{13}H_{28}$	5.6
8	26.890	Cyclotetradecane	196.3	$C_{14}H_{28}$	9.8
9	27.818	n-Tridecan-1-ol	200.3	$C_{13}H_{28}O$	1.3
10	30.450	3-Cyclopentene-1-butanol, beta,2,2,3-tetramethyl-delta-methylene-	208.3	$C_{14}H_{24}O$	2.8
11	30.930	E-11,13-Tetradecadien-1-ol	210.3	$C_{14}H_{26}O$	7.3
12	31.018	Dodecane, 2,6,10-trimethyl-	212.4	$C_{15}H_{32}$	13.5
13	32.594	Methyl 4-butyramido-3-methylbenzoate	235.3	$C_{13}H_{17}NO_3$	3.5
14	33.074	Phenol, 2,4-bis(1,1-dimethylethyl)-	278.5	$C_{17}H_{30}OSi$	8.1
15	35.135	1-Iodo-2-methylundecane	296.2	$C_{12}H_{25}I$	6.0
16	38.100	Heptadecane, 2,6,10,14-tetramethyl-	296.6	$C_{21}H_{44}$	7.1
17	41.185	2,4-Dit-butylphenyl 5-hydroxypentanoate	306.4	$C_{19}H_{30}O_3$	4.9
18	43.280	Phthalic acid, oct-3-yl pentyl ester	348.4	$C_{21}H_{32}O_4$	3.8

4 DISCUSSION

During enrichment with Bushnell Haas broth, five pure cultures were identified: Bacillus altitudinis (PHUB2), Serratia plymuthica (PHUB5), Achromobacter xylosoxidans (PHU7), Pseudomonas putida (PHU8), and Proteus vulgaris (PHU11), using 1% crude oil as the carbon substrate. Sivasubramanian et al., have isolated microorganisms present in the soil by enrichment technique using Bushnell Haas broth with used engine oil as sole carbon source (Sivasubramanian et al.2016).

Thirteen strains were isolated from hydrocarbon-affected leaves using 1% crude oil as the sole carbon source. Among these, five pure cultures were identified as potential bacteria. All these strains on gravimetric method (Luna et al.2009) showed greater dry cell biomass ranging from 1.79 to 2.01 g/L and degradation capability from 60 to 64 % on study with 10% crude oil extract as a carbon source.

All three methods—Neighbor Joining tree (Saitou and Nei, 1987), UPGMA tree (Sneath and Sokal, 1973), and Maximum Likelihood tree (Tamura and Nei, 1993)—were used to understand the evolutionary relationships among the following species: Bacillus altitudinis, Serratia plymuthica, Achromobacter xylosoxidans, Pseudomonas putida, and Proteus vulgaris. In the final dataset, these species showed the following positions: 1513, 1440, 1425, 1422, and 1436, respectively.

During the secondary screening of all five potential strains with 1% of individual BTEX compounds, the most prevalent bacteria identified was Pseudomonas putida HLPSC8, while the least prevalent was Serratia plymuthica HLPSC5. You et al. (2013) identified Pseudomonas putida as having the highest biodegradation ability, based on the 16S rDNA sequence analysis conducted on hydrocarbon-contaminated soil.

At varying concentrations of hydrocarbons, Pseudomonas putida HLPSC8 demonstrated significant cell biomass production at 7% for benzene, ethylbenzene, and m-xylene, while it reached 8% for toluene, xylene, and p-xylene compounds.

The factors influencing biodegradation activity include a pH of 7, a temperature of 35°C, agitation at 200 RPM, and the use of ammonium nitrate as a nitrogen source. You et al. (2013) revealed that the biodegradation of BTEX compounds is significantly affected by pH, temperature, and salinity.The presence of methyl groups in the aromatic rings makes it harder for microorganisms to oxidize xylene isomers. This difficulty can be overcome by the involvement of monooxygenases (El Naas et al.2014).
Gas chromatography revealed that Pseudomonas putida HLPSC8 is capable of m-xylene by 96.3%,. At the 168-hour mark, the analysis detected 18 compounds for m-xylene. The ability of P. putida BPI8, which was isolated on the basis of its ability to utilize either biphenyl or naphthalene as the sole carbon source, to utilize BTEX was tested by Baldwin et al. (2000). The strain utilizes all BTEX compounds as the sole carbon source. A biphenyl-grown strain transformed all substrates, i.e., benzene, toluene, ethylbenzene, o-, m- and p-xylene and the concentration of BTEX compounds was below the detection limit. It suggests a broad specificity for the aromatic metabolic pathway (Weigner et al. 2001).

5 CONCLUSION

The degradation of BTEX compounds poses significant challenges due to their complex chemical structures and environmental persistence. Research has demonstrated that only a limited number of specific bacterial populations possess the exceptional capability to effectively reduce these harmful substances. Notably, the specialized bacterium Pseudomonas putida HLPSC8 plays a critical role in bioremediation by efficiently breaking down BTEX compounds, thereby enhancing environmental health. While the presence of methyl groups within the aromatic rings complicates the oxidation of xylene isomers, our isolated bacterial strain, Pseudomonas putida HLPSC8, has exhibited promising potential. Under optimal conditions concerning pH, temperature, agitation, and nitrogen source, the degradation of m-xylene by this strain was found to be superior when compared to the other five BTEX compounds. This promising strain may contribute to efforts aimed at preventing environmental pollution and mitigating the associated carcinogenic effects. Furthermore, future research should focus on elucidating the underlying pathway mechanisms involved in this degradation process.

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