

Evaluation of Biosurfactant Production Capabilities in Bacterial Strains Isolated from Oil-Contaminated Soil

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ABSTRACT

This study aims to investigate the biosurfactant production capabilities of bacterial strains isolated from oil-contaminated soil samples. Employing a comprehensive methodological approach, we collected soil samples from thirty different fuel pumps and conducted an extensive screening of bacterial isolates using various tests such as hemolytic activity, emulsification index, blue agar plate method, and drop collapsing test. The results revealed significant biosurfactant production among certain isolates, specifically highlighting the effectiveness of two particular strains. This paper presents a detailed analysis of these strains, including their optimal growth conditions in terms of pH, temperature, carbon, and nitrogen sources. Our findings indicate a notable potential of these bacterial strains in biosurfactant production, with implications for environmental bioremediation, particularly in oil pollution contexts. The study also sheds light on the limitations encountered and underscores the originality of the research in exploring biosurfactant production in a novel context. This study contributes to the field by offering insights into the effective utilization of microbial strains for biosurfactant synthesis, which is crucial for sustainable and eco-friendly bioremediation practices.

Keywords: biosurfactant production, oil-contaminated soil, bacterial strains, bioremediation, environmental microbiology, biochemical screening, sustainable biotechnology.

INTRODUCTION

The escalating interest in the study of biosurfactants (BS) in recent years is largely driven by their potential applications in a wide array of industries, including but not limited to environmental remediation, the pharmaceutical industry, food processing, agriculture, petrochemicals, paper, and pulp (Vater et al., 2002; Wang et al., 2004; Romero et al., 2007; Mizumoto et al., 2007). Biosurfactants, which are amphiphilic compounds, have gained prominence due to their exceptional ability to reduce surface and interfacial tension, a property that makes them invaluable in processes such as emulsification, foaming, dispersion, and detergency (Rodrigues et al., 2006). This unique characteristic stems from

their structural makeup, containing both hydrophilic and hydrophobic moieties, which allows them to interact effectively at liquid interfaces (Mukherjee et al., 2005).

The importance of biosurfactants goes beyond their functional advantages; they are increasingly valued for their eco-friendly profile. Compared to traditional chemical surfactants, biosurfactants offer the advantages of low toxicity, environmental benignity, and biodegradability, making them a more sustainable and environmentally responsible choice for various industrial applications (Bach et al., 2002; Singh et al., 2004). This shift towards biosurfactants is part of a broader trend in scientific research and industrial practices that prioritize environmental preservation and sustainability. However, a significant gap in the field

remains the efficient and cost-effective production of biosurfactants. Identifying and utilizing microbial strains capable of prolific biosurfactant production remains a formidable challenge (Das and Mukharjee, 2007; Mullirigan et al., 2005). Our research addresses this issue by focusing on the evaluation of bacterial strains isolated from soil samples in oil-polluted areas, specifically around fuel pumps. The study aims to discover and optimize strains that demonstrate potent biosurfactant production capabilities. By examining these strains under various culture conditions, we seek to develop a deeper understanding of the biosynthesis processes in these microorganisms and their potential scalability for industrial applications.

The overarching goal of this research is twofold: to advance scientific knowledge in the field of biosurfactant production and to explore practical solutions for environmental challenges, particularly in managing oil pollution (Singh et al., 2007; Van Hamme et al., 2006). This study, therefore, not only contributes to the scientific understanding of microbial biosurfactant synthesis but also underscores the practical implications of these findings in environmental biotechnology, particularly in the context of bioremediation and sustainable environmental management.

MATERIAL AND METHODS

Soil sample collection and preliminary assessment

Understanding the unique challenges associated with soil contamination at gas stations, which are typically paved, our study adopted a strategic approach to sample collection. The selection of soil samples from beneath the paved surfaces of thirty distinct gas stations was based on the premise that oil and other hydrocarbon compounds can percolate through pavements and accumulate in the underlying soil. To ascertain the contamination levels, preliminary assessments were conducted using simple hydrocarbon detection methods. These methods provided a qualitative indication of oil contamination, guiding our selection of soil samples for further analysis.

Laboratory contamination of soil samples

In addition to collecting naturally contaminated soil samples, our study also involved artificially

contaminating soil samples in the laboratory. This step was included to create a controlled environment where the extent and type of contamination were precisely known, thus allowing for a more robust comparison of bacterial strain efficacy. The soil samples were contaminated with specific quantities and types of oil, mimicking the contamination levels typically found at gas station sites. This controlled setup helped in standardizing the conditions for bacterial growth and biosurfactant production studies.

Isolation and enrichment of microorganisms

Following the collection, both naturally and artificially contaminated soil samples were subjected to a microbial enrichment process. This process involved incubating the soil samples in sterile Mineral Salt Medium (MSM), providing an optimal environment for the growth of native microorganisms capable of surviving in hydrocarbon-rich conditions. The enrichment process was carefully monitored to ensure the development of a diverse microbial population, from which potential biosurfactant-producing strains could be isolated.

Screening for biosurfactant-producing bacteria

The enriched samples underwent a rigorous screening process to identify bacterial strains capable of producing biosurfactants. This screening involved several tests, such as hemolytic activity, emulsification index (E24), blue agar plate (BAP) method, and drop collapsing test. Each of these methods provided insights into the biosurfactant production capabilities of the bacterial isolates, allowing us to narrow down the strains with the most potential.

Optimization of physico-chemical parameters for biosurfactant production

Selected bacterial strains were further analyzed to determine the optimal conditions for biosurfactant production. This involved varying culture conditions such as pH, temperature, and the supply of carbon and nitrogen sources. The aim was to identify the physico-chemical parameters that maximized biosurfactant yield and efficacy, essential for potential scale-up and industrial application. Through this comprehensive approach, the study not only isolated potent

biosurfactant-producing bacteria but also optimized the conditions for their growth and biosurfactant synthesis, contributing valuable insights to the field of environmental biotechnology.

Collection of soil samples and enrichment of microorganisms

Samples of oil-contaminated soil were gathered from thirty distinct gas stations, and they were then enhanced in sterile Mineral Salt Medium (MSM). In summary, 15 mL of Minimal Salt Medium was inoculated with 30 mg of each soil sample, and the mixture was then cultured for 48 hours at 37°C at 100 rpm. Following incubation, sterile saline (0.85% NaCl) was used to serially dilute the samples from 10^{-1} to 10^{-4} .

Each sample's 10^{-4} dilutions were streaked on Nutrient Agar (NA) plates, and they were then incubated for 24 to 48 hours at 37°C. Several bacterial isolates were chosen and analysed for the ability to produce biosurfactant based on the shape of their colonies. Table 1 shows the locations of the 30 fuel stations that were chosen for sample collection.

Selection of bacterial isolates for screening process

The screening method included the selection of isolates with morphologically distinct colonies based on the morphology of their colonies. For screening purposes, ten sites were chosen from among the bacterial isolates. These locations were Vallabh Nagar 1, Nehrunagar, Chinchwad, Baner 1, Dapodi 1, Dapodi 2, Dapodi 3, Ajmera 1, Ajmera 2, Ajmera 3

Screening of biosurfactant producing organisms

We used four different techniques to examine the biosurfactant production of the isolated colonies: hemolytic activity, emulsification test (E24), blue agar plate (BAP) method, and drop collapsing test.

Drop collapsing test

To screen for biosurfactant synthesis, the qualitative drop-collapse test developed by Boudour and Maier (1998) was utilized. The process began with the application of 2µl of crude oil to each well location on a 96-well microplate cover, followed by a 24-hour acclimatization period. Bacterial cultures were grown for approximately 48 hours and then subjected to centrifugation at 12,000 g for 5 minutes. The supernatant from this process was then applied to the areas of the well covered with oil. After one minute, the size of the drop was assessed using a magnifying lens. A positive result for biosurfactant production was indicated if the drop appeared flat. Conversely, cultures that produced rounder droplets were considered negative, indicating low biosurfactant synthesis, as noted by Youssef et al. (2004). This method provides a straightforward and effective way to evaluate the biosurfactant-producing capabilities of different bacterial cultures.

Blue agar plate (BAP) method

To detect the presence of anionic biosurfactants, a method described by Satpute et al. (2008) was employed, which involved the use of cetyltrimethylammonium bromide (CTAB, 0.5 mg/mL)

Table 1. List of 30 different petrol pumps and location

Location	Petrol Pump	Location	Petrol Pump	Location	Petrol Pump
Chinchwad (C)	HP	Kasarwadi Chowk (K2)	HP	Pimpri Gaon (Main Chowk) (PG1)	HP
Chapekar Chowk (C1)	SHELL	Kasarwadi Bus stop (K3)	HP	Pimpri Gaon (PG2)	BP
Pimpri (P1)	HP	Dange Chowk (D1)	HP	Ajmera Road (AJ1)	HP
Pimpri Highway (P2)	HP	Dange Chowk (Signal) (D2)	HP	Ajmera (AJ2)	HP
Vallabh Nagar (V1)	SHELL	Pimple Gurav (Bridge) (PGu1)	IO	Ajmera Signal (AJ3)	SHELL
Vallabh Nagar Bus stop (V2)	SHELL	Pimple Gurav (PGu2)	HP	Dapodi (D1)	HP
Vallabh Nagar Chowk (V3)	IO	Banner (B1)	SHELL	Dapodi (D2)	SHELL
Tathawade (Near BioEra) (T1)	HP	Banner Phata (B2)	HP	Udhyamnagar (UD)	HP
Tathawade (T2)	HP	Dapodi Bridge (Signal) (DB1)	HP	Nehrunagar (N)	BP
Kasarwadi (K1)	BP	Dapodi Bridge (DB2)	SHELL	Morwadi (M)	HP

Note: HP – Hindustan petroleum, IO – Indian oil, BP – Bharat petroleum.

and methylene blue (MB, 0.2 mg/mL). These reagents were incorporated into a mineral salt agar medium that was supplemented with 2% glucose as a carbon source.

In this process, 30 µl of cell-free supernatant was deposited into wells created on a methylene blue agar plate using a 4 mm cork borer. The plate was then incubated for a period of 48 to 72 hours at a temperature of 37°C. The production of an anionic biosurfactant was indicated by the formation of a dark blue halo zone around the culture. This method is an effective way to qualitatively assess the production of anionic biosurfactants by microbial cultures.

Emulsification test (E24)

Each of the pure culture colony was placed in a test tube with two millilitres of MSM and allowed to incubate for a full day. Each tube received 2 mL of hydrocarbon (oil) upon incubation. After a minute of vigorous vortexing, the mixture was left to stand for twenty-four hours. This is the formula for calculating the emulsion index (E24) (Bodour et al., 2004).

$$\begin{aligned} \text{Emulsion index} &= \\ &= \left[\frac{\text{The height of the emulsion layer (cm)}}{\text{total height (cm)}} \right] \times 100\% \end{aligned} \quad (1)$$

Hemolytic activity

For the identification of bacterial isolates and their potential to produce biosurfactants, a process outlined by Carrillo et al. (1996) was followed. Initially, pure cultures of the bacterial isolates were streaked onto freshly prepared blood agar plates. These plates were then incubated for a period of 48 to 72 hours at 37°C to allow for bacterial growth and the development of hemolysis patterns.

The type of hemolysis exhibited by the colonies determined their classification:

- α -hemolysis was indicated by the presence of a greenish zone surrounding the colony.
- β -hemolysis was identified when the colony was surrounded by a clear white zone.
- γ -hemolysis occurred when the medium around the colony remained unchanged, showing no signs of hemolysis.

Following this screening process, isolates that tested positive for hemolysis were added to a mineral salt medium to encourage the production of biosurfactants. The strain that produced

the highest amount of biosurfactant was then selected as the optimal strain. This chosen strain underwent further identification through microscopic examination and biochemical testing, as prescribed in Bergey's Manual of Determinative Bacteriology. This comprehensive approach ensured the accurate selection and identification of the most effective biosurfactant-producing bacterial strains.

Purification of biosurfactants

The positive isolates containing biosurfactant were mixed well with 1 ml of distilled water, centrifuged at 7000 rpm for 30 minutes at 4°C, and then allowed to settle. After discarding the supernatant, the particle was given a 24-hour period to dry. The resultant dry pellet was the biosurfactant crude extract.

Optimization of physical and nutrient parameter

A standardised biosurfactant producing isolates inoculum (10%, v/v) was inoculated in 50 mL of MSM, supplemented with 1% (v/v) crude oil, for the purpose of measuring physical and nutritional parameters. For five days, the conical flask with the inoculum was incubated in an orbital shaker with agitation speed set to 150 rpm. MSM without an isolate inoculum was employed as a negative control.

Measurement of surface tension

To assess the physical and nutritional characteristics of biosurfactant-producing cultures, a methodology outlined by Pornsunthorntawe et al. (2008) was employed. This process began with a five-day incubation period for the culture. Post incubation, the culture was centrifuged at 10,000 rpm and a temperature of 4°C for 20 minutes. This step was crucial for separating the biosurfactants from the bacterial cells.

The supernatant, containing the biosurfactant, was then subjected to surface tension measurement using a tensiometer. The results of this measurement were recorded in dynes/cm, a unit that quantifies the force exerted per unit length on the surface of a liquid. To calculate the surface activity of the biosurfactant produced by the bacteria, a specific formula was used. This formula helped quantify the efficiency of the biosurfactant

in terms of its ability to decrease the surface tension of the medium.

This approach provided a quantitative assessment of the biosurfactant's effectiveness, an essential aspect in determining its potential for various applications, such as in environmental remediation or industrial processes.

Effect of pH and temperature on biosurfactant production

The standardised inoculum was inoculated in MSM at various pH values, namely 6, 7, 8, 9, and 10, and then incubated at 37°C on an orbital shaker operating at 150 rpm in order to determine the ideal pH. We found the isolates' optimal growth rate. Following the determination of the ideal pH, the bacteria were cultured in MSM at that pH and incubated for five days at various temperatures—33, 37, and 40°C—at 150 rpm on an orbital shaker.

Effect of carbon source

Different carbon sources (1%, v/v) including glycerol, coconut oil, groundnut oil, and olive oil were used to inoculate the bacterial inoculum in MSM. The cultures underwent five days of optimal temperature and speed incubation at 150 rpm. Following that, the carbon source that produced the greatest amount of biosurfactant and had the lowest surface tension was selected to vary at various carbon concentrations beginning at 0.5, 1, 3, and 4% (v/v).

Effect of different nitrogen source on isolates growth

To determine the optimal nitrogen source for the enhanced synthesis of biosurfactants, an experimental approach was employed where the total nitrogen in the Mineral Salt Medium (MSM), originally provided by NH_4Cl at approximately 4 g/L, was substituted with equivalent amounts of different nitrogen sources. This substitution aimed to evaluate the impact of various nitrogen sources on biosurfactant production.

The process involved incubating a standardized bacterial inoculum on an orbital shaker. This incubation was conducted for five days, under conditions with a predetermined optimized temperature and a constant shaking speed of 150 rpm. During this period, the inoculum was diluted with MSM adjusted to an optimized pH level. This medium was then supplemented with various

nitrogen sources, including ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$, sodium nitrate $[\text{NaNO}_3]$, ammonium chloride $[\text{NH}_4\text{Cl}]$, and ammonium nitrate $[\text{NH}_4\text{NO}_3]$. To further refine the conditions for maximum biosurfactant synthesis, these nitrogen sources were tested at different concentrations, ranging from 0.5 to 4.0 g/L. The selection of the most effective nitrogen source was based on its ability to produce the highest amount of biosurfactant, as indicated by the lowest surface tension activity of the culture medium. This approach allowed for the identification of the nitrogen source that most effectively promotes biosurfactant production, a crucial factor for optimizing the yield and efficiency of biosurfactant synthesis.

RESULT AND DISCUSSION

Collection of soil samples

Figure 1 shows the soil sample that was taken from several sites. For further research, the soil samples were gathered in collecting bags (Figure 1).

Screening of isolates for biosurfactant production

About 72 bacterial isolates were recovered from soil samples contaminated with oil using plate and dilution techniques. They received further biosurfactant activity screening utilising hemolytic, drop collapsing, blue agar, and emulsification index tests. Table 2 shows the results of many tests. Just five isolates yielded good findings across the four screening techniques. Nehrunagar, Banner 1, Ajmera 1, Dapodi 2, and Dapodi 3 are these isolates. Nehrunagar and Ajmera 1 isolates were chosen for further examination out of all the isolates that were deemed to be viable based on the greatest emulsification index. By inoculating these two isolates into the MSM medium, we were able to test them further for maximal biosurfactant synthesis. Bergey's manual of determinative bacteriology was followed in selecting the finest isolates by microscopic and biochemical examination.

Figure 2 presents the test findings. Results for the Hemolytic test, Blue Agar test, Drop Collapsing test, and Emulsification Index test were provided for the samples taken from Nehrunagar and Ajmera 1. In their research on biosurfactant production, Satpute et al. (2008) emphasized the importance of utilizing multiple screening methods



Figure 1. Soil samples collected from 30 different petrol pumps

to effectively identify potential biosurfactant producers. Following this recommendation, the current study employed four different screening tests to isolate biosurfactant-producing microorganisms. One of the employed methods was the blood agar test, the results of which were found to be consistent with those obtained by Mulligan et al. (1984, 1989). Mulligan and colleagues had

previously used this technique to isolate mutants overproducing biosurfactants, demonstrating its effectiveness. Another test used in this study was the drop collapse test, as suggested by Jain et al. (1991). The appearance of a flat drop in the microtiter plate was an indicator of a positive result in the drop collapse test. This outcome highlighted the utility of the drop collapse method as a simple

Table 2. Results of ten isolates’ screening for biosurfactant production*

Sr. no.	Test isolates	Hemolytic assay	Methylene blue agar plate	Drop collapsing test	Emulsification index (E24) %
1.	Vallabh Nagar 1	-	+	+	55.0
2.	Nehrunagar	+	+	+	57.5
3.	Chinchwad	+	-	+	53.5
4.	Banner 1	+	+	+	35.0
5.	Ajmera 1	+	+	+	60.0
6.	Dapodi 1	+	-	+	55.0
7.	Dapodi 2	+	+	+	50.0
8.	Dapodi 3	+	+	+	45.0
9.	Ajmera 2	-	+	+	50.0
10.	Ajmera 3	+	-	+	37.5

Note: * Test isolated mention in bold letters showed positive result for all screening test.

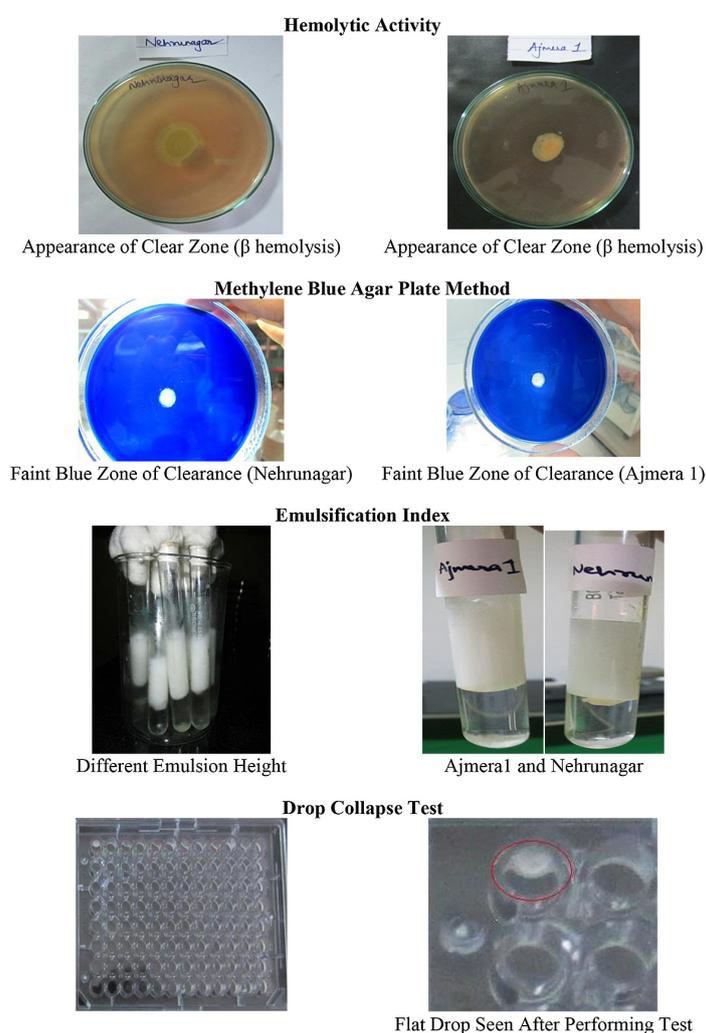


Figure 2. Screening of isolates for biosurfactant production

yet sensitive approach for detecting biosurfactant production. Additionally, the presence of an anionic biosurfactant was indicated by the formation of a dark blue halo zone on a methylene blue

agar plate treated with CTAB (cetyltrimethylammonium bromide). This result further validated the effectiveness of combining various screening methods to accurately identify and isolate

biosurfactant producers. By employing a range of tests, the study was able to more comprehensively assess the biosurfactant-producing capabilities of different microbial cultures.

Isolation of bacterial colonies

One method of obtaining pure culture was to streak loops full of culture on NA plates. Figure 3 displays the NA plates containing the sample isolates from Ajmera 1 and Nehrunagar. Table 3 provides the isolates' colony characteristics. Jaimera One isolated colony had an opaque 5 mm diameter lobate border and was round in shape. It has a damp surface and an elevated height. The Nehrunagar isolation colony has a 1.5 mm transparent colony with a circular shape and lobate edge. Its surface is wet and it has an umbiculated elevation.

Identification and characterization of isolates

In a study involving the isolation and characterization of bacterial strains, the Ajmera 1 isolate was found to test negative for bacteria. However, it exhibited positive results in several biochemical tests, indicating its metabolic capabilities. These tests included oxidase, citrate utilization, and the fermentation of various sugars such as glucose,

maltose, xylose, fructose, and sucrose. Additionally, the isolate showed positive results for nitrate reduction activity, further suggesting its diverse metabolic functions. On the other hand, the isolate from Nehrunagar tested positive for gram-negative pathogens. This was determined based on a series of positive test results, including the Monochrome Stain, oxidase, Catalase, Methyl Red (MR), Voges-Proskauer (VP), citrate utilization, gelatin liquefaction, and the fermentation of glucose, mannitol, mannose, fructose, and sucrose. These findings indicate the presence of a diverse metabolic profile in the Nehrunagar isolate, suggesting its potential for various biochemical processes.

All these test results were summarized in Table 4 of the study. This comprehensive analysis provided a detailed understanding of the biochemical characteristics and metabolic potentials of the isolated bacterial strains. Such information is crucial in determining the suitability of these strains for specific applications, such as in bioremediation, fermentation processes, or as indicators in environmental monitoring. Significant research has been dedicated to treating oil-contaminated soil effectively using biosurfactant treatments and microbial inoculation. A notable study by Noordam et al. (2002) highlighted that *Pseudomonas*



Figure 3. Pure culture of Nehrunagar and Ajmera 1 sample isolates

Table 3. Colony characteristics of the isolates

Colony characteristics	Ajmera 1 isolate	Nehrunagar isolate
Form	Circular	Circular
Size	5 mm	1.5mm
Elevation	Raised	Umbiculated
Surface	Moist	Moist
Edges	lobate	Entire
Opacity	Creamish	Pinkish
Colour	Opaque	Translucent

Table 4. Various test used for characterization of isolates

Sr. No.	Test	Ajmera 1 (AJ1)	Nehrunagar (N)
1.	Gram stain	-	+
2.	Monochrome stain	-	+
3.	Oxidase	+	+
4.	Catalase	-	+
5.	Indole production	-	-
6.	MR test	-	+
7.	VP test	-	+
8.	Citrate	+	+
9.	H ₂ S	-	-
10.	Gelatin liquefaction	-	+
11.	Glucose fermentation	+	+
12.	Mannitol fermentation	-	+
13.	Maltose fermentation	+	-
14.	Mannose fermentation	-	+
15.	Xylose fermentation	+	-
16.	Lactose fermentation	-	-
17.	Fructose fermentation	+	+
18.	Raffinose fermentation	-	-
19.	Sucrose fermentation	+	+
20.	Galactose fermentation	-	-
21.	Nitrate reduction	+	-

aeruginosa produces a rhamnolipid biosurfactant, which facilitates the absorption and subsequent degradation of hydrophobic compounds. This finding is crucial as it demonstrates the potential of biosurfactants in enhancing the bioavailability of otherwise hard-to-degrade pollutants.

In a similar vein, Das and Mukharjee (2007) conducted research showing the efficacy of certain strains of *Bacillus subtilis* (DM 04) and *Pseudomonas aeruginosa* (M and NM) in degrading crude petroleum-oil. These strains were isolated from soil contaminated with petroleum oil in Northeast India and were found to be proficient producers of biosurfactants. This study underscores the ability of these bacteria to not only produce biosurfactants but also to utilize them in breaking down complex hydrocarbon structures found in crude oil. One of the key advantages of using contaminated soil as a source for these bacteria is that it offers an inexpensive, natural, and non-toxic means of obtaining biosurfactants. These biosurfactants play a critical role in dissolving hydrophobic oil molecules, which is a preliminary step before their degradation. Furthermore, the same research by Das and Mukharjee (2007) also revealed that biosurfactants could

significantly increase the apparent solubility of toxic polyaromatic hydrocarbons like pyrene, enhancing it by a factor of five to seven. This increase in solubility, coupled with the observed changes in the hydrophobicity of bacterial cell surfaces, leads to improved absorption and utilization of pyrene by the bacteria.

These findings collectively suggest that biosurfactants are not only effective in cleaning oil-contaminated environments but also have the potential to transform the approach towards environmental remediation, particularly in the context of petrochemical pollution.

Effect of physicochemical parameters on biosurfactant production

At pH 7, both isolates exhibited optimal growth and biosurfactant synthesis. Surface tension decrease reached its highest percentage at pH 7. The optimal temperature was determined to be 37°C. There was the greatest percentage of surface tension drop at this temperature. Figure 4 shows the effects of different pH and temperature ranges. The MSM treated with 1% glycerol (v/v) showed the greatest decrease in surface

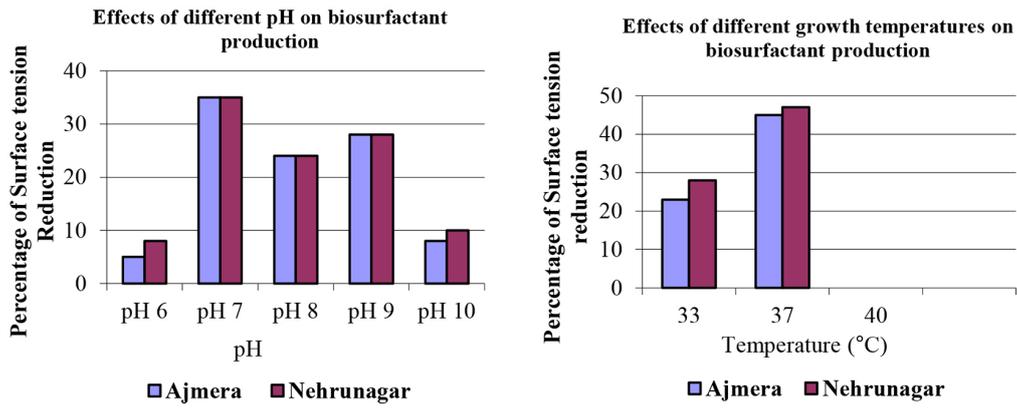


Figure 4. Effect of pH and temperature on the surface tension

tension. In comparison to other chosen concentrations, the MSM supplemented with % glycerol (v/v) concentration showed the greatest decrease in surface tension. Effects of adding various amounts of ammonium sulphate to MSM on the generation of biosurfactants. Five days of 37°C and 150 rpm shaking were required for the bacteria to thrive in MSM (pH 7.0). Use of ammonium sulphate as the

only nitrogen source resulted in the best growth for the formation of biosurfactants. Five days of 37°C and 150 rpm shaking were required for the bacteria to thrive in MSM (pH 7.0). The application of ammonium sulphate at a concentration of 1% resulted in optimal biosurfactant activity. The gramme positive isolate was determined to be Bacillus, and the gramme negative isolate to be Klebsiella. The blood

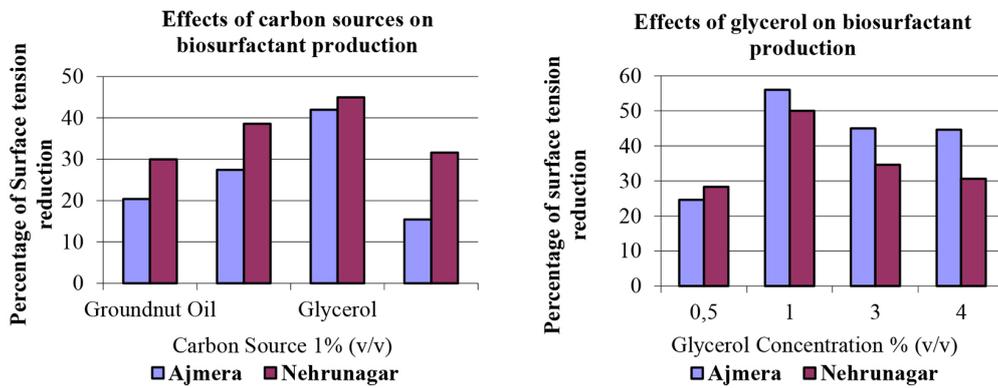


Figure 5. Effect of different carbon sources on the surface tension

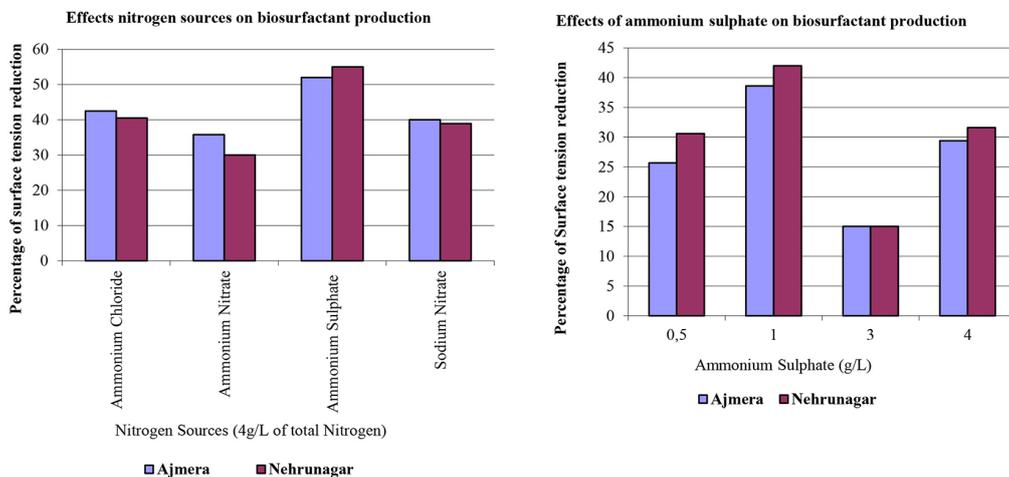


Figure 6. Effect of different nitrogen sources on the surface tension

hemolytic test revealed that both isolates were positive, and their excellent emulsification index (above 55%) was greater than that of previous studies. Up to now, the highest documented emulsification index has been between 47 and 53%.

CONCLUSIONS

Bioremediation, which leverages biosurfactants produced by bacterial colonies, has emerged as an effective method for treating oil pollution. This approach utilizes living microorganisms, predominantly bacteria, which possess the capability to metabolize various types of hydrocarbon compounds under specific conditions. Hydrocarbons, being rich in organic matter, serve as a viable carbon source for these bacteria, enabling them to break down these compounds. In the referenced study, two bacterial isolates were identified as having the highest levels of biosurfactant activity when optimized with various parameters. The identification of these isolates was achieved through a combination of physical characteristics, biochemical testing, and Gram staining. This comprehensive approach allowed for effective identification at the genus level, which is crucial for understanding their metabolic capabilities and potential application in bioremediation processes. Furthermore, the study explored the impact of varying concentrations of nitrogen and carbon sources on these isolates. The results from these experiments were promising, indicating that with the right balance of nutrients, these bacterial isolates could be effectively used in bioremediation processes. This finding is significant as it demonstrates the potential of using specific bacterial strains in targeted bioremediation strategies, particularly for environments contaminated with oil or hydrocarbon pollutants. The successful application of these isolates in bioremediation can lead to more environmentally friendly and efficient methods of managing oil pollution.

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