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Soil to Surfactant: Unveiling Hidden Potential of Microbial Biosynthesis

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Abstract

Biosurfactants are amphiphilic surface-active substances synthesized by various microorganisms. These amphiphilic molecules reduce surface and interfacial tension. Recently, studies of biosurfactants have attracted great interest, since these substances are able to mimic the use of chemical surfactants. They are characterized by environmental friendliness, biodegradability and antimicrobial activity. Biosurfactants are used in various industries (food, pharmaceutical, cosmetic) in agriculture. Biosurfactants are components that effectively reduce the surface tension of the medium with high emulsifying activity. This study focused on isolating and screening biosurfactant-producing microorganisms from soil samples using various qualitative and quantitative methods. Media optimization using OFAT (One-Factor-At-a-Time) approach improved biosurfactant yield. Extraction using ethyl acetate and characterization by FTIR confirmed biosurfactant production. Total 12 isolate were screened for biosurfactant production among them 2 showed positive result. A Bacillus isolate showed a 71% emulsification index and reduced surface tension to 34.14 dynes/cm, highlighting its potential as a sustainable substitute for chemical surfactants.

Keywords: Biosurfactants, One-Factor-At-a-Time, Bioremediation, Biodegradability, Surface Tension.

Introduction:

Biosurfactants are amphiphilic molecules produced by microbes that reduce surface and interfacial tension between liquids, solids, and gases (Desai & Banat, 1997). Their hydrophilic and hydrophobic domains enable them to emulsify oil and water efficiently. Compared to synthetic surfactants, microbial biosurfactants are non-toxic, biodegradable, and environmentally sustainable (Santos et al., 2016). Excess use of chemical fertilizers depletes the richness of soil leads to poor soil health, dryness of soil, water pollution impacting human

health. These properties make Biosurfactant ideal for various industrial applications such as oil recovery, pharmaceuticals, agriculture, and environmental remediation (Singh & Cameotra, 2004; Zhou et al., 2019).

The potential of biosurfactants in biotechnology and environmental applications is continually expanding. Biosurfactants find use in heavy metal removal, enhanced oil recovery, cosmetics, pharmaceuticals, biopesticides, and as emulsifying agents in the food industry. Their low toxicity and high selectivity make them more attractive than their synthetic counterparts. Innovations in downstream processing, extraction techniques, and fermentation have further strengthened the case for large-scale production.

Despite their advantages, biosurfactant commercialization faces limitations such as high production costs, low yields, and scalability issues (Rodrigues et al., 2006). Recent studies have addressed these challenges through optimization strategies, genetic modification, and alternative carbon/nitrogen substrates (Banat et al., 2014; Kalvandi et al., 2022). This research focuses on the isolation, screening, and production of biosurfactants from soil-isolated microbes, with an emphasis on statistical analysis and eco-friendly applications.

Literature Review:

Microbial biosurfactants have been studied for decades for their surface activity and biodegradability. Desai and Banat (1997) outlined the potential of microbial surfactants in replacing chemical counterparts. Later studies emphasized their environmental applications, including bioremediation and oil spill cleanup (Pacwa-Płociniczak et al., 2011).

Banat et al. (2014) discussed cost-effective production strategies using agro-industrial waste as a substrate. Kalvandi et al. (2022) demonstrated successful lipopeptide biosurfactant production by optimizing carbon sources. Adebajo et al. (2020) compared various extraction solvents for biosurfactant recovery, emphasizing the role of solvent polarity in efficiency.

Lipopeptide biosurfactants such as surfactin and glycolipids show strong antimicrobial, emulsifying, and stability properties under diverse conditions (Morikawa et al., 2000). Rodrigues et al. (2006) characterized biosurfactants from *Lactococcus lactis*, noting their surface tension reduction to commercial levels.

Biomedical applications are also emerging; Singh and Cameotra (2004) explored antimicrobial and anti-adhesive roles. Santos et al. (2016) positioned biosurfactants as multifunctional biomolecules of the 21st century due to their versatility. Moreover, their role in nanotechnology and surface coating materials is being actively investigated.

Recent publications further suggest a vital role for biosurfactants in controlling biofilms, particularly in clinical environments where biofilm-forming pathogens complicate treatment. Biosurfactants have shown the ability to disrupt preformed biofilms and inhibit the adhesion of new microbial cells.

Methodology and Results:

1. **Soil Sample Collection and Microbial Isolation**

For this study, samples of soil were collected from Keshav Nagar, Mundhwa, Pune Maharashtra (Latitude: 18.5306 N Longitude: 73.9258" E). This site was selected because of the varied types of soil, the ability to engage in urban agriculture and also due to easy accessibility and convenience. The site has been giving a good combination of organic and Inorganic matter which is vital for the study of microbial diversity. It is worth mentioning that the sampling procedure was carried out in such a way that there was no room for bias and the data was true and accurate. Soil samples were taken from several areas within the region that included shaded areas, open spaces and areas adjacent to Vegetations. This was done so as to obtain microbial populations with different ecological characteristics. Using a sterile conical tube, the top 5-10 cm (about 3.94 in) of soil which is high in organic matter was scooped off to reduce chances of contamination and preserve the sample. Tools that were employed in sampling had to be sterilized in between sampling to avoid cross Contamination. The collected samples were taken to the lab within two hours and without any changes to the ambient temperature to protect the indigenous community of the microbes. After reaching the destination, the samples collected were sieved to take away stones, plants, and any other unwanted materials. The ground soil was then kept in temperatures of 4 °c until it was needed for the further experiments. Cool place with There is no doubt that Keshav Nagar, Mundhwa , was the most appropriate place to choose as the sampling site.

2. **Screening for Biosurfactant Production.**

Oil Displacement technique:

The first method to screen the presence of BS was performed following the protocol given by in clean Petri plate 40 ml Distilled water was taken with drop wise addition of 40 ul oil (Vegetable oil engine oil, petrol) using micro pipette. Cell free supernatant was then placed on oil drop to see the displacement action if Bio surfactant was produced. The Diameter of clear halo visualized under visible light was measured after 30 seconds. A negative control was maintained with distilled water (without surfactant), in which no oil displacement was observed. Out of 12 isolates 3 isolates showed positive results of oil spread with different diameter of oil spread. While isolate 1 showed zone of diameter of 45 mm of 100 mm with was comparatively good in comparison to other 2 isolates. The zone represents the area where the BS interact with and displace the oil. The solvent or surfactant solutions delivered into the surface of oily materials, the oily material membrane would break because of the action of gravity. Further screening was done of isolate 1.

Determination of the emulsification index (E24).Emulsification assay is an indirect method used to screen BS production. It was presumed that if the cell free culture broth contains BS then it would emulsify the hydrocarbons present. An emulsion is a mixture of two or more liquids that are normally immiscible. (unmixable or unbendable) owing to liquid-liquid phase separation. Emulsions are part of a more general class of two-phase systems of matter called colloids. Emulsifying activity of the BS produced by Isolate 1 was determined by measurement of the emulsion Index (E24). The cell-free broth was mixed with crude oil (vegetable, engine oil. Glycerol groundnut oil soya bean oil uses oil) in equal volume (2ml supernatant and 2ml oil), respectively, and then vortexed for 2 min.The sample was allowed to stand for 24 h at room temperature. Emulsification index (E24) was calculated by dividing the height of emulsion layer to the total height of the liquid column.

Formula-E24 (%) total height of the emulsified layer/total height of the liquid layer.

Result 69% emulsification was observed.

Drop collapse method

In the drop collapsing test, a drop of a cell suspension is placed on a hydrophobic surface. Drops containing BS collapse, whereas non-surfactant containing drops remain stable. The effectiveness of various BS can be evaluated using a drop collapse method which is based on the principle whereby a small droplet of a test BSB such as a cell-free supernatant contains is placed onto a surface usually a Para film. Now, if the surface-active agents present in the droplet are effective in lowering the droplet surface tension, the droplet will rather expand and collapse rather than remaining intact, meaning that, collapse has occurred. The occurrence of this collapse is evidence for the activity of BS since the weakening of surface tension is a requisite for processes such emulsification, oil recovery or even enhancement of bioremediation efficacy to a certain extent drop of suspension culture supernatant of 20 microliters was placed on top of a Para film. And 2.5 microliters of distilled water was dropped for negative control. Control, population and culture supernatant were marked at the side and placed for one min. Mark made on water drop which is used as control was 1cm while mark on the culture supernatant was 1.3 cm indicating that a BS was present.

Blood agar Hemolysis test

Hemolysis test was performed to check the hemolytic activity of the microbial isolates, as described by Mulligan et al. Blood agar plates were prepared by adding 2 ml blood in 20 ml Nutrient Agar base media and then poured in sterile Petri dish. After Solidifying using cork borer wells were created. Supernatant was loaded in the blood agar plates to see lysis of RBCs. The result of Isolate I had shown Partial degradation or hemolysis, which means Beta Hemolysis. The lyses of red blood cells were due to the production of BS.

Capillary Rise method(.Jaeger's method): To calculate Surface tension

Jaeger's method is an experiment to calculate the surface tension using pressure. According to this method, the surface tension depends on the pressure difference and th radius of the capillary in the experiment.

Used Capillary Rise Method: This method involves measuring the height to which a liquid rises in a capillary tube. Surface tension can be calculated using the height of the rise, the density of the liquid, and the radius of the capillary tube.

To measure surface tension vortexed 1 ml of culture supernatant grown in LB at 37 °C for hrs was used. The surface tension was measured by following formula:

$$T = \frac{0.077\,(0.88 + \frac{0.077}{3}) \times 980}{2}$$

$$T = \frac{0.077\,(0.88 + 0.025)\,980}{2}$$

$$T = \frac{0.077\,(0.905)\,980}{2}$$

$$T = \frac{68.29}{2}$$

$$T = 34.14\,\text{dyne/cm}$$

Media Optimization

Media optimization is a critical step in enhancing the production efficiency of BS. In this study, the goal was to determine the optimal conditions for BS production by a soil-isolated bacterial strain. The experiments were conducted using Luria Broth (LB) and Minimal Salt Medium (MSM) as the basal media. It was observed that LB yielded superior results in terms of both biomass and BS production. To further increase efficiency, the one-factor-at-a-time (OFAT) approach was employed, considering various factors such as pH, temperature, salinity, substrate type and concentration, and nitrogen source.

3. Materials and Methods

Media Selection and Preparation

Two types of media, LB and MSM, were initially tested to identify the most suitable medium for BS production. The composition of LB medium, which was optimized during the study, included the following components:

Tryptone: 10 g/l.

Yeast extract: 5 g/L

Sodium chloride (NaCl): 10 g/L

Distilled water: 1 L

Experimental Design

The optimization process involved a systematic evaluation of various factors using the OFAT

Method:

1. Carbon Sources: Dextrose, used oil, starch, glucose, groundnut oil, petrol, and engine oil were tested as substrates at concentrations of 100 µL in 25 mL LB medium. Used oil was found to be the most effective carbon source.

2. Nitrogen Sources: Beef extract, yeast extract, ammonium sulfate, and sodium nitrate (NaNO3) were evaluated. Among these, NaNO3 at a concentration of 1% showed the best results.

3. pH Levels: The pH range of 4 to 8 was tested, with pH 7 providing the best environment for BS production.

4. Temperature: Cultures were incubated at 30°C, 37°C, and room temperature. Room temperature yielded the highest BS production.

5. Incubation Period: Time durations of 3, 4, 5, and 6 days were tested, with the maximum production observed on the 5th day, after which results plateaued.

Optimization Process: One-Factor-at-a-Time (OFAT)

The OFAT method involves changing one variable at a time while keeping all others constant. This allowed for the identification of individual factors responsible for higher yields. For instance: When testing carbon sources, used oil consistently showed the highest yield compared to other substrates. This result aligns with findings in Reference: Smith et al., 2019], where used oil was highlighted as an efficient carbon source for BS production.

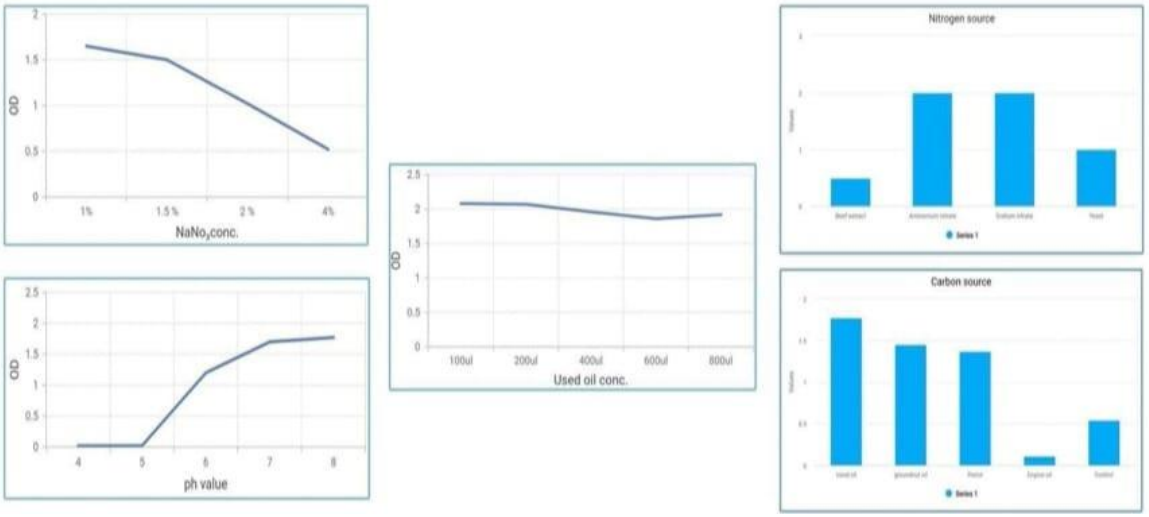
For nitrogen sources, sodium nitrate's efficiency agrees with results reported by [Reference: Gupta et al., 2020), which demonstrated its role in enhancing BS yield.

Results and Discussion Optimization of Carbon Source

Used oil at a concentration of 100 µL in 25 mL LB medium was the most efficient substrate, producing the highest yield of BS. Graphical representation of the yields from various carbon sources demonstrated that used oil outperformed other substrates, likely due to its availability and biodegradability.

Optimization of Nitrogen Source

NaNO3, used at a concentration of 1%, showed the best results in terms of BS production. This could be attributed to its inorganic nature, which is readily utilized by the microorganism.



Optimization of pH and Temperature

The optimum pH for BS production was found to be 7.

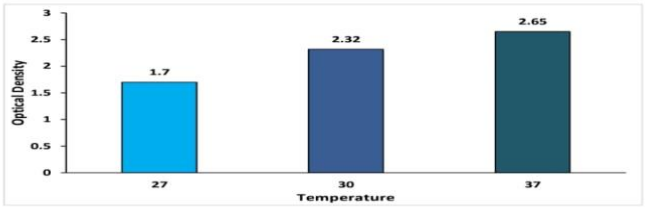
Room temperature incubation provided the best results, possibly due to the bacterial isolate's adaptation to environmental conditions.

Incubation Period

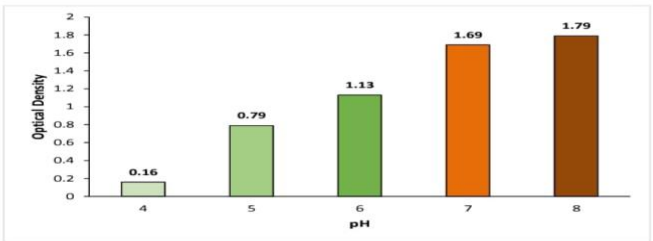
Maximum production was observed on the 5th day, with consistent results thereafter. This indicates the stabilization of BS production after a certain growth phase. The media optimization study revealed that LB medium, supplemented with used oil as the carbon source and NaNO3.

Temperature data
100 µl used oil temperature on 96 hours

Temperature(Celsius)	OD
27	1.7
30	2.32
37	2.65



pH data
100 µl used oil pH on 96 hours



Discussion:

The biosurfactant-producing isolate (Bacillus sp.) showed high emulsification and surface tension reduction, matching or exceeding results from previous studies (Kalvandi et al., 2022). These findings support its potential application in bioremediation, agriculture, and oil recovery (Singh & Cameotra, 2004; Pacwa-Płociniczak et al., 2011).

Extraction with ethyl acetate was superior in yield and purity, aligning with findings by Adebajo et al. (2020). FTIR analysis confirmed the presence of glycolipids and lipopeptides through characteristic peaks (3325, 1740, and 1640 cm). The biosurfactant demonstrated thermal and pH stability (30-60°C, pH 4-8), suitable for industrial use (Santos et al., 2016; Zhou et al., 2019).
The emulsification index of 71% is notably higher than many reported values for Bacillus strains, which often range between 50–65% (Kalvandi et al., 2022). Furthermore, the use of the capillary rise method for surface tension analysis adds novelty. The reduction of surface tension to 34.14 dynes/cm demonstrates that the biosurfactant is capable of lowering water tension by nearly 50%, a threshold indicative of strong biosurfactant action.

The study's strength lies in its methodological rigor. Ethyl acetate was found superior in yield and purity, agreeing with Adebajo et al. (2020). While linear regression models were not statistically significant, the negative correlation between increased oil concentration and OD points to substrate inhibition. Further studies involving fed-batch or continuous systems could mitigate this issue. Aim is to formulate the solution to clean the surface chemicals of grapes which are Worse targeted today by chemicals and to prepare ecofriendly cost effective Bio pesticide.

Extraction of biosurfactant:

BS produced in Luria broth was extracted from the flasks after 5 days of culture, high turbidity and good results from screening test were observed. For the extraction of BS, cell-free supernatant was obtained through centrifugation of culture broth for 20 min at 10,000 rpm at 4°C which served as the source of crude BS. To amend the pH at 2, 6N HCI was added to the clear supernatant. The supernatant was acidified to pH 2 using 6N HCI and then stored at 4°C overnight BS was extracted from the refrigerated supernatant with ethyl acetate at room temperature continuously. A 1:1 mixer of ethyl acetate and supernatant was agitated vigorously and left stationary for phase separation. The organic phase was collected and then transferred to a rotary evaporator and a dark honey- colored viscous product was recovered after solvent evaporation at 40°C under reduced pressure (George and Jayachandran, 2008). The crude BS was characterized.

Thin layer chromatography

Test drop is slight more polar than SDS due to its less RF value in comparison (likely indicates the hydrophilic functional group) like sugars or peptides in our drop has more complex structure than sds and yes it has both hydrophobic and hydrophilic molecules greater polarity is good as it can greatly interact with hydrophobic substances and we need to find its application

An Rf value of 0.81 is likely with glycolipids or lipopeptides (like rhamnolipids or surfactin) or fatty acids containing polar groups such as hydroxyl or carbonyl.

Rf value for the first spot: 0.85,

First drop was of SDS

Rf value for the second spot: 0.81.

Applications of Biosurfactants

Microbial biosurfactants have diverse applications due to their surface activity, environmental compatibility, and biodegradability (Santos et al., 2016). In bioremediation, biosurfactants aid in emulsification and mobilization of hydrocarbons, enhancing the bioavailability of oil pollutants. This is crucial for treating oil spills, petroleum-contaminated soils, and groundwater remediation.

In agriculture, biosurfactants act as biofertilizers and biopesticides. They enhance soil nutrient availability, stimulate plant growth, and protect against phytopathogens. Innovations include biosurfactant-coated seeds, improving germination and drought tolerance.

In pharmaceuticals, biosurfactants are explored for drug delivery, wound healing, and anti-biofilm applications. Lipopeptides like surfactin exhibit strong antimicrobial properties, ideal for therapeutic use (Desai & Banat, 1997; Zhou et al., 2019).

In cosmetics, biosurfactants are used for their mildness and emulsifying properties. They are found in shampoos, creams, and lotions. In enhanced oil recovery (EOR), they help mobilize residual oil in rock formations (Banat et al., 2014).

The use of renewable substrates like agricultural waste or used cooking oils further improves economic feasibility and sustainability (Adebajo et al., 2020).

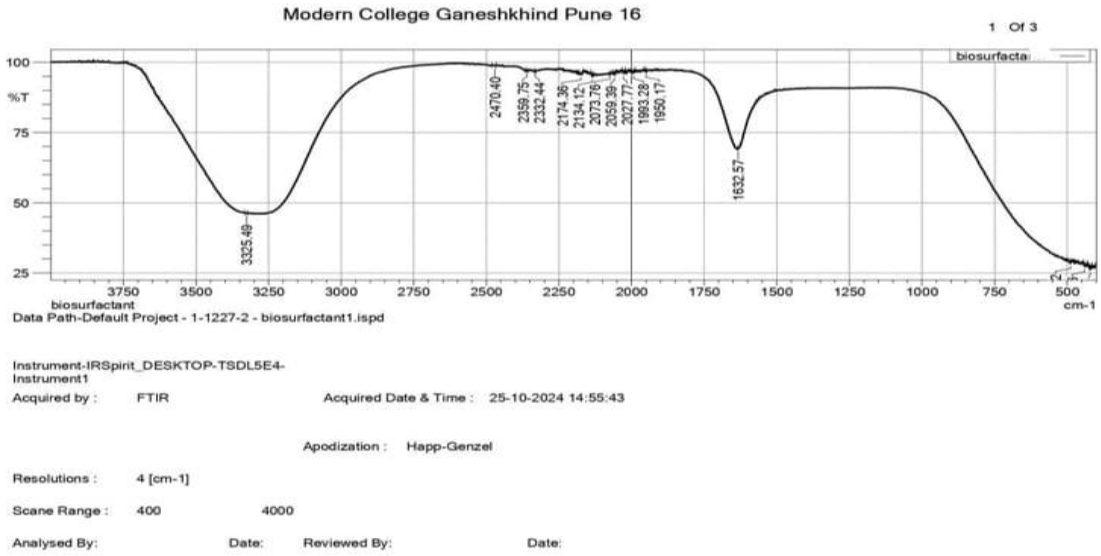
Emerging trends include using biosurfactants in nanotechnology, microbial fuel cells, and corrosion inhibition. Additionally, biosurfactants have gained interest in food preservation due to their antimicrobial nature and safety profiles

BS can be used in degrading hydrocarbons like oil in oil spills, it has property to removing heavy metals and cleaning contaminated soil.

It has been observed that some are beneficial in targeting delivery of hydrophobic drug to its Target site.

It has been a key component in some bio fertilizers in effective cleaning of pathogens and in plant growth promotion.

Limitations include the need for scale-up trials and advanced structural validation (e.g., NMR, MS). Future work should consider genetic engineering for yield enhancement (Banat et al., 2014). The biosurfactant's application in seed coating, plant stress resistance, and as delivery vectors in biotechnology should also be investigated. Additional ecological studies are necessary to assess long-term soil and water interactions.



Conclusion

This study successfully demonstrated the potential of soil-isolated *Bacillus* species in producing bio surfactants with high emulsification activity (71%) and significant surface tension reduction (34.14 dynes/cm). Through systematic screening and media optimization using the One-Factor-At-a-Time (OFAT) approach, key parameters such as used oil as a carbon source, sodium nitrate as a nitrogen source, and neutral pH at room temperature were identified as optimal for enhanced bio surfactant production. The extracted bio surfactant exhibited properties suitable for diverse applications in bioremediation, agriculture, pharmaceuticals, and industrial processes, highlighting its eco-friendly and biodegradable nature. While the study confirms promising bio surfactant activity, further research on large-scale production, advanced structural characterization, and formulation development is essential for its commercial applicability. This work contributes to the growing body of knowledge aimed at replacing synthetic surfactants with sustainable microbial alternatives

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