Nat. Volatiles & Essent. Oils, 2022; 9(1): 1766-1776

NVEO
Natural Volatiles & Essential Oils

Screening Of Water And Soil Derived Microbial Consortia For The Production Of Biosurfactant

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ABSTRACT: The present study is aimed at screening the microbial consortia from polluted effluents and soil samples taken from dumping sites of textile industries and extracting some effective Biosurfactants with promotable textile dye degrading ability. Soil and water samples were collected from designated locations in and around Thane District. Enrichment medium was prepared with paraffin oil as the sole carbon source. A suitable nutrient agar medium supplied with oil was used for the isolation of bacterial species. To detect the Biosurfactant-producing bacteria, haemolysis test was used. Isolated were streaked onto superimposed human blood agar plates, incubated, and checked for clearance zones. Oil Spreading Technique was used to detect their oil displacement activity. The emulsification index (EI) was calculated and the ability of the extracted Biosurfactant to emulsify the hydrocarbons was determined. Chemical characterization of the extracted Biosurfactant and evaluation of its dye degradation efficacy will be done in the later part of this study.

KEYWORDS: Biosurfactants, Characterization, Emulsification, Effluent, Microbial consortia

1. INTRODUCTION

Biosurfactants are structurally diverse surface-active substances produced by micro-organisms. All Biosurfactants are amphiphiles consisting of two parts; a polar (hydrophilic) moiety containing mono, oligo, or polysaccharides, peptides or proteins, and a non-polar (hydrophobic) moiety usually consist of saturated, unsaturated, and hydroxylated fatty acids or fatty alcohols [1]. Due to their amphiphilic structure, Biosurfactants are exploited as excellent emulsifiers, foaming agents, and dispersing agents in Agriculture, Food Production, Chemistry, Cosmetics, Pharmaceuticals, and Textile industries [2]. Further, Biosurfactants are environment-friendly, biodegradable, less toxic, non-hazardous, active at extreme temperatures, pH, and salinity, and can be produced from industrial wastes or by-products. When compared to chemical or synthetic surfactants, Biosurfactants have gained several advantages including their bioavailability, biocompatibility, and digestibility [3].

1.1. Advantages of Biosurfactants

Biosurfactants are more superior than their chemically synthesized equivalents and some its advantages are as follows:

1.1.1. Physical factors: Biosurfactants remain unaffected by factors such as pH, temperature, and ionic strength.

1.1.2. Low toxicity: Biosurfactants exhibit higher toxicity than chemical-based surfactants. It was also reported that biosurfactants showed higher EC 50 values than similar synthetic dispersants [4].

1.1.3. Easy availability of raw materials: Biosurfactants can be produced from easily accessible and inexpensive raw materials in large volumes. The carbon source can be used separately or in combination with each other which may come from hydrocarbons, carbohydrates, or lipids [5].

1.1.4. Biodegradability: Biosurfactants are easily degraded by microorganisms [6].

1.1.5. Other advantages: Biosurfactants are used in pharmaceuticals, cosmetics, and food additive industries because of their biocompatibility and digestibility [5].

1.2. Factors affecting Biosurfactant Production

The constitution and emulsifying activity of the biosurfactant depends on producer strain and culture conditions, thus, the nature of the carbon source, nitrogen source as well as the C:N ratio, nutritional limitations, chemical and physical parameters such as temperature, aeration, and pH affect the type and amount of biosurfactant produced [7].

1.2.1. Carbon source: The nature of carbon substrate can influence the product quality and quantity of biosurfactants [8]. We can use diesel, crude oil, glucose, sucrose, glycerol as a source of carbon substrate in the production of biosurfactants [4].

1.2.2. Environmental factors: The yield and characteristics of any biosurfactant depend on environmental factors. It is necessary to optimize the bioprocess as the product may be affected by changes in temperature, pH, aeration, or agitation speed to obtain the maximum yield. Zinjarde and Pant reported that the best production of biosurfactant was observed when the pH was 8.0 which is the natural pH of seawater [9]. Most biosurfactants are produced at a temperature of 25-30^{o} C [4].

1.2.3. Aeration and Agitation: The transfer of oxygen from the gas phase to the aqueous phase is facilitated by aeration and agitation consequently affecting the production of biosurfactants. It may be associated with the physiological function of microbial emulsifier, it has been suggested that the manufacture of bio-emulsifiers can increase the solubility of the water-insoluble substrate and therefore facilitate nutrient transport to microorganisms.

Synthetic dyes are broadly used in textile, paper, food, cosmetics, and pharmaceutical industries with the textile industry as the largest consumer [2]. This is because of the ease and cost-effectiveness of their synthesis, stability, and variety of colours [3,10]. Several emerging technologies such as electrochemical destruction, advanced oxidation, and absorption techniques have the potential for dye decolorization but they are expensive [11]. An alternative to these techniques is inexpensive biological treatment methods viz. Phytoremediation and bacterial biodegradation in environmental conditions involving fungi, yeast, algae [12]. However, for large-scale treatment of dye-loaded wastewater, the bacterial system has shown good biodegradation and decolorization capability in many studies.

The focus of this project is to make economical production of Biosurfactants from well-known strains of organisms. An attempt to utilize their potential in different aspects of environmental biotechnology and bioremediation of textile industrial effluent which exude tons of textile dyes every year will be made. The successful completion of

this project will pave way for the use of Biosurfactants in environmental clean-up by degradation and detoxification of azo dyes industrial effluents and in bioremediation of contaminated soil.

2. MATERIALS AND METHODS

The steps carried out for the said project are described below:

2.1. Sample Collection: Soil and Water samples were collected into plastic bags and quartz sampling vials respectively from the locations in and around textile industrial units in Thane District. Sampling was done particularly from the strategic locations in the vicinity of Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar. The samples were carried to the laboratory using an icebox and stored at -20 °C until further use.

2.2. Isolation of Bacterial Cultures: Soil sample (1.0 g) from each bag was dried and water sample (10 mL) from each vial was suspended in sterile distilled water. Samples were diluted and plated on nutrient agar. Antibiotics (1.0 mg/mL) were added to the plates to suppress or eliminate any fungal growth. The plates were incubated at a suitable temperature for 24-48 h.

2.3. Morphology Studies: Colony characteristics of fully grown colonies will be noted for the isolated cultures. The prepared smears will be observed and the organism types will be noted using standard key identifying features.

2.4. Screening or Qualitative Estimation: The standard protocol to detect Biosurfactant producing bacteria by haemolysis test was used. Suitable nutrient medium was used for all the bacterial species isolated from the designated water and soil samples. The isolates were streaked onto sheep blood agar plates in a sterile environment. The plates were incubated at a suitable temperature (37°C) for 24-48 hrs. After the incubation period, the plates were checked for zones of clearing (haemolysis) around the colonies, indicative of Biosurfactant production. The bacterial isolates showing haemolytic activity were taken further for confirmation using the oil spreading technique.

2.5. Oil Spreading Technique: The bacterial isolates were tested for their oil displacement activity which is based on the ability of Biosurfactant to alter the contact angle at the oil-water interface. The surface pressure of the Biosurfactant can displace the oil. Different oils viz. coconut oil, Groundnut oil, Castor oil, and other crude oils were added to the surface of distilled water on a petri plate. The oil layer was formed on water; a minute quantity of the cultured supernatant was gently placed at the centre of the oil layer. The displacement of oil and clear zone formation was an indication of the presence of Biosurfactant. The displaced diameter was measured after some time. The isolate showing positive tests were further used for bulk extraction of Biosurfactant.

2.6. Fermentation of Positive Culture: The seed inoculum was prepared in sterile Nutrient broth by inoculating positive culture from the slant. The culture was incubated for 24-48 hours. A suitable production medium was used for fermentation. Crude coconut oil (from a local refinery) is used as a carbon source at a suitable concentration. Seed culture was inoculated in a bulk quantity of production media. After inoculation, the flask was kept under suitable conditions for fermentation.

2.7. Optimisation Studies: The bioprocess was optimized for bulk extraction of the biosurfactant. Optimization Studies projected maximum production under static condition at e 37^{o}C and pH 7.0 respectively when Paraffin oil was used as a substrate.

2.8. Extraction of the Biosurfactant: The vast majority of high molecular weight biosurfactants are produced by ammonium sulphate precipitation method followed by dialysis to remove any small molecules that may be present (Rosenberg et al., 1979). The methods reported for isolation of the high molecular biosurfactants are quite varied and generally specific to the actual biosurfactant present. Other techniques for high molecular weight biosurfactant isolation include TCA/acetone precipitation, acid ethanol and chloroform/methanol. The ammonium sulphate precipitation method below is based on using 100 ml of culture broth and can be adjusted according to the starting volume.

A 100 ml of culture broth was taken and cells were removed by centrifuging at 10,000 rpm for 15 minutes. The supernatant solution was cooled at 4°C and 23.34 g of ammonium sulphate was added with stirring to obtain a 40% saturated solution. The solution was kept overnight at 4°C and centrifuged at 10,000 rpm for 15 minutes. It was re-suspended in a 40% solution of ammonium sulphate and centrifuged again at 10,000 rpm for 15 minutes to obtain pellet. The pellet was dissolved in 20 mL water and extracted with equal volume of hexane, three times in a separating funnel to remove the residual non-polar lipids. By using dialysis tubing with a molecular weight cut off point of 5 kDa clamp one end and tubing was rinsed with distilled water to check for leaks. The tubing was filled with product from step 5 and clamp top. A beaker was placed with dialysis buffer of distilled water on a stirring plate in a cold room overnight. The dialysis buffer was changed after 6h. The sample was removed after the completion of dialysis and lyophilized to obtain the biosurfactant. The organic layers obtained after extraction was pooled together, filtered, and concentrated. The extracted Biosurfactant was refrigerated and used for further experiments.

2.9. Measurement of Emulsification Index (EMI): The ability of the extracted Biosurfactant to emulsify hydrocarbons was determined by using the technique as described by Cooper and Goldenberg (1987). Briefly, a small quantity of extracted Biosurfactant was dissolved in a suitable solvent and different hydrocarbons viz. Hexane, Xylene, Crude oil, etc was added and vortexed. The emulsification activity was checked after allowing it to settle for 24-36h. The emulsification index was calculated by measuring the emulsion layer, expressed as a percentage of total height of the mixture in the tube. The emulsification power of a mixture of equal volumes of 1.0 mg/mL SDS (Sodium Dodecyl Sulphate) and the hydrocarbon was used as the control. EI was calculated by using the following formula:
Emulsification Index EI = Height of the Emulsion Layer / Total Height x 100

3. RESULT AND DISCUSSION

3.1. Haemolysis Experiment:

The results obtained in the haemolytic experiment are depicted below:

Table 1: Results of Haemolysis Experiment

Sample Code	Particulars
V_s1	Beta haemolysis
V_s3	Alpha haemolysis
V_s4	Alpha haemolysis
V_s5	No haemolysis
V_s7	Alpha haemolysis
V_s8	No haemolysis

V_s9	No haemolysis
V_w1	No haemolysis
V_w4	Alpha haemolysis
V_w7	No haemolysis

Youssef et al., (2004) reported the development of clear zones around the colony by Microbacterium esteraromaticum IITR47 and Pseudomonas aeruginosa IITR48 [13].

Anandaraj and Thivakaran (2010) also reported β-haemolysis in the blood agar plate by a biosurfactant-producing strain that was isolated from oil spilled soil [14].

In a previous study, Ebrahimi and Tashi (2012) stated that the relationship between haemolytic activity and biosurfactant production appeared to be a good screening criterion to limit the number of samples that were subjected to the biosurfactant activity test [15].

Shah et.al (2016) in their studies on Biosurfactant types, detection methods, importance and applications also checked for the zone of clearance (haemolysis) around the colonies, indicative of Biosurfactant production. Both observed alpha, beta and gamma haemolysis. α-haemolysis was observed when spotted colony gave greenish zone around its inoculation, β-haemolysis was observed when a clear white zone was observed around the inoculated colony and γ-haemolysis was recorded when no change was observed around the spotted colony [16].

Eldin et.al (2019) observed all isolates exhibited no haemolysis that gives Gamma haemolysis as there were no changes in the medium surrounding their colonies in their work reinforced that yeast isolates showed no haemolytic activity indicating that produced anionic Biosurfactant with possible glycolipid structure [17]. In Farhah Husna Mohd Nor et.al. (2021) studies, the Kurthia gibsonii KH2 exhibited (β-haemolysis) represents a complete breakdown of the haemoglobin of the red blood cells in the vicinity of a bacterial colony clear zone indicates the presence of biosurfactant producing organisms. This strain completely lysed blood with diameters of >1 cm. These clear zones demonstrate the ability of the strain to produce biosurfactants [18].

3.2. Oil Spreading Experiment: The oil spreading technique measured the surface activity of the solution-containing surfactant against the oil–water interface [19]. A large clear zone diameter allowed high surface activity and excellent properties to reduce the surface tension of oil [20]. The results of strain KH2 from the oil spreading technique showed that it had a high surface activity as it was able to spread oil when the culture supernatant dropped into the oil. The clear zone diameter for strain KH2 was 50 mm. [18].
Out of all the five isolates, two isolates V_s1 and V_w4 showed displacement of oil and clear zone formation which indicates the presence of biosurfactant.

3.3. Emulsification Index:
The data obtained by following the procedure as explained in materials and method section is depicted as follows:

Table 2: Total height of the mixture in the tube

Sample Code	Coconut Oil (cm)	Groundnut oil (cm)	Castor oil (cm)	Olive oil (cm)	Diesel (cm)	Xylene (cm)
V_s1	2.8	2.6	3.0	2.9	2.9	2.9

V_s3	2.7	2.6	2.9	2.8	2.9	2.9
V_s4	2.7	2.8	2.9	2.9	2.8	2.8
V_s7	2.8	2.7	3.0	2.8	2.9	2.9
V_w4	2.9	2.7	2.8	2.8	2.9	2.9
Control	2.6	2.5	2.8	2.8	2.9	2.9

Table 3: Height of the Emulsification layer in the tube

Sample Code	Coconut oil (cm)	Groundnut oil (cm)	Castor oil (cm)	Olive oil (cm)
V_s1	1.1	1.0	1.3	0.5
V_s3	1.1	0.2	1.1	0.8
V_s4	1.1	0.2	0.9	0.9
V_s7	1.1	0.2	0.9	1.0
V_w4	1.2	0.2	0.9	0.9

Table 4: Calculations For EMI

Sample Code	Coconut Oil (cm)	Groundnut oil (cm)	Castor oil (cm)	Olive oil (cm)
V_s1	39.28	38.46	43.33	17.24
V_s3	40.40	7.69	37.93	28.57
V_s4	40.40	7.14	31.03	31.03
V_s7	39.28	7.40	30.00	35.71
V_w4	46.15	7.40	32.14	32.14

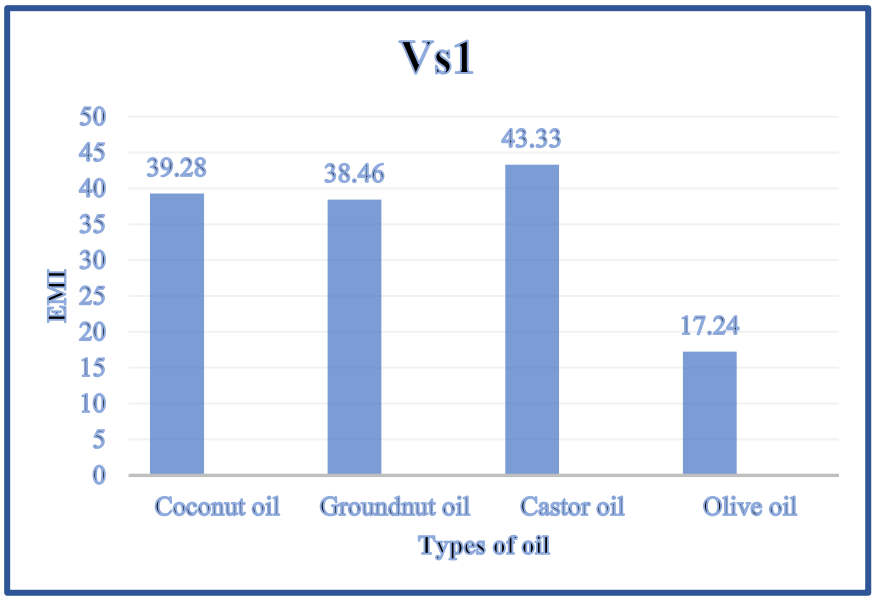

Figure 1: Emulsification Indices - Vs1

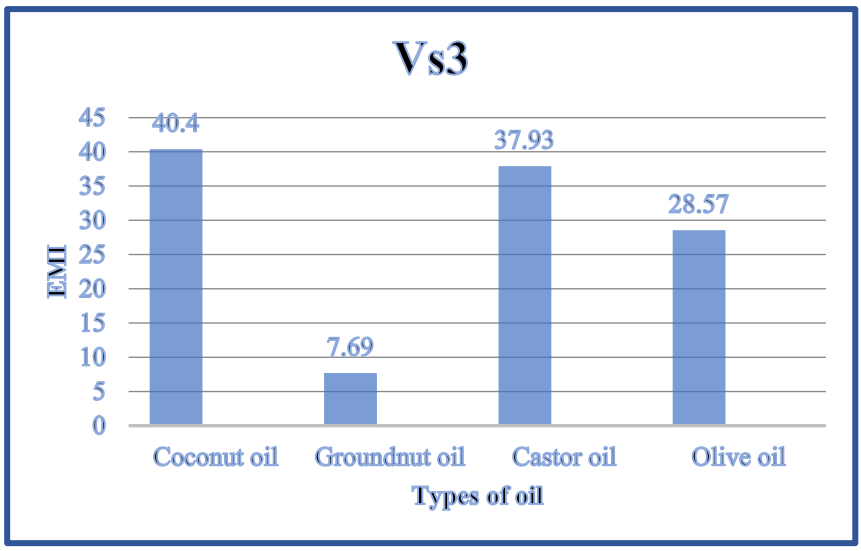

Figure 2: Emulsification Indices - Vs3

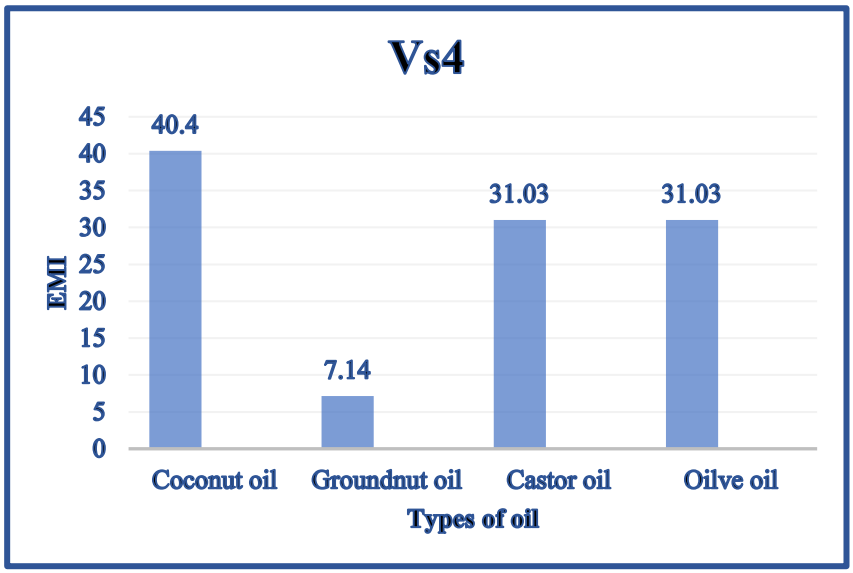

Figure 3: Emulsification Indices - Vs4

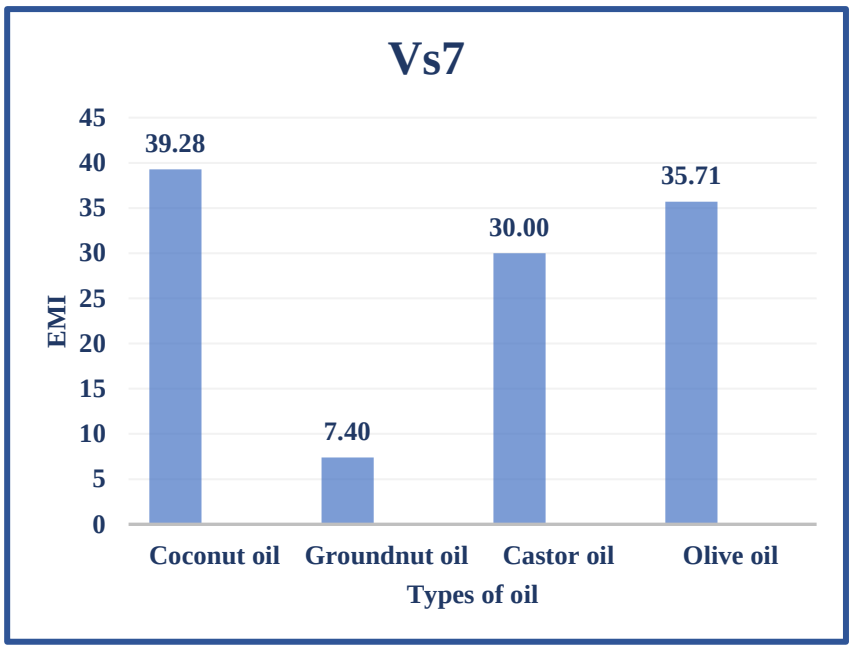

Figure 4: Emulsification Indices - Vs7

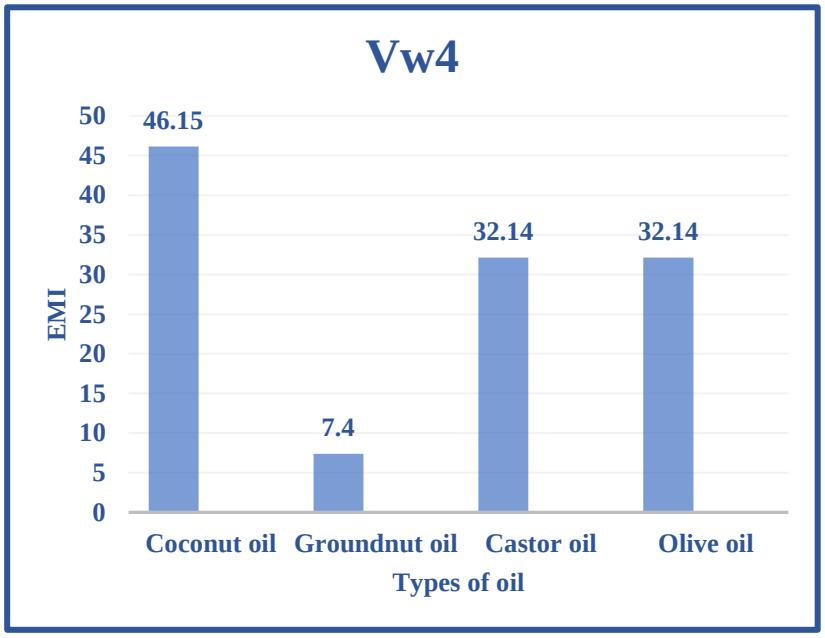

Figure 5: Emulsification Indices - Vw4

Balan et al. (2017) used the emulsification index method to determine the ability of the cell-free supernatant which contained biosurfactants in emulsifying two immiscible liquids to mix and form a single, clear emulsified layer [21]. The cell-free supernatant of strain KH2 emulsified with n-hexane, palm oil, olive oil, vegetable oil, and sunflower-seed oil.

Morais et al. (2017) discovered that the emulsifier produced by Lactobacillus jensenii P6A and Lactobacillus gasseri P65 had significant emulsifying activity against various hydrocarbons and oils including kerosene, toluene, hexane, xylene, cotton oil, olive oil, sunflower-seed oil, gasoline oil, and diesel fuel. As the results of the screening tests

were consistent and mutually supportive, it could be argued that the bacterial strains that were used in this study could produce biosurfactants and secrete them extracellularly in the growth medium [22].

Eldin et.al. (2019) reported that maximum emulsification indexes of 59.65, 56.14, 52.63 and 47.37%, the yeast isolates 263, 27(1), 20(2) and 24(1) effectively emulsified soybean oil than other isolates, giving statistically (LSD = 1.65) the highest emulsification indices (E24%) among the 25 isolates to be 59.65, 56.14,52.63 and 47.37%, respectively [18].

In contrast, Ibrahim et al. (2020) found that there was less emulsion with toluene due to the lower molecular weight of this compound. The highest E24 value displayed by the strain KH2 was 63% when it was emulsified with the palm oil [23].

4. CONCLUSION

4.1. Summary

This research work found that the soil sample collected was rich in microbial diversity. A total of 20 samples were collected, 10 soil and 10 water samples. After enrichment, only 10 isolates were screened qualitatively and quantitatively. From which only five isolates were found to be biosurfactant producers. These isolates were further tested and only one isolate was selected for lab-scale production and further analysis. After primary, qualitative, and quantitative screening the V_s1 isolate was found to be potent biosurfactant producers. V_s1 sample was the most efficient of all isolates and thus was selected for further studies. The Biosurfactant production process was optimized with above isolates. All the findings from the present study will facilitate the use of designated microbial strains as effective tools in various environmental cleaning applications, particularly in the remediation of soil and water resources near textile industry sites as well as the comparative studies with commercially available surfactant on samples from various locations.

4.2. Blueprint for future

The biosurfactants will be characterized by Thin Layer Chromatographic Studies, Fourier Transform Infrared Spectroscopic analysis (FTIR) and hyphenated Gas Chromatographic - Mass Spectroscopic analysis (GC - MS) analysis. The biosurfactant would further be checked for its azo dye degrading ability. The employment of Biosurfactant-producing and hydrocarbon-utilizing microbes will further be expected to enhance the effectiveness of bioremediation as Biosurfactant plays a key role by making hydrocarbons available for degradation. The work will concoct some new series of Biosurfactants that can be effectively used in handling textile industrial emulsions, control over toxic dyes spill-out from industries, biodegradation, and detoxification of polluting industrial effluents as well as in bioremediation of contaminated soil. This project will pave way for the successful implementation of some novel strategies for microbial degradation of azo dyes by utilizing carbon sources available in the effluent of textile industries which will further reduce the CO_2 load in the natural water resources. The successful completion of this project will further endow us with a way of large-scale production of some new series of Biosurfactants, précised for revamping of degradation of water and soil natural resources because of industrial effluents form textile industries and this project will strengthen our current understanding of cell-molecule interaction by understanding the mechanism of action of Biosurfactants in degradation of hazardous textile dyes. This understanding will help us to prepare chemical analogues of effective natural substances present in Biosurfactants.

ACKNOWLEDGEMENT

Chemicals and infrastructural facilities provided by the Department of Chemistry at Smt. C.H.M College for carrying out this research work are gratefully acknowledged.

CONFLICT OF INTEREST

The authors declare that they have no competing interests that could have appeared to influence the work reported in this paper.

REFERENCES

1. Bheemaraddi, M. C., Patil, S., Shivannavar, C. T., & Gaddad, S. M. (2014). Isolation and characterization of Paracoccus sp. GSM2 capable of degrading textile azo dye reactive violet 5. The Scientific World Journal, 2014.
2. Cheriaa, J., Khaireddine, M., Rouabhia, M., & Bakhrouf, A. (2012). Removal of triphenylmethane dyes by bacterial consortium. The Scientific World Journal, 2012.
3. Aamr, A., & Cuiling, J. (2012). Bacterial influence on textile wastewater decolorization. Journal of Environmental protection, 2012.
4. Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. Microbiology and Molecular biology reviews, 61(1), 47-64.
5. Kosaric, N. (2001). Biosurfactants and their application for soil bioremediation. Food Technology and Biotechnology, 39(4), 295-304.
6. Mohan, P. K., Nakhla, G., & Yanful, E. K. (2006). Biokinetics of biodegradation of surfactants under aerobic, anoxic and anaerobic conditions. Water Research, 40(3), 533-540.
7. Salihu, A., Abdulkadir, I., & Almustapha, M. N. (2009). An investigation for potential development on biosurfactants. Biotechnology and Molecular Biology Reviews, 4(5), 111-117.
8. Rahman, P. K., & Gakpe, E. (2008). Production, characterisation and applications of biosurfactants-Review. Biotechnology.
9. Zinjarde, S. S., & Pant, A. (2002). Emulsifier from a tropical marine yeast, Yarrowia lipolytica NCIM 3589. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms, 42(1), 67-73.
10. Asad, S., Amoozegar, M. A., Pourbabaee, A., Sarbolouki, M. N., & Dastgheib, S. M. M. (2007). Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Bioresource technology, 98(11), 2082-2088.
11. Chen, K. C., Wu, J. Y., Liou, D. J., & Hwang, S. C. J. (2003). Decolorization of the textile dyes by newly isolated bacterial strains. Journal of Biotechnology, 101(1), 57-68.
12. Levin, L., Melignani, E., & Ramos, A. M. (2010). Effect of nitrogen sources and vitamins on ligninolytic enzyme production by some white-rot fungi. Dye decolorization by selected culture filtrates. Bioresource technology, 101(12), 4554-4563.
13. Youssef, N. H., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M., & McInerney, M. J. (2004). Comparison of methods to detect biosurfactant production by diverse microorganisms. Journal of microbiological methods, 56(3), 339-347.
14. Anandaraj, B., & Thivakaran, P. (2010). Isolation and production of biosurfactant producing organism from oil spilled soil. J Biosci Tech, 1(3), 120-126.
15. Ebrahimi, A., & Tashi, N. (2012). Isolation of biosurfactant producing bacteria from poultry breast skin. Jundishapur journal of natural pharmaceutical products, 7(3), 93.
16. Shah, N., Nikam, R., Gaikwad, S., Sapre, V., & Kaur, J. (2016). Biosurfactant: types, detection methods, importance and applications. Indian Journal of Microbiology Research, 3(1), 5-10.
17. Eldin, A. M., Kamel, Z., & Hossam, N. (2019). Isolation and genetic identification of yeast producing biosurfactants, evaluated by different screening methods. Microchemical Journal, 146, 309-314.

18. Nor, F. H. M., Abdullah, S., Yuniarto, A., Ibrahim, Z., Nor, M. H. M., & Hadibarata, T. (2021). Production of lipopeptide biosurfactant by Kurthia gibsonii KH2 and their synergistic action in biodecolourisation of textile wastewater. Environmental Technology & Innovation, 22, 101533.
19. Ibrahim, H. M. (2018). Characterization of biosurfactants produced by novel strains of Ochrobactrum anthropi HM-1 and Citrobacter freundii HM-2 from used engine oil-contaminated soil. Egyptian Journal of Petroleum, 27(1), 21-29.
20. Chandran, P., & Das, N. (2011). Characterization of sophorolipid biosurfactant produced by yeast species grown on diesel oil. International journal of science and nature, 2(1), 63-71.
21. Balan, S. S., Kumar, C. G., & Jayalakshmi, S. (2017). Aneurinifactin, a new lipopeptide biosurfactant produced by a marine Aneurinibacillus aneurinilyticus SBP-11 isolated from Gulf of Mannar: Purification, characterization and its biological evaluation. Microbiological research, 194, 1-9.
22. Morais, I. M. C., Cordeiro, A. L., Teixeira, G. S., Domingues, V. S., Nardi, R. M. D., Monteiro, A. S., ... & Santos, V. L. (2017). Biological and physicochemical properties of biosurfactants produced by Lactobacillus jensenii P 6A and Lactobacillus gasseri P 65. Microbial cell factories, 16(1), 1-15.
23. Ibrahim, S., Abdul Khalil, K., Zahri, K. N. M., Gomez-Fuentes, C., Convey, P., Zulkharnain, A., ... & Ahmad, S. A. (2020). Biosurfactant production and growth kinetics studies of the waste canola oil-degrading bacterium Rhodococcus erythropolis AQ5-07 from Antarctica. Molecules, 25(17), 3878.

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