ISOLATION, MOLECULAR CHARACTERIZATION OF BIOSURFACTANT-PRODUCING BACTERIA FROM PETROLEUM-CONTAMINATED SITES AND THEIR APPLICATION IN MESOCARBON-AMALGAMATED OIL ABSORPTION SYSTEMS.

ABSTRACT

Petroleum hydrocarbon contamination poses a persistent environmental threat, necessitating sustainable and efficient remediation strategies. The present study investigated the isolation and molecular characterization of biosurfactant-producing bacteria from petroleum-contaminated soil and water collected from Ennore, Chennai, and Ranimookanur, Thiruvallur, and explored their application in mesocarbon-based oil-absorbing materials. Bacterial isolates were obtained by serial dilution and purification on nutrient agar and subsequently screened for biosurfactant production using oil spreading, emulsification index (E24), and foam-forming assays. Growth was evaluated in mineral salt medium supplemented with different hydrocarbon sources, including coconut, castor, palm, diesel, and engine oils. Among the tested substrates, engine oil supported the highest growth and biosurfactant activity. The most efficient isolate was molecularly identified as *Stenotrophomonas maltophilia* and deposited in GenBank under accession number PX624000. The crude biosurfactant was incorporated with KOH-activated mesocarbon to fabricate flexible sheets using agar-PVA and spherical beads using sodium alginate-calcium chloride ionic gelation. Both materials exhibited porous structures and effective oil uptake, with the sheet showing comparatively higher absorption performance than the beads. FTIR analysis revealed characteristic hydrocarbon-associated functional groups, including C–H, C=C, C–O, and C–O–C groups, supporting hydrocarbon adsorption. Photomicroscopic observations further demonstrated surface roughness, interconnected pores, and oil retention within the material matrix. Overall, the findings demonstrate the potential of biosurfactant-functionalized mesocarbon sheets and beads as promising, biodegradable, and environmentally compatible materials for oil-spill remediation and wastewater treatment. The developed approach integrates microbial biosurfactant production with porous carbon engineering, offering a potentially low-cost strategy for sustainable hydrocarbon removal and recovery from contaminated aquatic environments.

Keywords: Biosurfactant, *Stenotrophomonas maltophilia*, petroleum hydrocarbon, mesocarbon, oil absorption, oil-spill remediation, FTIR, bioremediation.

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INTRODUCTION

Petroleum hydrocarbon contamination is a major environmental problem associated with rapid industrialization, petroleum processing, transportation, accidental oil spills, and improper disposal of petroleum-derived wastes. Hydrocarbons released into terrestrial and aquatic ecosystems can persist for prolonged periods because of their complex chemical composition and limited natural bioavailability. Their accumulation adversely affects soil properties, microbial communities, aquatic organisms, and plant growth, while several hydrocarbon fractions, particularly polycyclic aromatic hydrocarbons, may exert toxic effects and cause long-term ecological disturbances (Das & Chandran, 2011; Kumar et al., 2021). Consequently, the development of efficient, economical, and environmentally sustainable approaches for the removal of petroleum hydrocarbons has become increasingly important.

Conventional approaches for oil-contaminated environments include mechanical recovery, adsorption, chemical dispersants, incineration, landfilling, and chemical oxidation. Although these techniques can reduce contamination, their effectiveness may be limited by high operational costs, incomplete pollutant removal, and the possible formation of secondary pollutants. Bioremediation has therefore emerged as an environmentally compatible alternative that exploits the metabolic capabilities of microorganisms to transform or degrade hazardous contaminants into less harmful products (Vidali, 2001). Hydrocarbon-degrading bacteria are particularly important in this process because they possess diverse enzymatic systems capable of utilizing petroleum hydrocarbons as carbon and energy sources. Members of *Pseudomonas*, *Bacillus*, *Rhodococcus*, and *Acinetobacter* have been widely recognized for their ability to degrade different hydrocarbon fractions (Varjani, 2017).

A major limitation of microbial hydrocarbon degradation is the poor water solubility and low bioavailability of petroleum compounds. Microorganisms can overcome this limitation through the production of biosurfactants, amphiphilic biomolecules containing hydrophilic and hydrophobic domains. Biosurfactants accumulate at oil–water interfaces, reduce surface and interfacial tension, promote emulsification, and increase the accessibility of hydrophobic substrates to microbial cells (Banat et al., 2010; Mehta et al., 2025). Their biodegradability, relatively low toxicity, and functional stability under diverse environmental conditions make them attractive alternatives to conventional synthetic surfactants (Satpute et al., 2010).

Biosurfactant-producing microorganisms are therefore of considerable interest for improving the efficiency of hydrocarbon bioremediation.

Bacteria are among the most efficient microbial producers of biosurfactants. Several genera, including *Pseudomonas*, *Bacillus*, *Rhodococcus*, and *Enterobacter*, produce surface-active compounds when cultivated on suitable carbon substrates, including hydrocarbons, petroleum derivatives, vegetable oils, and other hydrophobic compounds (Mehta et al., 2025). The major classes of bacterial biosurfactants include glycolipids, lipopeptides, phospholipids, polymeric biosurfactants, and particulate biosurfactants. Rhamnolipids produced by *Pseudomonas* and lipopeptides such as surfactin produced by *Bacillus* are among the extensively investigated microbial surfactants because of their strong surface activity, emulsification capacity, and antimicrobial properties (Santos et al., 2016). The production of these compounds can enhance hydrocarbon accessibility and consequently support microbial degradation of petroleum pollutants.

Hydrocarbon-contaminated soils, wastewater, and marine environments represent important sources for the isolation of biosurfactant-producing bacteria. Microorganisms inhabiting such environments are frequently exposed to hydrophobic pollutants and may develop metabolic adaptations that facilitate hydrocarbon utilization. Preliminary screening of bacterial isolates for biosurfactant production can be performed using assays such as the drop collapse test, oil spreading assay, haemolysis test, and emulsification index (E24). These methods provide a practical means of identifying potential biosurfactant-producing strains and evaluating their surface-active properties (Rizvi et al., 2024). Biosurfactant-producing bacteria have also been recovered from oil-contaminated marine environments using techniques including oil displacement, emulsification assays, and CTAB agar-based screening, highlighting their potential for petroleum bioremediation (Valli et al., 2025).

Following production, extraction and characterization are essential for determining the physicochemical properties and potential applications of microbial biosurfactants. Recovery from fermentation broth commonly involves centrifugation, acid precipitation, and solvent extraction, depending on the chemical nature of the biosurfactant (Karthikeyan et al., 2021). Characterization may include assessment of emulsification activity, surface tension reduction, and stability under different environmental conditions. Analytical approaches such as Fourier Transform Infrared Spectroscopy (FTIR), Thin Layer Chromatography (TLC), and Gas Chromatography–Mass Spectrometry (GC-MS) can provide information regarding functional

groups and chemical constituents, thereby supporting the identification and characterization of the extracted biosurfactant (Patel et al., 2022; Hossain et al., 2023).

Beyond their role in hydrocarbon degradation, microbial biosurfactants possess considerable potential in environmental, industrial, pharmaceutical, and biomedical applications. Their antimicrobial, antifungal, anti-adhesive, and biofilm-inhibitory properties have encouraged their investigation in pharmaceutical formulations, medical coatings, cosmetics, and other biotechnological applications (Nitschke and Costa, 2007; Varjani and Upasani, 2016). However, large-scale biosurfactant production remains constrained by factors such as production cost, substrate availability, and comparatively low yields. The utilization of inexpensive substrates, agricultural residues, and industrial by-products, together with advances in fermentation technology and microbial biotechnology, may help overcome these limitations and improve commercial feasibility (Mukherjee et al., 2006; Mohanty et al., 2021).

Biosurfactants can also contribute to the development of improved oil-absorption and oil-remediation systems. Oil-absorbing sheets, pads, beads, and related materials provide extensive surfaces for capturing petroleum contaminants from water and soil. Incorporating biosurfactants into such materials can enhance oil–material interactions by modifying interfacial properties and promoting the spreading of oil droplets, thereby improving pollutant capture (Rahman et al., 2020; Chakraborty et al., 2022). Sheet-based materials can be applied over broad contaminated surfaces, whereas bead-based systems may offer advantages in filtration and packed-column applications. Combining microbial biosurfactants with absorbent materials therefore represents a promising strategy for developing efficient and environmentally compatible oil-spill remediation systems (Das et al., 2023).

In this context, the present study focuses on the isolation and screening of oil-degrading bacteria from hydrocarbon-contaminated soil, evaluation of their biosurfactant-producing potential, extraction and characterization of the produced biosurfactant, and enhancement of hydrocarbon degradation through microencapsulation techniques. Immobilization of bacterial cells using suitable polymeric carriers can improve cell stability, protect microorganisms against environmental stress, and facilitate their application and recovery during bioremediation (Malik et al., 2020). The integration of hydrocarbon-degrading bacteria, biosurfactant production, and microencapsulation may therefore provide a sustainable and cost-effective approach for improving petroleum hydrocarbon remediation.

MATERIALS AND METHODS:-

Chemicals

All chemicals used for the study were of AR grade, procured from Merck Ltd.,SRL chemicals, Hi Media Laboratories Pvt Ltd.

Collection of samples

Petroleum-contaminated soil and water samples were collected from Ennore, Chennai district and Ranimookanur, Thiruvallur district. The collected samples were transferred into sterile zip-lock covers, sealed, and transported to the laboratory for further processing and analysis.

Isolation and maintaining pure cultures from collected samples

The soil and water samples were diluted in distilled water and subjected to serial dilution. Nutrient agar plates were prepared, and the microorganisms were inoculated onto the plates. The isolates were subcultured repeatedly to obtain distinct pure cultures.

Standardization of media for Bacterial growth:-

Nutrient agar and Mineral Salt Medium (MSM) were prepared and sterilized by autoclaving at 121°C for 15 minutes. After cooling, the media were poured into sterile Petri plates under aseptic conditions. The bacterial isolates were inoculated onto the media and incubated at 37°C for 24–48 hours. The growth of microorganisms in nutrient agar and MSM was observed and compared to determine their suitability for microbial growth and biosurfactant production.(Watanabe, 2010)

Preparation of Mineral Salt Medium(MSM)

Mineral Salt Medium(MSM) was prepared in distilled water. The pH of the medium was adjusted to around 7.0. The medium was sterilized by autoclaving at 121°C for 15 minutes. After cooling, 3% of different hydrocarbon source (Coconut oil, castor oil, Palm oil, engine oil and diesel oil) was added as the sole carbon source. The medium was then inoculated with the bacterial culture and incubated under shaking conditions for biosurfactant production for 7 days(Rizvi et al., 2024; Varjani, 2017).

Composition

Na_2HPO_4	–	6%
KH_2PO_4	–	3%
NaCl	–	0.5 %
NH_4Cl	–	1%
$MgSO_4 \cdot 7H_2O$	–	0.2 %
$CaCl_2$	–	0.02%
Glucose	-	10%
Distilled water	-	1000ml

Extraction of Biosurfactant by bacteria

The bacterial culture was centrifuged at high speed (10,000 rpm for 10–15 minutes) to separate cells. The supernatant was collected and its pH was adjusted to 2.0 using HCl to precipitate the biosurfactant. The precipitate was kept at 4°C overnight and then collected by centrifugation. It was allowed to dried to obtain crude biosurfactant and store it in refrigerator for further purpose.(Pruthi et al.,2003)

Screening Assays for of Biosurfactant producing Bacteria

The Isolates were screened for biosurfactant production using different assays. In the oil spreading test, oil was added to water surface followed by culture supernatant, and clear zone formation was observed. In emulsification index (E24), equal volumes of culture supernatant and oil were mixed and left undisturbed for 24 hours, and emulsion height was measured. Foam Forming test done by shaking biosurfactant in medium with different hydrocarbon sources. Isolates showing higher activity in these tests were selected (Brinda et al., 2024).

Selection of best biosurfactant producing bacterium

Based on the screening results, the isolate showing maximum oil displacement, highest emulsification index and strong Foam forming activity was selected as the best biosurfactant-producing bacterium for further studies.

Molecular Identification of the Selected Bacterium

PCR Amplification

Molecular identification of the selected bacterial isolate was performed by PCR amplification of the target DNA using specific primers. The PCR reaction was prepared using 5 µL of isolated DNA, 1.5 µL each of forward and reverse primers, 5 µL deionized water, and 12 µL of 2X Taq Master Mix. The master mix contained Taq DNA polymerase, dNTPs, $MgCl_2$, and bromophenol blue.

Primer details:

Primer	Sequence (5′–3′)	Bases
ITS1	TCCGTAGGTGAACCTGCGG	19
ITS4	TCCTCCGCTTATTGATATGC	20

PCR amplification was carried out with an initial denaturation at 95°C for 2 min, followed by 25 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min, followed by a hold at 4°C.

PCR Product Purification and Sequencing

The amplified PCR products were purified using the Montage PCR Clean-up Kit (Millipore) to remove unincorporated primers and dNTPs. The purified products were subjected to sequencing using the ABI PRISM® BigDye™ Terminator Cycle Sequencing Kit with AmpliTaq® DNA polymerase (Applied Biosystems). Fluorescently labelled sequencing products were purified by ethanol precipitation and analyzed using an ABI 3730xl sequencer.

Sequence Analysis

The obtained sequence was compared with available sequences using the NCBI BLAST similarity search tool. Closely related sequences were selected for phylogenetic analysis and aligned using MUSCLE 3.7 (Edgar, 2004). Poorly aligned and divergent regions were removed using Gblocks 0.91b (Talavera and Castresana, 2007). Phylogenetic analysis was performed using PhyML 3.0 aLRT with the HKY85 substitution model. The resulting phylogenetic tree was rendered using TreeDyn 198.3 (Dereeper et al., 2008).

Preparation of Mesocarbon:

To Prepare Mesocarboncoconut shell powder was washed with distilled water and dried at

100–110°C to remove moisture. The dried material was then placed in a closed crucible and subjected to carbonization in a muffle furnace at 400–500 °C for about 1–2 hours under limited oxygen conditions to obtain primary carbon. The carbonized sample was then impregnated with potassium hydroxide (KOH) in a 1:1 ratio and allowed to soak for several hours. After drying, the sample was again heated in the muffle furnace at 500–700 °C for 1 hour to enhance pore formation. The activated product was cooled, washed with dilute hydrochloric acid and distilled water until neutral pH was achieved, and finally dried at 100 °C to obtain mesocarbon with improved porosity and adsorption properties (Marsh and Rodríguez-Reinoso, 2006; Lua and Yang, 2004).

Preparation of Mesocarbon Amalgamated Sheet incorporating Bacterial Biosurfactant:

The extracted biosurfactant was mixed with 2% (w/v) agar + PVA solution and stirred continuously to obtain a homogeneous mixture. To this, mesocarbon powder (1–2% w/v) was added and mixed uniformly. The mixture was poured onto sterile Petri plates and spread evenly to form a thin layer. It was allowed to dry at room temperature or in a hot air oven at 40–50°C until a firm sheet was formed. The dried sheets were carefully peeled off and stored in sterile conditions for further use.(Yang et al., 2021; Jiang et al., 2024).

Preparation of Mesocarbon Amalgamated Beads incorporating Bacterial Biosurfactant:

A 2% (w/v) sodium alginate solution was prepared and mixed with the extracted biosurfactant. Mesocarbon (1–2% w/v) was added and mixed thoroughly to obtain a uniform mixture. This mixture was loaded into a syringe and added dropwise into 0.1 M calcium chloride ($CaCl_2$) solution. Upon contact, spherical beads were formed due to ionic gelation. The beads were allowed to harden in the solution for 30–60 minutes, then collected, washed with distilled water, and stored at 4°C for further analysis.(Hassan et al., 2014)

Evaluation of Oil Absorption Efficiency of prepared mesocarbon sheet and beads with bacterial biosurfactant

Experiment was planned to use the surfactant incorporated sheets to remove oil in water body, (in lab scale). Turf A was prepared with 500 mL of water containing 5 mL of floating oil. The sheets were cut into 3 cm^2 pieces and introduced into Turf A. After 5 minutes, the sheets were removed and transferred to another container (Turf B) containing 20 mL of water. After 3 minutes of immersion, the sheets were again transferred back to Turf A. This procedure was repeated several times until the oil present on the surface of Turf A was

completely removed (visibly).

The water in Turf B was then evaporated to recover the absorbed oil. The recovered oil was measured and recorded, and this was used to determine the oil holding capacity of the prepared sheets.The oil efficiency of mesocarbon beads also done as per the protocol mentioned above (Adebajo et al., 2003)

Fourier Transform Infrared Spectroscopy (FTIR) of prepared mesocarbon sheet and beads with bacterial biosurfactant

Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out to identify functional groups present in oil-absorbed sheets and beads. The samples were dried and analyzed in the range of 4000–400 cm^{-1} to confirm interaction between biosurfactant and oil(Stuart, 2004).

Photomicroscopic Observation of Prepared Mesocarbon Sheet and Beads with Bacterial Biosurfactant

The oil-absorbed sheets and beads were observed under a photomicroscope. Surface morphology, porosity and oil retention characteristics were examined and recorded.

RESULT AND DISCUSSION:-

Collection of Samples

Petroleum-contaminated soil and water samples collected from from Ennore, Chennai district and Ranimookanur, Thiruvallur district. were found to be a suitable source for isolating hydrocarbon-degrading microorganisms in Fig 1. The presence of oil contamination in the sampling site favours the growth of microorganisms capable of utilizing hydrocarbons.

Isolation and maintaining pure cultures from collected samples

The serial dilution of soil and water samples followed by inoculation on nutrient agar plates resulted in the development of well-isolated microbial colonies in Fig 2 . Colonies with distinct morphological characteristics were observed, indicating the presence of diverse microbial populations. Repeated subculturing of these colonies led to the successful isolation and maintenance of pure cultures. This confirms that the applied isolation technique was effective in separating individual microorganisms from mixed samples, making them suitable for further analysis.

Standardization of media for Bacterial growth

The growth of bacterial isolates was assessed on Nutrient Agar and Mineral Salt Medium (MSM). Nutrient agar showed luxuriant growth with well-developed colonies, whereas comparatively limited growth was observed in MSM. This indicates that nutrient agar supports rapid bacterial growth due to its rich nutrient composition, while MSM, lacking an easily available carbon source, allows selective growth of hydrocarbon-utilizing bacteria. Hence, MSM was found to be suitable for screening potential biosurfactant-producing bacteria (Madigan et al., 2018; Bento et al., 2005).

Preparation of Mineral Salt Medium(MSM)

The bacterial isolates exhibited growth in Mineral Salt Medium (MSM) supplemented with different hydrocarbon sources such as coconut oil, castor oil, palm oil, engine oil, and diesel oil, indicating their ability to utilize these substrates as a carbon source. Variations in growth and biosurfactant production were observed among the different oils, suggesting substrate-specific preference of the isolates. The use of MSM facilitated selective growth of hydrocarbon-degrading bacteria, thereby confirming their potential for biosurfactant production. Incubation under shaking conditions further enhanced microbial activity and biosurfactant yield (Mulligan, 2005).

Growth and Extraction of Biosurfactant produced by bacteria

The bacterial isolates exhibited varying levels of growth and biosurfactant production in Mineral Salt Medium (MSM) supplemented with different hydrocarbon sources. Among the substrates tested, coconut oil showed no significant growth, while castor oil supported moderate growth. Palm oil exhibited good growth, whereas diesel showed very good growth

The highest level of growth and biosurfactant production was observed in engine oil, indicating it as the most suitable carbon source among those tested. Following incubation, the culture was processed for biosurfactant extraction, and the successful recovery of crude biosurfactant further confirms the ability of the bacterial l isolates to utilize hydrocarbons effectively. These observations suggest that the type of hydrocarbon source significantly influences bacterial growth and biosurfactant yield

Screening Assays for of Biosurfactant producing Bacteria

The Isolates were screened for biosurfactant production using different assays.

The bacterial isolates were screened for biosurfactant production using oil spreading,

emulsification index (E24), and foam formation tests. In the oil spreading assay, the formation of clear zones indicated the presence of surface-active compounds produced by the bacterial isolates. The emulsification index (E24) showed variations among the isolates, reflecting differences in their ability to stabilize oil–water emulsions.

In the foam formation test, stable foam formation upon shaking further confirmed biosurfactant production. Based on the results of these assays, isolates which showing higher activity were selected as efficient biosurfactant producers in Fig 3, 4, 5 and Tab 2, 3. These findings demonstrate that the applied screening methods were effective in identifying potential biosurfactant-producing bacteria (Biktasheva et al., 2024)

Selection and molecular identification of biosurfactant producing Bacterium

Based on the screening results, the isolate exhibiting maximum oil displacement, highest emulsification index (E24), and strong foam-forming activity was selected as the most efficient biosurfactant-producing bacterium for further studies. Molecular identification of the selected isolate confirmed it as *Stenotrophomonas maltophilia*. The high biosurfactant activity observed in this isolate may be attributed to its ability to effectively utilize hydrocarbons and produce surface-active compounds. These results indicate that *Stenotrophomonas maltophilia* is a promising candidate for biosurfactant production and hydrocarbon degradation applications

Phylogenetic analysis of bacterial isolate was performed using ITS region sequencing. The isolate was identified as *Stenotrophomonas maltophilia*. The sequence showed significant similarity with existing sequences in the NCBI database, confirming its identity. The isolate was deposited in NCBI corresponding GenBank Submission SUB15757844 subsequently it was allotted PX624000 as deposit number .The phylogenetic tree was constructed based on the obtained sequence data, demonstrating the relationship of the isolate with other closely related bacterial species.

Stenotrophomonas maltophilia

GGGAGAAAATCCCAGCCAAACCTCGGTGGCCACACATCAAGTCGAAAACAG
GACCGGAAAGCTCCCTTCCGGGTGGCGAGTGGCGGACGGGTGAGGAATACA
TCGGAATCTACTCTGTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACC
GCATACGACCTACGGGTGAAAGCAGGGGACCTTCGGGCCTTGCGCGATTGA
ATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGGCG
ACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACAC

GGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGC
AAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAA
GCCCTTTTGTTGGGAAAGAAATCCAGCCGGCTAATACCCGGTTGGGATGACG
GTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATAC
GAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTG
GTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTG
GATACTGGGCGACTAGAATGTGGTAGAGGGTAGCGGAATTCCTGGTGTAGCA
GTGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTG
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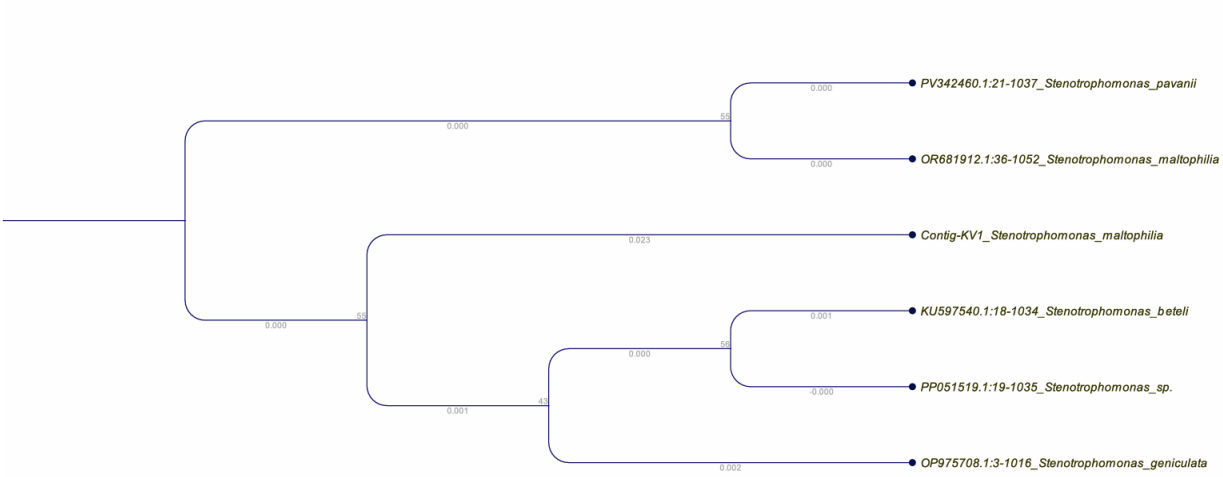


TGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGAGAGTACGG
TCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GTATGTGTTTTAATTCCATGCAACGCGAAAAACTTACCTGGCCTTGACATGTC
GAAAACTTTCCAAAAATGAATGGGTCCTTCGGGAACTCGAACAGAGTGGTGC
AAGCGGCCGACGCTAGACGAAAAGTGGATAAGACCGCAACAAGAGACCCCG
TTGTCTTTGTTGCCGTC

Preparation of Mesocarbon:-

The Mesocarbon prepared by muffle furnace-assisted carbonization and KOH activation was obtained as a fine black, porous material. The prepared mesocarbon exhibited improved porosity and surface characteristics, which were evident from its lightweight nature and increased adsorption capacity. The material showed good affinity towards oil/biosurfactant absorption, indicating the development of micro- and mesoporous structures. The washing process ensured the removal of residual KOH, resulting in a neutral pH of the final product.

Overall, the synthesized mesocarbon demonstrated enhanced adsorption efficiency and was found to be suitable for further applications such as biosurfactant immobilization and oil absorption studies.

Preparation of Mesocarbon Amalgamated Sheet incorporating Bacterial Biosurfactant :

The Mesocarbon sheets incorporated with Bacterial Biosurfactantwere successfully prepared using bacterial biosurfactant. The obtained sheets were uniform, flexible, and structurally stable, indicating proper blending of agar, PVA, and mesocarbon within the matrix Fig 7. The incorporation of mesocarbon enhanced the surface properties of the sheets, providing increased porosity and adsorption sites(Li et al., 2022).These modified sheets exhibited improved oil absorption efficiency compared to sheets without mesocarbon, suggesting the positive role of carbon incorporation in enhancing performance. The stability and integrity of the sheets further support their suitability for repeated use in oil removal applications. Thus, the combination of bacterial biosurfactant and mesocarbon significantly improves the functional properties of the prepared sheets

Preparation of Mesocarbon Amalgamated Beads incorporating Bacterial Biosurfactant:-

The Mesocarbonbeads incorporated with Bacterial Biosurfactant were successfully formed through the ionic gelation process. The beads obtained were spherical, uniform in size, and mechanically stable, indicating effective crosslinking of sodium alginate with calcium ions Fig 8. The incorporation of mesocarbon within the bead matrix enhanced surface roughness and porosity, providing additional active sites for oil adsorption.

These modified beads exhibited improved oil absorption and retention capacity compared to beads without mesocarbon. The structural stability of the beads also supports their reusability and effectiveness in oil removal applications(Chen et al., 2023)

Overall, the combination of bacterial biosurfactant and mesocarbon significantly enhances the adsorption efficiency and functional performance of the prepared beads.

Evaluation of Oil Absorption Efficiency of prepared mesocarbon sheet and beads with Bacterial biosurfactant

The surfactant-incorporated sheets prepared using bacterial biosurfactant were evaluated for their oil removal efficiency in a laboratory-scale water system. The sheets effectively

absorbed the oil present on the water surface, and repeated transfer between the oil-containing system and clean water facilitated continuous removal of oil. Over successive cycles, a visible reduction in the oil layer was observed, indicating the efficiency of the sheets in oil uptake.

Similarly, the biosurfactant-based beads prepared from bacterial isolates also exhibited effective oil absorption and retention capacity. The absorbed oil was released into the secondary container (Turf B), and upon evaporation of water, the recovered oil was measured. Both sheets and beads demonstrated good oil holding capacity and reusability(Gudina et al., 2015). These results Tab 4 indicates that bacterial biosurfactant incorporation significantly enhances the oil absorption efficiency of the materials, making them suitable for oil spill remediation applications Fig 9, 10.

Fourier Transform Infrared Spectroscopy (FTIR) of prepared mesocarbon sheet and beads with bacterial biosurfactant

The FTIR analysis of the mesocarbon-incorporated sheets showed characteristic peaks corresponding to hydrocarbon functional groups Fig 11, 12. . Prominent peaks observed around 2924 cm^{-1} and 2854 cm^{-1} indicate C–H stretching vibrations of alkanes, confirming the presence of hydrocarbon chains. The peak near 1450 cm^{-1} corresponds to C–H bending, further supporting aliphatic hydrocarbon structures. Additionally, the peak around 1029 cm^{-1} indicates C–O stretching, suggesting the presence of alcohol or ester groups associated with the biosurfactant matrix.

These functional groups confirm that the prepared sheets effectively adsorbed hydrocarbons from the oil phase. The presence of strong alkane peaks clearly indicates successful oil absorption by the sheets, demonstrating their efficiency in hydrocarbon removal applications (Coates, 2000).

The FTIR spectrum of the biosurfactant-based beads also revealed characteristic peaks corresponding to hydrocarbon functional groups. Peaks observed around 2924 cm^{-1} and 2854 cm^{-1} confirm C–H stretching vibrations of alkanes, indicating the presence of absorbed hydrocarbons. The peak near 1637 cm^{-1} suggests C=C stretching, which may be attributed to aromatic or unsaturated hydrocarbons. Further peaks around 1415 cm^{-1} and 1122 cm^{-1} correspond to C–H bending and C–O stretching vibrations, respectively Tab 1.5, 1.6.

These observations confirm that the beads effectively adsorbed both aliphatic and aromatic hydrocarbons. The FTIR results clearly demonstrate the ability of the beads to retain

hydrocarbon compounds, highlighting their potential in oil spill remediation (Stuart, 2004).

Photomicroscopic Observation of Prepared Mesocarbon Sheet with BacterialBiosurfactant

The oil-absorbed mesocarbon sheets and beads were examined using a photomicroscope to evaluate their surface morphology, porosity, and oil retention behaviour.

Photomicroscopic observations of the mesocarbon sheets revealed a rough and heterogeneous surface morphology with numerous micro-pores and irregular cavities Fig13 . These pores appeared to be well distributed across the sheet surface, forming a network capable of trapping oil within the matrix. After oil absorption, the surface showed darkened regions and filled pores, indicating the retention of hydrocarbons within the porous structure.

The presence of such interconnected porous architecture enhances capillary action and increases the contact area between the material and oil, thereby improving adsorption efficiency. Similar observations have been reported where porous carbon-based materials exhibit enhanced oil uptake due to increased surface roughness and pore volume (Zhang et al., 2021; Li and Zhao, 2020).

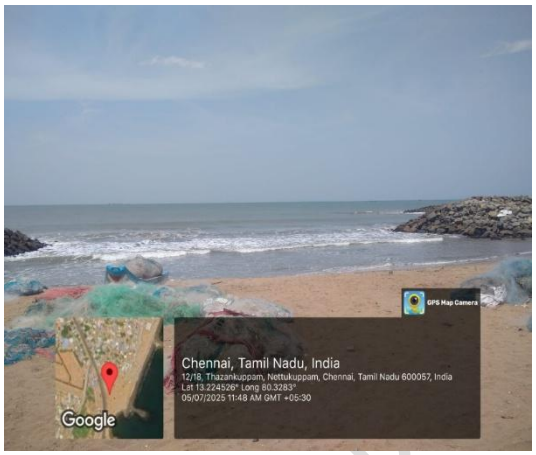
Additionally, the biosurfactant incorporation likely contributed to improved wettability and dispersion of hydrocarbons across the sheet surface, facilitating deeper penetration into pores. This behaviour aligns with previous findings that biosurfactants reduce surface tension and enhance hydrocarbon interaction with solid matrices (Banat et al., 2014).

Photomicroscopic Observation of Prepared Mesocarbon Beads with Bacterial Biosurfactant

The photomicroscopic analysis of mesocarbon beads showed a comparatively more uniform and spherical morphology with visible surface pores and micro-channels. The beads exhibited a compact structure with internal porosity, which plays a crucial role in oil entrapment Fig 14.

After oil exposure, the bead surfaces displayed adsorbed oil layers and partially filled pore structures, confirming the ability of the beads to retain hydrocarbons both on the surface and within internal cavities. The spherical nature of beads provides mechanical stability and reusability, while the internal pore network supports efficient oil absorption.

Studies on carbonaceous and polymeric beads have demonstrated that porous spherical structures enhance oil sorption capacity due to combined surface adsorption and internal



451 diffusion mechanisms (Wang et al., 2022; Kumar et al., 2021). The observed morphology in
452 this study is consistent with such reports.

453 Both mesocarbon sheets and beads exhibited well-developed porous structures and significant
454 oil retention characteristics under photomicroscopic observation. The sheets showed higher
455 surface roughness and pore accessibility, favouring rapid adsorption, while the beads
456 demonstrated controlled absorption with structural stability.

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467 **Fig 1 Collection site of Petroleum contaminated water and soil samples**

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Fig 2 Isolated colonies from the collected samples.

Fig 3 Foam Forming Activity of bacterial biosurfactant

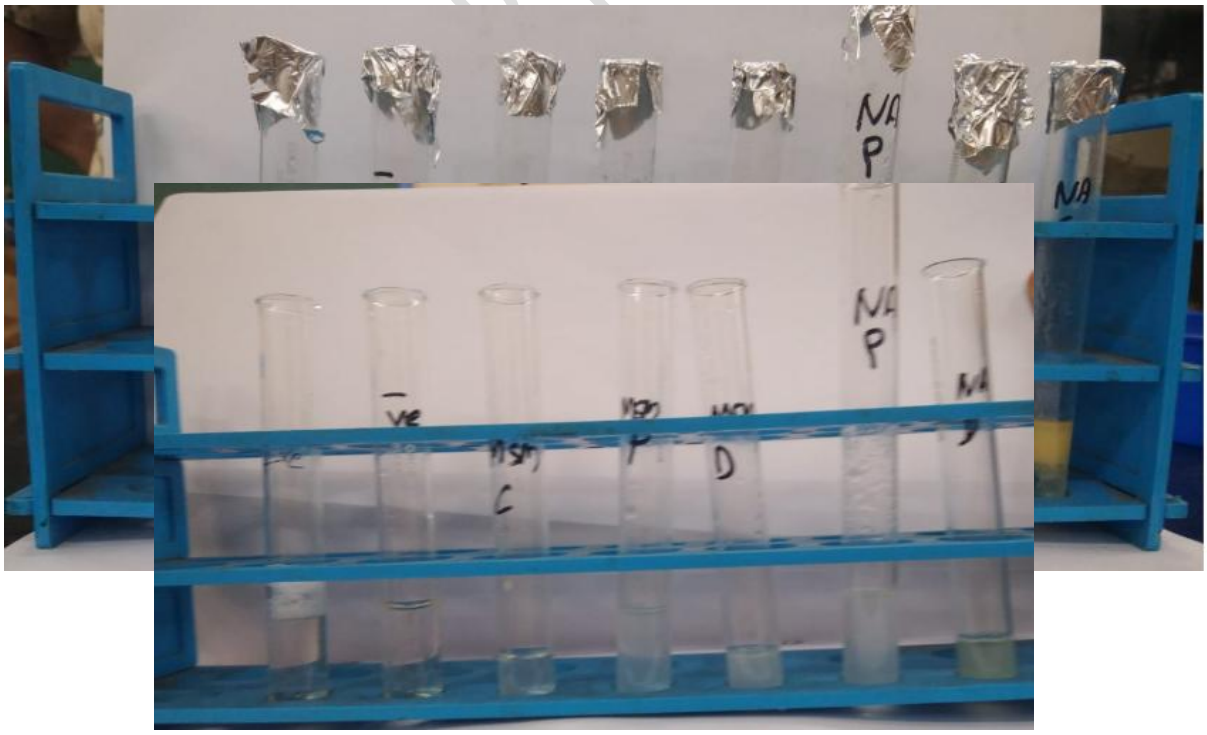

Fig 4Emulsification Activity of bacterial biosurfactant

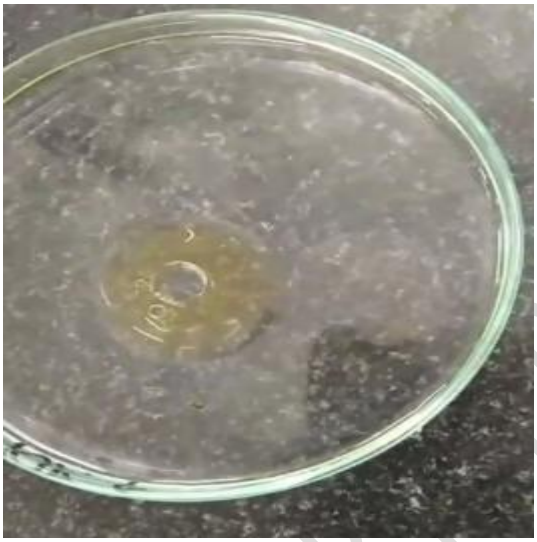

Fig 5 Oil Displacement Activity of bacterial biosurfactant

Fig 6 Single colony of *Stenotrophomonas maltophilia*

522 **Fig 7 Bacterial Biosurfactant Fig 8 Bacterial Biosurfactant**

523 **Mesocarbo**

nSheetMes

ocarbonBe

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Turf A

533 **Turf B**

534 **Fig 9 Evaluation of Oil Absorption Efficiency of prepared mesocarbon sheet with**

535 **Bacteria biosurfactant**

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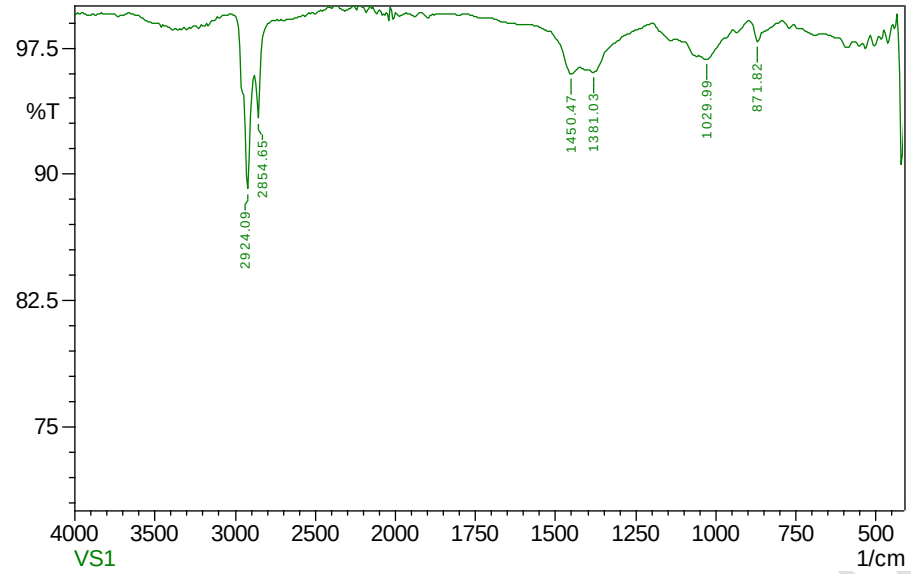

Turf A

Turf B

Fig 10 Evaluation of Oil Absorption Efficiency of prepared mesocarbon beads with Bacterial biosurfactant

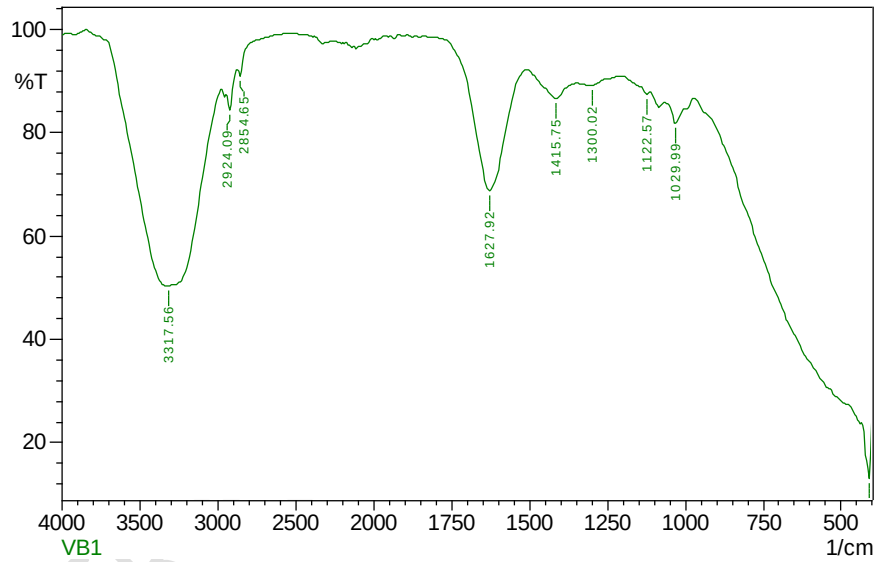

Fig 11Fourier Transform Infrared Spectroscopy (FTIR) of prepared mesocarbonSheet with Bacterial biosurfactant

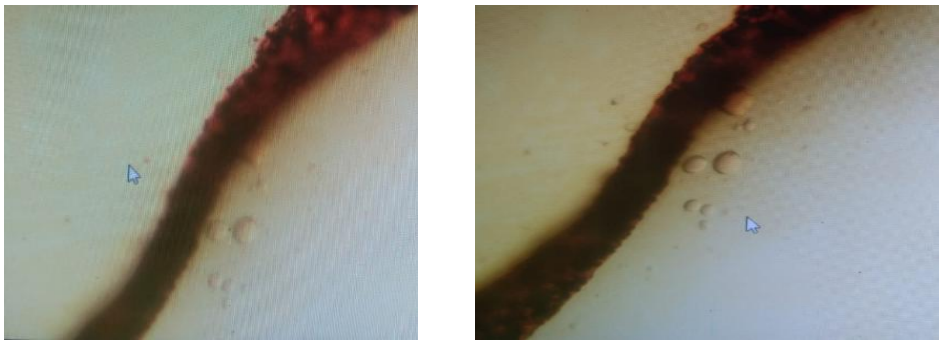

Fig 12Fourier Transform Infrared Spectroscopy (FTIR) of prepared mesocarbonBeads with Bacterial biosurfactant

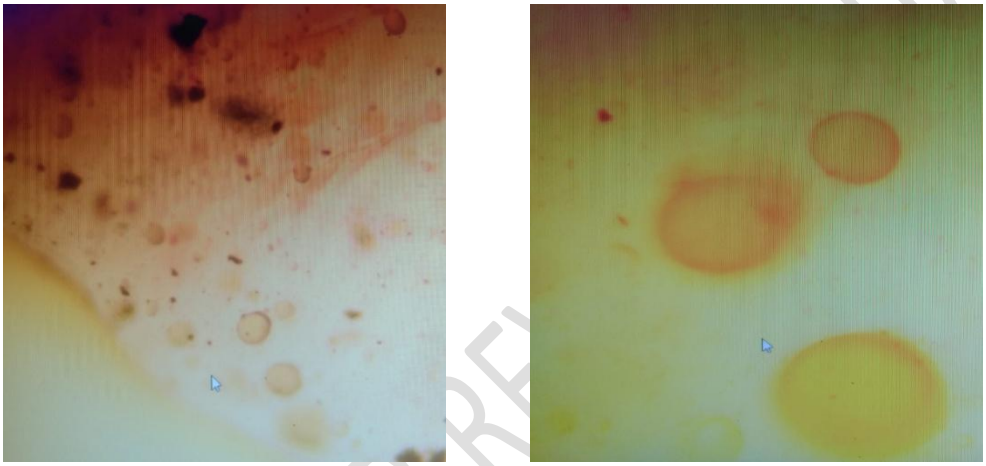

Fig 13 Photomicroscopic Observation of Prepared Mesocarbon Sheet with Bacterial Biosurfactant

Fig14 Photomicroscopic Observation of Prepared Mesocarbonbeads With Bacterial Biosurfactant

S.NO	TYPES OF HYDROCARBONS	GROWTH INTENSITY
1	Coconut Oil	No Growth
2	Castor Oil	Moderate
3	Palm Oil	Good
4	Diesel Oil	Very Good
5	Engine Oil	Excellent

Table 1 Growth intensity of bacterial biosurfactant in different hydrocarbon sources

S.no	Hydrocarbon sources	Total height	0 mins	5 mins	15 mins
1	**Castor oil**	1.4 cm	1.4 cm	1.4 cm	1.4 cm
2	**Palm oil**	1.5cm	1.5cm	1.5cm	1.5cm
3	**Diesel oil**	1.2cm	1.4cm	1.5cm	1.5cm
4	**Engine oil**	2.2cm	2.4cm	2.9cm	3.2cm

604 **Table 2 Form Forming test of Bacterial biosurfactant**

S.no	Hydrocarbon sources	Total height	0 mins	10 mins	Emulsification index
1	**Castor oil**	1.9 cm	2.2 cm	2.2 cm	52
2	**Palm oil**	2.2 cm	3.5cm	3.5cm	68
3	**Diesel oil**	2cm	2.4cm	2.4cm	82
4	**Engine oil**	3.5 cm	3.8cm	3.8cm	90

605 **Table 3Emulsification activity of Bacterial biosurfactant**

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Material	Initial weight(g)	Final weight(g)	Oil absorbed (g)	Absorption capacity(%)	Total oil used (ml)	Oil recovered	System Efficiency(%)
Sheet	**0.05**	**0.16**	**0.11**	**220**	**3**	**0.33**	**11**
Beads	**0.05**	**0.14**	**0.99**	**180**	**3**	**0.25**	**8.3**

Table 4 Oil Absorption Efficiency of prepared mesocarbon sheet and beads

Table 5Fourier Transform Infrared Spectroscopy (FTIR) of prepared mesocarbon sheet with Bacterial biosurfactant

S.no	Absorbance	Peak / Functional group
1.	3317.56	O-H stretching (alcohols/phenols) or N-H stretching (amines/amides)
2.	2924.09	C-H stretching (asymmetric)
3.	2854.65	C-H stretching (symmetric)
4.	1627.92	C=C stretching (alkenes/aromatics)
5.	1415.75	O-H bending (carboxylic acids)
6.	1300.02	C-O stretching or C-N stretching (amines)
7.	1122.57	C-O stretching (esters/ethers)
8.	1029.99	C-O-C stretching

Table 6Fourier Transform Infrared Spectroscopy (FTIR) of prepared mesocarbonbeads with bacterial biosurfactant

S.no	Absorbance	Peak / Functional group
1.	2924.09	Alkane (C-H stretch)
2.	2854.65	Alkane (C-H stretch)
3.	1450.47	Alkane (C-H bend)
4.	1381.03	Alkane (C-H bend)
5.	1029.99	C-O stretch/C-C stretch
6.	871.82	Aromatic C-H bend

CONCLUSION

The present study demonstrated an integrated biological and material-based approach for the removal of petroleum hydrocarbons using a biosurfactant-producing bacterium and mesocarbon-based sorbent materials. Bacterial isolates obtained from petroleum-contaminated samples were screened for biosurfactant production using oil-spreading

activity, emulsification index (E24), and foam formation. Among the tested hydrocarbon substrates, engine oil showed the highest emulsification activity (90%), followed by diesel oil (82%), palm oil (68%), and castor oil (52%). These results indicate that the selected isolate produced surface-active compounds with the ability to interact with different hydrocarbon substrates and enhance their dispersion.

The most efficient isolate was molecularly identified as *Stenotrophomonas maltophilia* based on sequence similarity analysis, and the sequence was deposited in GenBank under accession number PX624000. The selected bacterium demonstrated growth and biosurfactant activity in mineral salt medium supplemented with different hydrocarbon sources, with comparatively higher activity observed in diesel and engine oil. This adaptability to petroleum-derived substrates highlights the potential of *S. maltophilia* as a microbial resource for hydrocarbon-associated environmental applications.

A major outcome of the study was the incorporation of the bacterial biosurfactant with activated mesocarbon to develop functional oil-absorbing materials. KOH-activated mesocarbon was prepared through carbonization and activation, producing a porous carbonaceous matrix with potential adsorption sites. The material was subsequently incorporated into polymeric matrices to fabricate flexible sheets and spherical beads. This strategy combined the surface-active properties of the biosurfactant with the porous structure of mesocarbon, providing a multifunctional system for hydrocarbon removal.

The mesocarbon-amalgamated sheets exhibited a flexible and structurally stable form with a relatively large exposed surface area. The oil-removal experiments demonstrated visible reduction of the oil layer following treatment, indicating effective oil uptake and retention. The comparatively higher absorption performance of the sheets may be associated with their greater surface exposure, pore accessibility, and direct contact with the floating oil layer. Therefore, the sheet formulation may be particularly suitable for rapid surface oil-spill remediation.

The mesocarbon-amalgamated beads, prepared by ionic gelation using sodium alginate and calcium ions, exhibited a relatively uniform spherical structure with good mechanical stability. Surface pores and internal regions provided potential sites for hydrocarbon retention. Although the beads showed comparatively lower oil-absorption performance than the sheets, their compact structure and stability may provide advantages in applications involving particulate sorbents, filtration systems, or packed-bed treatment.

FTIR analysis supported the chemical characteristics of the developed materials, with characteristic bands corresponding to C–H stretching, C=C stretching, O–H bending, and C–O/C–N-related functional groups. These functional groups indicate the chemical complexity of the biosurfactant–mesocarbon matrix and support its potential interaction with hydrocarbon molecules. Microscopic observations further revealed rough surfaces, pores, and irregular cavities in the sheets, while the beads exhibited spherical morphology with surface pores and internal microchannels. Following oil exposure, darkened regions and filled pores were observed in both materials, providing visual evidence of hydrocarbon retention within the porous structures.

The comparatively better performance of the sheets demonstrates that material configuration plays an important role in oil-absorption efficiency. The sheets provide greater surface exposure and direct interaction with the oil layer, whereas the beads offer improved structural stability and controlled retention. Thus, the two formulations may have complementary applications depending on the intended remediation system.

Overall, the study demonstrates the feasibility of integrating microbial biosurfactant production with activated mesocarbon technology for petroleum hydrocarbon removal. The biosurfactant contributes biological surface activity, while mesocarbon provides a porous matrix for hydrocarbon adsorption and retention. This combination offers a promising alternative to conventional approaches based entirely on synthetic surfactants or non-functionalized sorbents.

Further studies are required to optimize biosurfactant concentration, mesocarbon loading, sheet thickness, bead size, and crosslinking conditions. Adsorption kinetics, regeneration capacity, repeated-use performance, and quantitative analysis of residual hydrocarbons should also be investigated. Field-based studies using naturally contaminated water and different petroleum products would further establish the practical applicability of the developed materials.

In conclusion, the successful isolation of *Stenotrophomonas maltophilia*, demonstration of biosurfactant activity, fabrication of mesocarbon sheets and beads, and confirmation of their porous and hydrocarbon-interacting properties collectively establish a promising platform for petroleum hydrocarbon removal. The superior performance of the sheet formulation indicates its potential for surface oil-spill remediation, while the bead formulation provides an alternative for applications requiring mechanically stable sorbents. The integration of

microbial biosurfactants with activated mesocarbon and polymeric matrices therefore represents a promising sustainable strategy for oil-spill remediation and hydrocarbon-contaminated wastewater treatment.

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