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# Exploration of Biosurfactant Producing Microorganism from Garage Soil: Production, Characterization, and its Application

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## ABSTRACT

***Biosurfactants are bioactive surface molecules produced by microorganisms, gaining notoriety for their environmentally friendly and biodegradable characteristics. This research emphasizes the extraction, production, and analysis of biosurfactants from hydrocarbon-polluted soils collected from a garage and truck terminal in the Yeshwanthpur industrial region. The samples were enriched using Mineral Salt Medium (MSM), and bacterial strains were isolated through serial dilution and pour plate methods. The identification of biosurfactant-producing bacteria was performed utilizing drop collapse, oil displacement, and emulsification assays. Among the isolates, isolate 2 exhibited the most promising results and was chosen for further research. Gram staining, endospore staining, and biochemical tests revealed the organism to be Bacillus cereus. Optimization of biosurfactant production was achieved by adjusting pH, temperature, incubation duration, inoculum volume, and nutrient sources. The maximum biosurfactant yield was attained with 250 µl of inoculum and with optimum physical parameters of pH 6 and temperature 35°C at a 24-hour incubation period, with glucose and peptone as carbon and nitrogen sources, respectively. The biosurfactants were extracted through acid precipitation followed by solvent extraction using chloroform and methanol. The characterization of the crude biosurfactant was performed. The antimicrobial properties against selected bacterial and fungal strains were assessed using the agar diffusion method, and bioremediation potential was evaluated. Distinct zones of inhibition confirmed the antimicrobial efficacy of the biosurfactant. These results imply that Bacillus cereus isolated from garage soil contains effective biosurfactant-producing potential and can be used in environmental bioremediation and antimicrobial property, offering a sustainable substitute for synthetic surfactants.***

**Keywords:** *Biosurfactants, Antimicrobial Properties, Bioactive Compounds, Bioremediation, Biodegradability.*

## 1. INTRODUCRION

Surfactants are compounds that reduce surface and interfacial tension between individual molecules at the surface and interface, respectively. They are widely used in many industrial applications, such as cosmetics, pharmaceuticals, petroleum, paper, textile, leather, food, agriculture, and mining. Chemically synthesized surfactants are toxic and non-biodegradable, and their production costs are high. Hence, there is a growing interest in microbial surfactants, known as biosurfactants, due to their lower toxicity, higher biodegradability, and eco-friendly nature. Biosurfactants are structurally diverse surface-active compounds produced by various microorganisms. They include glycolipids, lipopeptides, phospholipids, neutral lipids, fatty acids, and polymeric compounds. These compounds can be produced from renewable resources such as waste materials, making them economically and environmentally beneficial. Biosurfactants have applications in oil recovery, bioremediation, agriculture, pharmaceuticals, and food industries.
The increasing demand for eco-friendly and biodegradable surfactants has led to the exploration of novel biosurfactant-producing microorganisms.

Garage soils, which are exposed to hydrocarbon pollutants such as oils and greases, provide a favourable environment for the growth of biosurfactant-producing bacteria that can utilize hydrocarbons as their carbon source. Biosurfactants are better than synthetic surfactant in many aspects like, Environmental safety and biodegradability, microorganisms produce biosurfactants, which easily

break down into byproducts like water and $CO_2$. Petroleum-based synthetic surfactants are typically non-biodegradable and pollute the environment as they build up (Sharma, et.al.,2023). Biosurfactants are significantly less harmful to both aquatic and land life. For instance, it has been discovered that glycolipid biosurfactants are roughly 50% less hazardous than synthetic surfactants such as Tween 80. Certain artificial surfactants may be detrimental to human health and ecosystems. (Ravichandran,2024). A lot of biosurfactants, like rhamnolipids, can lower the surface tension of water below 30 mN/m (to about 26 mN/m). This is frequently better than any other synthetic surfactants like SDS (about 25 mN/m). Additionally, their critical micelle concentrations are lower, which indicates that they are efficient.

Biosurfactants are more versatile than synthetic surfactants because of their complex molecular structures, which enable them to carry out particular tasks like emulsification, antimicrobial activity, and environmental remediation. (Ravichandran,2024). Biosurfactants are adaptable for a range of industrial applications because they maintain their stability and activity over a wide range of pH values, temperatures, and salinities. (Sharma, et.al. 2023). Biosurfactants are made biologically through fermentation from renewable raw materials, which lowers their carbon footprint and dependency on fossil fuels. This is in contrast to synthetic surfactants, which are made from non-renewable petrochemicals. (Sharma, et al. 2023) (Ravichandran, 2024). Biosurfactant has wide range of application in all the sectors like, Environmental and bioremediations in encouraging the decrease of surface tension between the oil and rock, biosurfactants effectively mobilize immobile hydrocarbons by lowering the capillary forces that prevent oil from passing through rock pores (Rawat, et.al 2020, Khademolhosseini, et.al 2019). Oil cleanup and bioremediation, which are frequently required due to accidents and the resulting hydrocarbon contamination of the environment, can benefit from the same qualities that make oil exploration possible. Because they can sustain a high rate of biodegradation in contaminated soils, biosurfactants are a great ecological substitute for synthetic surfactants, according to bioremediation techniques. (Chandankere, et.al, 2013). Biosurfactants are technically efficient and can be released in situ, where they can exert their effects with less subsequent handling effort than their synthetic counterpart. (Zou, et.al, 2014, da Silva, et.al 2020).

Bacterial biosurfactants, which break down microbial membranes and prevent the formation of biofilms, cause many chronic infections. When it comes to multidrug-resistant pathogens, they exhibit antibacterial, antifungal, antiviral, and anti-adhesive properties. (de Paniagua-Michel, et al. 2014). Some biosurfactants are cytotoxic to cancer cells, preventing them from proliferating and differentiating. As a result, they are promising candidates for new anticancer treatments. (Elsheikh. 3 et.al.,2017). Biosurfactants act as biocompatible drug carriers and improve the solubility and bioavailability of hydrophobic medications. By inhibiting pathogen colonization, their antimicrobial and anti-biofilm qualities aid in the promotion of wound healing. (Gudia et al. 2013) Industrial and agriculture application of biosurfactant in, food industry biosurfactants are generally used to enhance texture and shelf life, they are utilized as natural emulsifiers, stabilizers, and foaming agents. They are also Used in skin care and cosmetic products because of their antimicrobial, anti-inflammatory, and moisturizing properties as well as their capacity to create stable emulsions. These biosurfactants also improves nutrient availability and managing phytopathogens, biosurfactants—also used as biopesticides and biofertilizers—promote plant growth in agriculture field. (Elsheikh. et.al.,2017). They are environmentally friendly substitutes that work well in harsh pH, salinity, and temperature environments in cleaning supplies and detergents. (Elsheikh. et.al.,2017), (Gudia, et al., 2013). The research project focuses on identifying and characterizing Biosurfactant producing microorganisms found in garage soil, with the goal of exploring their potential industrial applications. This study will involve isolating these microorganisms examining their ability to produce biosurfactants and analysing the properties of the biosurfactants they generate. Ultimately, the research aims to demonstrate the potential of garage soil as a source of these valuable compounds for various sectors.

## 2. MATERIALS & METHODS

The project was carried out at the Scientific & Industrial Research Centre (SIRC), Gorguntepalya, Yeshwanthpur Industrial area, Bangalore, Karnataka.

**2.1. Collection of soil sample:** 50g of 2 different garage soil samples, contaminated with crude oil, were procured at Yeshwanthpur Industrial area, in zip lock pouches.

**2.2. Enrichment and Isolation of potent bacteria using mineral salt medium:** Biosurfactant-producing bacteria was enriched using MSM Broth composed of (g/l), $KH_2PO_4$ (0.3), $K_2HPO_4$(4.4), NaCl (1.1), KCl (1.1), $NaNO_3$(15), $FeSO_4$ (0.0002), $MgSO_4$(0.5), and yeast extract (0.5). The broth was prepared and dispensed into two (500ml) screwcap flasks, by adding 225ml of MSM Broth. The media was autoclaved and allowed to cool at room temperature, 25g of each soil sample was added, and kept for incubation at 37 °C for 48 hours. 1ml of $10^{-4}$ dilution was inoculated via the pour plate method. The plates were kept for incubation at 37 °C for 48 hours. After incubation, four different colonies were selected and pure culture was obtained by streaking. (Faisal. et.al., 2023)

**2.3. Screening of biosurfactants:**100ml MSM Broth was prepared, and 25ml of broth was dispensed into 4 test tubes labelled as isolates (1, 2, 3, 4). A loopful of colony (1, 2, 3, & 4) was inoculated into respective tubes and incubated at 37°C for 48 hours.

**2.3.1. Drop collapse test:** 2ml of crude petroleum oil was taken in 4 glass vials with screw cap. A drop of isolates 1, 2, 3, and 4 inoculum was added to each vial and allowed to incubate for 2minutes (Jain et.al., 1991)

**2.3.2. Oil displacement test:** A thin layer of oil is placed on 4 petri dishes, and a drop of each culture inoculum is added and observed for the clear zone to appear. The diameter of the clear zone is measured in mm at different time intervals (Morikawa, et.al., 2000).

**2.3.3. Emulsification test:** 1ml of crude oil was taken in 4 glass vials with screw cap and 2 ml of 4 inoculum samples was added to each vial respectively and was mixed vigorously to form the foam. The initial height was measured, and the mixture was allowed to stand at room temperature for 24 hrs, and the final length was measured. (Faisal, et.al., 2023)

**2.4. Identification of potential bacteria:** Various biochemical test was performed to identify the bacterial strain like Gram staining, endospore staining, indole test, methyl red test, vogues Proskauer test, catalase test, oxidase test, starch hydrolysis test, hydrogen sulphide test, urease test, citrate test and carbohydrate test (Cappuccino,2013)

**2.5. Optimization: -**

**2.5.1. Inoculum preparation:** 1000ml of MSM broth was prepared and distributed into 4 test tubes equally and autoclaved and cooled the tubes, a loop full of inoculum was added and incubated at 37° °C for 48 hours. 10ml of each sample was transferred into centrifuge tubes and centrifuged at 5000rpm for 15 minutes. Supernatant was collected and this is known as crude biosurfactant.

**2.5.2. Extraction of biosurfactant:** 10ml of crude biosurfactant was acidified to pH 2.0 using 6N HCl and Incubated at 4°C for 1hr. Then it was transferred into separating funnel to which 20ml chloroform and 10ml of methanol was added and mix vigorously. solvent layer was collected in pre-weighted evaporating dish. Evaporate on a water bath and cool the dish, then record the final weight of the dish.

Calculation: - Percentage of biosurfactant= $\frac{W_2-W_1}{W_1} \times 100$

Where, $W_1$= pre weight of the evaporating dish

$W_2$= final weight of the evaporating dish with biosurfactant.

Optimization was performed to provide efficient environmental factors for microorganisms to enhance the productivity by changing one variable at a time keeping other factors fixed at a specific set of condition. 500ml of MSM broth was prepared and used in optimization process under different factors considering in this experiment are shown in Table 1. All of the procedures were conducted in boiling test tubes containing 25ml of sterile MSM broth. (Faisal, et.al., 2023)

***Table-1: Optimization of factors to increase the yield of biosurfactant for identified strain***

| Factors | Range |
|---|---|
| pH | 4,5,6,7,8 |
| Temperature (°C) | 25,30,35,40 |
| Incubation period (hrs) | 24,48,72 |
| Inoculum size (µl) | 63,188,250,313 |
| Carbon | Glucose, Sucrose |
| Nitrogen | Peptone, Yeast extract |

**2.6. Production of biosurfactant:**

100ml of MSM broth with optimum pH was prepared, autoclaved and cooled. Then optimum inoculum size culture was added and incubated at optimum temperature and time period. After incubation extraction of biosurfactant was done using above mentioned steps (2.5.2). The obtained biosurfactant was collected in the vials for further process (Qazi et.al., 2013).

**2.7. Characterization of biosurfactant:**

The obtained biosurfactant was dissolved using DMS (dimethyl sulfoxide). Extracted crude biosurfactant from cell free culture were analysed by TLC (thin Layer Chromatography) using silica gel. Chloroform- methanol-water (65:15:2 v/v) solvent system was used. Various colour developing reagents such as ninhydrin 0.2% in ethanol 16 for lipopeptide, 1-5% of H2SO4 followed by heating at 110° C for 20 minutes for glycolipids and iodine vapours for lipids were used to identify the type of biosurfactant (Sun, et.al., 2018 & Chaurasia, et.al., 2020)

**2.8. Applications:**

**2.8.1. Antimicrobial activity of obtained crude biosurfactant:** 100ml nutrient agar and 100ml CPZ agar was prepared was set to 7 and autoclaved. Sterile media was cooled at room temperature and poured to petriplates. Solidified nutrient agar plates were inoculated with test bacteria like *Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus* and *Staphylococcus aureus.* CPZ agar plates were inoculated with test fungi *Fusarium, Penicillium, Trichoderma* and *Aspergillus niger* swabbing method. Wells were punched using well borer and 100µl of dissolved biosurfactant was added. Nutrient agar plates were incubated at 37°C for 24hrs and CPZ agar plates were incubated for 4 days at 30°C and observed for the zone of clearance. (Ohadi, M et.al., 2020: jiang, et.al., 2019)

**2.8.2. Bioremediation property:** Three sample of soil and water was taken to measure the oil and grease present in it before and after biosurfactant treatment by gravimetrically. 50ml of sample was taken in beaker and acidified to pH 2. Then it was transferred to separating funnel followed by 30ml of hexane was added to sample and mixed thoroughly by releasing pressure eventually. The solvent layer was collected to evaporating dish by passing through sodium sulphite. The empty weight of evaporating dish was measured and later after evaporation the weigh was measured. The steps were repeated for after biosurfactant treatment where 100µl of biosurfactant was added and repeated the procedure. (Adebajo, et.al., 2020).

The bioremediation property was calculated using standard formula:

$$\text{Percentage of oil(mg/lt)} = \frac{W_2 - W_1 \text{ X } 1000 \text{ X } 1000}{\text{Volume of sample}}$$

## 3. RESULT AND DISCUSSION:

**3.1. Isolation of bacteria:** Several colonies were obtained on MSM agar, four different bacteria (YS1, YS2, YS3 & YS4) were selected by enrichment technique and pure culture was obtained. The process was performed at many steps to ensure bacterial isolate that was obtained at the end of the enrichment cycle could be better biosurfactant producers which can degrade crude oil or hydrocarbons.

**3.2. Screening of biosurfactant**

**3.2.1. Drop collapse test:** Drop Collapse test is a qualitative test which is quick and simple method used to screen for biosurfactant producing microorganism, in which the drop collapses at the bottom of the screw cap bottle in the experiment out of four isolates, YS2 collapsed quickly due to lower interfacial tension between the inoculum and the oil surface. While isolate 1, 3, and 4 remained stable for brief period of time. Therefore YS2, active biosurfactant bacteria was used for further studies. Researcher have recommended the use of drop collapse test during the isolation of biosurfactant producer. Most of the biosurfactant producers give positive results in this test, suggesting that it is one of the most reliable methods. The stability of the drop depends on surfactant concentration and corelates with surface and interfacial tensions (Faisal, et.al., 2023).

***Table 2: Drop collapse test for the isolated samples.***

| Sample | YS1 | YS2 | YS3 | YS4 |
|---|---|---|---|---|
| Drop collapse test (Time in minutes) | More than 5 | Less than 2 | More than 5 | More than 5 |

**3.2.2. Oil displacement test:** It is a quantitative reliable and rapid method used assess the surface activity of biosurfactant produced bacteria by observing the clear zone formed when inoculum is added to an oil - water interface a clear zone diameter indicates biosurfactant activity. YS2 showed high oil – water interface forming larger zone of diameter, while other 3 isolates inoculum were not potentially active compared to YS2. Zeena Ghazi Faisal and her colleagues in their experiment results concluded that, different biosurfactant producer showed diameter of displaced circle ranging from 3.66 ± 1.33mm to 77.66 ± 0.33mm and no clear zone was observed with water. (Faisal, et.al., 2023).

***Table 3: Oil displacement test for the isolated samples.***

| Oil displacement | Initial diameter (in cm) | Final diameter (in cm) | | |
|---|---|---|---|---|
| | | 5mins | 10mins | 15mins |
| YS1 | 0.4 | 0.5 | 0.6 | 0.7 |
| YS2 | 0.4 | 0.6 | 0.7 | 0.7 |
| YS3 | 0.4 | 0.4 | 0.5 | 0.7 |
| YS4 | 0.4 | 0.6 | 0.6 | 0.7 |

**3.2.3. Emulsification test:** It is a test which evaluates biosurfactants ability to stabilize emulsion. It involves mixing the biosurfactant with an oil and measure the height of the stable emulsion layer for different time period. Out of the 4 isolates, YS2 has shown higher emulsification index of 2cm at 24 hours, compared to other 3 isolates. Zeena Ghazi Faisal and colleagues in their experiment result showed a variable emulsification activity ranging from 9.66 ± 0.66% to 64.66 ± 0.33% indicating the ability of bacterial isolates to produce different amounts of biosurfactants that enhance oil contact with water (Faisal, et.al., 2023).

***Table 4: Emulsification test for isolated samples***.

| Samples | Initial height of emulsified liquid (in cm) | Final height of emulsified liquid (in cm) |
|---|---|---|
| YS1 | - | 1.4 |
| YS2 | 1.5 | 2.0 |
| YS3 | - | 1.8 |

| | | |
|---|---|---|
| YS4 | 0.6 | 0.9 |

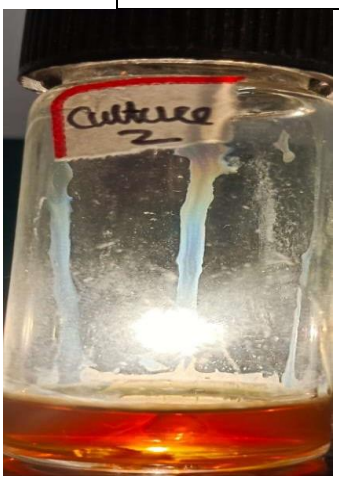


*Figure 1: Drop collapse test*

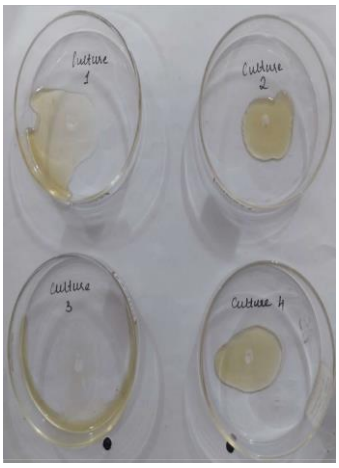


*Figure 2: Oil Displacement test*


*Figure 3: Emulsification test*

**3.3. Staining techniques**

**3.3.1. Gram staining: -** Gram staining was performed to identify the Gram characteristics of biosurfactant producing bacteria. YS2 was identified as Gram positive bacilli in chains (fig 1).

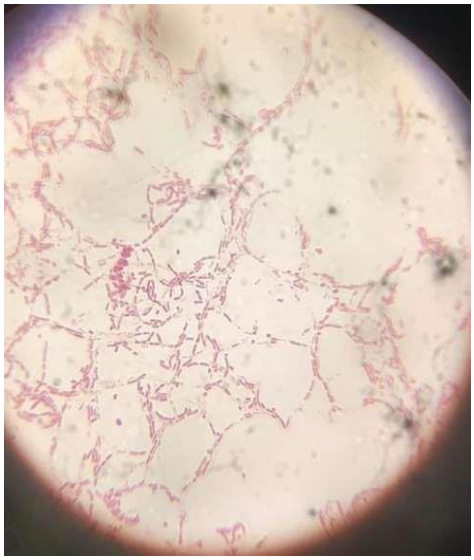

***Figure 4: Gram staining- Gram positive Bacilli in chain.***

**3.3.2. Endospore staining: -** Endospore staining was performed by Schaffer-Fulton method. YS2 showed the presence of endospores stained green and vegetative cells were pink (fig 2).

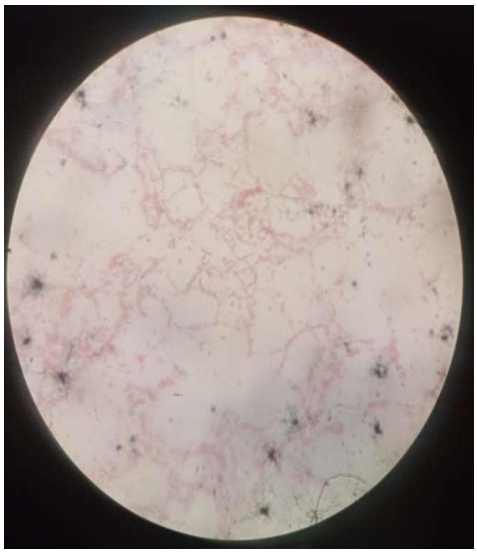

*Figure 5: Endospore staining*

### 3.4. BIOCHEMICAL TEST:

*Table 5: The results of various biochemical tests are tabulated below*

| Sl. no | Biochemical test | RESULT |
|---|---|---|
| 1 | Indole test | Negative |
| 2 | Methyl red test | Negative |
| 3 | Vogus Proskauer Test | Positive |
| 4 | Citrate test | Positive |
| 5 | Hydrogen sulphide Test | Negative |
| 6 | H2S Motility Test | Positive |
| 7 | Carbohydrate fermentation test | Lactose- negative<br>Maltose- negative<br>Sucrose- negative |
| 8 | Urease test | Positive |
| 9 | Oxidase test | Positive |
| 10 | Catalase test | Positive |

Based on Gram staining, endospore staining and biochemical tests, the isolate was found to be Gram positive endospore forming bacilli, the results gave positive result for Voges Proskauer, citrate, urease, oxidase, and catalase tests. The bacterium exhibited motility and did not ferment common sugars (lactose, maltose, sucrose), and did not produce hydrogen sulphide. A dichotomous key method (based on Bergey's manual) was used to characterize the organism up to species level and the organism was identified as Bacillus cereus (Holt, et.al., 1994)

### 3.5. Optimization

*Table 6: Result for Optimization of factors to increase the yield of biosurfactant for identified strain*

| Factors | Optimum | Yield (%) |
|---|---|---|
| pH | 6 | 0.084% |
| Temperature (°C) | 35 | 0.075% |
| Incubation period (hrs) | 24 | 0.022% |
| Inoculum size (µl) | 250 | 0.33% |
| Carbon | Glucose | 0.196% |
| Nitrogen | Peptone | 0.087% |

**3.5.1. pH optimization:** pH is crucial environmental factor that significantly impacts microbial growth and survival of different microorganisms have specific pH growth ranges and optima, influencing distribution and activity. Thus the optimum pH of potent biosurfactant producing bacteria was found to be pH 6 with the highest biosurfactant yield of 0.084% for 10 ml of crude sample.

**3.5.2. Temperature optimization:** Temperature plays an important role in bacterial growth and also significantly impact the production of metabolites. Optimizing temperature is directly proportional to the yield. Out of 4 different temperatures, 35°C had maximum yield of 0.075% surfactant than at other temperatures.

**3.5.3. Incubation period:** It involves finding the right time period for microorganism to achieve log phase which doubles the number of microorganisms. Incubation period directly maximizes product yield and simultaneously minimizes waste and energy consumption. Hence, in the experiment conducted the optimum incubation period at which the bacteria produce highest yield of biosurfactant was found to be 24 hours than compared to other incubation time.

**3.5.4. Inoculum size:** Inoculum size is generally defined as the amount or volume of microorganisms added into a growth medium. Optimization of inoculum size plays a significant role in fermentation as appropriate size of inoculum in suitable amount of media

maximize the yield of metabolites or microbial product. In the present study it was found that 250 µl of inoculum per 25ml of MSM broth(substrate) produced highest yield of biosurfactant (0.33%), whereas lower and higher inoculum size decreased the yield.

**3.5.5. Carbon and nitrogen sources:** Both carbon and nitrogen are 2 major components which take part in various metabolic activity from generation of energy to synthesis of metabolites and overall growth of culture. In the present study significant increase of biosurfactant was seen where basal media was incorporated with glucose as carbon source and peptone as nitrogen source at optimum temperature and pH as compared to other components.

## 4. CHARACTERIZATION OF BIOSURFACTANT

TLC (Thin Layer Chromatography) is an analytical technique used for the separation and identification of the molecules from mixture using thin layer of absorbent material (stationary phase) and a mobile phase (solvent). The molecules separate due to capillary action based on the difference in affinities of the compounds for the stationary and mobile phases. TLC analysis conducted for characterization of crude biosurfactant showed reddish pink spot for ninhydrin test indicating presence of lipopeptide (fig. 6), brown spot for glycolipid by $H_2SO_4$ test (fig. 7) and brown spot for lipids in iodine vapours test (fig. 8)

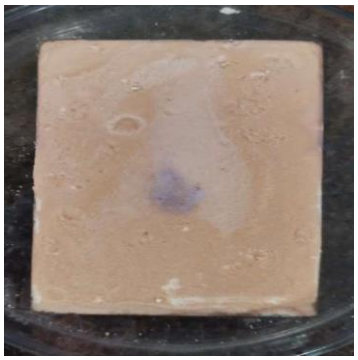

*Figure 6: Ninhydrin Test*

*Figure 7: $H_2SO_4$ Test*

*Figure 8: Iodine vapours test*

## 5. ANTIMICROBIAL ACTIVITY:

Antimicrobial property refers to the inhibitory action of a compound towards microbial growth, it means that either the microbial growth is inhibited or completely killed. Antimicrobial activity of biosurfactant is one of the important applications which makes biosurfactant, a potent candidate in medical, pharmaceutical, and agriculture fields, supressing the pathogenic activity by either disrupting cell membrane, inhibiting cell wall synthesis, or by interfering with other cellular processes. In the present study, the bacterial biosurfactant has shown antimicrobial activity against Escherichia coli of 2mm of zone of clearance and Staphylococcus aureus of 1mm of zone of clearance (fig. 9), fungal colonies of Fusarium of 30mm, Trichoderma of 20mm and A.niger of 21mm zone of clearance, the minimum inhibitory concentration is recorded (fig. 10).

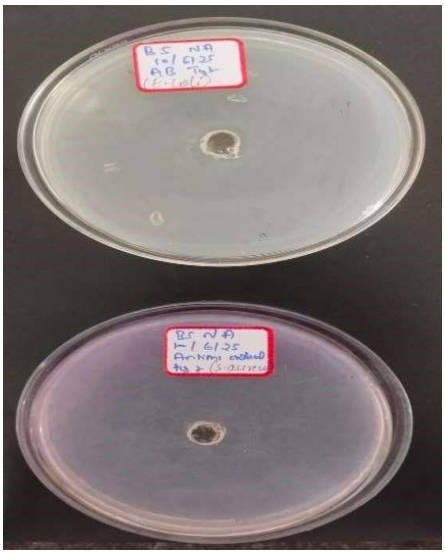

*Figure 9: Antibacterial activity of biosurfactant*

*Figure 10: Antifungal activity of biosurfactant*

## 6. BIOREMEDIATION:

Bioremediation is an eco-friendly approach which uses living microorganisms to clean up polluted soil and water which are contaminated with oil pollutants. Bioremediation is one of the most important applications of biosurfactant which has shown promising result in decontamination of hydrocarbon contaminated soil and water. In the present study bioremediation activity of

produced biosurfactant was analysed. Where effluent water and soil samples contaminated with hydrocarbons in soil and water samples were found to be decreased. From the table 6, it was clearly observed that after adding biosurfactant, there was a significant reduction in the concentration of crude oil and grease residues in soil and effluent water samples.

***Table 7: Effect of biosurfactant on bioremediation***

| Sources | Before biosurfactant(mg/l) | After biosurfactant(mg/l) |
|---|---|---|
| Soil (2226) | 240 | 120 |
| Soil (2491) | 40 | 40 |
| Effluent water (4071) | 7000 | 160 |
| Effluent water (4067) | 500 | 140 |
| Effluent water (4070) | 320 | 20 |

## 7. CONCLUSION:

This study focuses on the isolation and characterization of biosurfactant -producing bacteria from hydrocarbon-contaminated garage soil. Although biosurfactant can be derived from fungi, yeasts, and various bacterial species, the present research emphasizes bacterial biosurfactant production due to its eco-friendly nature and potential to replace synthetic surfactants in industrial, agricultural, and medical fields. Soil samples were enriched using mineral salt medium (MSM) broth, leading to the isolation of four bacterial colonies. These isolates were screened using drop collapse, emulsification index, and oil displacement tests. One potent strain demonstrating the highest biosurfactant activity was selected for further analysis. The isolate was identified as Bacillus cereus.

Optimization studies revealed the best production conditions: pH 7, temperature 35°C, a 24-hour incubation period, and an inoculum size of 25 µL. Glucose and peptone served as the most effective carbon and nitrogen sources, respectively. Under these optimized conditions, the strain was cultivated for large-scale biosurfactant production in MSM broth. The crude biosurfactant was extracted and dissolved in DMSO for antimicrobial testing. The compound showed strong antibacterial and antifungal activity against selected pathogens. Thin-layer chromatography (TLC) analysis confirmed the biosurfactant as lipid-type.

This investigation confirms that garage soil harbours efficient biosurfactant-producing bacteria with substantial antimicrobial and bioremediation potential. These findings underscore the possibility of using indigenous bacterial strains as a sustainable, non-toxic alternative to synthetic surfactants in environmental and industrial applications.

## REFERENCE

[1] Adebajo, S. O., Akintokun, P. O., Ojo, A. E., Akintokun, A. K., & Badmos, O. A. (2020). Recovery of biosurfactant using different extraction solvents by rhizospheric bacteria isolated from rice-husk and poultry waste biochar amended soil. Egyptian Journal of Basic and Applied Sciences, 7(1), 252–266. B6. Microbial Pathogenesis, 138, 103806. https://doi.org/10.1016/j.micpath.2019.103806

[2] Chandankere, R., Yao, J., Choi, M. M. F., Masakorala, K., & Chan, Y. (2013). An efficient biosurfactant-producing and crude-oil emulsifying bacterium Bacillus methylotrophicus Utsa isolated from petroleum reservoir. Biochemical Engineering Journal, 74, 46–53.

[3] Chaurasia, L. K., Tamang, B., Tirwa, R. K., & Lepcha, P. L. (2020). Influence of biosurfactant-producing Bacillus tequilensis LK5.4 isolate of kinema, a fermented soybean, on seed germination and growth of maize (Zea mays L.). 3 Biotech, 10(7), 297. https://doi.org/10.1007/s13205-020-02281-7

[4] da Silva, I. G. S., de Almeida, F. C. G., da Rocha Silva, N. M. P., Casazza, A. A., Converti, A., & Sarabu, L. A. (2020). Soil bioremediation: Overview of technologies and trends. Energies, 13, Article 6028. https://doi.org/10.3390/en13226028.

[5] de Paniagua-Michel, J. J., Olmos-Soto, J., & Morales-Guerrero, E. R. (2014). Algal and microbial exopolysaccharides: New insights as biosurfactants and bioemulsifiers. Advances in Food and Nutrition Research, 73, 221–257. https://doi.org/10.1016/B978-0-12-800268-1.00013-6

[6] Elsheikh, M., Moya-Ramírez, I., Moens, H., Roelants, S., Soetaert, W., Marchant, R., & Banat, I. M. (2017). Rhamnolipids and laconic chlorolipids: Natural antimicrobial surfactants for oral hygiene. Journal of Applied Microbiology, 1235), 1111–1123.

[7] Faisal, Z. G., Mahdi, M. S., & Khalid, K. (2023). Optimization and chemical characterization of biosurfactant produced from a novel Pseudomonas guguanensis Iraqi ZG.K.M. International Journal of Microbiology, 2023. https://doi.org/(insert actual DOI if available).

[8] Jain, D. K., Collins-Thompson, D. L., Lee, H., & Trevors, J. T. (1991). A drop-collapsing test for screening surfactant-producing microorganisms. Journal of Microbiological Methods, 13(4), 271–279. https://doi.org/10.1016/0167-7012(91)90064-M

[9] Gudia, E. J., Rangarajan, V., Sen, R., & Rodrigues, L. R. (2013). Potential therapeutic applications of biosurfactants. Trends in Pharmacological Sciences, 34(12), 667–675.

[10] Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994). Bergey's Manual of Determinative Bacteriology (9th ed.). Williams & Wilkins.
[11] Jiang, X., Yan, X., Gu, S., Yang, Y., Zhao, L., He, X., Chen, H., Ge, J., & Liu, D. (2019). Biosurfactants of Lactobacillus helveticus for biodiversity inhibit the biofilm formation of Staphylococcus aureus and cell invasion. Future Microbiology, 14, 1133–1146.
[12] Khademolhosseini, R., Jafari, A., Mousavi, S. M., Hajfarajollah, H., Noghabi, K. A., & Manteghian, M. (2019). Physicochemical characterization and optimization of glycolipid biosurfactant production by a native strain of Pseudomonas aeruginosa HAK01 and its performance evaluation for the MEOR processes. Advanced Materials Research, 9, 7932–7947.
[13] Morikawa, M., Hirata, Y., & Imanaka, T. (2000). A study on the structure–function relationship of lipopeptide biosurfactants. Biochimica et Biophysica Acta (BBA) - Molecular Cell Biology of Lipids, 1488(3), 211–218. https://doi.org/10.1016/S1388-1981(00)00123-4
[14] Ohadi, M., Forootanfar, H., Dehghannoudeh, G., Eslaminejad, T., Ameri, A., Shakibaie, M., & Adeli-Sardou, M. (2020). Antimicrobial, anti-biofilm, and antiproliferative activities of lipopeptide biosurfactant produced by Acinetobacter junii
[15] Qazi, M., Malik, Z., Qureshi, G., Hameed, A., & Ahmed, S. (2013). Yeast extract as the most preferable substrate for optimized biosurfactant production by rhlB gene positive Pseudomonas putida SOL-10 isolate. Journal of Bioremediation & Biodegradation, 4(2). https://doi.org/10.4172/2155-6199.1000187
[16] Ravichandran, A., (2024). Biosurfactant: sustainable alternative to synthetic surfactants and their applications. International Journal of Applied Pharmaceuticals, 16(2), 34-43.
[17] Rawat, G., Dhasmana, A., & Kumar, V. (2020). Biosurfactants: The next generation biomolecules for diverse applications. Environment Sustainability, 3, 353–369.
[18] Sharma, J., Sundar, D., & Srivastava, P. (2023). Advantages and disadvantages of biosurfactants over other synthetic surfactants. In Biosurfactants (pp. 505–523).
[19] Sun, W., & Cao, W. (2018). Isolation and characterization of biosurfactant producing diesel oil degrading Pseudomonas sp. CQ2 from Changing oil field, China. RSC Advances, 8(69), 39540–39547. https://doi.org/10.1039/C8RA06349K
[20] Zou, C., Wang, M., Xing, Y., Lan, G., Ge, T., Yan, X., & Gu, T. (2014). Characterization and optimization of biosurfactants produced by Acinetobacter baylyi ZJ2 isolated from crude oil-contaminated soil sample toward microbial enhanced oil recovery applications. Biochemical Engineering Journal, 90, 49–58.