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Comparative Analysis of Hydrocarbon-Degrading Microbes and Pineapple Fruit-Peel Biosurfactants in Engine Oil Biodegradation

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ABSTRACT

This study evaluated the potential of pineapple fruit peel-derived biosurfactants to enhance the biodegradation of engine oil-contaminated liquid medium using a bacterial consortium. Laboratory-scale microcosm experiments were conducted over nine days with treatments including bacterial isolates alone, pineapple biosurfactant alone, and combined bacteria + biosurfactant at 50%, 75%, and 100% concentrations. Three bacterial isolates—Pseudomonas spp., Bacillus spp., and Staphylococcus spp.—were identified, with biosurfactant activity highest in Pseudomonas. The crude biosurfactant yield from pineapple peel was 0.42 g/g, indicating the substrate's suitability for biosurfactant production. Key parameters assessed included Total Petroleum Hydrocarbon (TPH), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), turbidity, pH, and microbial counts. TPH decreased from 482.1 ± 2.5 mg/L in the control to 25.5 ± 1.9 mg/L in the 75% consortium + 75% biosurfactant treatment. BOD and COD declined from 25.3 ± 0.3 to 12.0 ± 0.2 mg/L and 46.2 ± 0.5 to 6.5 ± 0.6 mg/L, respectively, while turbidity decreased from 50.3 ± 0.4 to 27.4 ± 0.3 NTU. The pH dropped from 7.18–7.22 to 6.35–6.82, with combined treatments showing the greatest reductions, reflecting intensified microbial metabolism. Heterotrophic and hydrocarbon-degrading bacterial counts increased substantially in combined treatments, demonstrating a synergistic effect of the consortium and biosurfactant in promoting microbial growth and hydrocarbon degradation. Pineapple peel biosurfactant significantly enhanced microbial activity, accelerated hydrocarbon breakdown, and improved physicochemical properties of the engine oil-contaminated medium, highlighting its potential as a sustainable and low-cost approach for hydrocarbon bioremediation.

1. INTRODUCTION

Petroleum hydrocarbons are complex mixtures of aliphatic, aromatic, resinous, and asphaltene fractions whose environmental behaviour is governed by molecular structure, physicochemical properties, and interactions with soils and sediments (Varjani, 2017). Low molecular weight alkanes are readily biodegraded, whereas polycyclic aromatic hydrocarbons (PAHs) and high molecular weight fractions are recalcitrant, bioaccumulative, and pose chronic ecotoxicological risks (Satpute *et al.*, 2010). Hydrocarbon aging, sorption to soil organic matter, and sequestration in micropores further reduce bioavailability, impairing biodegradation (SelvaFilho *et al.*, 2023). In oil-producing regions like the Niger Delta, persistent petroleum contamination leads to

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ecological and socio-economic impacts, including reduced crop yields, groundwater pollution, and human health risks (Okoye *et al.*, 2020; Obire *et al.*, 2023). Conventional remediation methods—such as excavation, thermal desorption, chemical oxidation, and solvent extraction—can remove contaminants rapidly but are costly, energy-intensive, and disruptive, often transferring pollutants rather than detoxifying them (Elayaperumal *et al.*, 2025). Synthetic surfactants improve hydrocarbon mobilization but may persist environmentally and pose ecotoxicological risks. Consequently, bioremediation has emerged as an environmentally sustainable alternative, relying on hydrocarbon-degrading bacteria to mineralize hydrocarbons into less toxic products such as carbon dioxide, water, and biomass (SelvaFilho *et al.*, 2023; Satpute *et al.*, 2010).

2. LITERATURE REVIEW

Hydrocarbon-degrading bacteria (hydrocarbonoclasts), including *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Rhodococcus*, and *Alcanivorax*, utilize enzymatic systems—mono- and dioxygenases, dehydrogenases, and hydrolases—to oxidize aliphatic and aromatic hydrocarbons under aerobic and anaerobic conditions (Aruotu *et al.*, 2023; Obire *et al.*, 2023). Indigenous consortia from chronically polluted sites often exhibit robust degradation capabilities (Aruotu *et al.*, 2023; Obire *et al.*, 2023). However, microbial efficiency is constrained by hydrocarbon bioavailability, nutrient limitation, oxygenation, temperature, and pH (Zhang *et al.*, 2024). Some bacteria produce biosurfactants in situ, enhancing hydrocarbon solubilization and uptake, but yields are often insufficient for optimal remediation (Satpute *et al.*, 2010). Biosurfactants—amphiphilic compounds such as glycolipids, lipopeptides, phospholipids, and polymers—reduce surface tension, emulsify hydrophobic compounds, and increase bioavailability (Begum *et al.*, 2023; SelvaFilho *et al.*, 2023). Their environmental benefits include biodegradability, low toxicity, and sustainability. Exogenous biosurfactant addition can accelerate hydrocarbon removal, though effects are dose-dependent: low to moderate concentrations enhance biodegradation, while excessive levels may sequester hydrocarbons in micelles, reducing microbial uptake (Elayaperumal *et al.*, 2025; Rico *et al.*, 2020).

Low-cost feedstocks are crucial for economic viability, and fruit peels are particularly promising substrates. Pineapple peel contains fermentable sugars, organic acids, and micronutrients that support microbial growth and biosurfactant production, notably lipopeptides with high surface activity (Rico *et al.*, 2020; Wang *et al.*, 2023). While orange and watermelon peels also yield functional biosurfactants, comparative studies highlight pineapple peel as especially effective for hydrocarbon biodegradation due to its composition and fermentability (Wang *et al.*, 2023). Previous studies indicate that combining biosurfactants with hydrocarbonoclastic bacteria can produce synergistic effects, accelerating biodegradation more effectively than either treatment alone (Zhang *et al.*, 2024; SelvaFilho *et al.*, 2023). Yet, the influence of biosurfactant type, concentration, and microbial compatibility on remediation efficiency remains context-dependent, particularly in tropical oil-impacted regions such as the Niger Delta. Laboratory evaluations measuring Total Petroleum Hydrocarbon (TPH), microbial population dynamics, and physicochemical parameters are therefore essential to identify optimal treatment conditions and support sustainable, cost-effective bioremediation strategies. This study investigates the effectiveness of pineapple peel-derived biosurfactants, hydrocarbon-degrading bacteria, and their

combination in degrading engine oil under laboratory conditions.

3. MATERIALS AND METHODS

3.1 Collection and Preparation of Experimental Samples

Soil samples were collected from two locations within the staff quarters of the Petroleum Training Institute (PTI), Effurun, Delta State (5.5709° N, 5.8054° E). A hand-dug soil auger was used to obtain samples from a depth of 0.5–1.0 m to target the subsurface zone likely impacted by hydrocarbons (Aruotu *et al.*, 2023; Ojo *et al.*, 2021). Triplicate samples were placed in sterilized, labeled polyethylene bags and transported immediately to the laboratory for physicochemical and microbiological analyses (Cheesbrough, 2016).

Fresh pineapple (*Ananas comosus*) fruits were purchased from Effurun Market, Delta State. Fruits were washed thoroughly with clean water to remove dirt and residues (Aruotu *et al.*, 2023). Peels were carefully separated, chopped into small pieces, and oven-dried at 50 °C until crisp (Aruotu *et al.*, 2023). The dried peels were ground into fine powder using a clean electric blender and stored in airtight containers at room temperature until required for biosurfactant production (Okoye *et al.*, 2020).

3.2 Isolation of Hydrocarbon-Degrading Bacteria

During the enrichment of hydrocarbon-degrading bacteria, ten grams of soil were transferred aseptically into 250 mL Erlenmeyer flasks containing 90 mL of sterile Mineral Salt Medium (MSM) supplemented with 1% (v/v) used engine oil as the sole carbon and energy source (Okoye *et al.*, 2020; Satpute *et al.*, 2010). Flasks were incubated at 30 °C on a rotary shaker (120–150 rpm) for 5–7 days under aerobic conditions to promote microbial growth and hydrocarbon degradation. Development of turbidity and emulsification was used as an indicator of microbial activity. Enriched cultures were subsequently used as inocula for isolation and biodegradation studies.

Enriched cultures were serially diluted using sterile physiological saline (0.85% NaCl) in a tenfold dilution series (10^{-1} to 10^{-6}) (Cappuccino and Sherman, 2014). Aliquots (0.1 mL) from dilutions 10^{-3} to 10^{-5} were spread onto MSM agar plates containing 1% (v/v) used engine oil. Plates were incubated at 30 °C for 3–5 days. Distinct colonies with different morphologies were subcultured using the streak plate method to obtain pure isolates (Aruotu *et al.*, 2023). Purity was confirmed through repeated streaking. Pure isolates were preserved for biochemical characterization and biodegradation assays. Pure bacterial isolates were maintained on Nutrient Agar (NA) following initial enrichment on MSM–oil medium.

3.3 Screening for Biosurfactant Production

Isolates capable of growing in MSM supplemented with 1% (v/v) used engine oil were screened for biosurfactant production. Cultures were incubated at 30 °C on a rotary shaker (120–150 rpm) for 48–72 hours, followed by centrifugation at 5,000 rpm for 20 minutes to obtain cell-free supernatants. A thin layer of crude oil was spread over a clean glass slide. A 5 µL drop of cell-free supernatant was placed on the oil-coated surface. Collapse of the drop within 1–2 minutes indicated positive biosurfactant activity, while a stable, rounded drop indicated a negative result (Satpute *et al.*, 2010).

Fifty milliliters of distilled water were poured into a Petri dish, and 20 µL of engine oil was added to form a uniform oil layer. Twenty microliters of cell-free supernatant were placed at the center of the oil film. The diameter of the clear zone formed, measured in millimeters, indicated biosurfactant activity; a larger zone

reflected higher activity (Satpute *et al.*, 2010; Rico *et al.*, 2020). Isolates showing strong positive results in both tests were selected as potential biosurfactant producers for further experiments using pineapple peel-derived biosurfactants.

3.4 Identification of Bacterial Isolates

Morphological, microscopic, spore staining, and biochemical characterization were used to identify bacterial isolates. Pure bacterial isolates were examined for colony morphology on freshly prepared Nutrient Agar (NA) plates after 24 hours of incubation at 30 °C. Observations included size, shape, margin, elevation, surface texture, pigmentation, and overall appearance, providing initial genus-level differentiation (Cheesbrough, 2006). Microscopic examination involved Gram staining of heat-fixed smears from 24-hour-old cultures. Slides were observed under a compound light microscope at 1000× magnification using oil immersion. Isolates were classified as Gram-positive or Gram-negative and further described by cell shape (rod or cocci) and arrangement (single, chains, clusters, or pairs), which guided subsequent biochemical testing. Gram-positive rod-shaped isolates suspected to belong to *Bacillus* spp. were subjected to Schaeffer–Fulton spore staining (Harley and Prescott, 2002). Smears were flooded with malachite green, steamed for five minutes, rinsed, and counterstained with safranin. Under microscopy, vegetative cells appeared red, while endospores retained green coloration, confirming spore-forming ability.

For biochemical characterization, representative isolates with distinct morphological traits were subjected to standard biochemical tests following Cheesbrough (2016), Cappuccino and Sherman (2014), and Holt *et al.* (1994):

- Catalase Test: Tested for catalase activity by adding 3% H_2O_2 to a bacterial smear; effervescence indicated a positive reaction.
- Oxidase Test: Detects cytochrome c oxidase using moistened oxidase paper; color change to deep purple/blue within 30 seconds indicated positive activity.
- Motility Test: Assessed using semi-solid agar (0.4%); diffuse growth radiating from stab line indicated motility.
- Urease Test: Inoculation in urea broth; pink coloration after 24–48 h indicated urease production.
- Sugar Fermentation Test: Phenol red broth tubes containing glucose, lactose, sucrose, or mannitol and inverted Durham tubes were inoculated; yellow color change indicated acid production, gas in Durham tube indicated fermentation with gas.

Morphological, microscopic, and biochemical data were combined to assign probable taxonomic identities. Gram reaction, cell shape, motility, and enzymatic/metabolic activity (catalase, oxidase, urease, carbohydrate fermentation) were compared with standard descriptions in Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) to determine probable genus and species of the isolates (Varjani, 2017; Das and Chandran, 2011).

3.5 Production of Biosurfactants

Biosurfactants were produced using both microbial fermentation and fruit peel-based extraction to allow comparative evaluation of biologically- and agro-waste-derived surfactants. All procedures were conducted under aseptic conditions.

Bacterial isolates were cultured in Mineral Salt Medium (MSM) supplemented with 1% (v/v) used engine oil as the sole carbon source (Atlas, 2010).

MSM composition (g/L) included: KH_2PO_4 (1.0), K_2HPO_4 (1.0), NH_4NO_3 (1.0), $MgSO_4 \cdot 7H_2O$ (0.2), $CaCl_2 \cdot 2H_2O$ (0.02), $FeSO_4 \cdot 7H_2O$ (0.05), NaCl (1.0), with pH adjusted to 7.0. Each 250 mL Erlenmeyer flask containing 100 mL sterile MSM was inoculated with 1 mL of an 18–24 h actively growing bacterial culture and incubated at 30 ± 2 °C on a rotary shaker at 150 rpm for 72 h. Cultures were centrifuged at 10,000 rpm for 15 minutes at 4 °C to obtain cell-free supernatants, which served as crude microbial biosurfactants and were stored at 4 °C for subsequent analysis (Mukherjee *et al.*, 2006; Satpute *et al.*, 2010). This method allowed isolates to metabolize hydrocarbons while secreting extracellular biosurfactants to aid emulsification and solubilization (Banat *et al.*, 2014).

Fruit peels (pineapple, orange, watermelon) were used as renewable substrates for biosurfactant extraction due to their carbohydrate, lipid, and phenolic content (Rahman and Gakpe, 2008). Peels were washed with tap and distilled water, chopped into small pieces, oven-dried at 50 °C until constant weight, ground into fine powder, and stored in airtight containers at room temperature (Makkar *et al.*, 2011). Solid–liquid extraction was performed using 70% ethanol at a 1:10 (w/v) ratio. Fifty grams of peel powder were mixed with 500 mL solvent and shaken at 150 rpm for 24 h. The mixture was filtered through muslin cloth and Whatman No. 1 paper, re-extracted once, and combined filtrates concentrated using a rotary evaporator at 45 °C under reduced pressure (Thavasi *et al.*, 2011; Olasanmi and Thring, 2018). Concentrated extracts were air-dried at 40–45 °C, re-dissolved in sterile distilled water, and stored at 4 °C. For long-term storage, lyophilization was performed and samples kept in desiccators to prevent moisture absorption (Gudiña *et al.*, 2015). This approach provided an eco-friendly, low-cost alternative to synthetic surfactants (Banat *et al.*, 2010).

Biosurfactant yield was expressed as total weight of crude biosurfactant per mass of culture medium or substrate. All measurements were performed in triplicate (Satpute *et al.*, 2010). Oven-dried fruit peel extracts were weighed, and yield calculated using standard formulae (Rahman and Gakpe, 2008; Makkar *et al.*, 2011).

3.6 Inoculum Application

Standardized bacterial inoculum was immediately applied to the biodegradation treatments. A known volume (1–5% v/v) of inoculum was introduced into each flask containing liquid MSM and engine oil, according to the experimental design. This ensured consistent microbial introduction across treatments, minimizing variability and promoting reproducible hydrocarbon degradation (APHA, 2017; Aruotu *et al.*, 2023).

3.7 Comparative Experimental Treatments

To evaluate the effect of fruit peel-derived biosurfactants on hydrocarbon degradation, six treatment conditions were established:

- T_1 – Control: MSM + engine oil (no bacteria, no biosurfactant)
- T_2 – Bacterial Consortium Only: MSM + engine oil + hydrocarbon-degrading bacterial consortium
- T_3 – Biosurfactant Only: MSM + engine oil + biosurfactant (no bacteria)
- T_4 – Low Biosurfactant Dose: MSM + engine oil + bacterial consortium (75%) + biosurfactant (50%)
- T_5 – Standard Biosurfactant Dose: MSM + engine oil + bacterial consortium (75%) + biosurfactant (75%)
- T_6 – High Biosurfactant Dose: MSM + engine oil + bacterial

consortium (75%) + biosurfactant (100%)

Each treatment was prepared in duplicate to ensure reproducibility. This design allowed for comparative assessment of microbial degradation under varying biosurfactant concentrations. Treatments were incubated at 30°C for 9 days. Samples were aseptically withdrawn at 0 (baseline), 3, 6, and 9 days to monitor biodegradation progression. Subsamples were collected for physicochemical analysis (soil pH, turbidity, COD, BOD, and TPH concentration) and microbial enumeration (total heterotrophic and hydrocarbon-degrading bacterial counts) (Aruotu *et al.*, 2023; Okoye *et al.*, 2020).

3.8 Laboratory Analysis

3.8.1 Determination of pH

pH was measured using a digital pH meter (Hanna HI2211). A 10 mL aliquot of each sample was measured, and calibration was performed using standard buffers (pH 4, 7, 10). Triplicate readings were recorded, and variations were used to infer microbial activity and hydrocarbon degradation (APHA, 2017; Ezeonu *et al.*, 2021).

2.8.2 Determination of Turbidity

Turbidity, an indicator of suspended particulates and emulsification, was measured using a Nephelometric Turbidity Meter (HACH 2100N). Results were expressed in NTU. Increased turbidity indicated biosurfactant-mediated hydrocarbon solubilization (APHA, 2017; Cappuccino and Sherman, 2014).

3.8.3 Determination of Chemical Oxygen Demand (COD)

COD was determined using the closed reflux dichromate method. Samples were digested with $K_2Cr_2O_7$ and H_2SO_4/Ag_2SO_4 at 150°C for 2 hours and titrated with 0.025 N FAS using ferroin indicator:

$$COD\ (mg/L) = \frac{(A-B)\times N\times 8000}{Sample\ Volume\ (mL)} \quad (2)$$

Where A = FAS volume for blank, B = FAS volume for sample, and N = normality of FAS. Higher COD reflected higher chemically oxidizable organic matter (APHA, 2017; Ezeonu *et al.*, 2021).

3.8.4 Determination of Biological Oxygen Demand (BOD_5)

The 5-day BOD test measured microbial oxygen consumption. Samples were incubated at 20 ± 1°C in the dark for 5 days. Initial (DO_1) and final (DO_5) dissolved oxygen were measured:

$$BOD\ (mg/L) = DO_1 - DO_5 \quad (3)$$

Higher BOD indicated greater microbial activity and hydrocarbon biodegradation potential (APHA, 2017; Cheesbrough, 2006).

3.8.5 Determination of Total Petroleum Hydrocarbon (TPH)

TPH was determined gravimetrically. Samples (10 mL) were extracted with 20 mL n-hexane, filtered through anhydrous sodium sulfate, and evaporated at 60°C. TPH was calculated as:

$$TPH\ (mg/L) = \frac{(W2-W1)\times 1000}{Sample\ Volume\ (mL)} \quad (4)$$

Where W_1 = weight of empty beaker, W_2 = weight of beaker + extract residue. Decreasing TPH over time indicated hydrocarbon degradation (Ezeonu *et al.*, 2021; IjahandAntai, 2003).

3.9 Microbial Enumeration

3.9.1 Total Heterotrophic Bacterial Count (THBC)

Samples were serially diluted to 10^{-6}, plated on Nutrient Agar, incubated at 37°C for 24–

48 h, and colonies counted as CFU/mL (Cheesbrough, 2006).

3.9.2 Hydrocarbon-Degrading Bacterial Count (HDBC)

Samples were plated on Mineral Salt Agar with 1% engine oil, incubated at 30°C for 5–7 days. Colony counts indicated the density of hydrocarbon-utilizing bacteria (Atlas, 1995; Holt *et al.*, 1994).

3.9.3 Calculation of CFU/mL

Only plates with 30–300 colonies were used for accuracy, and duplicate results were averaged (Cappuccino and Sherman, 2014).

$$CFU/mL = \frac{Number\ of\ Colonies \times Dilution\ Factor}{Volume\ Plated\ (mL)} \quad (5)$$

3.10 Data Analysis

Results were expressed as mean ± SD. ANOVA was used to compare means, and post hoc Tukey's HSD test identified pairwise differences ($p < 0.05$). Analyses were performed using SPSS v25 and Microsoft Excel 2019 for preliminary data organization (Laerd Statistics, 2023; Zar, 2010).

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Morphological and Biochemical Characterization of Bacterial Isolates

Table 1 shows the biochemical and biosurfactant characteristics of the bacterial isolates. Three isolates were identified as Pseudomonas spp. (B1), Bacillus spp. (B2), and Staphylococcus spp. (B3) based on their morphology, Gram reaction, biochemical profiles, and sugar fermentation patterns. Pseudomonas spp. (B1) was a Gram-negative rod, catalase- and oxidase-positive, motile, and exhibited oxidative metabolism with no sugar fermentation. It showed strong biosurfactant activity with an oil displacement diameter of 4.2 cm and drop-collapse diameter of 7 mm. Bacillus spp. (B2) was a Gram-positive rod, catalase- and oxidase-positive, variably motile, and urease-positive. It fermented sugars with acid and gas production and showed moderate biosurfactant activity with 3 cm oil displacement and 5 mm drop collapse. Staphylococcus spp. (B3) was a Gram-positive coccus, catalase- and oxidase-positive, non-motile, and produced acid without gas during fermentation. It showed weak biosurfactant activity with 1.7 cm oil displacement and 3 mm drop collapse. Overall, biosurfactant activity followed the order Pseudomonas spp. > Bacillus spp. > Staphylococcus spp (Table 2).

Table 1: Biochemical Characterization of isolates with bio-surfactant activity

	Shape	**Gram Reaction**	Biochemical Test				**SugarFermentation**	**Likely Genus**
			Catalase	**Oxidase**	**Motility**	**Urease**		
B1	Rod	-	+	+	M	-	Oxidative (no fermentation)	*Pseudomonas* spp.
B2	Rod	+	+	V	M	+	Fermentative (acid ± gas)	*Bacillus* spp.
B3	Cocci	+	+	-	NM	V	Fermentative (acid, no gas)	*Staphylococcus* spp.

NB: V = variable; M = Motility; NM = Non-Motile

Table 2: Bio-surfactant Screening Assay of isolates with bio-surfactant activity

			Mean Oil Displacement (cm)	**Mean Drop Collapse (mm)**	**Bio-surfactant Activity**
B1	Rod	*Pseudomonas* spp.	4.2	7	Strong
B2	Rod	*Bacillus* spp.	3	5	Moderate
B3	Cocci	*Staphylococcus* spp.	1.7	3	Weak

4.1.2. Crude Biosurfactant Yield

The yield of crude biosurfactant from pineapple peel was calculated based on the weight of oven-dried extract obtained relative to the mass of dried fruit powder used. From 50 g of dried pineapple peel powder, 21.12 g of crude biosurfactant was obtained, corresponding to a yield of 0.42 g/g."

$$Yield\ (g/g) = \frac{Weight\ of\ dried\ extract\ (g)}{Weight\ of\ fruit\ peel\ powder\ used\ (g)} = \frac{21.12}{50} = 0.42 g/g \quad (6)$$

4.1.3. Heterotrophic Bacterial Count (Pineapple Treatment)

Heterotrophic bacterial counts increased steadily across all pineapple treatments from Day 0 to Day 9 (Figure 1). The control (T_1) showed the lowest counts (2.30 ± 0.08 to 2.95 ± 0.06 log_{10} CFU/mL), indicating limited indigenous activity. Moderate increases were observed in T_2 (consortium only) and T_3 (biosurfactant only), rising from 5.58 ± 0.06 to 6.46 ± 0.07 log_{10} CFU/mL and 5.28 ± 0.06 to 6.27 ± 0.08 log_{10} CFU/mL, respectively. The combined treatments (T_4–T_6) recorded the highest bacterial proliferation, with final counts of 6.46 ± 0.07 to 6.47 ± 0.07 log_{10} CFU/mL, demonstrating a strong synergistic effect between the pineapple biosurfactant and the microbial consortium.

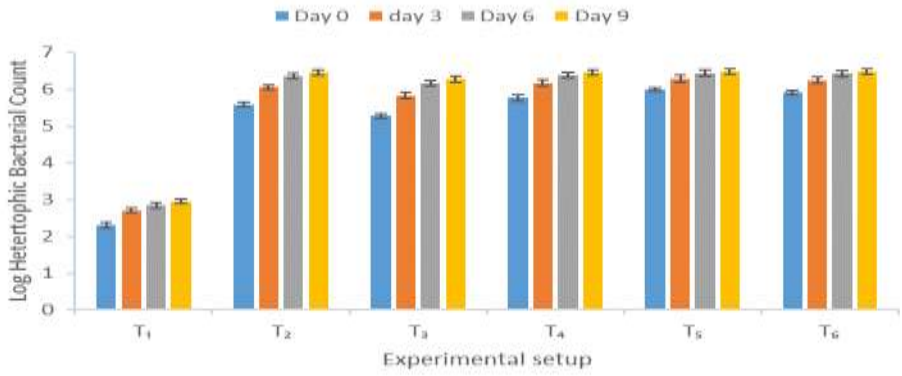


Figure 1: Heterotrophic bacterial counts in treatments supplemented with pineapple peel biosurfactant over a 9-day incubation period.

4.1.4. Hydrocarbon-Degrading Bacterial Count (Pineapple Treatment)

Hydrocarbon-degrading bacterial (HDBC) counts on pineapple treatments varied over time. The control (T_1) remained low, starting at 2.05 ± 0.09 log_{10} CFU/mL on Day 0, slightly increasing to 2.38 ± 0.06 on Day 3, and decreasing to 2.25 ± 0.07 by Day

9, indicating minimal degradation activity. Individual treatments with the bacterial consortium (T_2) and biosurfactant alone (T_3) showed higher initial counts (5.55 ± 0.06 and 5.14 ± 0.10, respectively), peaking around Day 3 before a slight decline by Day 9.Combined treatments (T_4–T_6) exhibited the highest HDBC proliferation, with T_5 (75% consortium + 75% biosurfactant) achieving the maximum activity: 5.92 ± 0.06 on Day 0, peaking at 6.24 ± 0.09 on Day 3, and maintaining 5.88 ± 0.08 log_{10} CFU/mL by Day 9, demonstrating the most effective hydrocarbon degradation.

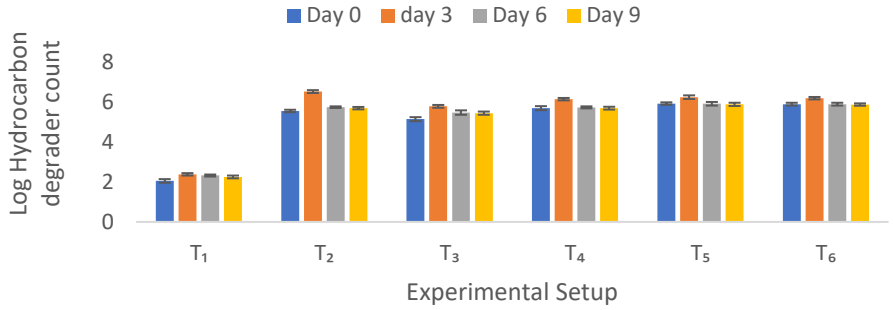


Figure 2: Hydrocarbon degrader count in treatments supplemented with Orange peel biosurfactant over a 9-day incubation period.

4.1.5. pH Variation (Pineapple Treatment)

Over the 9-day incubation, pH values of pineapple treatments decreased gradually, reflecting microbial metabolism and hydrocarbon degradation (Figure 3). The control (T_1) showed minimal change, declining slightly from 7.21 ± 0.015 (Day 0) to 6.85 ± 0.015 (Day 9). Individual treatments T_2 (bacterial consortium) and T_3 (biosurfactant alone) decreased from 7.19 ± 0.015 to 6.60 ± 0.015 and 7.18 ± 0.014 to 6.98 ± 0.015, respectively. Combined treatments (T_4–T_6) exhibited more pronounced reductions, with T_5 (75% consortium + 75% biosurfactant) declining from 7.18 ± 0.014 to 6.35 ± 0.015, and T_6 from 7.22 ± 0.015 to 6.82 ± 0.014, indicating enhanced microbial activity and increased acidification associated with hydrocarbon degradation.

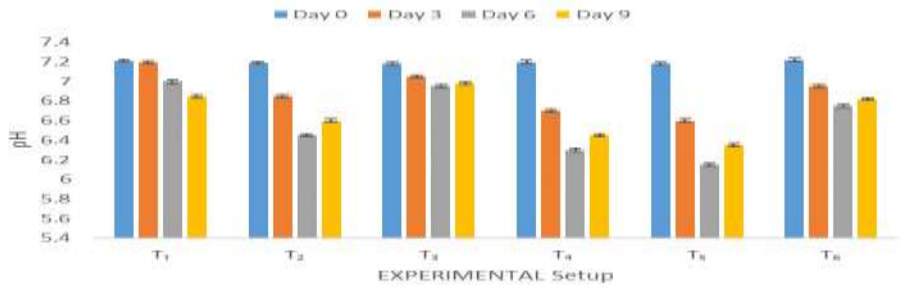


Figure 3: Changes in pH of Pineapple Peel During Hydrocarbon Biodegradation

4.1.6. Chemical Oxygen Demand (COD) – Pineapple Treatment

Over the 9-day incubation, COD values for pineapple treatments decreased progressively, indicating active hydrocarbon degradation (Figure 4). The control (T_1) showed minimal reduction from 46.2 ± 0.5 mg/L (Day 0) to 41.0 ± 1.0 mg/L (Day 9). The bacterial consortium alone (T_2) reduced COD more effectively,

from 47.0 ± 0.6 mg/L to 19.5 ± 1.2 mg/L. Combined treatments (T_4–T_6) achieved the greatest reductions, with T_5 (75% consortium + 75% biosurfactant) lowering COD from 47.2 ± 0.6 mg/L to 6.5 ± 0.6 mg/L, indicating the most efficient hydrocarbon degradation, while T_6 reduced COD to 9.0 ± 0.7 mg/L. These results demonstrate the synergistic effect of biosurfactant and bacterial consortium in enhancing hydrocarbon breakdown.

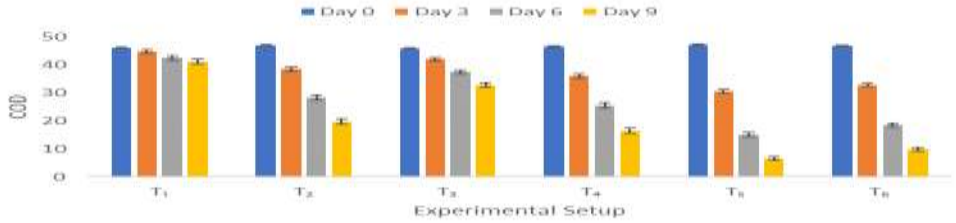


Figure 4: Reduction of COD in pineapple peel during hydrocarbon biodegradation under different treatments

4.1.7. Biological Oxygen Demand (BOD) – Pineapple Treatment

BOD measurements in the pineapple-treated medium (Fig. 5) increased initially from Day 0 to Day 6 across all treatments, reflecting active microbial hydrocarbon oxidation. The control (T_1) showed a moderate rise from 25.3 ± 0.8 mg/L to 29.0 ± 0.7 mg/L by Day 6, followed by a slight decrease to 22.4 ± 0.5 mg/L on Day 9, indicating limited biodegradation. Treated samples exhibited higher initial BOD rises, followed by notable declines by Day 9, demonstrating progressive substrate utilization. T_5 (75% consortium + 75% biosurfactant) recorded the sharpest decrease from 27.0 ± 0.3 mg/L to 12.0 ± 0.2 mg/L, indicating efficient hydrocarbon degradation. Similarly, T_4 and T_6 reduced BOD to 15.4 ± 0.3 mg/L and 14.2 ± 0.3 mg/L, respectively. The overall downward trend by Day 9 confirms effective microbial oxidation and stabilization of the medium, highlighting the enhancing effect of pineapple peel biosurfactant on biodegradation.

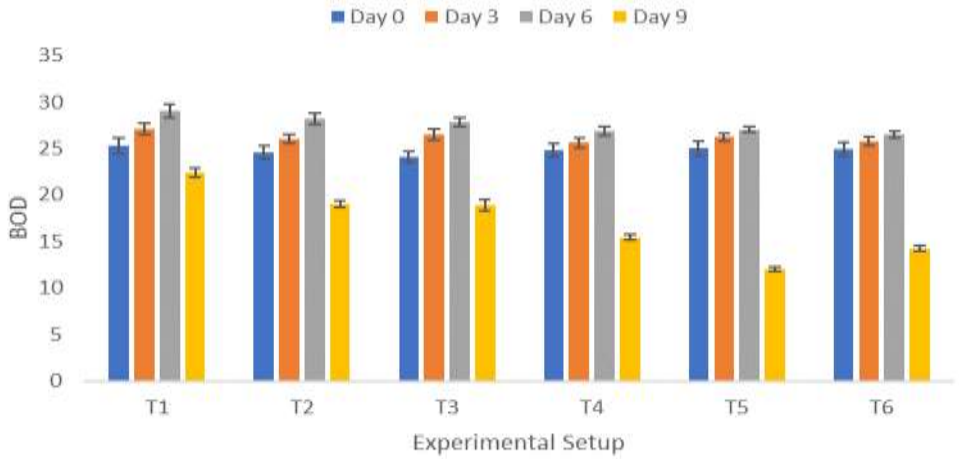


Figure 5: Effect of Different Treatments on BOD Reduction of Pineapple Peel During Biodegradation

4.1.8. Total Petroleum Hydrocarbon (TPH) – Pineapple Treatment

TPH concentrations in the pineapple-treated medium (Fig. 6) declined steadily across all treatments over the 9-day period, indicating progressive hydrocarbon biodegradation. The control (T_1) showed only a slight decrease from 482.1 ± 2.53 mg/L to 423.0 ± 3.07 mg/L, reflecting minimal natural degradation. Combined treatments demonstrated the highest reduction, with T_5 (75% consortium + 75% biosurfactant) achieving the most substantial decrease from 479.1 ± 3.04 mg/L to 25.5 ± 1.91 mg/L. T_4 and T_6 also showed marked reductions to 51.8 ± 2.33 mg/L and 35.6 ± 2.05 mg/L, respectively. The pronounced TPH decline in these treatments highlights the synergistic effect of the bacterial consortium and pineapple peel biosurfactant in enhancing hydrocarbon degradation efficiency.

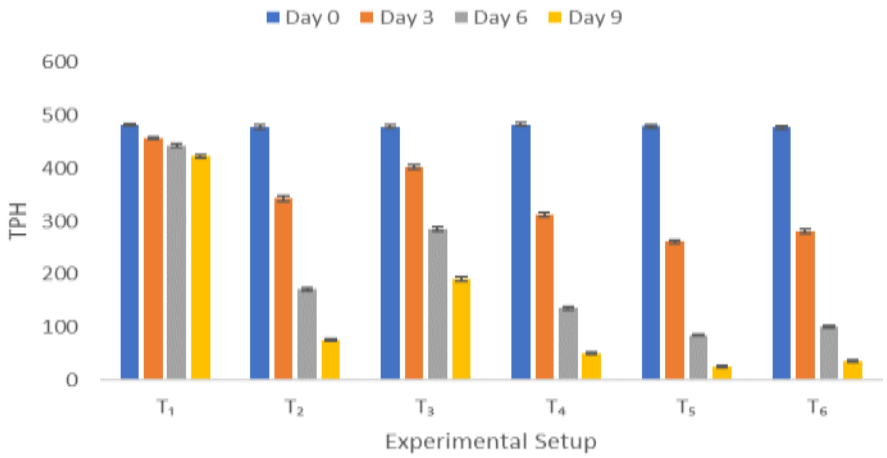


Figure 6: Changes in Total Petroleum Hydrocarbon (TPH) Concentration during Biodegradation Using Pineapple Peel Extract

4.1.8. Turbidity – Pineapple Treatment

In the pineapple-treated medium (Fig. 7), turbidity remained nearly constant in the control (T_1), from 45.0 ± 0.3 NTU on Day 0 to 45.2 ± 0.2 NTU on Day 9, indicating minimal microbial activity. Treatments T_2–T_6 exhibited a distinct pattern of initial turbidity increase by Day 3, peaking at 63.1 ± 0.5 NTU in T_5, reflecting active microbial growth and biomass dispersion. By Day 9, turbidity declined sharply to 27.4 ± 0.3 NTU in T_5, suggesting effective hydrocarbon utilization and medium clarification. The highest peak and lowest final turbidity in T_5 highlight strong microbial proliferation and the synergistic effect of the bacterial consortium and pineapple peel biosurfactant, while the control remained essentially unchanged.

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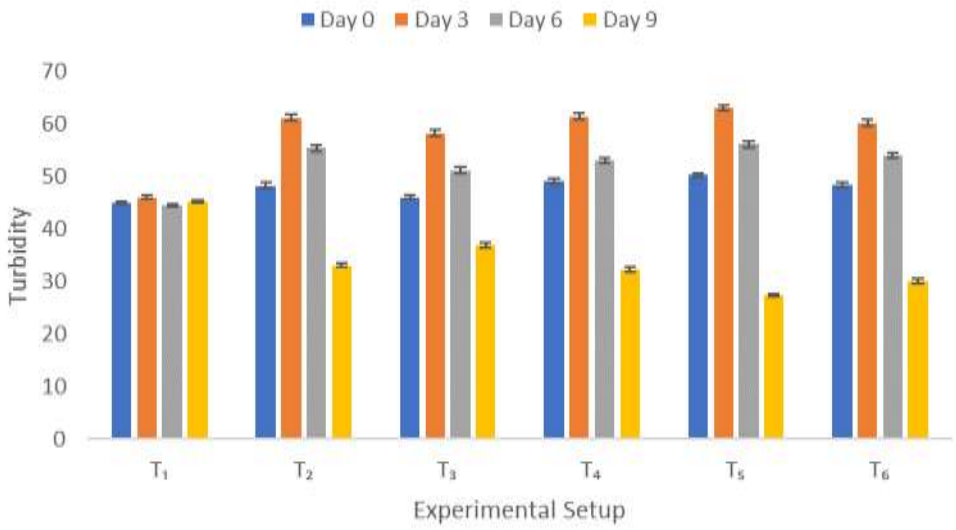


Figure 7: Turbidity Trends in Pineapple Peel Treatments During Biodegradation

4.2 Discussion

Three hydrocarbon-degrading bacterial isolates—Pseudomonas spp. (B1), Bacillus spp. (B2), and Staphylococcus spp. (B3)—were identified based on morphological, biochemical, and sugar fermentation characteristics. Pseudomonas spp. was Gram-negative, motile, and oxidative, showing the highest biosurfactant activity (oil displacement: 4.2 cm; drop-collapse: 7 mm). Bacillus spp. exhibited moderate activity (3 cm; 5 mm), while Staphylococcus spp. showed weak activity (1.7 cm; 3 mm). The results suggest that hydrocarbon biodegradation efficiency correlates with biosurfactant production, with Pseudomonas spp. providing superior emulsification and substrate accessibility (Cappuccino and Sherman, 2014).

The crude biosurfactant yield from pineapple peel was 0.42 g/g (21.12 g from 50 g dried powder), indicating high substrate suitability. This yield aligns with reports for other fruit wastes, such as orange and banana peels, which range from 0.2–0.5 g/g (Satpute *et al.*, 2010; Rahman and Gakpe, 2008; Makkar *et al.*, 2011; Ismail *et al.*, 2019). The high yield supports the potential of pineapple peel as a low-cost, sustainable substrate for biosurfactant production, providing sufficient quantities to enhance microbial hydrocarbon degradation in contaminated media.

Heterotrophic bacterial counts increased progressively over the 9-day period, with the control (T_1) showing minimal growth, confirming limited indigenous microbial activity. Treatments with either the bacterial consortium (T_2) or pineapple peel biosurfactant (T_3) showed moderate increases, while combined treatments (T_4–T_6) exhibited the highest counts, highlighting a synergistic effect between microbial inoculum and biosurfactant. This pattern aligns with previous reports that biosurfactants enhance microbial access to hydrophobic substrates, promoting proliferation and biodegradation (Banat *et al.*, 2014; Mulligan, 2005).

Hydrocarbon-degrading bacteria followed similar trends, with T_1 remaining low throughout. Treatments T_2 and T_3 showed moderate growth, whereas combined treatments (T_4–T_6), particularly T_5 (75% consortium + 75% biosurfactant), demonstrated the highest counts, indicating optimal conditions for hydrocarbon utilization. This underscores the importance of combining biosurfactants with microbial consortia to improve substrate emulsification and biodegradation efficiency (Satpute *et al.*, 2010).

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Medium pH declined across all treatments, reflecting microbial metabolism and hydrocarbon breakdown. Controls decreased minimally (7.21 → 6.85), while T_2 and T_3 showed moderate reductions. Combined treatments, especially T_5, exhibited the largest decrease (7.18 → 6.35), indicating enhanced microbial activity and acidification due to metabolic by-products. These results confirm that biosurfactant-assisted microbial treatments accelerate biodegradation (Rahman *et al.*, 2002; Satpute *et al.*, 2010).

COD values decreased progressively, reflecting hydrocarbon degradation. The control showed minimal reduction (46.2 → 41.0 mg/L), while the bacterial consortium alone (T_2) reduced COD more effectively (47.0 → 19.5 mg/L). The greatest reduction occurred in T_5 (47.2 → 6.5 mg/L), demonstrating a synergistic effect of the consortium and biosurfactant, consistent with previous studies on emulsification-enhanced microbial uptake (Rahman *et al.*, 2003; Banat *et al.*, 2010).

BOD initially increased up to Day 6, corresponding to active microbial respiration, then declined by Day 9 as hydrocarbons were depleted. T_5 exhibited the most pronounced reduction (27.0 → 12.0 mg/L), reflecting efficient microbial oxidation and stabilization of the medium. These trends demonstrate that pineapple peel biosurfactant enhances hydrocarbon bioavailability, promoting microbial degradation (Varjani, 2017; Makkar *et al.*, 2011).

TPH concentrations declined steadily, with the control showing minimal reduction (482.1 → 423.0 mg/L). Combined treatments, particularly T_5, exhibited the highest TPH removal (479.1 → 25.5 mg/L), indicating effective hydrocarbon utilization. The results highlight the synergistic action of bacterial consortia and biosurfactants in improving substrate accessibility and accelerating biodegradation, corroborating previous studies on biosurfactant-assisted remediation (Banat *et al.*, 2014; Obayori *et al.*, 2009).

Turbidity in T_1 remained nearly constant (45.0 → 45.2 NTU), whereas treatments T_2–T_6 showed an initial peak by Day 3 followed by a sharp decline by Day 9. T_5 exhibited the highest peak and lowest final turbidity (63.1 → 27.4 NTU), indicating active microbial growth, biomass dispersion, and subsequent hydrocarbon consumption. This pattern confirms that the combination of pineapple peel biosurfactant and bacterial consortium enhances both microbial proliferation and medium clarification (Banat *et al.*, 2010; Obayori *et al.*, 2009).

5. CONCLUSION

Pineapple peel biosurfactant significantly enhances the biodegradation of engine oil-contaminated media when applied with a bacterial consortium. The synergistic effect accelerates hydrocarbon breakdown, reduces organic load, and stabilizes the medium, demonstrating its potential as a low-cost, sustainable bioremediation agent.

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