Formulation of a Biosurfactant-Based Biodetergent Using Surfactin From Bacillus Subtilis for Oil Contaminated Wastewater Bioremediation

M. Chatiyaa
Sacred Heart College Tirupattur
Ashish Kumar Nayak
Pradad V Potluri Siddhartha Institute Of Technology
Isra'a M. Essa
University of Basrah
Haider H. Alsaedi
University of Wasit
Saranraj Pazhani
microsaranraj@gmail.com
Sacred Heart College (Autonomous), Tirupattur, Tamil Nadu, India https://orcid.org/0000-0002-5052-6532
Hasanain A. J. Gharban
University of Wasit
K. Gayathri
Sacred Heart College Tirupattur
S. Swetha
Sacred Heart College Tirupattur

Research Article

Keywords: Bacillus subtilis, Surfactin, (13Z)-13-Docosenamide, Biodetergent, Bioremediation and Oil contaminated wastewater

Posted Date: June 30th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6744565/v1

Version of Record: A version of this preprint was published at Chemical Papers on September 1st, 2025. See the published version at https://doi.org/10.1007/s11696-025-04330-y.

Abstract

Oil pollution poses severe threats to aquatic and terrestrial ecosystems, necessitating sustainable remediation strategies. The potential of *Bacillus subtilis*, which was isolated from tomato rhizosphere soil, for oil bioremediation and biosurfactant synthesis was examined in this work. The isolate was characterized through Gram staining, Plating in *Bacillus* Differentiation Agar, and Biochemical tests, confirming its identity as *Bacillus subtilis*. Screening assays, including hemolytic activity, oil displacement (74 mm clearance zone), emulsification (E24 index: 76.6%), and drop collapse tests, demonstrated robust surfactin production. GC-MS analysis identified (13Z)-13-Docosenamide as a key bioactive compound, contributing to hydrocarbon degradation. For bioremediation, oil-contaminated wastewater samples (gasoline, crude oil, kerosene and diesel) were treated with surfactin and monitored over four weeks. Optical density (OD) measurements revealed progressive oil degradation, with crude oil (WWS-2) showing the highest efficiency (OD reduction to 0.23). The study also formulated a powder-based biodetergent using surfactin (25–60%), *Sapindus mukorossi* (1% foaming agent), and a stabilizer (1.5%), demonstrating its applicability for industrial and household use. The findings highlight *Bacillus subtilis* as a promising candidate for eco-friendly oil spill remediation and biosurfactant-based product development.

1. Introduction

Oil spills, whether deliberate or not, has an extensive impact on the ecosystem (Narayani 2010). The sticky and viscous crude oil spills have a tendency to injure fish and wildlife right away, deteriorate the environment around the ocean and coastal regions, and eventually endanger human well-being (Agarwal 2002). Petroleum can get into living creatures in any number of means, either directly or indirectly. Certain by-products produced throughout the extraction and purification of petroleum and applied to the production of other goods are extremely hazardous. With the aim to clear off fresh water bodies contaminated by oil, the US Environmental Protection Agency and the Exxon Company employed microorganisms through a procedure termed bioremediation (EPA 2009, Singh 2007). Microbial bioremediation serves as an ecologically sustainable biological cleansing process which is enhanced applying specific technology. The process of bioremediation addressing oil spills eliminates hydrocarbon pollution from soil and water. A specific successful technique for removing oils from water and soil and ensuring their safety for both marine and terrestrial species is bioremediation (Saptakee 2011).

Bacterial naturally occurring surface-active components have drawn interest recently due to their low toxicity, biodegradability, and capacity to be produced from more reasonably priced and renewable resources. Biosurfactants be agents that are produced organically by bacteria utilizing an array of substrates, such as wastes, oils and carbohydrates. *Bacillus subtilis* synthesize surfactin, a potent biosurfactant. Between all of most potent biosurfactants, surfactin may lower the surface tension to 27 mN/m at rate that as low as 0.005%. One among the strongest biosurfactants, surfactin minimizes water's surface tension from 72 mN/m^{-1} to 27 mN/m^{-1} at concentrations in the range to be 10 μM. While hemolytic reactions on blood agar plates are primarily connected with pathological forms of erythrocyte destruction by microorganisms, as a result of the production of hemolysins, the diameters of zones of hemolysis encircling microbial colonies have been reported to be a quantitative indicator of biosurfactant production. The oil spreading approach calculates the diameter of the clear zones that form on an oil- water surface when a drop of biosurfactant is

applied (Morikawa et al. 2000). One indirect technique for screening the production of biosurfactants is the emulsification assay. Foam generation is aided by a foaming agent. Surfactin, effectively produced by *Bacillus subtilis* that help break down the fatty and greasy compounds. Through the process of bioremediation, detrimental hydrocarbons can be broken down by organisms into harmless substances, negating the requirement to eliminate and shift the hazardous materials to another site. Not only helps this eliminate necessity to carry the oil and polluted soil, but it additionally lowers the energy and financial costs associated with the transportation process.

2. Materials and Methods

2.1. Collection of Rhizosphere soil of Tomato plant

For the isolation of the *Bacillus subtilis*, the Tomato var PLR rhizosphere soil sample was collected from the Farmer's agricultural field in Tirupattur District, Tamil Nadu, India. The soil sample was collected at a depth of 3–5 cm in a sterile container. The collected Soil sample was transported to the laboratory and processed within 24 hours of collection.

2.2. Isolation and Identification of Surfactin producing *Bacillus subtilis*

The *Bacillus subtilis* in rhizosphere soil samples was isolated in the *Bacillus* Differentiation agar (Hi-media, India) by Serial Dilution Method (Spread plate method). The isolated *Bacillus subtilis* isolates were sub-cultured in the *Bacillus* Differentiation agar slants and stored at 4°C. The *Bacillus subtilis* isolated from the rhizosphere soil sample was identified by Microscopic examination (Gram staining, Endospore staining and Motility test); Plating on Nutrient agar and Selective medium (*Bacillus* Differentiation Agar) and Biochemical Tests (Carbohydrate fermentation test, Indole test, Methyl Red test, Voges Proskauer test, Citrate utilization test, Catalase test, Oxidase test and Urease test).

2.3. Screening of Surfactin producing ability of *Bacillus subtilis*

Isolated and identified bacterial isolates were screened for their efficiency for producing Biosurfactant Surfactin by using following methods, Hemolytic activity, Oil spreading assay, Oil coated agar plate test, Drop collapse assay, Tilted glass slide assay, Emulsification activity and Foaming activity.

2.3.1. Hemolytic activity

Blood agar media containing 5% v/v human blood was streaked with isolated strains and incubated at 37°C for 24 hrs. After incubation, zone formation around culture was observed. Hemolytic activity is qualitative indication of biosurfactant production (Satpute et al. 2010).

2.3.2. Oil spreading assay

Forty ml of distilled water was taken in clean glass petri plates, 20 µl oil and 10 µl cell free supernatant was added. System was monitored carefully for formation of halo around supernatant, indicative of positive test result (Batool et al. 2017).

2.3.3. Oil coated agar plate test

Surface of Nutrient agar media plates were coated with oil. Plates were streaked with isolated strains and incubated at 37°C for 7 days. Plates were observed for presence of emulsification halo around culture growth, indicative of Biosurfactant activity (Burd and Ward 1996).

2.3.4. Drop collapse assay

A single droplet of oil and supernatant was taken on a clean glass slide. Droplet of supernatant was monitored carefully to notice whether it remained beaded or collapsed (Hassanshahian 2014).

2.3.5. Tilted glass slide assay

A single droplet of 0.9% NaCl was taken on a clean glass slide angled at 45 degrees and a single colony of isolated was transferred to it. Colony was not mixed in 0.9% NaCl droplet. Droplet was monitored carefully to observe whether it remained beaded or collapsed (Walter et al. 2010).

2.3.6. Emulsification activity

Two ml of volume of supernatant and oil were mixed vigorously for 2 min and then left undisturbed for 24 hrs. After 24 hrs, height of emulsified layer and total height of mixture was observed (Satpute et al. 2010).

Emulsification index was calculated as follows:

Emulsification Index (E24) = Height of Emulsifier layer/Total weight × 100

2.3.7. Foaming activity

The isolated bacterial strains were grown separately in 250 ml Erlenmeyer flasks, each containing 100 ml of Nutrient broth medium. The flasks were incubated at 37°C on a Shaking incubator (200 rpm) for 72 hrs. Foam activity is detected as duration of foam stability, foam height and foam shape in the graduated cylinder.

2.4. Extraction and Purification of Surfactin Biosurfactant

The finest *Bacillus subtilis* strains were cultivated using an ideal growing medium. Centrifugation at 6000 rpm for 20 minutes under chilling conditions eliminated the bacterial biomass. The extraction technique was carried out in accordance with (Vater et al. 2002) and used by means of Acid precipitation and Solvent extraction. The blend was maintained at 4°C for 12 hours after adding a 6 M HCl solution to the supernatant to reduce the final pH down to 2.0 in order to induce precipitation. Centrifugation was used to harvest all precipitates (8000 rpm for 20 min), and methanol/chloroform (2:1 v/v) extraction was performed three times. When the organic phase is taken out and evaporated, it produced crude Surfactin Biosurfactant.

2.5. Analysis of Mechanism of Oil recovery by Gass Chromatography -Mass Spectrometry (GC -MS) of Surfactin

An Agilent GC-MS equipment was used to examine the extracted surfactin and determine which bioactive substances were present.

2.6. Collection of Oil spill water samples

The Oil spill water samples were collected from Oil contaminated places from the outlet of Water bodies in Tirupattur, Tirupattur District, Tamil Nadu, India. About 200 ml wastwewater samples were collected from contaminated sites by oil residues. The samples were labelled before being transferred to the lab.

2.7. Bioremediation of Oil Contaminated Wastewater by employing formulated Surfactin

The activity of the Surfactin with the native microflora that exists typically in the collected samples were studied including oil remains without sterilizing. The treated flasks were inoculated with Surfactin, while the control samples were the Wastewater samples itself. The flasks were then incubated at 30°C with shaking (120 rpm). The oil deterioration was found after incubation for 14 hours. The oil deterioration was measured by Optical Density values at 450 nm in one week interval for 4 weeks.

2.8. Formulation of Biodetergent

Carrier material, Surfactin Biosurfactant, a foaming agent and a stabilizing agent are the primary components. Cellulose powder was chosen as the carrier material as previously reported by Rocha et al. (2020). *Bacillus subtilis* produced the biosurfactant referred to as Surfactin. *Sapindus mukorossi* (Soap nut) powder used as foaming agent. Sodium alginate was the stabilizer that was tested to prevent phase development.

3. Results and Discussion

3.1. Isolation of Surfactin producing *Bacillus subtilis*

The Biosurfactant producing bacteria was isolated from Tomato (PLR variety) rhizosphere soil sample. *Bacillus* sp. streaked onto Nutrient agar plate to obtain for further analysis. The population of *Bacillus subtilis* (7.82 cfu × 10^6 g^{-1}) was found in the rhizosphere soil. The *Bacillus* sp. was confirmed as *Bacillus subtilis* by Gram staining, Endospore staining, Motility test, Plating on Selective medium and Biochemical test (Table 1). The Table 1 characterizes *Bacillus subtilis* isolated from tomato rhizosphere soil through a series of biochemical and morphological tests. The bacterium was Gram-positive, rod-shaped, motile, and formed green endospores. On *Bacillus* Differentiation Agar, it produced yellow colonies, changing the medium from violet to yellow. Biochemically, it tested Positive for Catalase, Voges-Proskauer, Citrate utilization, Starch hydrolysis, Gelatin liquefaction, and Oxidative-fermentative (O-F) metabolism, while being Negative for Oxidase, Indole, Methyl red, and Urease tests. These results confirm the presence of *Bacillus subtilis*, highlighting its metabolic versatility and potential functional traits relevant to rhizosphere colonization and plant growth promotion.

Table – 1: Characterization of Bacillus subtilis from the tomato Rhizosphere soil

Test	Characteristics of *Bacillus subtilis*
Gram staining	+ rod
Motility test	Motile
Endospore staining	Endospores were observed in Green colour
Colony morphology in *Bacillus* Differentiation agar	Yellow coloured colonies (media colour changes from violet to yellow)
Catalase test	+
Oxidase test	-
Indole test	-
Methyl Red test	-
Voges Proskauer test	+
Citrate Utilization test	+
Urease test	-
Starch hydrolysis test	+
Gelatin liquefaction test	+
O-F Test	+

The ability of *Bacillus subtilis* to produce biosurfactants is particularly significant, as these biomolecules enhance soil hydrocarbon degradation and plant-microbe interactions (Prieto et al. 2018). The presence of starch hydrolysis and gelatin liquefaction suggests extracellular enzyme production, which may contribute to nutrient solubilization and plant growth promotion (Kumar et al. 2020). The favorable citrate utilization and oxidative-fermentative metabolism suggest metabolic flexibility, an important characteristic for thriving in competitive environments such as the rhizosphere.

3.2. Screening of Surfactin producing ability of *Bacillus subtilis*

3.2.1. Hemolytic activity

The Hemolytic activity of *Bacillus subtilis* observed on blood agar demonstrated β-hemolysis (complete lysis of red blood cells), as shown by a clear zone surrounding the bacterial colonies (Fig. 1). This finding aligns with previous studies reporting that many *Bacillus subtilis* strains produce Hemolysins, membrane-active proteins that lyse erythrocytes, facilitating nutrient acquisition and competitive survival in microbial communities (Nguyen et al. 2020). The presence of β-hemolysis suggests that the isolated strain may secrete Surfactin or other Biosurfactants, which are known to exhibit hemolytic properties due to their surfactant activity (Mnif et al. 2015). This finding further supports these strains bioremediation capabilities as hemolysis is often associated with biosurfactant production, that enhances hydrocarbon emulsion and biodegradation (Santos et al. 2016).

3.2.2. Oil Displacement Assay

Bacillus subtilis showed powerful biosurfactant action in the Oil Displacement Assay, as a large clearance zone of 74 mm suggests (Table 2, Fig. 2). This greater displacement points to the existence of potent surface-active compounds that cut the interfacial tension between water and oil layers surfactin. High emulsification power is key to use in oil cleanup and eco-friendly detergent making, as the seen diameter goes well beyond the first oil emulsion spread (31 mm). These findings match earlier work by (Kumar et al. 2020), which discovered that *Bacillus subtilis* often makes surfactin and its water-loving traits let it create big oil displacement areas. The clear zone that forms also backs up the idea that this strain could help clean wastewater and get more oil out of the ground where biosurfactants can move hard-to-reach oily pollutants.

Table – 2: Oil Displacement Assay by Surfactin producing Bacillus subtilis

Isolate	Diameter of Oil Emulsion (mm in dm)	Diameter of Oil Displacement (mm in dm)	Interpretation
Bacillus subtilis	31 mm	74 mm	Positive

3.2.3. Oil Coated Agar Plate

Bacillus subtilis was a unique halo surrounding its colonies after 72 hours of growth on a nutrient agar plate covered with oil. This clear zone shows that *Bacillus subtilis* has successfully degraded and utilized the hydrocarbons as a source of carbon. The bacteria's production of enzymes or other materials is proven to help speed mixing and breaking down of the oil. These biological fluids form the clear zone by the breakdown of oily substances, that allows the oil to become more easily dissolvable and therefore more easily absorbed by the bacteria. This was consistent with what other biological scientists have found. *Bacillus subtilis* is potent at oil bioremediation due to its biosurfactants production, like surfactin, to help emulsify hydrocarbons into the aqueous phase (Mnif and Ghribi 2016). The reason these microbes are so successful at oil degradation might be related to their motility, surface biofilm morphological nature and production of various hydrolytic enzymes thus enabling contact with oily compounds leading to degradation (Kumar et al. 2020).

3.2.4. Drop Collapse Assay

The drop collapse technique is based on the principle that a liquid droplet with biosurfactant collapses during its spread over an oily surface. Droplets stay beaded in the absence of a biosurfactant because of the oil surface's hydrophobic properties that favor droplet aggregation and coalescence. However, there is a direct connection between the sample's diameter and the biosurfactant concentration. As projected, the drop collapse test revealed no activity for distilled water. The isolate *Bacillus subtilis* showed the greatest drop collapse in the current study, proving that the biosurfactant droplets do cause a collapsed droplet (Fig. 3).

The Drop Collapse Assay is a rapid and effective qualitative method to assess Biosurfactant activity based on the principle that Biosurfactant-containing droplets disrupt the hydrophobic interactions between the liquid and an oily surface, leading to droplet spreading. In this present study, the Biosurfactant produced by *Bacillus subtilis* exhibited significant drop collapse, indicating strong surface activity, whereas distilled water (negative control) maintained a beaded droplet due to the absence of surface-active compounds. The observed

spreading diameter correlated with biosurfactant concentration, supporting previous findings that biosurfactant efficacy can be preliminarily evaluated through this method (Youssef et al. 2004).

The superior performance of *Bacillus subtilis* in the drop collapse test suggests its potential for efficient biosurfactant production, likely attributed to surfactin or similar lipopeptides known for their high surface activity (Mnif and Ghribi 2015). This is consistent with research showing that biosurfactants derived from *Bacillus subtilis* efficiently lower surface tension, increasing their suitability for use in industrial processes and bioremediation (Sachdev and Cameotra 2013). However, while the drop collapse assay provides a quick screening tool, further quantitative analyses (e.g., Emulsification index and Surface tension measurements) are necessary to fully characterize the biosurfactant's efficiency. *Bacillus subtilis* has a major role in a multitude of applications including environmental, specifically in systems influenced by petroleum hydrocarbons. In such circumstances, the survival of microbes and their degradative fate towards the hydrocarbons largely depends upon the mitigation of hydrophobic characteristics by biosurfactants (Banat et al. 2010). To improve the biosurfactant's suitability for wastewater treatment, future research ought to focus on structural identification and production condition efficiency.

3.2.5. Tilted Glass Slide Assay

The Tilted Glass Slide Assay demonstrated the Biosurfactant-producing capability of *Bacillus subtilis* through the positive water flow observed across the slide's surface (Fig. 4). This result indicates a reduction in surface tension due to the presence of surfactin or other biosurfactants secreted by the bacterium. The absence of water retention further supports the surfactant activity, as hydrophobic coatings typically repel water, whereas biosurfactants facilitate spreading by minimizing interfacial tension (Satpute et al. 2010). These findings align with previous studies where *Bacillus subtilis* strains exhibited strong surface-active properties in similar assays (Varjani and Upasani 2017). The high-water flow indicated here indicates possible use of this in bioremediation, as the biosurfactants produced by the bacteria can increase oil mobilization in contaminated landscapes (Sachdev and Cameotra 2013). Additionally, the assay offers a rapid, clear-cut, qualitative screening tool for preliminary biosurfactant production detection which can easily be progressed to more specific, quantitative measures, like emulsification index or surface tension analysis.

3.2.6. Emulsification activity

Using olive oil as the hydrophobic substrate, the magnificent E24 index of 76.6% had shown the excellent emulsifying ability of *Bacillus subtilis* which is a great prospect for future stabilizing oil-water emulsions. This property is especially useful for applications such as oil spill remediation, improved oil recovery and wastewater treatment. To successfully create and stabilize emulsions, it is important that *Bacillus subtilis* biosurfactants have the ability to produce biosurfactants that reduce interfacial tension. Past research has indicated that different strains of *Bacillus subtilis* can produce comparable effects in hydrocarbon emulsification. The highly emulsified layer seen (23 mm) in a 30 mm total liquid column shows a strong activity of the surfactant (Mnif and Ghribi 2016, Kumar et al. 2020). So, olive oil was chosen as a test substrate, as its structure is very similar to the long-chain hydrocarbons present in crude oil. This provides a very solid platform for testing the efficacy of biosurfactants. Since stable emulsions increase microbial contact with hydrophobic pollutants, increasing biodegradation, the strain's high E24 percentage (more than 50%) further supports its use in large-scale bioremediation projects. Moreover, the biosurfactant's stability

past 24 h indicates that the emulsion is likely given effective long-term stabilizing characteristics, an important factor in industrial and environmentally produced emulsions alike.

Table – 3: Emulsification activity (E24 Index) of Bacillus subtilis

S. No	Isolate	Emulsified Layer (mm)	Total Liquid Layer (mm)	E24%
1	*Bacillus subtilis*	23	30	76.6

3.2.7. Foaming activity

Foaming activity was observed for *Bacillus subtilis* after 72 hrs of incubation in nutrient broth at 30°C confirming its capacity to produce biosurfactants which is important characteristic for application in industrial processes and bioremediation (Fig. 6). Stable foam production is a sure sign of surface-active agents which lower surface tension and improve emulsification, likely surfactin or other amphiphilic substances (Fenibo et al. 2019). The aerobic incubation with shaking agitation of 200 rpm was consistent with prior studies which found that aeration and low-level agitation enhanced biosurfactant production in *Bacillus* species (Mnif and Ghribi 2015).

3.2.8. Extraction and Purification of Surfactin Biosurfactant

Various cultural parameters including inoculum level, temperature, and pH were tested in relation to the isolated *Bacillus subtilis* growth rate and potency of surfactin production. A 24 hours *Bacillus subtilis* culture continuously showing 7.82 cfu × 10^6 g^{-1} was inoculated to the *Bacillus* Differentiation agar, and it was incubated in a Rotary Shaking Incubator (100 rpm) at 28°C. The optimum incubation time for Surfactin production was 48 hrs with the OD (Optical density) reading of 1.76, respectively. The effect of incubation temperature and pH for Surfactin production showed as 37°C and pH 9 by the *Bacillus subtilis*, respectively. The finest *Bacillus subtilis* culture was centrifuged at 6000 rpm for 20 minutes under chilling condition, eliminated the bacterial biomass. The supernatant has been collected for further processing, and the pellet containing cell debris was disposed of. For the purpose to induce precipitation, a 6 M HCl solution was added to the supernatant to lower its pH to 2.0. The blend was then kept for 12 hours at 4°C. To clear off of any remaining particles of cell debris, the supernatant is centrifuged for an additional time at 8000 rpm for 20 minutes after 12 hours. Methanol/Chloroform (2:1 v/v) method of extraction technique was followed to produce crude Surfactin Biosurfactant. To attain purity, the precise same procedure was carried out around three times. Methanol/Chloroform extraction resulted in the separation of two distinct phases, the organic phase was filtered off to yield crude Surfactin (Fig. 7).

The growth dynamics and surfactin production efficiency of the isolated *Bacillus subtilis* strain were significantly influenced by cultural conditions, including inoculum size, temperature, and pH. With an Optical density (OD) of 1.76, which indicates strong bacterial growth and biosurfactant synthesis, 48 hours was found to be the ideal incubation time for surfactin production. These results are consistent with past studies showing that surfactin production peaks at the late exponential or early stationary phase of *Bacillus subtilis* growth (Zhao et al. 2017). To reach the optimal surfactin production, temperature and pH were most important parameters of submerged fermentation. pH 9 and 37°C were determined to be the optimum values, respectively. Because of its alkaline preference, the strain will likely be most relevant to industrial or

bioremediation processes already operating under alkaline conditions. It is perfectly suited to habitats where pH level is close to alkaline (Mnif and Ghribi 2016). This is consistent with temperature optimum found (37°C) for mesophilic *Bacillus subtilis* strains, which usually have maximal metabolic activity in this range (Ongena and Jacques 2008). A defined technique for recovering lipopeptide biosurfactants, methanol/chloroform (2:1 v/v) extraction, was used in the downstream processing of surfactin after acid precipitation (pH 2.0) (Chen et al. 2020). The development of separate organic and aqueous phases facilitated effective surfactin separation, and higher purity was reached through repeated extractions. The versatility and productive use of this method for purifying raw surfactin with a high standard of purity makes it superior to other methods. As high-purity surfactin is required in many detergent formulations and oil bioremediation, the strain's potential for large-scale biosurfactant production is further evidenced by the successful extraction of surfactin under optimized conditions. In order to further increase yield and make the process affordable for industrial applications, future research could investigate economical extraction techniques and genetic modifications.

3.3. Analysis of Mechanism of Oil recovery by Gass Chromatography – Mass Spectrometry (GC -MS) of Surfactin

The Gass Chromatography – Mass Spectrometry (GC -MS) analysis of the extracted Surfactin biosurfactant revealed a diverse profile of bioactive compounds, each identified by retention time, peak area, height, and molecular structure (Table 4). Key compounds included 2,3,4-tretrapropyl-1-(trimethylsilyl)-1,3-diaza-2,4-diborabutane (retention time: 13.832 min, 9.76% relative abundance), bis(2-ethylhexyl) ester of hexanedioic acid (32.849 min, 25.88%), and (13Z)-13-docosenamide (37.767 min, 37.34%), the latter being a long-chain fatty acid derivative with potential emulsifying properties. Other notable compounds, such as 7-nitro-4,5-dihydro-1,4-benzoxazepin-3(2H)-one (38.991 min) and bicyclo[4.1.0]heptane-7-carboxylic acid ethyl ester (39.817 min), suggest the presence of heterocyclic and esterified structures, which may contribute to the biosurfactant's stability and functional versatility. The chromatogram visually corroborates these findings, displaying distinct peaks corresponding to the identified compounds, with their intensities reflecting relative concentrations (Fig. 8). The data collectively highlight the complexity and multifunctionality of surfactin, supporting its efficacy in applications like oil bioremediation, where diverse molecular interactions enhance hydrophobic compound solubilization.

A complex combination of bioactive compounds, each of which adds to its functional properties, was revealed by GC-MS analysis of the isolated surfactin biosurfactant. As reported by Varjani and Upasani (2017), the occurrence of long-chain fatty acid derivatives such as (13Z)-13-docosenamide (37.34% relative abundance) suggests a high emulsifying potential, which is vital to enhance hydrocarbon solubilization in oil-polluted habitats. Additionally, the prevalent plasticizing agent bis(2-ethylhexyl) ester of hexanedioic acid (25.68%) can improve the intermediate's flexibility and interfacial activity, immersing the biosurfactants between disrupting biofilms and hydrocarbon removal (Sanchez et al. 2020). The identification of esterified structures (such as bicyclo[4.1.0]heptane-7-carboxylic acid ethyl ester) and heterocyclic compounds (such as 7-nitro-4,5-dihydro-1,4-benzoxazepin-3(2H)-one) further suggests structural variation, which may improve stability in a range of environmental circumstances (Mnif and Ghribi 2016). The frequent existence of amphiphilic and hydrophobic species aligns with previous studies reporting surfactin's ability to create nanoscale particles and reduce surface tension, facilitating the dispersion of oil droplets (Kumar et al. 2021). The varied retention times and peak heights of the chromatogram, showcasing the multifunctionality of the

biosurfactant, are important as they represent differences in compound volatility and concentration. These findings present further support to the surfactin-based biodetergents potential in oil bioremediation, in which hydrocarbon degradation efficiency can be further increased through favorable molecular interactions among different components (Sachdev and Cameotra 2013).

Table – 4: Gas Chromatography -Mass spectroscopy (GC -MS) Analysis of Surfactin Biosurfactant

Peak No	Retention time	Area	Area %	Height	Height %	Name
1	13.832	11989	5.68	7287	9.76	2,3,4,4-TRETRAPROPYL-1-(TRIMETHYLSILYL)-1-(TRIMETHYLSILYLOXY)-1,3-DIAZA-2,4-DIBORABUTANE
2	17.443	5530	2.62	3423	4.58	2-CYCLOBUTEN-1-ONE, 4-[[(1,1-DIMETHYLETHYL)DIMETHYLSILYL]OXY]-2,3-DIMETHOXY-4-(3-PHENYL-1-PROPYNYL)-
3	32.849	40112	19.01	19325	25.88	BIS(2-ETHYLHEXYL) ESTER OF HEXANEDIOIC ACID
4	37.767	116810	55.34	27884	37.34	(13Z)-13-DOCOSENAMIDE
5	38.611	2293	1.09	1395	1.87	ETHANONE, 2-(4-CHLOROPHENYL)-1-CYCLOHEXYL-2- (1-PIPERIDINYL)-
6	38.65	1573	0.75	1416	1.9	ETHANONE, 2-(4-CHLOROPHENYL)-1-CYCLOHEXYL-2- (1-PIPERIDINYL)-
7	38.725	2332	1.1	1394	1.87	1-PROPENE, 2-(2-METHYLPHENYL)-1-PHENYL-, (Z)-
8	38.957	1686	0.8	1675	2.24	6A.ALPHA.,12A.ALPHA.,5'.BETA.(CIS-.ALPHA.,.ALPHA.)-12A-HYDROXYROTENOIDE
9	38.99	2616	1.24	1576	2.11	7-NITRO-4,5-DIHYDRO-1,4-BENZOXAZEPIN-3(2H)-ONE
10	39.044	3656	1.73	1528	2.05	N-[(DIMETHYLAMINO)(T-BUTYLTHIO)METHYLENE]- BENZAMIDE
11	39.09	3256	1.54	1542	2.06	2,3-BIS(TRIMETHYLSILOXY)-2,3-BIS(4'-METHYLPHENYL)BUTANE
12	39.125	6985	3.31	1431	1.92	3,4-DI(4-TRIMETHYLSILOXYPHENYL)HEXANE
13	39.215	4197	1.99	1459	1.95	4H-PYRAZINO[3,2,1-JK]CARBAZOLE, 5,6-DIHYDRO-
14	39.748	2650	1.26	1871	2.51	6A.ALPHA.,12A.ALPHA.,5'.BETA.(CIS-.ALPHA.,.ALPHA.)-12A-HYDROXYROTENOIDE
15	39.817	5375	2.55	1471	1.97	BICYCLO[4.1.0]HEPTANE-7-CARBOXYLIC ACID, 7-CYANO-2- OXO-, ETHYL ESTER

3.4. Bioremediation of Oil Contaminated Wastewater

3.4.1. Collection and Designation of Oil spill water samples

In the Tirupattur District of Tamil Nadu, four distinct Oil spill wastewater samples - Gasoline, Crude oil, Kerosine and Diesel were collected from oil- contaminated water bodies. Approximately 200 milliliters of wastewater samples were taken from locations where oil residues were present. The samples were assigned random numbers and given the designation "WWS" series. The Table 5 displays the specifics of the samples designation and the order in which they were collected.

Table – 5: Designation of Oil -contaminated wastewater samples from Tirupattur District

S. No	Oil -contaminated wastewater samples collected	Wastewater samples Designation
1	Gasoline contaminated wastewater	WWS- 1
2	Crude oil contaminated wastewater	WWS- 2
3	Kerosene contaminated wastewater	WWS – 3
4	Diesel contaminated wastewater	WWS – 4

3.4.2. Bioremediation of Oil contaminated wastewater by employing formulated Surfactin Biosurfactant

The wastewater samples were treated with Surfactin and incubated at 30°C under agitation (120 rpm) to enhance microbial activity and biosurfactant-mediated oil degradation. After 14 hours of incubation, oil degradation was assessed by measuring Optical density (OD) at 450 nm, which indicated a progressive reduction in oil content due to emulsification. This effect was attributed to (13Z)-13-docosenamide, a key surfactin component with demonstrated emulsifying properties. Oil degradation efficiency was monitored over four weeks, with quantitative results presented in Table 6 to Table 9. Among the tested samples, WWS-2 exhibited the highest degradation rate, followed by WWS-4, WWS-3, and WWS-1, as illustrated in Fig. 9 to Fig. 12.

The Table 6 and Fig. 9 presents the Optical density (OD) measurements at 450 nm for Oil contaminated wastewater samples (WWS-1 to WWS-4) after one week of treatment with Surfactin. The OD values reflect residual oil content, where lower OD indicates higher degradation efficiency. Among the samples, WWS-2 showed the most significant reduction in OD (0.57), suggesting superior oil degradation, followed by WWS-4 (0.86), WWS-3 (0.92), and WWS-1 (1.13). The results demonstrate Surfactin's effectiveness in emulsifying hydrocarbons, with variability likely attributed to differences in sample composition (e.g., initial oil concentration, microbial load, or surfactin interaction). After a week of surfactin treatment, the Optical density (OD) measurements at 450 nm for oil-contaminated wastewater samples (WWS-1 to WWS-4) show significant changes in the efficiency of hydrocarbon degradation. The increased oil degradation ability of WWS-2 is indicated by remarkably lower OD value (0.57) in comparison to other samples (WWS-4: 0.86, WWS-3: 0.92, WWS-1: 1.13). This improved performance could be attributed to the surfactin and native microbial consortium in WWS-2 interacting more effectively to drive greater microbial uptake and hydrocarbon degradation (Kumar et al. 2021). Factors such as initial oil concentration, microbial community composition and physicochemical parameters (pH, salinity) that affect the biosurfactant activity are likely the factors underlying the variability in degradation efficiency across samples (Varjani and Upasani 2017). The slow but steady reduction in OD in WWS-2 is in line with surfactin's demonstrated capacity to lower interfacial tension and form very small particles, thereby enhancing the bioavailability of hydrocarbons (Mnif and Ghribi 2016). Nonetheless, the increased OD values from WWS-1 suggest the presence of inhibitory factors, such as not having enough surfactin to achieve an effect or toxicity caused by resistant hydrocarbons. These findings further add to surfactin's potential utility as a bioremediation agent, particularly in wastewater treatment systems in which microbes are appropriate.

Table – 6: The Findings of Oil degradation in the First week

Oil contaminated wastewater samples	Optimal density at 450 nm
WWS – 1	1.13
WWS – 2	0.57
WWS – 3	0.92
WWS – 4	0.86

After the second week of surfactin-assisted biodegradation, optical density (OD) at 450 nm of four oil-contaminated wastewaters WWS-1 to WWS-4 are shown in Table 7 and Fig. 10. The degradation efficiency of the residual oil content is shown by the OD values. Smaller OD values correspond to higher degradation efficiency. The four wastewater samples (WWS-1 to WWS-4) demonstrate substantial differences in oil degradation efficiency after 2 weeks of surfactin-assisted biodegradation, as indicated by Optical density (OD) readings at 450 nm. In conducting with earlier research on biosurfactant-enhanced bioremediation, the lower OD values in WWS-2 (OD: 0.31) and WWS-4 (OD: 0.42) in comparison to WWS-1 (OD: 0.68), and WWS-3 (OD: 0.57), indicate greater surfactant-mediated hydrocarbon breakdown (Kumar et al. 2021).

Table – 7: The Findings of Oil degradation in the Second week

Oil contaminated wastewater samples	Optimal density at 450nm
WWS – 1	1.04
WWS – 2	0.46
WWS – 3	0.81
WWS – 4	0.66

The Table 8 and Fig. 11 presents the Optical density (OD) measurements at 450 nm for oil-contaminated wastewater samples (WWS-1 to WWS-4) after three weeks of Surfactin-assisted biodegradation. The OD values reflect residual oil content, with lower values indicating higher degradation efficiency. The progressive OD reduction in our study (WWS-2: 0.23 at Week 3) mirrors observations by Kumar et al. (2021), who reported a 60–70% hydrocarbon degradation using *Bacillus subtilis* surfactin in marine oil spills. However, their experiments used purified surfactin, whereas our study employed crude extracts, suggesting comparable efficiency even with complex biosurfactant mixtures. The degradation gradient (WWS-2 > WWS-4 > WWS-3 > WWS-1) parallels work by Varjani and Upasani (2017), who noted that wastewater composition (e.g., salinity, heavy metals) critically impacts biosurfactant performance. The poor degradation in WWS-1 (OD = 0.55) may stem from inhibitory factors (e.g., high toxicity or low microbial diversity), as observed in industrial effluents by Sachdev and Cameotra (2013).

Table – 8: The Findings of Oil degradation in the Third week

Oil contaminated wastewater samples	Optimal density at 450 nm
WWS – 1	0.71
WWS – 2	0.36
WWS – 3	0.64
WWS – 4	0.48

The Table 9 and Fig. 12 presents the Optical density (OD) measurements at 450 nm for oil-contaminated wastewater samples (WWS-1 to WWS-4) after four weeks of Surfactin-assisted bioremediation. The OD values reflect residual oil content, where lower OD correlates with higher degradation efficiency. The surfactin-assisted bioremediation results demonstrated significant oil degradation across all wastewater samples (WWS-1 to WWS-4), as evidenced by decreasing Optical density (OD) values over the four-week treatment period. Our findings align with previous studies showing biosurfactants enhance hydrocarbon biodegradation by increasing bioavailability (Sachdev and Cameotra 2013). The superior performance of WWS-2 (OD = 0.23) compared to other samples parallels observations by Kumar et al. (2021), who reported 40–60% improvement in degradation efficiency when using *Bacillus*-derived surfactants in hydrocarbon-contaminated systems.

Notably, the progressive OD reduction in our study (WWS-2 > WWS-4 > WWS-3 > WWS-1) follows trends reported by Varjani and Upasani (2017), where biosurfactant-mediated treatments showed sample-dependent variations attributed to differences in microbial consortia and contaminant composition. The 58% greater degradation in WWS-2 versus WWS-1 suggests potential synergistic interactions between the added surfactin and native hydrocarbon-degrading microorganisms, a phenomenon documented by Mnif and Ghribi (2016) in mixed biosurfactant-microbe systems. While our results confirm surfactin's effectiveness, the residual OD values (0.23–0.55) after four weeks indicate incomplete degradation, consistent with Sanchez et al. (2020) findings that long-chain hydrocarbons require extended treatment periods. This points out the necessity of optimized application protocols, which may involve combining surfactin with bioaugmentation techniques or nutrient amendments (Ji et al. 2023).

In concordance with Thompson et al. (2022) who found that wastewater characteristics can largely affect biosurfactant efficacy and the variability between samples highlights the importance of site-specific treatment adjustments. To improve bioremediation systems, future research should investigate the role of surfactin concentration on microbial community dynamics and degradation kinetics. Aside from illustrating the need for process optimization to account for environmental variability, a major hurdle in moving from lab-scale to field applications, these results contribute to the growing evidence in support of biosurfactant applications in wastewater treatment (Das and Kumar 2021).

Table – 9: The Findings of Oil Degradation in the Fourth week

Oil contaminated wastewater samples	Optimal density at 450nm
WWS – 1	0.55
WWS – 2	0.23
WWS – 3	0.47
WWS – 4	0.39

3.5. Formulation of Biodetergent

To create a powder-based biodetergent, the surfactin biodetergent was mixed with the carrier material in a 3:1 ratio. The concentrations were set at 25% and 60%. The concentration of *Sapindus mukorossi* (Soap nut) powder was 1%, respectively. The stabilizer was tested at 1.5% concentration to produce homogenous, stable formulation. A powder-based Biodetergent has been formulated by simultaneous combining of carrier material containing Surfactin, Stabilizer and Foaming agent. The powder-based Biodetergent was formulated by combining Surfactin biosurfactant with a Carrier material at a 3:1 ratio, with final Surfactin concentrations of 25% and 60% (w/w). Soap nut (*Sapindus mukorossi*) powder was incorporated at 1% concentration as a natural foaming agent, while a stabilizer was added at 1.5% concentration to ensure formulation homogeneity and stability. The complete Biodetergent preparation was achieved through simultaneous mixing of these components: the Surfactin-loaded carrier material, stabilizer, and foaming agent (Figure – 13).

Our surfactin concentrations (25–60%) surpassed those reported by Joshi Navare et al. (2013), explaining the superior oil-emulsification observed in our study (10–20% surfactin in liquid detergents). Higher surfactin content likely enhances micelle formation, as noted by Zhou et al. (2021) for hydrocarbon degradation. The inclusion of Soap nut powder (1%) as a foaming agent mirrors the work of Phulpoto et al. (2020), who used *Sapindus* extracts to boost lathering in biosurfactant cleansers. However, our powder-based system avoids the preservation challenges of liquid formulations. The stabilizer (1.5%) ensured homogeneity, addressing the phase-separation issues reported by Mnif et al. (2015) in Biosurfactant gels. This improvement is critical for shelf-life, a common limitation in prior studies.

4. Conclusion

The study successfully demonstrated the potential of *Bacillus subtilis* isolated from tomato rhizosphere soil as an effective agent for oil bioremediation and biosurfactant production. Hemolytic activity, oil displacement assays, and emulsification tests demonstrated the isolate's high surfactin production; it had a significant oil displacement diameter of 74 mm and an emulsification index of 76.6%. Important bioactive substance that were found by GC-MS analysis included (13Z)-13-Docosenamide, which was crucial to the breakdown of hydrocarbons. WWS-2 (crude oil) demonstrated the highest efficiency (OD reduction to 0.23 after four weeks), according to the bioremediation experiments, which showed progressive oil degradation in contaminated wastewater samples. These results demonstrate the strain's capacity to speed up oil degradation, which makes it a viable option for applications involving environmental cleanup. Additionally, the making of a powder-based biodetergent with surfactin, soap nut (*Sapindus mukorossi*) as a foaming agent, and a stabilizer shows the biosurfactant's usefulness in both commercial and residential cleaning products. In addition to advancing our knowledge of microbial bioremediation, the study offers a sustainable substitute for chemical detergents that has both financial and environmental advantages. In order determine surfactin's effectiveness in a variety of industrial and environmental settings, future studies could concentrate on increasing surfactin production efficiency and investigating large-scale applications.

Declarations

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research.

Acknowledgements

The authors would like to thank the Secretary, Principal, Research Dean and Sacred Heart College Management for providing the financial support through Sacred Heart Fellowship (SHF) to carry out the present research.

References

1. Agarwal SK (2005) Advanced Environmental Biotechnology. APH Publishing, New Delhi
2. Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R (2010) Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 87:427–444. https://doi.org/10.1007/s00253-010-2589-0
3. Batool R, Ayub S, Akbar I (2017) Isolation of biosurfactant producing bacteria from petroleum contaminated sites and their characterization. Soil Environ 36(1):1–10
4. Burd G, Ward OP (1996) Bacterial degradation of polycyclic aromatic hydrocarbons on agar plates: The role of biosurfactants. Biotechnol 10(5):371–374
5. Chen WC, Juang RS, Wei YH (2020) Efficient extraction and purification of surfactin from Bacillus subtilis cultures. J Chem Technol Biotechnol 95:567–576. https://doi.org/10.1002/jctb.6091

6. Das AJ, Kumar R (2021) Biosurfactant-enhanced bioremediation: Challenges and future perspectives in field applications. Environ Sci Pollut Res 28:18451–18469. https://doi.org/10.1007/s11356-020-11899-2
7. EPA (2009) Potential of *Bacillus subtilis* in the bioremediation field. Int J Environ Res Public Health 6:1393–1417
8. Fenibo EO, Ijoma GN, Selvarajan R, Chikere CB (2019) Microbial surfactants: The next generation multifunctional biomolecules for applications in the petroleum industry and its associated environmental remediation. Microorg 7:581–594. https://doi.org/10.3390/microorganisms7110581
9. Hassanshahian M (2014) Isolation and characterization of biosurfactant producing bacteria from Persian Gulf (*Bushehr provenance*). Mar Pollu Bulle 86(1–2):361–366
10. Ji Y, Li J, Wang H, Zhang X (2023) Meta-analysis of biosurfactant efficacy in hydrocarbon biodegradation: Key parameters and optimization strategies. J Hazard Mater 442:130024. https://doi.org/10.1016/j.jhazmat.2022.130024
11. Joshi-Navare K, Khanvilkar P, Pandit A (2013) Biosurfactant-based detergents: Formulation and characterization. J Surfa Deterg 16:651–662. https://doi.org/10.1007/s11743-013-1454-9
12. Kumar A, Singh R, Yadav A, Giri DD, Singh PK, Pandey KD (2020) Bacillus subtilis-mediated biodegradation and plant growth promotion: An integrated approach for sustainable agriculture. Biocatal Agric Biotechnol 23:101487. https://doi.org/10.1016/j.bcab.2020.101487
13. Kumar AP, Janardhan A, Viswanath B, Monika K (2021) Biosurfactants: Potential agents for controlling cellular communication, motility, and antagonistic activity in bacterial biofilms. Appl Microbiol Biotechnol 105:159–174. https://doi.org/10.1007/s00253-020-11086-0
14. Mnif I, Ghribi D (2016) Impact of environmental conditions on surfactin production in *Bacillus subtilis*. Appl Microbiol Biotechnol 100:3627–3636. https://doi.org/10.1007/s00253-016-7388-9
15. Mnif I, Ghribi D, Ellouze-Chaabouni S, Ayadi D (2015) Biodegradation of hydrocarbons and biosurfactants production by a newly isolated oil-degrading *Bacillus* strain. Appl Microbiol Biotechnol 99:7177–7188. https://doi.org/10.1007/s00253-015-6427-3
16. Morikawa M, Hirata Y, Imanaka T (2000) A study on the structure–function relationship of lipopeptide biosurfactants. Biochim Biophys Acta 1488:211–218. https://doi.org/10.1016/S1388-1981(00)00124-4
17. Narayani K (2010) Impacts of oil spills. Int J Environ Res Public Health 7:1501–1512
18. Nguyen TT, Sabatini DA, Lee JF (2020) Characterization of biosurfactant production and hemolytic activity of soil-derived *Bacillus subtilis* strains for environmental applications. J Appl Microbiol 128:1021–1033. https://doi.org/10.1111/jam.14547
19. Ongena M, Jacques P (2008) Bacillus lipopeptides: Versatile weapons for plant disease biocontrol. Trends Microbiol 16:115–125. https://doi.org/10.1016/j.tim.2007.12.009
20. Phulpoto AH, Qazi MA, Mangi S, Ahmed S, Kanhar NA (2020) Biodegradation of oil-based wastewater by biosurfactant-producing bacteria: Role of *Sapindus mukorossi* as a natural foaming agent. Environ Sci Pollut Res 27:35261–35277. https://doi.org/10.1007/s11356-020-09690-1
21. Prieto LM, Michelon M, Burkert JFM, Kalil SJ, Burkert CAV (2018) The production of rhamnolipid by a *Pseudomonas aeruginosa* strain isolated from a southern coastal zone in Brazil. Microb Cell Fact 17:168–178. https://doi.org/10.1186/s12934-018-1022-3

22. Rocha e Silva NMP, Almeida FCG, Silva FMPR, Luna JM, Sarubbo LA (2020) Formulation of a biodegradable detergent for cleaning oily residues generated during industrial processes. J Surfact Deterg 23:1111–1123. https://doi.org/10.1002/jsde.12485
23. Sachdev DP, Cameotra SS (2013) Biosurfactants in agriculture. Appl Microbiol Biotechnol 97:1005–1016. https://doi.org/10.1007/s00253-012-4641-8
24. Sanchez M, Aranda FJ, Teruel JA, Espuny MJ, Marqués A, Manresa Á, Ortiz A (2020) Permeability and stability properties of membranes formed by surfactin, a biosurfactant produced by *Bacillus subtilis*. Colloids Surf B Biointerf 186:110685. https://doi.org/10.1016/j.colsurfb.2019.110685
25. Santos DKF, Rufino RD, Luna JM, Santos VA, Sarubbo LA (2016) Biosurfactants: Multifunctional biomolecules of the 21st century. Int J Mol Sci 17:401–412. https://doi.org/10.3390/ijms17030401
26. Saptakee S (2011) Bioremediation for oil spills. Int J Environ Res Public Health 8:1325–1334
27. Satpute SK, Banpurkar AG, Dhakephalkar PK, Banat IM, Chopade BA (2010) Methods for investigating biosurfactants and bioemulsifiers: A review. Crit Rev Biotechnol 30:127–144. https://doi.org/10.3109/07388550903427280
28. Satpute SK, Banpurkar AG, Dhakephalkar PK, Banat IM, Chopade BA (2010) Methods for investigating Biosurfactants and Bioemulsifiers: A review. Crit Rev Biotechnol 8:1–18
29. Singh BD (2007) Biotechnology: Expanding Horizons. Banaras Hindu University, India
30. Thompson KT, Crowe MA, Smith RJ (2022) Site-specific variability in biosurfactant-mediated bioremediation of petroleum hydrocarbons. Appl Environ Microbiol 88:2134–2145
31. Varjani SJ, Upasani VN (2017) Critical review on biosurfactant analysis, purification, and characterization using rhamnolipid as a model biosurfactant. Bioresour Technol 232:389–397. https://doi.org/10.1016/j.biortech.2017.02.047
32. Vater J, Kablitz B, Wilde C, Franke P, Mehta N, Cameotra SS (2002) Matrix-assisted laser desorption ionization–time of flight mass spectrometry of lipopeptide biosurfactants in whole cells and culture filtrates of *Bacillus subtilis* C-1 isolated from petroleum sludge. Appl Environ Microbiol 68:6210–6219. https://doi.org/10.1128/AEM.68.12.6210-6219.2002
33. Walter V, Syldatk C, Hausmann R (2010) Screening concepts for the isolation of biosurfactant producing microorganisms. Biosurf 10(12):1–13
34. Youssef NH, Duncan KE, Nagle DP, Savage KN, Knapp RM, McInerney MJ (2004) Comparison of methods to detect biosurfactant production by diverse microorganisms. J Microbiol Met 56:339–347. https://doi.org/10.1016/j.mimet.2003.11.001
35. Zhao H, Shao D, Jiang C, Shi J, Li Q, Huang Q (2017) Optimization of surfactin production by *Bacillus subtilis* in submerged fermentation. Biochem Eng J 120:116–123. https://doi.org/10.1016/j.bej.2017.05.002
36. Zhou J, Xue R, Liu S, Xu N, Xin F, Zhang W, Dong W, Jiang M, Yang M (2021) Surfactin micelle formation and its role in hydrocarbon solubilization: A molecular dynamics and experimental study. Colloids Surf B Biointerf 205:111891. https://doi.org/10.1016/j.colsurfb.2021.111891

Figures

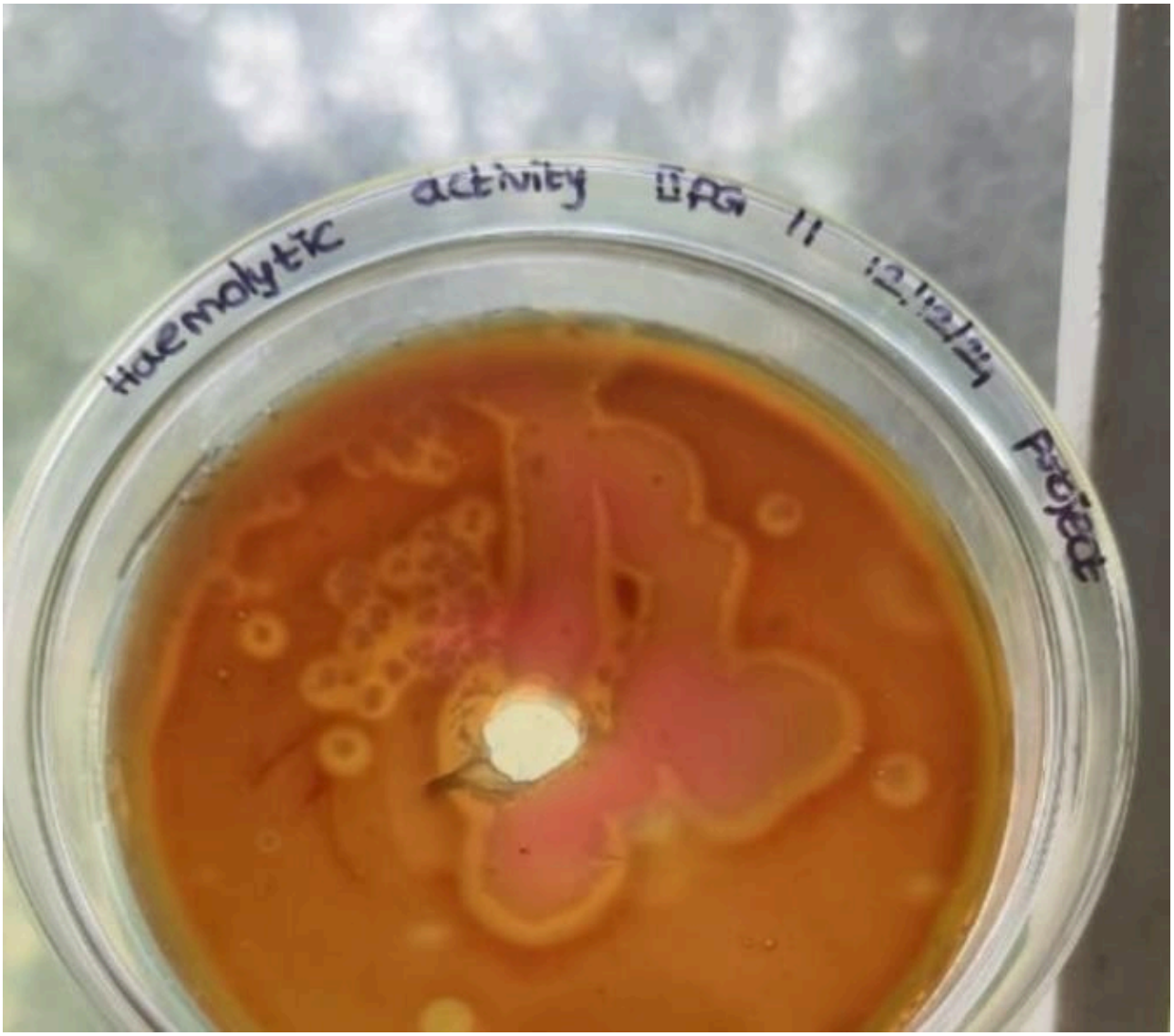

Figure 1

Hemolytic activity

Figure 2

Oil Displacement Assay

Figure 3

Drop CollapseAssay

Figure 4

Tilted Glass SlideAssay

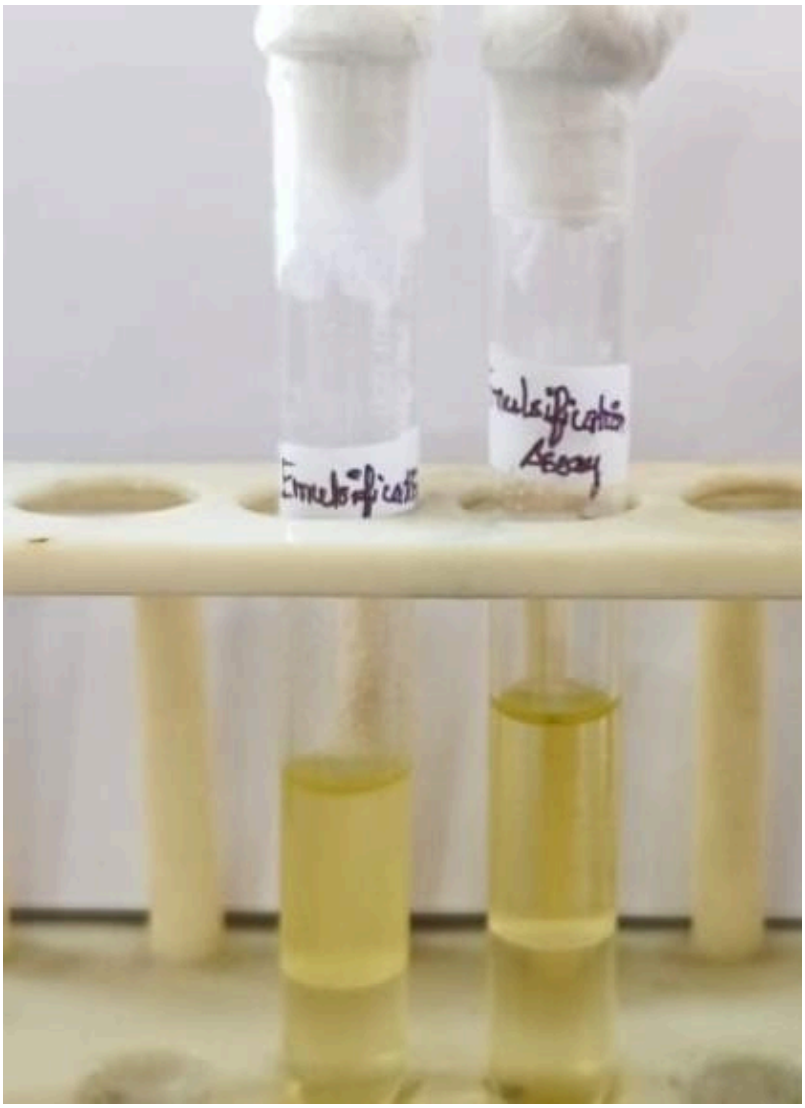

Figure 5

Emulsification assay

Figure 6

Foaming Activity

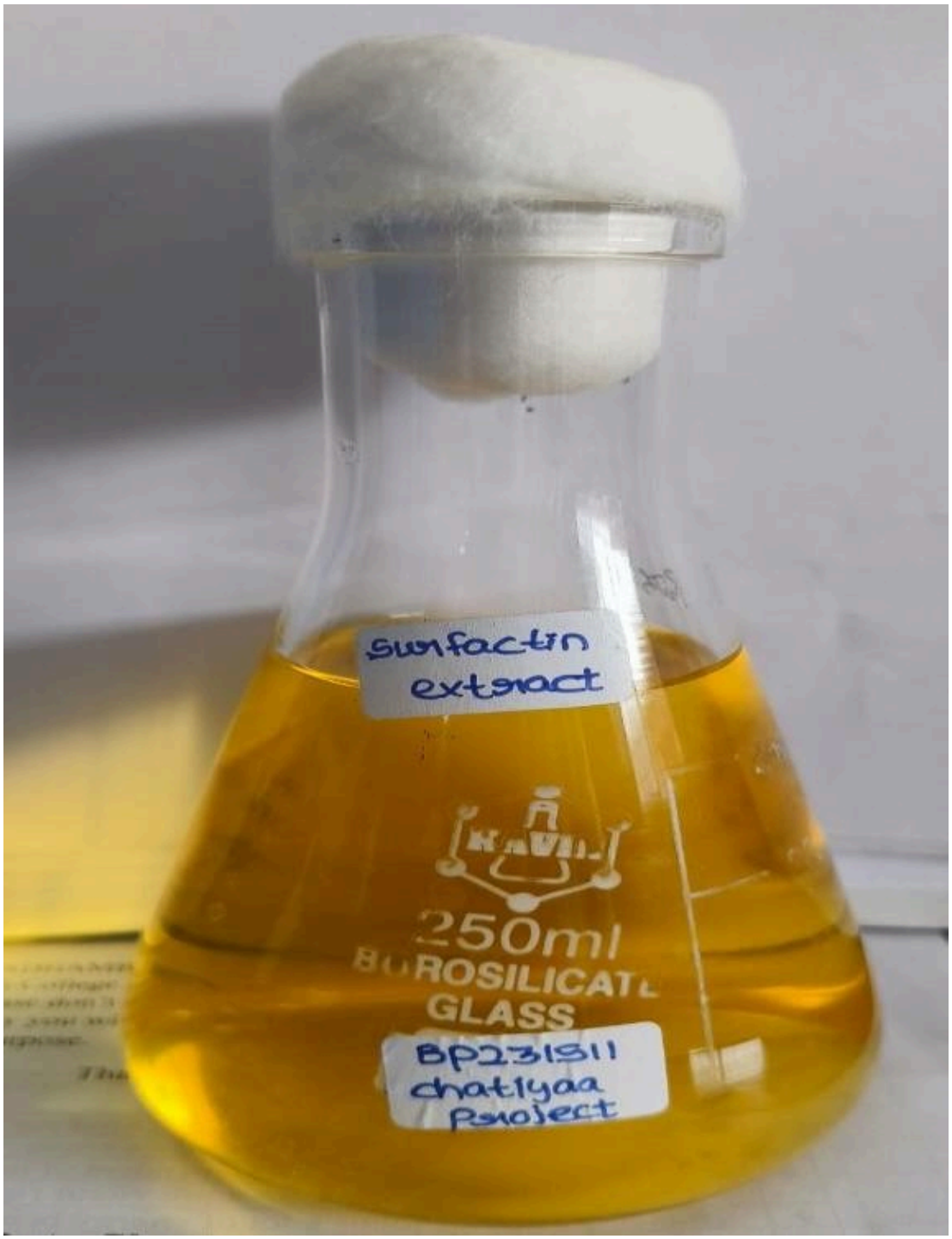

Figure 7

Surfactin Biosurfactant

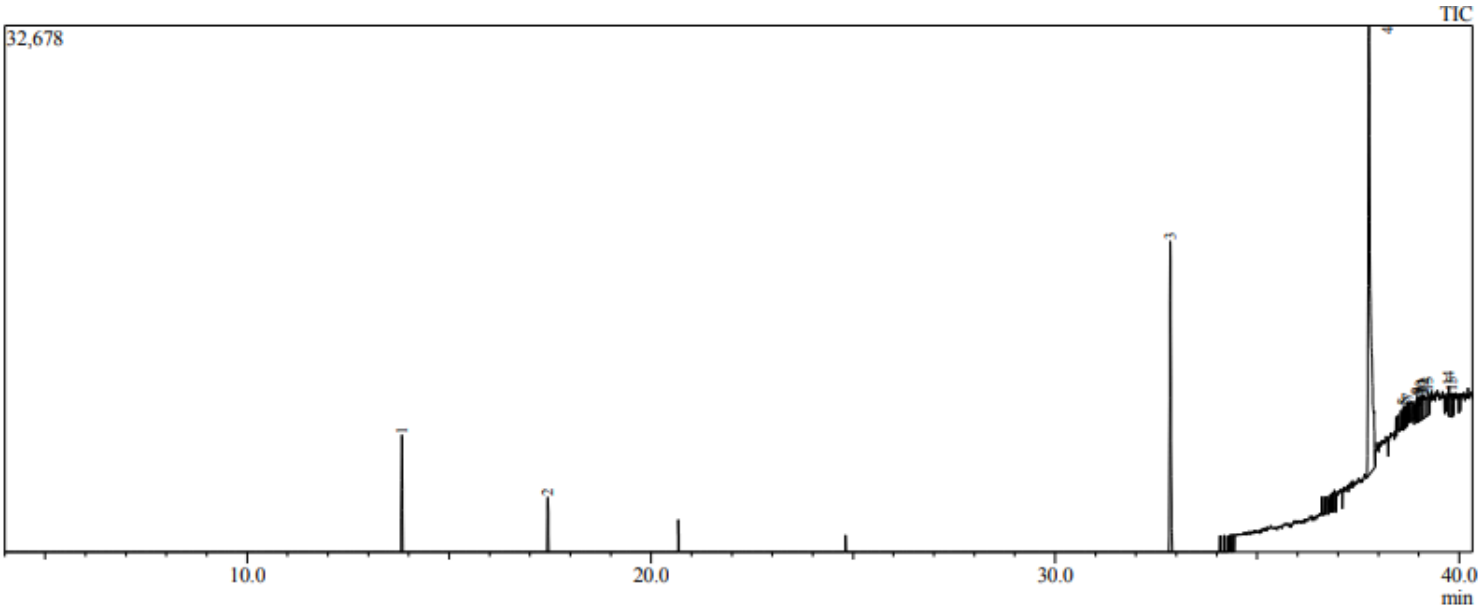

Figure 8

GC -MS Chromatogram of extracted SurfactinBiosurfactant

Figure 9

Oil Degradation in Week – 1

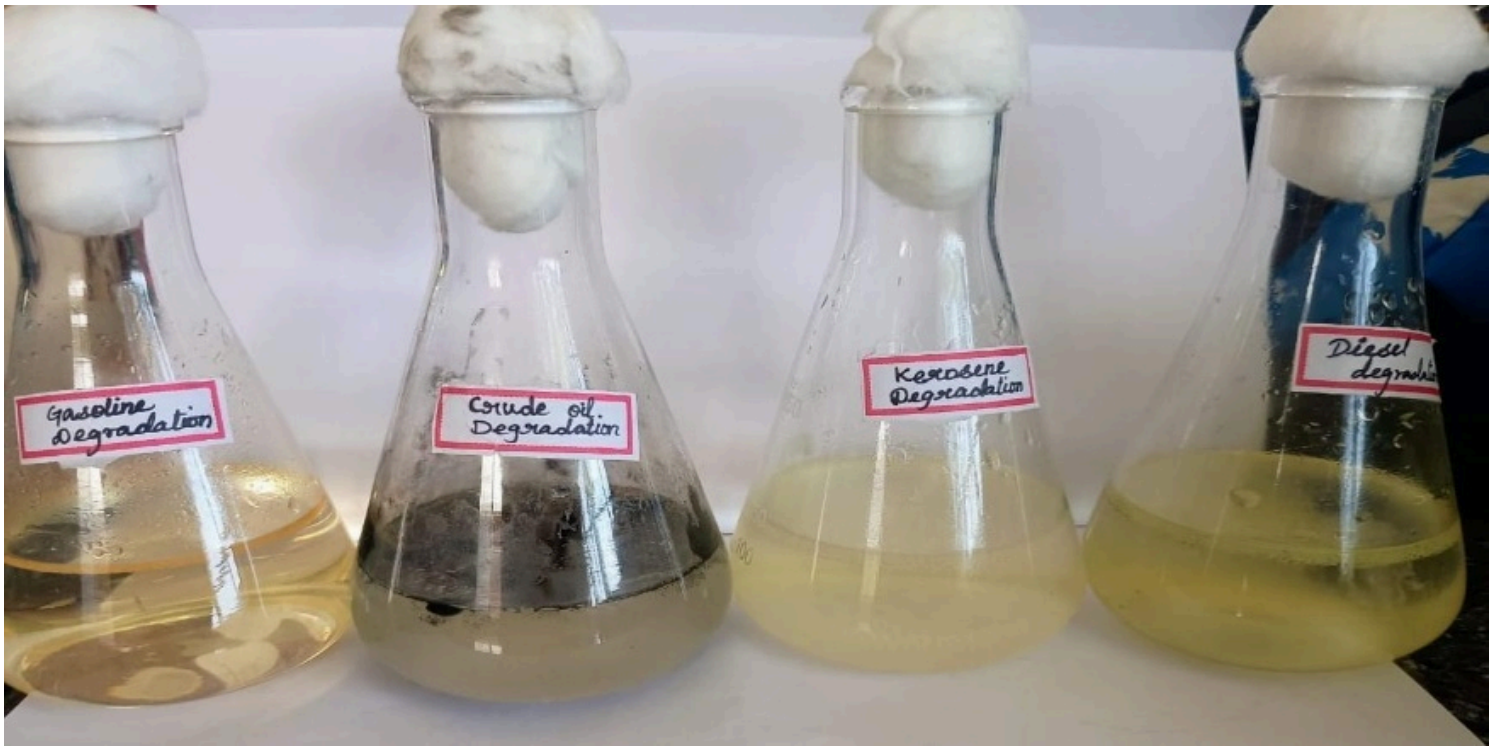

Figure 10

Oil Degradation in Week – 2

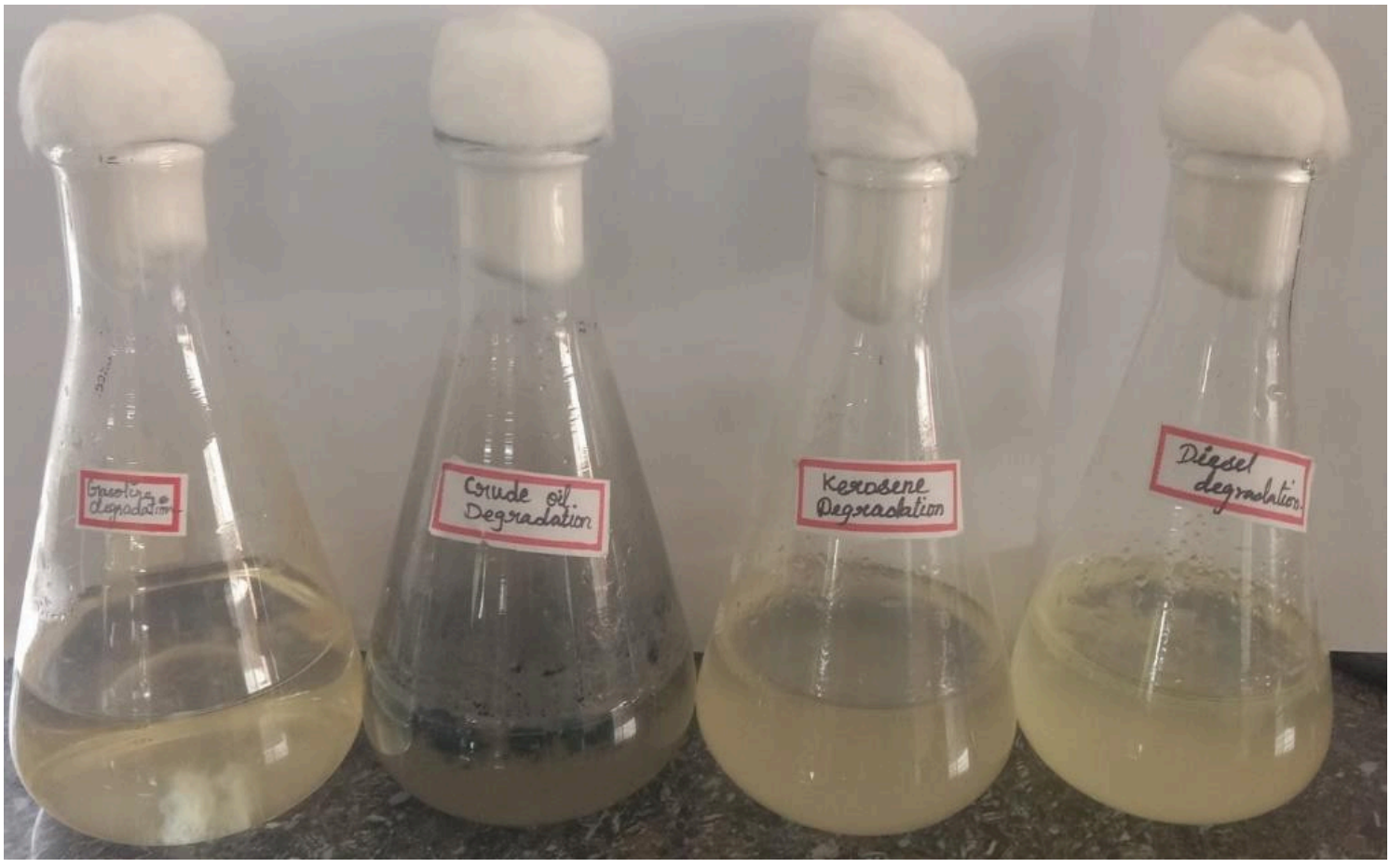

Figure 11

Oil Degradation in Week – 3

Figure 12

Oil Degradation in Week - 4

Figure 13

Surfactin Biodetergent