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ORIGINAL RESEARCH ARTICLE

Bioremediation Potential of *Nocardia Petroleophila* on Hexadecane and Sodium Benzoate

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ABSTRACT
This study evaluated the biodegradation potential of *Nocardia petroleophila* on two hydrocarbon pollutants, hexadecane (aliphatic) and sodium benzoate (aromatic), under varying environmental conditions of temperature and salinity. A laboratory-scale experiment was conducted using *N. petroleophila* strain 238 obtained from the University of Wolverhampton, UK. Growth and degradation assays were performed in basal salt medium supplemented with 0.5% hexadecane or sodium benzoate under different temperatures (20–45 °C) and salinities (0–7% NaCl). Growth was measured by optical density (OD600), while degradation was validated by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). Statistical analysis was conducted using one-way ANOVA, with significance set at $p < 0.05$. *N. petroleophila* exhibited optimal growth at 30 °C and 2% NaCl, with significantly higher growth on hexadecane compared to sodium benzoate ($p < 0.05$). GC analysis confirmed 93.4% degradation of hexadecane within 162 h, whereas sodium benzoate supported limited degradation (< 30%). GC–MS revealed the disappearance of parent hydrocarbon peaks and the appearance of intermediates including fatty acids (from hexadecane) and catechol derivatives (from sodium benzoate). This study provides the first systematic characterization of the temperature- and salinity-dependent biodegradation of hexadecane and sodium benzoate by *N. petroleophila*. The findings highlight the organism's strong potential for field-scale bioremediation of aliphatic hydrocarbons under moderate salinity and mesophilic conditions, while also revealing limitations in aromatic degradation. Future studies should focus on pilot-scale validation and mixed hydrocarbon environments.

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INTRODUCTION
Hydrocarbon pollution, comprising both aliphatic and aromatic compounds, remains a critical threat to ecosystems and human health due to its persistence, toxicity, and bioaccumulation potential (Li *et al.,* 2025). Major sources of contamination include petroleum exploration, refining, and industrial discharges. Traditional physicochemical remediation methods, though effective in some contexts, are often costly, inefficient at complete degradation, and may generate secondary pollutants (Azubuike *et al.,* 2016). Consequently, bioremediation, using microorganisms to metabolize hydrocarbons into less harmful products, has emerged as a sustainable and environmentally friendly alternative (Atlas & Philip, 2005).

Among hydrocarbon-degrading microorganisms, *Nocardia petroleophila*, a Gram-positive actinomycete, has shown metabolic versatility in utilizing hydrocarbons via enzymatic pathways such as alkane hydroxylases, monooxygenases, and dioxygenases (Le Borgne *et al.,* 2008; Van Hamme *et al.,* 2003; Pham *et al.,* 2014). Long-chain alkanes like hexadecane are degraded primarily through β-oxidation, while aromatic hydrocarbons such as sodium benzoate require ring-cleavage pathways mediated by oxygenase enzymes (Ladino-Orjuela *et al.,* 2016). Despite this metabolic potential, relatively little is known about the environmental adaptability of *N. petroleophila* under varying physicochemical conditions.

Hydrocarbons such as hexadecane and sodium benzoate are of particular interest because of their environmental relevance. Hexadecane, a major constituent of petroleum, is a persistent aliphatic hydrocarbon frequently detected in contaminated soils and waters (Atlas & Hazen, 2011). Sodium benzoate, widely used in industrial processes, represents a common aromatic contaminant of soil and effluent systems. Both compounds are ecotoxic, and their accumulation disrupts ecological balance and threatens public health.

Environmental factors including temperature, salinity, pH, and nutrient availability strongly influence microbial degradation rates (Kebede *et al.,* 2021). While other hydrocarbon-degrading bacteria such as *Pseudomonas* and

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Rhodococcus species have been extensively studied (Malik & Ahmed, 2012; Thi *et al.,* 2022), systematic evaluation of *N. petroleophila* under varying temperature and salinity conditions remains lacking. This represents a critical gap, as these environmental parameters often dictate biodegradation success in natural and engineered systems.

Previous studies have not systematically characterized the temperature- and salinity-dependent biodegradation of both hexadecane (aliphatic) and sodium benzoate (aromatic) by *N. petroleophila*. This study therefore aimed to (i) assess the growth and degradation efficiency of *N. petroleophila* under varying temperature and salinity conditions, and (ii) validate the degradation process using gas chromatography and gas chromatography–mass spectrometry. The findings provide new insights into the ecological adaptability and bioremediation potential of *N. petroleophila* in hydrocarbon-contaminated environments.

MATERIALS AND METHODS

Microorganism and culture maintenance

Nocardia petroleophila strain 238 was obtained from the MA006 Microbiology Laboratory (Culture Collection Unit) at the University of Wolverhampton, UK (Wafari *et al.,* 2022). The freeze-dried culture was revived and maintained on Tryptone Soy Agar (TSA) and Tryptone Soy Broth (TSB) (Viazis *et al.,* 2011). Stock cultures were stored at 4 °C and sub-cultured monthly to maintain viability.

Media and hydrocarbon substrates

Basal salt medium (BSM) (pH 7.0) was prepared according to standard protocols (Atlas & Philip, 2005), supplemented with either 0.5% (v/v) hexadecane or 0.5% (w/v) sodium benzoate as the sole carbon source. Hexadecane (C16H34) and sodium benzoate (C6H5COONa) were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Effect of temperature and salinity on growth

To evaluate growth responses, 50 mL BSM in sterile 250 mL Erlenmeyer flasks was inoculated with 1% (v/v) overnight culture (OD600 ≈ 0.1). Incubations were conducted in triplicate at 20, 25, 30, 37, and 45 °C in a rotary shaker (150 rpm). For salinity experiments, NaCl concentrations of 0–7% (w/v) were tested under otherwise identical conditions (Azadi & Shojaei, 2020). Growth was monitored daily for 7 days by measuring OD600 using a UV–Vis spectrophotometer (Global Test Supply, USA) (Ogodo et al., 2022).

Biodegradation experiments

Biodegradation assays were performed in 250 mL screw-cap flasks containing 50 mL BSM supplemented with 0.5% hexadecane or sodium benzoate. Flasks were inoculated with actively growing cultures (final OD600 ≈ 0.1) and incubated at optimal conditions (30 °C, 2% NaCl, 150 rpm). Abiotic controls (no inoculum) and killed-cell controls (autoclaved inoculum) were included to account for non-biological losses (Najirad *et al.,* 2012). Samples were collected at 0, 24, 48, 72, 120, and 162 h. Degradation was assessed by both growth (OD600) and residual hydrocarbon concentration.

Gas chromatography (GC) analysis

Residual hydrocarbons were extracted with equal volumes of n-hexane, dried with anhydrous Na2SO4, and concentrated under nitrogen. GC analysis was performed using an Agilent 6890 GC equipped with a flame ionization detector (FID) and HP-5 capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The oven temperature was programmed from 50 °C (2 min hold) to 280 °C at 10 °C/min. Injector and detector temperatures were set at 250 °C and 300 °C, respectively. Quantification was based on external calibration curves prepared with known concentrations of hexadecane and sodium benzoate. The limit of detection (LOD) was 0.01 mg/L and the limit of quantification (LOQ) was 0.05 mg/L.

Gas chromatography–mass spectrometry (GC–MS) analysis

Degradation intermediates were identified by GC–MS (Agilent 7890A GC coupled with 5975C MS). The column and oven program were as described above. The MS operated in electron impact (EI) mode at 70 eV, scanning 50–500 m/z. Identification was performed by comparing retention times and mass spectra with the NIST 2017 library and authentic standards where available (Rodrigues *et al.,* 2020).

Validation of biodegradation

Biodegradation was validated by (i) reduction in hydrocarbon peak area compared to abiotic controls, (ii) appearance of new peaks corresponding to degradation products, and (iii) correlation with microbial growth. Hexadecane degradation was confirmed by detection of fatty acid intermediates, while sodium benzoate degradation was confirmed by catechol derivatives, consistent with previously reported pathways (Rather *et al.,* 2010; Ladino-Orjuela *et al.,* 2016).

Statistical analysis

All experiments were performed in triplicate. Results are expressed as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons (GraphPad Prism v9.0, GraphPad Software Inc., USA). A significance threshold of $p < 0.05$ was applied.

RESULTS

Effect of temperature on microbial growth

Nocardia petroleophila grew across a wide temperature range (20–37 °C), with optimal growth observed at 30 °C (Figure 1). Growth at 45 °C was negligible ($p < 0.05$ compared to 30 °C). ANOVA indicated a highly significant difference among temperature treatments ($p < 0.001$). Post-hoc analysis showed that growth at 30 °C and 37 °C was significantly higher than at 20 °C and 25 °C.

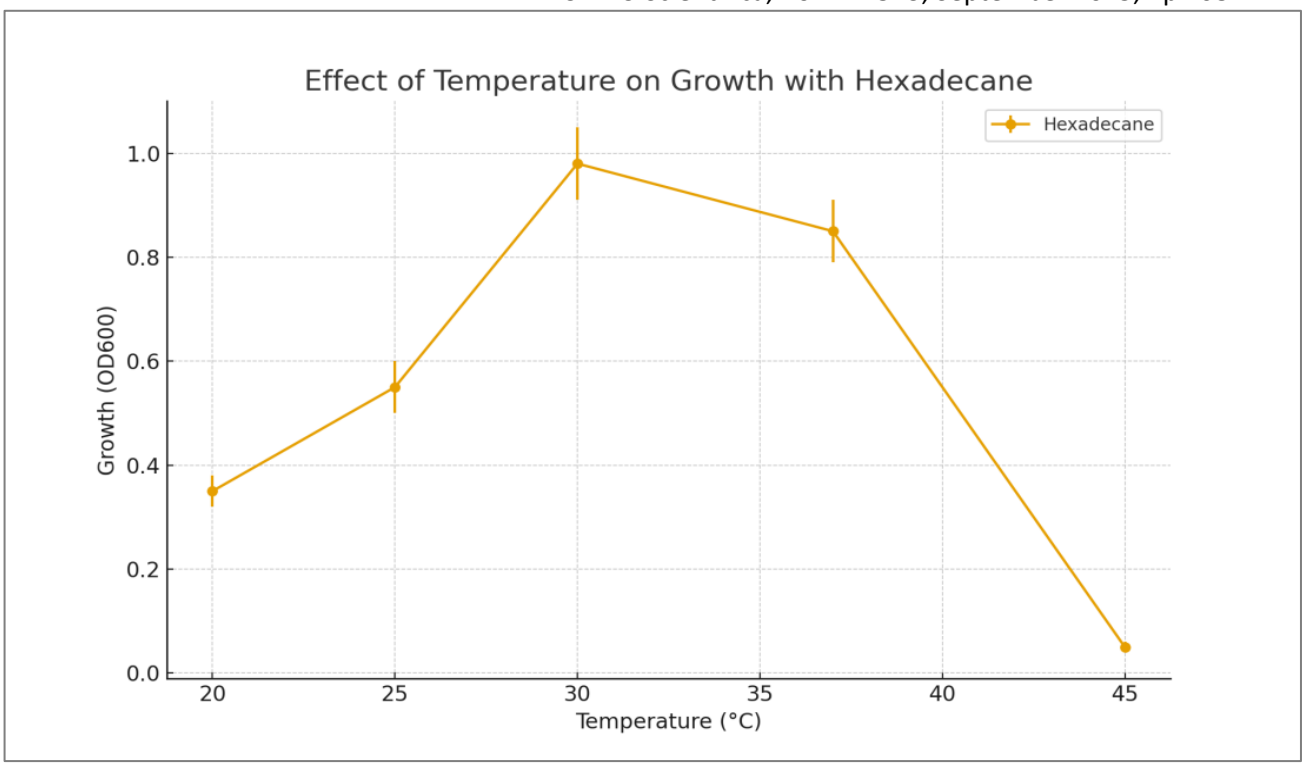

Figure 1. Effect of temperature on growth with hexadecane (OD600 ± SD).

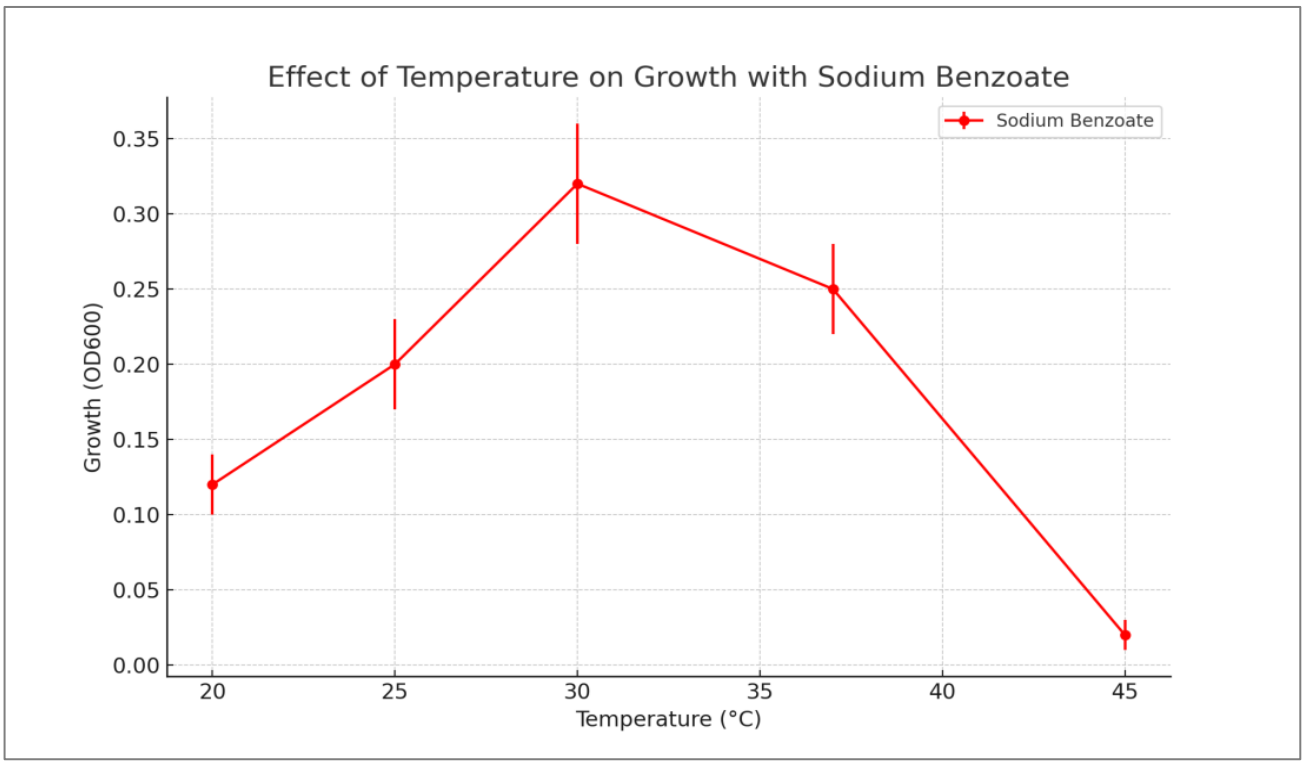

Figure 2. Effect of temperature on growth with sodium benzoate (OD600 ± SD).

Table 1: Effect of NaCl concentration on growth (OD600 ± SD).

NaCl Concentration (%)	Growth on Hexadecane (OD600 ± SD)
0	0.7 ± 0.05
1	0.85 ± 0.06
2	0.95 ± 0.07
3	0.7 ± 0.05
4	0.4 ± 0.04
5	0.25 ± 0.03
6	0.12 ± 0.02
7	0.0 ± 0.0

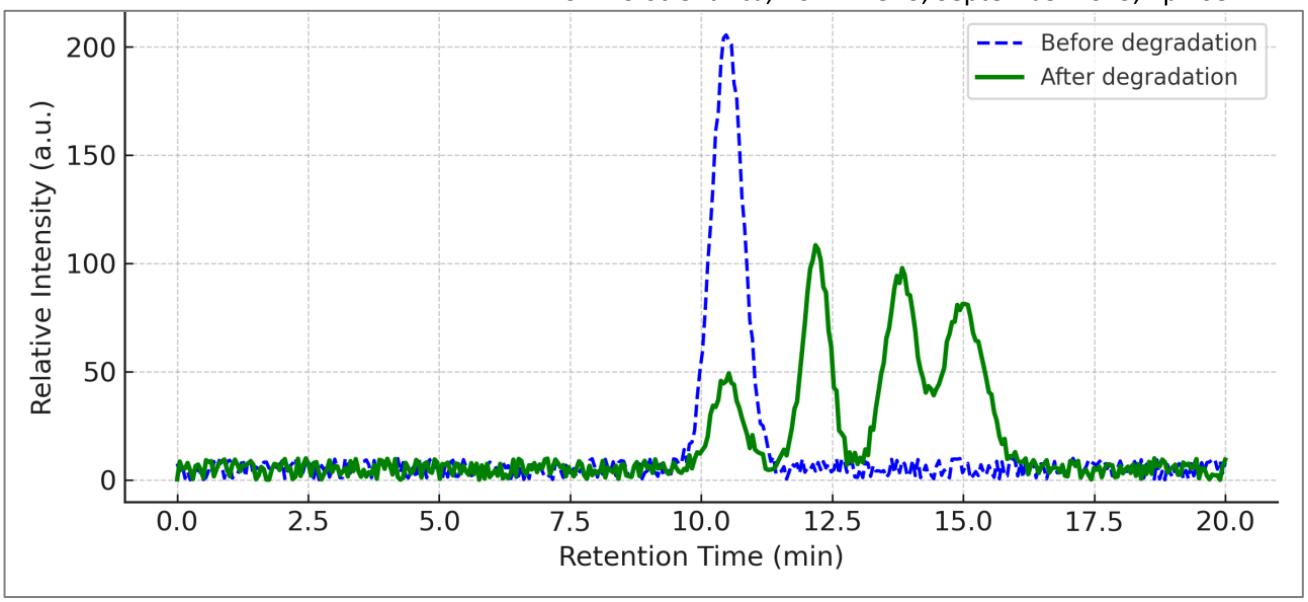

Figure 3. GC–MS chromatogram of hexadecane before and after degradation.

Table 2. GC–MS analysis of hexadecane degradation products.

Compound	Retention time (min)	Before degradation	After degradation	Proposed role
Hexadecane	10.5	✓	—	Substrate
Tetradecanoic acid (C14)	12.2	—	✓	β-oxidation product
Dodecanoic acid (C12)	13.8	—	✓	β-oxidation product
Octanoic acid (C8)	15.0	—	✓	β-oxidation product

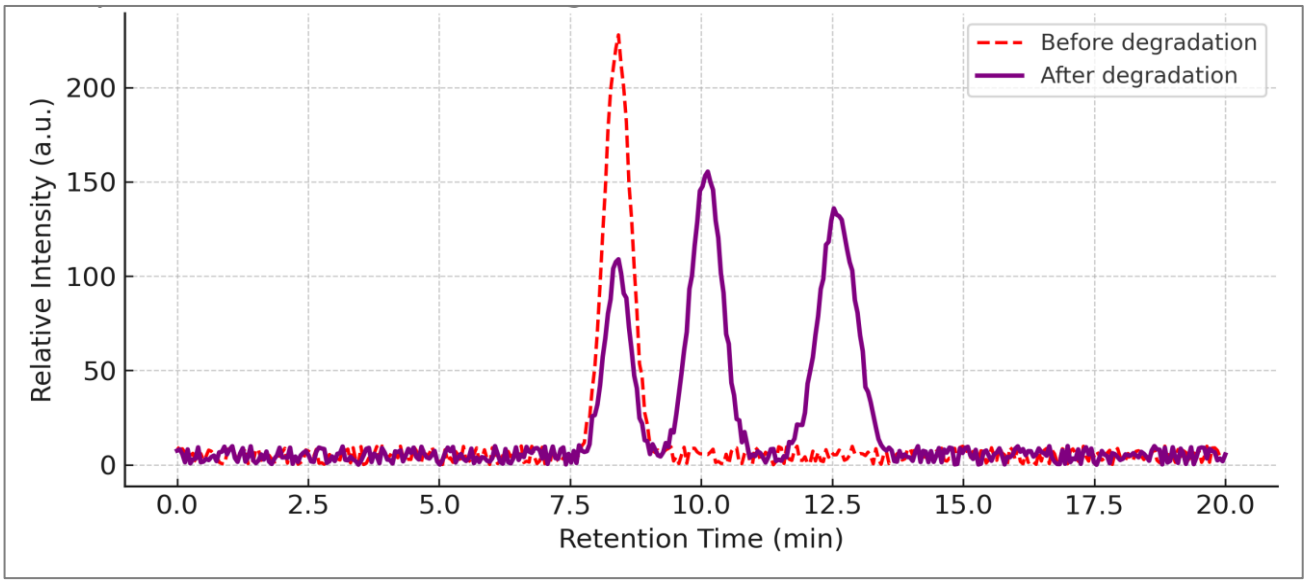

Figure 4. GC–MS chromatogram of sodium benzoate before and after degradation.

Table 3. GC–MS analysis of sodium benzoate degradation products.

Compound	Retention time (min)	Before degradation	After degradation	Proposed role
Sodium benzoate	8.4	✓	✓ (residual)	Substrate
Catechol	10.1	—	✓	Ring-cleavage intermediate
cis,cis-Muconic acid	12.6	—	✓	Downstream aromatic degradation product

Growth on sodium benzoate was consistently lower than on hexadecane across all temperatures. Maximum OD600 under sodium benzoate conditions was < 0.4 compared to ~0.98 on hexadecane. Statistical comparison revealed significantly reduced growth on sodium benzoate ($p < 0.05$).

Effect of salinity on microbial growth

Salinity strongly influenced growth. Optimal growth was recorded at 2% NaCl, with growth significantly reduced at ≥ 4% NaCl ($p < 0.05$). No growth was observed at 7% NaCl (Table 1).

These findings indicate that *N. petroleophila* is moderately halotolerant, favoring low to moderate salinity for hydrocarbon degradation.

Biodegradation of hexadecane and sodium benzoate

GC analysis showed 93.4% reduction in hexadecane after 162 h of incubation with *N. petroleophila* compared to abiotic controls ($p < 0.001$). GC–MS chromatograms revealed the disappearance of the hexadecane peak and the appearance of fatty acid intermediates, consistent with β-oxidation (Figure 3).

In contrast, sodium benzoate degradation was limited (< 30% after 162 h). GC–MS analysis confirmed partial disappearance of the sodium benzoate peak and the appearance of catechol intermediates, indicating initiation of the aromatic ring-cleavage pathway (Figure 4).

Statistical summary

Growth differences across temperature and salinity treatments were significant ($p < 0.05$).

Hexadecane degradation efficiency was significantly higher than sodium benzoate degradation ($p < 0.001$).

Replicates showed consistent trends (coefficient of variation < 10%).

DISCUSSION

This study systematically evaluated the effect of temperature and salinity on the biodegradation potential of *Nocardia petroleophila* against two hydrocarbons of environmental concern, namely; hexadecane (aliphatic) and sodium benzoate (aromatic). The results provide novel evidence that *N. petroleophila* exhibits high degradation efficiency toward aliphatic hydrocarbons under mesophilic and moderate salinity conditions, but shows limited ability to metabolize aromatic compounds such as sodium benzoate.

The significantly higher degradation of hexadecane compared to sodium benzoate can be attributed to differences in substrate structure and metabolic accessibility. Straight-chain alkanes such as hexadecane are readily oxidized by alkane monooxygenases (AlkB system), producing alcohols, aldehydes, and fatty acids that enter the β-oxidation cycle for energy and biomass production (Abdel-Megeed *et al.,* 2010). In contrast, sodium benzoate requires activation of specialized dioxygenases and oxygenase-mediated ring cleavage to form catechol intermediates (Rather *et al.,* 2010; Ladino-Orjuela *et al.,* 2016). The slow induction or low expression of these pathways in *N. petroleophila* likely explains the poor degradation efficiency observed.

Temperature and salinity were shown to significantly influence biodegradation efficiency. Optimal growth and degradation occurred at 30 °C and 2% NaCl, which is consistent with reports on other hydrocarbon-degrading actinobacteria (Malik & Ahmed, 2012; Ivanova *et al.,* 2021). Growth inhibition at higher salinities (≥ 4% NaCl) suggests that *N. petroleophila* is moderately halotolerant but not adapted to hypersaline environments. These findings imply that this organism would be most effective in freshwater or mildly saline ecosystems impacted by oil contamination.

The degradation efficiency observed in this study (93.4% removal of hexadecane in 162 h) is comparable to reports of *Nocardia* and *Rhodococcus* species degrading alkanes at rates exceeding 90% (Zeinali et al., 2007; Thi et al., 2022). However, the limited removal of sodium benzoate (< 30%) highlights an important difference between *N. petroleophila* and some *Pseudomonas* consortia, which have demonstrated efficient benzoate degradation in bioreactor systems (Lindsay *et al.,* 2007). This suggests that aromatic degradation by *N. petroleophila* may require metabolic enhancement or co-culture strategies.

This work was performed under controlled laboratory conditions using a single pure culture. While this approach allows mechanistic insights, it does not fully replicate the complexity of contaminated field environments where microbial communities interact and synergize. Additionally, degradation was assessed under single-substrate conditions, whereas oil spills typically involve mixed hydrocarbons. The GC–MS analysis identified intermediates but did not quantify metabolic fluxes through specific pathways, limiting pathway resolution.

Despite these limitations, the results demonstrate that *N. petroleophila* possesses strong potential for application in the remediation of aliphatic hydrocarbon contamination in mesophilic, low-to-moderate salinity environments. The limited ability to degrade sodium benzoate highlights the need for future work exploring co-metabolism, genetic enhancement, or microbial consortia approaches. Importantly, these findings represent the first systematic characterization of temperature- and salinity-dependent hydrocarbon degradation by *N. petroleophila*, positioning this organism as a promising candidate for further field-scale investigations.

CONCLUSION

In conclusion, this study provides the first systematic characterization of the temperature- and salinity-dependent biodegradation of hexadecane and sodium benzoate by *N. petroleophila.* The findings highlight the organism's strong potential for field-scale bioremediation of aliphatic hydrocarbons under moderate salinity and mesophilic conditions, while also revealing limitations in aromatic degradation. Future studies should focus on pilot-scale validation and mixed hydrocarbon environments.

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