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Toluene Biodegradation by Bacteria Isolated from Hydrocarbon Contaminated Sites in Bangalore

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Abstract

Gasoline stations in Bangalore, Karnataka, India, were found to pollute groundwater with BTEX (benzene, toluene, ethylbenzene, and xylenes) compounds due to leakage from subterranean storage tanks. Prior global research has isolated several BTEX degraders from petroleum-contaminated soils. The success of earlier studies prompted the isolation of bacterial species from Bangalore's gasoline-contaminated groundwater and soil samples for BTEX removal. *Pseudomonas* sp. and *Bacillus haynesii* were isolated from gasoline-contaminated soil and groundwater samples by enriching the cultures with toluene. Identification of aerobic bacteria isolates from soil and groundwater samples was performed by the 16S rRNA method. The *B. haynessii*, isolated from the hydrocarbon-contaminated groundwater, is a novel culture that has not been used as a BTEX degrader. Toluene was selected owing to its occurrence in Bangalore's groundwater and the hydrocarbon's potential to create major health issues.

Greater cell reduction was observed with *B. haynesii* implying them to be less tolerant to toluene contamination. The *Pseudomonas* sp. bacteria degraded toluene at a steady rate of 0.127 h^{-1}, leading to 38%, 50%, 84%, and 100% mineralization after 2 h, 6 h, 12 h, and 24 h, respectively. Combining the findings of this study and of other research indicated that the rate of toluene decomposition is retarded by the presence of co-compounds, increased toluene concentrations, and depleted dissolved oxygen levels. Compared to *B. haynesii, Pseudomonas sp.* aerobically digested greater amounts of toluene (concentration range = 5 to 300 mg/L), suggesting that it is a superior choice for biodegradation of Bengaluru's toluene-contaminated groundwater (concentration range = 0.062 to 0.153 mg/L) by using appropriate field technologies. The study also showed that Bangalore's BTEX degraders can mineralize toluene concentrations encountered in contaminated environments or those used in previous laboratory investigations.

Categories: Environmental Engineering, Water Resources Engineering
Keywords: batch tests, physio-chemical parameters, aerobic, bioremediation, contaminated material, groundwater, hydrocarbons, pollution, soil, bacteria

Introduction

The petrochemical, chemical, and solvent industries use BTEX (benzene, toluene, ethylbenzene, and xylenes) compounds. Leakage from gasoline storage tanks and the release of products like paints, thinners, pesticides, herbicides, and industrial waste during their storage, manufacture, transport, use, and disposal are the main ways of BTEX entering the environment [1-3]. The U.S. EPA classifies benzene as a Class A pollutant, and toluene, ethylbenzene, and xylenes are classified as Class D pollutants [4], necessitating their removal from polluted water and soil environments.

Among several options (adsorption, membrane filtration, and chemical treatments), bioremediation is preferred for remediating BTEX-contaminated groundwater, wastewater, and subsurface soils, as the process is eco-friendly, non-toxic, and has good remediation capability [5-7]. In the bioremediation process, microbes use BTEX as food along with substrates like oxygen, nitrates, and sulphates to break down the hydrocarbons. Whether to use aerobic or anaerobic bioremediation processes depends on what electron acceptors are available and the types of nutrients present in the polluted soil or groundwater.

Aerobic processes rely on dissolved oxygen as the final electron acceptor, while anoxic and anaerobic processes use nitrate, sulphate, ferrous ion, Mn (IV), and methane as electron acceptors for BTEX biodegradation [8]. The breakdown of BTEX compounds by bacteria happens according to first-order or Monod models when there is only one type of carbon source available [9-10]. The degradation efficiency of aerobic bacteria depends on their substrate concentration, oxygen availability, bacterial type, pH, temperature, and nutrient requirements [1,11,12].

Several studies have identified effective BTEX-degrading bacterial strains isolated from diverse contaminated environments. *Pseudoxanthomonas spadix*, isolated from gasoline-contaminated soil in South Korea, exhibited significant potential for BTEX degradation [13]. *Janibacter* sp. SB2, isolated from

sea tidal flat sediments near the Taean coast in South Korea [12], demonstrated comparable degradation capabilities. Research in Brazil [14] found *Pseudoxanthomonas* sp. and *Serratia* sp. in polluted industrial sites in Cubatão, while *Paraburkholderia* sp. taken from soil at a gas station in South Korea [15] showed it could break down 45% to 83% of BTEX, naphthalene, and aliphatic hydrocarbons in just 3 to 10 days. Kaur et al. [7] found the bacterial strains *Microbacterium esteraromaticum and Bacillus infantis* in geothermal borehole soils taken from depths of 3-73 m in Canada. The isolated strains exhibited superior degradation efficiencies: 67.98% and 65.2% for benzene, 72.8% and 71.02% for toluene, 77.52% and 76.44% for ethylbenzene, and 74.58% and 74.04% for xylenes, respectively. Grishchenkov et al. [16] found bacteria (*Pseudomonas* sp. BS2201, BS2203 and *Brevibacillus* sp. BS2202) from oil-polluted soil in Western Siberia that could break down 20-25% of the total extractable material, including 90-95% of all alkanes (n-C10-C35) under aerobic conditions.

The effective ability of bacterial strains from various polluted areas to break down BTEX shows strong support for creating specific bioremediation methods, which could lead to good solutions for cleaning up BTEX-contaminated sites in different locations and environments.

Scope of the present study

Earlier studies from South Korea, Brazil, Canada, and Russia have brought out that microorganisms isolated from petroleum-contaminated sites are capable of BTEX bioremediation. Researchers found that gasoline stations in Bangalore, Karnataka, India, pollute groundwater with BTEX compounds due to leakage from subterranean storage tanks [17]. The success of previous research prompted the isolation of bacterial species from Bangalore's gasoline-contaminated groundwater and soil samples for BTEX removal. Enrichment of bacterial cultures is performed in minimal media with toluene as a carbon source. Identification of aerobic bacteria isolates from soil and groundwater samples was performed by the 16S rRNA method. Scanning Electron Microscopy (SEM) studies were performed to characterize the morphological features of the bacteria. The efficacy of the BTEX degraders was examined by performing toluene biodegradation batch experiments. Toluene is classified as a Class D pollutant, and chronic exposure to toluene causes damage to the central nervous system, the cardiovascular system, and the hepatic system [2,4]. A typical gasoline mixture contains several hydrocarbons, including BTEX compounds, making toluene a useful indicator of gasoline contamination in groundwater. An earlier study has shown [6] that microbial consortia acclimatized to toluene can degrade other BTEX compounds.

Materials And Methods

Sample collection

Gasoline contaminated soil was collected at a depth of 20 mm from a gasoline station (13°00′34″ N latitude and 77°33′45″ E longitude, Figure *1*) in the northern part of Bengaluru city. A groundwater sample was collected from a tube well located in a gasoline filling station (12°57′28″ latitude and 77°33′44″ E longitude, Figure *1*) that had shown notable BTEX contamination [17]. The soil and groundwater samples were aseptically collected in sterile bottles and stored in the laboratory at 4°C till further use.

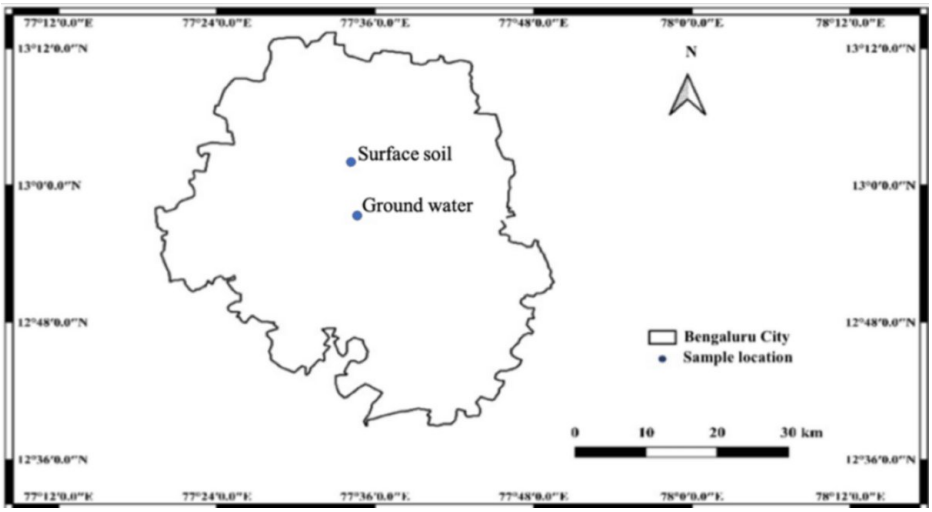

FIGURE 1: Sampling locations of soil and groundwater samples

The DO values in groundwater samples were measured using an Eutech Cyberscan DO-110 probe connected to a handheld portable meter (Cyberscan PD300 model). Redox potential (E_h) was measured using a pre-calibrated Sure Flow Combination Redox/ORP electrode (Thermo Electron Corporation model

9678BNWP) connected to an Orion 4-star bench-top meter. Nitrate ion concentration was measured by ion selective electrode (Orion 9700BNWP Ion Plus Sure Flow Electrode) connected to Orion 4-star bench-top meter. The pH was measured using a pH probe (Hanna Instruments Model pH213) connected to a bench-top meter. The sulphate ion concentration was measured using the turbidimetric method at 420 nm absorbance using a UV-Visible spectrophotometer (Thermo Scientific Evolution 201). The measured DO and E_h values were 2.5 mg/L and -21.3 mV, respectively. The pH, sulphate, and nitrate concentrations in the groundwater sample were 6.69, 2 mg/L, and 0 mg/L, respectively.

Isolation and enrichment of bacteria from soil and groundwater samples

Isolation and enumeration of bacteria from the soil and groundwater samples were performed in Luria Bertani (LB) broth and agar (Himedia, Bengaluru). One gram of soil was inoculated in 50 mL of sterile LB broth contained in 120 mL bottles. Likewise, 1 mL of groundwater was inoculated in 49 mL of sterile LB broth contained in 120 mL bottles. After being crimped, the bottles were incubated for 24 h at 25°C while being agitated. All experiments were performed in duplicate. The colony-forming unit technique and turbidimetry were used to estimate the bacterial growth at the conclusion of the fourth cycle [18]. The bacterial isolates that were separated from the soil and groundwater samples are designated S1 and S2, respectively.

Aerobic bacterial enrichment was performed in minimal media using toluene as the only carbon source for the S1 (soil) and S2 (groundwater) cultures. A 50 mL batch of minimal media is made up of the following salts: disodium hydrogen phosphate (1.5 g/L), potassium dihydrogen phosphate (1 g/L), sodium carbonate (0.1 g/L), calcium chloride hexahydrate (0.01 g/L), manganese sulphate heptahydrate (0.02 g/L), ferrous sulphate heptahydrate (0.01 g/L), magnesium sulphate heptahydrate (0.02 g/L), ammonium chloride (2 g/L), and sodium chloride (1.0 g/L). The pH of the minimal media was adjusted to 7.0 ± 0.02. Bacteria isolated from contaminated samples were acclimatized to toluene concentrations up to 10 mg/L. Only one type of bacterial colony, designated as S1 culture, was found in gasoline-contaminated soil when bacterial isolates were plated at a toluene concentration of 10 mg/L. Likewise, a single type of bacterial colony designated as S2 culture was found growing from the groundwater sample. As single colonies were extracted from the soil and groundwater samples, they were used for toluene degradation. Additional research is needed to assess the performance of S1 and S2 cultures within a mixed culture environment.

Serial dilutions and the spread plate method [19] were performed to enumerate, identify, and count the toluene degraders grown from the contaminated samples. One mL of the bacterial culture (S1 or S2) was mixed with 99 mL of 0.9% sterile saline (NaCl) solution. The bacteria-enriched saline solution was diluted with sterile saline in five steps. In each step, 1 mL of the bacteria-enriched saline solution was diluted to 10 mL with 0.9% sterile saline solution. A total of 0.1 mL samples of the diluted bacteria were spread on three LB agar plates, which were then covered with parafilm and kept at 37°C for 24 h. The plates having 30 to 300 bacterial colonies were selected, and colony-forming units (CFU/mL) were enumerated.

Characterization of bacterial cultures

Identification of bacterial isolates was performed on duplicate samples. The reliability of the identification procedure was demonstrated by the consistent matching of bacterial isolates across duplicate samples. The 16S rRNA was performed for aerobic bacteria isolates cultured from soil (S1 isolates) and groundwater (S2 isolates) samples. The 16SrRNA sequences were aligned with relative strains using the Clustal W program, and a phylogenetic tree was constructed using the neighbor-joining method within the software to identify the bacterial strains. SEM analysis was performed to characterize the morphological features of the bacteria. Samples for SEM analysis were prepared using dehydration with an ethanol gradient and sputter coated with gold (120 s of sputter time) using the critical point drying method and imaged using SEM (Ultra-55 FE-SEM Karl Zeiss EDS) equipment.

Toluene biodegradation batch experiments

Bioremediation experiments were set up in 120 mL serum bottles containing 1 mL of bacterial culture (S1 or S2) and 48 mL of sterile minimal media. Calculated volumes of toluene stock solution (5000/10,000 mg/L) were added to individual bottles to yield toluene concentrations of 5, 10, 50, 100, 250, and 300 mg/L, respectively. Each bottle containing a 50-mL solution had enough headspace to provide oxygen for the growth and sustenance of bacteria. No efforts were made to replenish dissolved oxygen consumed by the bacteria during the aerobic process. The bottles were crimped with Teflon-coated septa. To account for volatilization losses, an additional 40% of the respective toluene concentration was added to each bottle. Two control samples were used in the study. Control A is composed of 49 mL of sterile media and toluene solution of desired volume and concentration. It was used to monitor toluene loss due to adsorption by the surface of the glass container and volatilization. Control B is composed of 49 mL of sterile media and 1 mL of bacterial culture. Throughout the study period, it tracked the growth and decay of bacteria. The crimped bottles containing test and control samples were incubated at 25°C for 24 h in a rotary incubator shaker at 120 rpm. Triplicate experiments were performed with test samples at each toluene

concentration.

After 24 h, 10 mL samples from the test and control setups were filtered through a 2-µm cellulose acetate filter. A 5 mL of the filtered liquid were put into a GC vial with 1 g of NaCl salt; the vial was sealed, and the amount of toluene was measured using a Gas Chromatography with Flame Ionization Detection (GC-FID), which was connected to an auto sampler (Shimadzu QP 2010-HS 20). Statistical analysis of the data was performed using standard deviation. For the biodegradation studies using S1 and S2 isolates, the standard deviation ranged from 5% to 9%. In the biokinetics study, the standard deviation varied between 10% and 15% [20].

The spread plate technique was used to count bacteria in test samples and control B to see how the growth rate of bacterial colonies changed during toluene degradation.

Kinetics of toluene degradation

Toluene degradation kinetics of the S1 isolate were examined at the substrate concentration of 50 mg/L. For each time point, 1 mL of bacteria culture (S1) and 0.5 mL of 5000 mg/L toluene stock solution were added to 48.5 mL of minimal media in a 120-mL serum bottle. The bottles were crimped and agitated at 25°C (120 rpm, using a rotary incubator shaker) for the desired incubation periods. Separate bottles were prepared for incubation periods of 2, 6, 12 and 24 h, respectively. At the end of each incubation period, samples were drawn from the bottle to measure toluene concentration using GC-FID. Enumeration of bacteria, variation in pH, redox potential, and dissolved oxygen were also performed after each incubation period with test and Control B samples.

Results

Morphological and phylogenetic characterization of bacteria

The optical densities at 450 nm for the S1 and S2 cultures were 1.66 and 0.81, respectively, and their viable counts were 3.23×10^8 and 8.9×10^7 CFU/mL. The S1 isolate multiplied quickly, reaching 1.6 optical density in 16 h. After 24 h, the S2 isolate reached a reduced optical density (0.81) and showed a lag phase.

The scanning electron microscopy study indicated that the S1 culture colonies were characterized by (1) fibrous coatings on their surfaces, (2) curved rods with smooth surfaces, and (3) jointed curved rods (Figure 2).

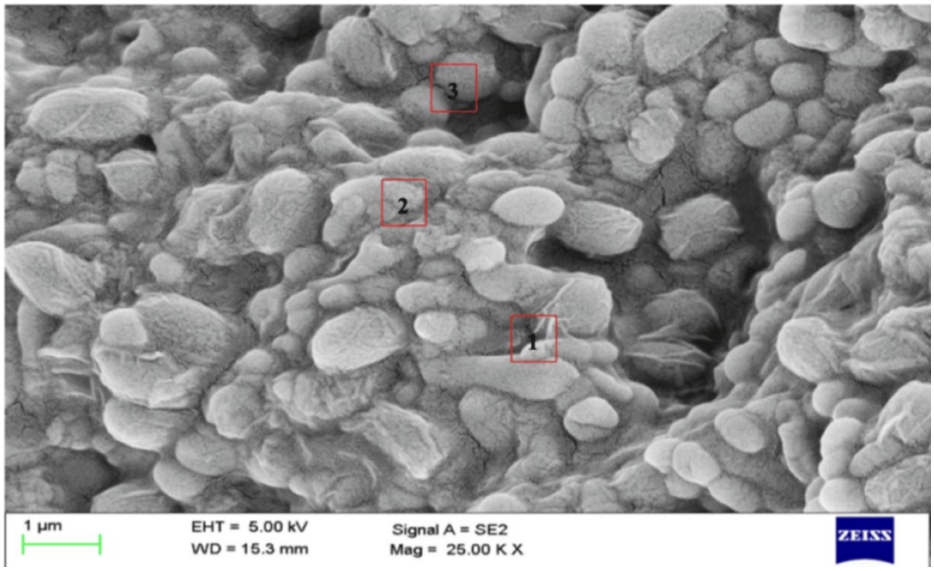

FIGURE 2: SEM image of S1 culture: 1-fibrous coatings on surface, 2-curved rods with fine surface, 3-joined curved rods

SEM, Scanning Electron Microscopy

The size of bacterial cells ranged from 0.59 to 1.56 µm and exhibited spherical, rod-shaped, and elongated morphologies. The S2 culture bacteria exhibited singular ellipsoidal morphology encapsulated in spores, and their cell sizes ranged from 1.02 to 1.59 µm (Figure 3).

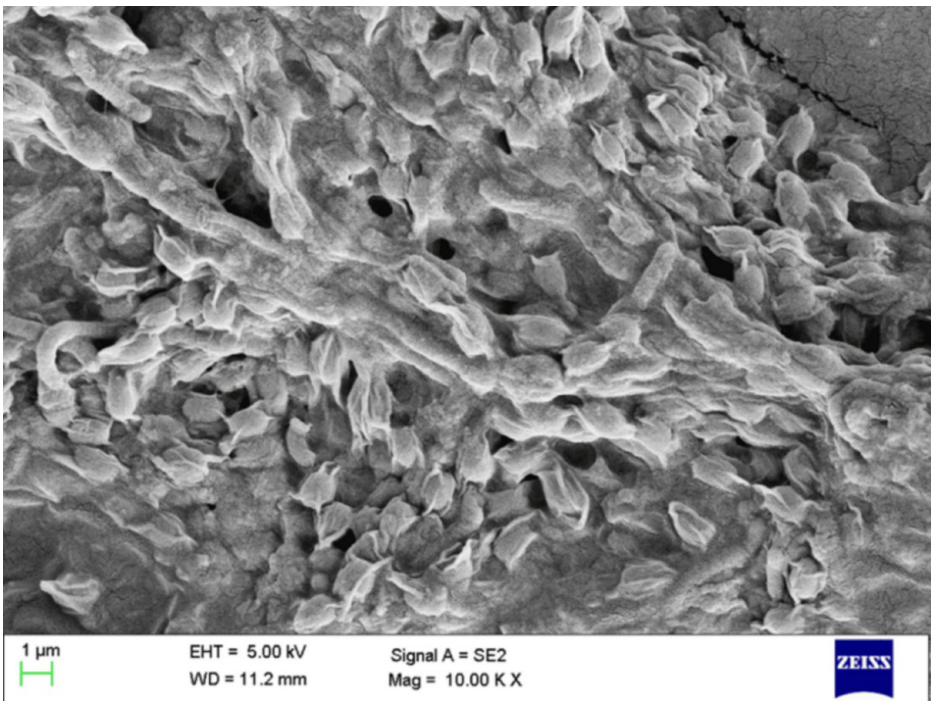

FIGURE 3: SEM image of S2 culture

SEM, Scanning Electron Microscopy

The 1500 bp (base pairs) of 16S rRNA was Polymerase Chain Reaction amplified, and consensus sequences for the isolates were generated. Based on the phylogenetic tree, bacterial cells in the S1 culture have a strong similarity with *Pseudomonas* sp. The bacterial cells in the S2 culture have a strong similarity to the *Bacillus haynesii* strain. *Pseudomonas* sp. is gram-negative, rod-shaped, aerobic, and motile bacteria of the *Proteobacteria phylum* [21]. *Bacillus haynesii* is a gram-positive, rod-shaped, facultative anaerobic bacterium belonging to the *Firmicutes phylum* [22]. Notably, there have been no reports of using *B. haynesii* to bioremediate BTEX.

Toluene degradation studies

The percent toluene degradation by S1 culture at varying (C_0 = 5 to 300 mg/L) toluene concentrations was obtained as:

$$\% \text{ toluene degradation} = (x_t - x_0 / x_0) * 100\% \quad (1)$$

In this context, x_0 and xt represent the toluene concentrations at time t = 0 h and t = 24 h, respectively. An initial cell concentration of 1.2×10^7 CFU/mL was maintained in all experiments. Complete toluene breakdown occurs at low hydrocarbon levels (5-10 mg/L), but at higher levels (25 to 300 mg/L), the ability of microbes to break it down decreases from 81% to 40% (Figure *4*).

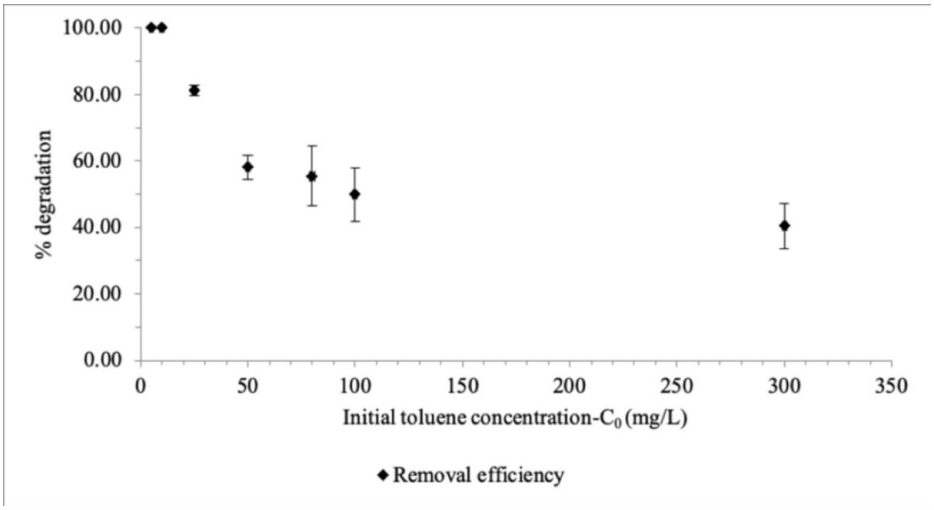

FIGURE 4: The % toluene degradation by S1 culture at various initial toluene concentrations

The variations in S1 culture cell concentrations spiked with different toluene concentrations are depicted in Figure 5. The initial toluene concentration of 10 mg/L caused a 10-fold increase in cell concentration. Thereafter, a progressive decrease in cell concentration occurred at the higher toluene concentrations. Cell counts of 7.4×10^6, 5.3×10^6, and 5.5×10^5 CFU/mL were measured at toluene concentrations of 25, 100, and 300 mg/L, respectively.

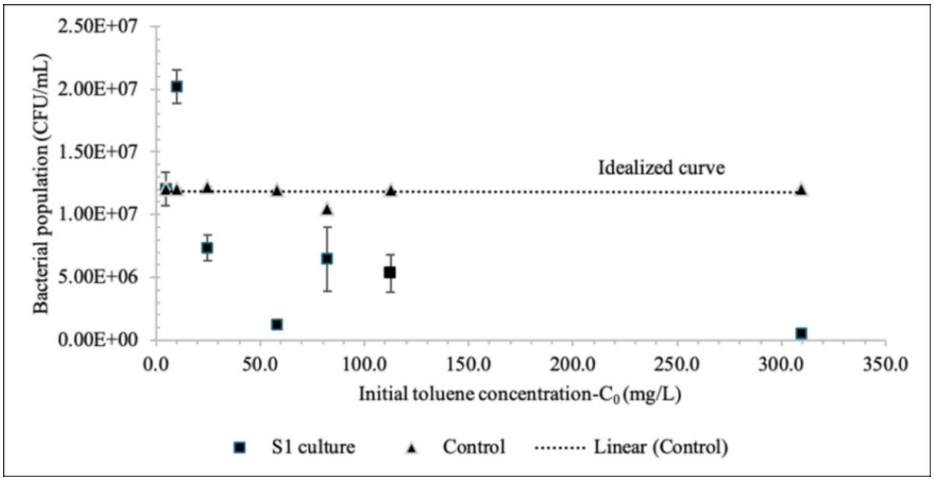

FIGURE 5: Effect of initial toluene concentration on cell population of S1 culture

The Control B sample is characterized by a DO concentration and E_h of 3.57 mg/L and 281 mV, respectively. In toluene-spiked cultures, the DO concentrations and E_h values decreased to 1.76 and 1.28 mg/L and 152 and 118 mV, respectively, when exposed to toluene concentrations of 50 and 300 mg/L.

Figure 6 compares the toluene degradation ability of S1 and S2 cultures at various toluene concentrations. The S1 culture accomplished 40-100% toluene degradation, while the S2 culture achieved less toluene mineralization (22-66%).

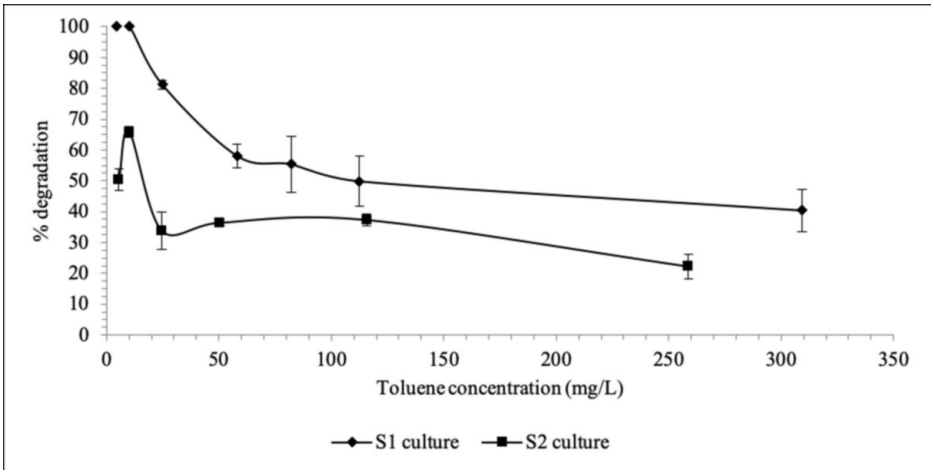

FIGURE 6: Toluene degradation efficiency of S1 and S2 cultures

Figure 7 compares the cell counts and DO levels of S1 and S2 cultures that were spiked with various toluene concentrations. The spiked S1 culture is characterized by larger cell counts (2×10^7 to 5×10^5 CFU/mL) than the S2 culture (4.8×10^6 to 4×10^5 CFU/mL). In comparison, the DO levels of the S2 culture (1.67-2.62 mg/L) are larger than those of the S1 culture (1.76-1.28 mg/L).

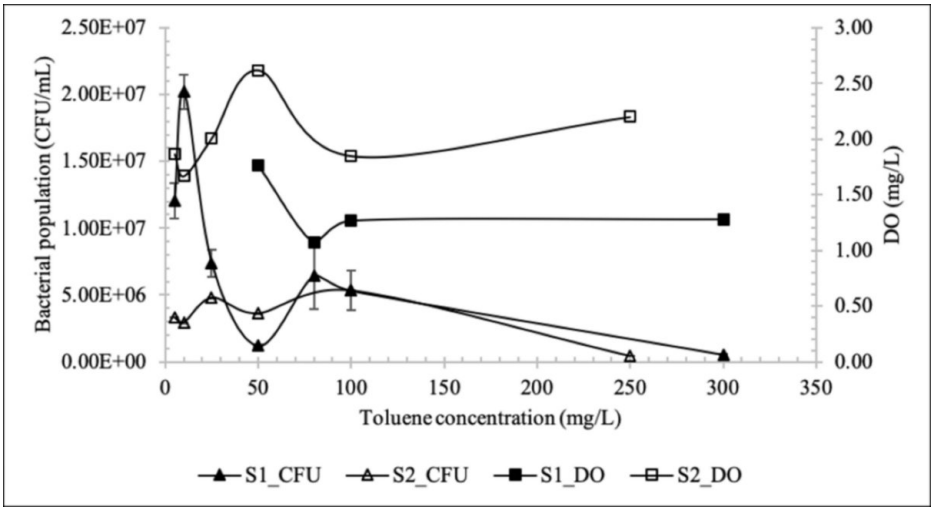

FIGURE 7: Comparison of cell counts and DO levels of S1 and S2 cultures at various toluene concentrations

Figure 8 compares the pH of S1 and S2 cultures at various toluene concentrations. The pH of S1 and S2 cultures ranges from 6.3 to 7.18 at all toluene concentrations and falls in the optimal pH range [5] for bacterial growth (6.5-8.0). Hence, the adverse pH environment did not contribute to the lower toluene removal abilities of either culture.

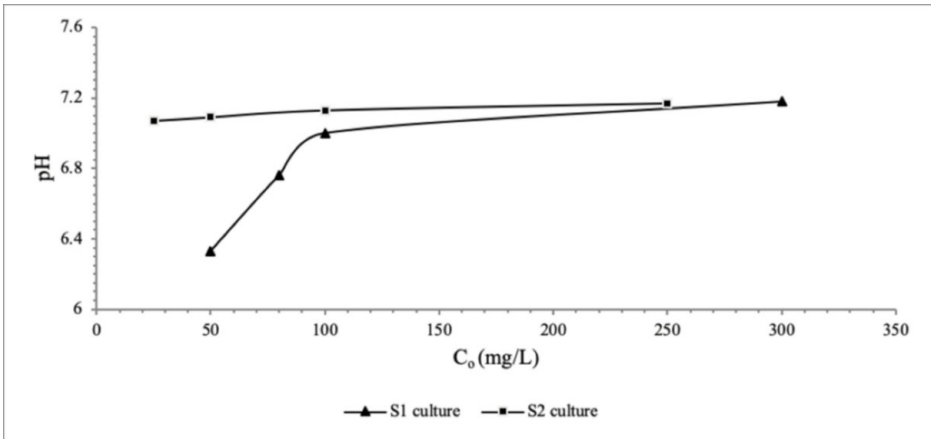

FIGURE 8: pH of S1 and S2 cultures exposed to various toluene concentrations

Toluene degradation with time

Figure 9 displays how much toluene was degraded and the residual toluene concentration (Ce) for the S1 culture (*Pseudomonas* sp.) over time, beginning with an initial toluene concentration of 50 mg/L.

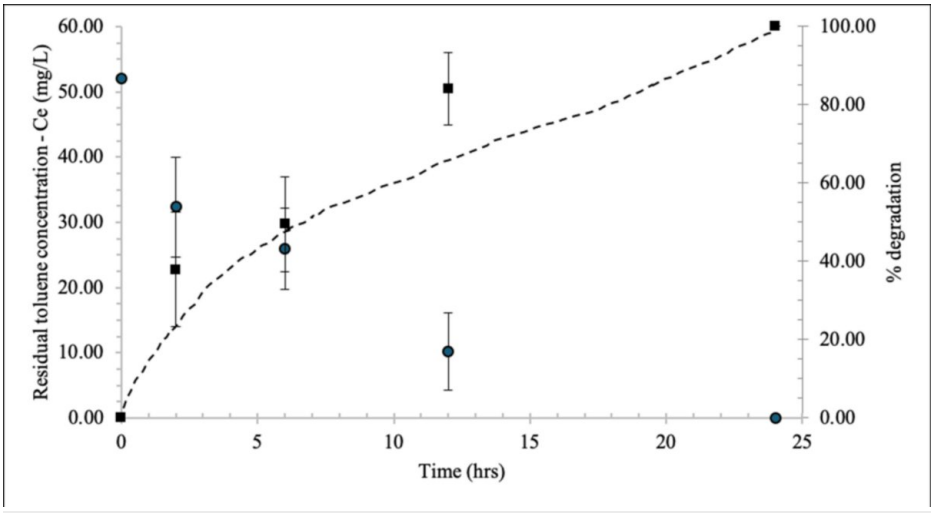

FIGURE 9: Degradation of toluene by S1 culture with time

The process proceeded rapidly at first, with 50% of the toluene degrading in 6 h, and the remaining substrate took 18 h to do so. The secondary Y-axis of Figure 9 depicts that 38%, 50%, 84% and 100% degradation occurred after 2 h, 6 h, 12 h, and 24 h, respectively. The DO levels in the spiked S1 culture were between 1.14 mg/L and 1.94 mg/L, while in the Control B sample, they ranged from 2.38 mg/L to 2.68 mg/L during the reaction (Figure 10).

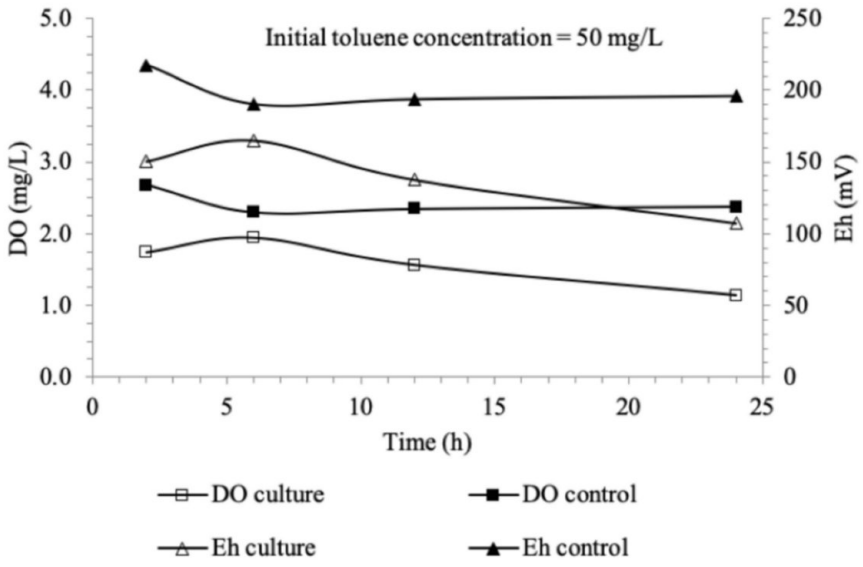

FIGURE 10: Variation of DO and Eh with time during toluene degradation by S1 culture

Toluene degradation by the S1 isolate followed first-order kinetics. The first-order rate constant is given as:

$$\ln C_t = \ln C_0 - K_1 t \quad (2)$$

Where C_t is the toluene concentration (mg/L) at time t (h), C_0 is the initial toluene concentration (mg/L), and K_1 is the first-order rate constant (h^{-1}).

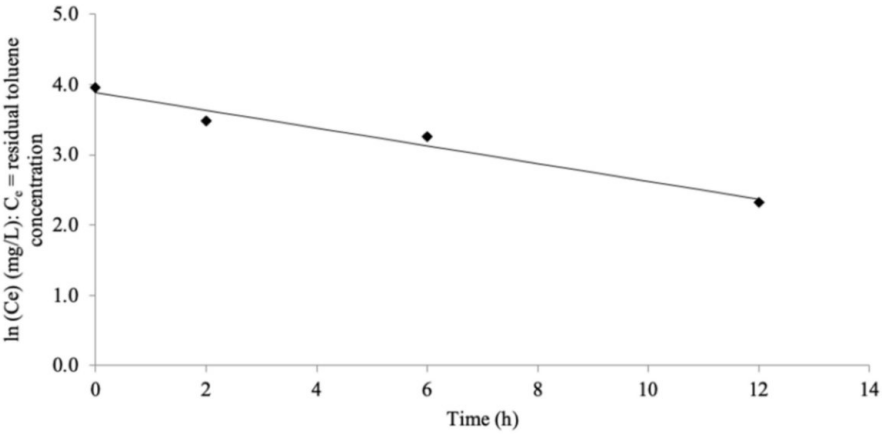

FIGURE 11: First-order kinetics for toluene degradation by S1 culture

Discussion

Toluene degradation studies

Rajamanickam et al. [23] used toluene concentrations of 50 to 500 mg/L; Singh and Celin [24] had examined higher toluene concentrations of 8.67-8670 mg/L, and Mukherjee and Bordoloi [25] had examined toluene concentrations of 29-348 mg/L. Hendrickx et al. [26] had reported a notable toluene concentration (10 mg/L) in contaminated groundwater at an oil refinery site in Northern Bohemia, Czech Republic. The toluene concentration range used in this study (5-300 mg/L) falls within the range of other researchers. The toluene concentrations in Bengaluru groundwater samples ranged from 0.062 mg/L to

0.153 mg/L [17]. A complete breakdown of toluene was seen at concentrations of 5-10 mg/L (Figure *4*), which means *Pseudomonas* sp. could help clean up toluene-polluted groundwater in Bengaluru with the right technology. Pilot-scale or field simulation tests were not conducted to validate laboratory findings.

Pseudomonas sp. (S1 culture) and *B. haynesii* (S2 culture) were isolated from gasoline-contaminated soil and groundwater samples by enriching the cultures with toluene. As single colonies were extracted from the soil and groundwater samples, they were used for toluene degradation. Additional research is required to assess the performance of S1 and S2 cultures within a mixed culture environment. The growth in *Pseudomonas* sp. at toluene concentrations <10 mg/L (Figure *5*) suggested that these microbes can flourish at low toluene concentrations. The decrease in cell growth at higher toluene levels in Figure *5* was likely due to the substrate limiting the bacteria's growth. Shim et al. [27] similarly reported that an increase in BTEX concentration inhibited bacterial growth.

The results in Figure *6* demonstrated that *Pseudomonas* sp. (S1 culture) has greater toluene removal efficiency than *B. haynesii* (S2 culture). A deeper exploration of the physiological and biochemical differences between the two strains is provided for greater insight. *Pseudomonas* belongs to the *Pseudomonadaceae* family. It is a gram-negative, non-spore-forming, flagellated, rod-shaped bacterium. *Pseudomonas* sp. can thrive in a wide range of temperatures from 4°C to 42°C, tolerate pH levels between 4.0 and 8.0, and survive in both oxygen-rich and low-oxygen environments (with 0.5 to 8 mg/L of dissolved oxygen), which is why it has a larger population than *B. haynesii* even at toluene levels of 30 mg/L, as shown in Figure *7*. In addition, *Pseudomonas* sp. degrades petroleum hydrocarbons and aromatic compounds due to its complex genetic components. It is characterized by monooxygenase and dioxygenase degradation pathways, containing functional genes such as todE, tmoA, xylMA, and ND6, which activate enzymes for toluene and other hydrocarbon degradation via the TOL and TOD pathways [28-30].

Bacillus is a saprophytic, gram-positive, spore-forming organism capable of thriving in extreme environments [31]. However, limited data are available for *B. haynesii* [32,33]. BTEX degradation by *Bacillus* is limited to certain species such as *B. cereus, B. stratosphericus*, *B. subtilis*, and *B. pumilus*. The BTEX degradation process by *Bacillus* is not well investigated [34]. This study is the first to report the hydrocarbon degradation activity of *B. haynesii*.

The presence of genetic material in *Pseudomonas* sp. that can break down toluene and other hydrocarbons, along with its high physiological adaptability, may have contributed to a greater ability to degrade these substances.

Toluene degradation with time

Figure *6* showed that *Pseudomonas* sp. broke down more toluene (40-100%) than *Bacillus haynesii* (22-66%); therefore, *Pseudomonas* sp. was selected to study biodegradation kinetics. The microbes required 24 h to completely degrade 50 mg/L of toluene substrate (Figure *9*). During the microbial breakdown of toluene, the amount of dissolved oxygen dropped by 48-72%, creating conditions that favor anoxic processes. The first-order plot in Figure *11* gave a graphical C_0 value of 48 mg/L (y-axis intercept) and was defined by a rate constant of 0.127 h^{-1}. The strong regression coefficient (R^2 = 0.97) and the close match between the experimental and graphical C_0 values (51 and 48 mg/L) showed that the *Pseudomonas* sp. followed first-order kinetics while breaking down toluene.

Figure *12* compares this study's findings with those of others [23,35,36] and contextualizes the results. Additional aerobic toluene degradation investigations from [23] are included in Figure *12*. The S1 culture showed a reduced rate of degradation (0.127 h^{-1}) at comparable toluene concentrations in comparison to literature values. One possible reason for the slower rate of *Pseudomonas* sp. could be that there is not enough dissolved oxygen available, which is necessary for aerobic processes. The literature data (Figure *12*) exhibit a reduction in rate constants with an increase in toluene concentration. An explanation for the slower rates of degradation could be the inhibition of microbes when toluene concentrations are high. Rajamanickam et al. [23] reported rate constants ranging from 0.020 h^{-1} at 3 mg/L to 0.44 h^{-1} at 500 mg/L for toluene degradation by various bacterial isolates.

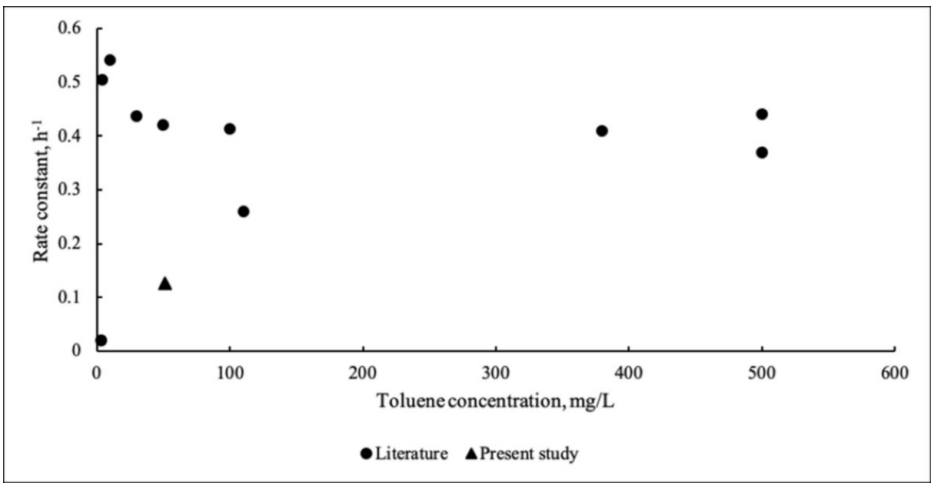

FIGURE 12: Kinetic parameters for toluene degradation from literature

A comparison between the findings of the current study and of Wongbunmak et al. [34] highlights the impact of co-compounds on the rate constant. The slower rate of toluene breakdown (0.019 h^{-1}) by *Bacillus amyloliquefaciens* compared to the current study (0.127 h^{-1}) is likely due to the interference of other BTEX compounds. A first-order rate constant of 0.021 h^{-1} was obtained by Careghini et al. [36] using a combination of facultative and aerobic species.

Xin et al. [35] examined how quickly *Mycobacterium* sp. CHXY119 and *Pseudomonas* sp. YATO411 biodegrade toluene, along with the effects of oxygen-releasing compounds. The biodegradation rate for *Mycobacterium* sp. CHXY119 was 0.087 h^{-1}. The addition of oxygen carriers accelerated the rate of toluene degradation to 1.113 h^{-1}, indicating that oxygen was a critical parameter for toluene degradation. BTEX biodegradation by a *Pseudomonas putida* strain acclimatized to unleaded gasoline confirmed the expression of tod genes responsible for synthesizing dioxygenases that catalyze toluene degradation [31].

Future research directions

Different types of bacteria, such as *Pseudomonas*, *Bacillus*, *Rhodococcus*, *Janibacter*, *Mycobacterium*, *Acinetobacter*, and *Microbacterium*, can break down BTEX at levels between 0.5 mg/L and 500 mg/L. Multiple studies on BTEX degradation indicate that bacteria capable of degrading toluene often have the potential to degrade benzene, ethylbenzene, and total xylenes. However, more research is needed to understand how low levels of dissolved oxygen, nutrients, changing amounts of hydrocarbons, and the recalcitrant nature of BTEX compounds can influence the breakdown of mixed substrates. Furthermore, research should find the best-suited oxygen carriers to improve the breakdown process and compare how well single bacteria perform against groups of bacteria in breaking down BTEX. The metabolism of toluene by *B. haynesii* needs elucidation.

Conclusions

The *Pseudomonas* sp. and *B. haynesii* strains occurred in the bacterial isolates of gasoline-contaminated soil and groundwater, respectively. *Pseudomonas* sp. outperformed *B. haynesii* in toluene degradation. *Pseudomonas* sp. broke down 40-100% of the available materials at toluene levels of 5-300 mg/L, while *B. haynesii* only processed 22-66% of the materials.

Cell counts in both microbe cultures decreased at toluene concentrations greater than 10 mg/L. As *B. haynesii* bacteria were less resistant to toluene, the microbes experienced greater cell loss, which led to lesser toluene degradation.

The toluene degradation process by *Pseudomonas* sp. was initially rapid, with 50% toluene degradation occurring in 6 h. The remaining 50% reduction occurred over 18 h.

Toluene degradation by *Pseudomonas* sp. followed first-order kinetics and was characterized by a rate constant of 0.127 h^{-1}.

The comparison of the kinetic data with other studies shows that having co-compounds, higher toluene levels, and lower dissolved oxygen levels slows down how quickly toluene breaks down.

The study's findings show that using the right aerobic field technology, native *Pseudomonas* sp. can clean

up toluene-contaminated groundwater. Pilot-scale or field simulation tests were not conducted to validate laboratory findings.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Sudhakar M. Rao, Rita Evelyne

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Disclosures

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