VOLUME 8 (SPECIAL ISSUE) 2022

ISSN 2454-3055

Manuscripts under Special issue are published with the Theme
"Modern Perspectives of Biological Sciences"

Guest Editor: Dr. S. Mohanasundaram
Assistant Guest Editor: Dr. S.S. Syed Abuthahir

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 8, Special Issue, 126-135 (2022)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Isolation and Characterization of Bio-surfactant Producing Bacteria

Nirmalkumar R., Uma S. and Indumathi Parameswaran*

Post Graduate Department of Biotechnology, Dwaraka Doss Goverdhan Doss Vaishnav College, Arumbakkam, Chennai 600 106, Tamil Nadu, India

**Corresponding Author*

Received: 14th August, 2022; **Accepted:** 24th September, 2022; **Published online:** 10th October, 2022

https://doi.org/10.33745/ijzi.2022.v08i0s.016

Abstract: Oil spillages are so common in many fields which lead to contaminated soil. Soil polluted land filling contains bacteria that produce a broad spectrum of bio-surfactants. Bio-surfactant as an efficient biological surface-active agent may provide an alternative solution for the removal of heavy metals from industrial wastes. The bio-surfactant has more potential efficiency in the process of remedying natural resources. Surfactin, Iturin and Fengycin are widely studied bio-surfactants. The present study was aimed to isolate and characterize the bio-surfactant producing bacteria from the soil samples collected from the petrol bunk. Two isolates obtained were used for the further analysis. Catalase, Lactose fermentation, Citrate utilization tests were positive for isolate 1 and identified as *Staphylococcus* sp. Starch hydrolysis, Methyl red test, Catalase, Lactose fermentation, Citrate utilization tests and Nitrate reduction were positive for isolate 2 and identified as *Bacillus* sp. The samples were anlayzed for the Bio-surfactant property using Haemolytic activity, oil drop assay, oil collapse activity and emulsification test. The functional group was detected using the FTIR analysis. *Bacillus* sp. strains have more potent biosurfactant property than *Staphylococcus* sp. and they act as bio-surfactants in removing the stains. Biochemical characterization of isolated samples, determination of bio-surfactant producing organism by oil spreading activity, drop collapse assay, oil spreading activity and biofilm formation were confirmed by *Bacillus* species bacteria.

Keywords: Bio-surfactant, Drop collapse assay, Oil spreading activity, Biofilm, *Bacillus*, *Staphylococcus*

Citation: Nirmalkumar R., Uma S. and Indumathi Parameswaran: Isolation and characterization of bio-surfactant producing bacteria. Intern. J. Zool. Invest. 8(Special Issue): 126-135, 2022.

https://doi.org/10.33745/ijzi.2022.v08i0s.016

Introduction

Microbes produce active agents known as bio-surfactant and are amphipathic in nature (Mannanov and Sattarova, 2001). Bio-surfactants are glycolipids, Trehalose lipid, Rhamnolipids, Mannosylerythritol lipid, lipopeptide, BS20 Rhamnolipid, BN56 Trehalose tetraester (Lamichhane *et al*., 2017). Bio-surfactants are detergents that breaks hydrocarbons particles and has antifungal (Besson *et al*., 1978; Vanittanakom *et al*., 1986), antimicrobial, antiviral (Katz and Demain 1977; Razafindralambo *et al*., 1998) and antitumour (Sivapathasekaran *et al*., 2010)

activities. Bio-surfactants are widely used in petroleum industry, cosmetics, antimicrobial agents, medicine and bioremediation (Singh *et al*., 2008; Bharathi *et al*., 2016). Nowadays they replace the chemical surfactants in food and biomedical industries (Arun *et al*., 2014; Shah *et al*., 2016; Wang *et al*., 2022). Microbial Bio-surfactants has biodegradability, biocompatability and digestibility to eradicate the toxic substances in the environment (Olivera *et al*., 2003). Bio-surfactants synthesized by microorganism are safe like *Lactobacilli* and they have emulsifying, antiadhesive and antimicrobial activities which is also cost effective (Mohanasundaram *et al*., 2012; Vijayakumar and Saravana, 2015). The poly-anionic Bio-surfactants produced by *Acinetobacter calcoaceticus* RAG-1 are surface active components that degrades oil known as emulsan which forms and stabilizes oil-water emulsions with a variety of hydrophobic substrates (Amiriyan *et al*., 2004; Mohanasundaram *et al*., 2019).

The present study was aimed to isolate and characterize the bio-surfactant producing bacteria from the soil samples collected from the petrol bunk. Two isolates obtained were used for the further analysis.

Materials and Methods

Collection and Isolation of Bacteria:

The soil sample was collected from the petrol pump near Avadi, during the month of February 2022. The soil samples were collected from 3-6 cm depth and stored in a sterile container. 1 g of the soil sample was weighed accurately and serially diluted to 10^{-1} to 10^{-8} using normal saline and plated in nutrient agar by spread plate method. The colonies obtained was preserved in nutrient agar slant and stored at 4^0C until use.

Chemicals:

50 mM Tris buffer, TritonX-100, BBL tm Trypticase tm Soy Agar, 5% sheep blood, L.B Medium, Ethanol, Crystal violet stain, Ammonium sulphate, Dialysis bag, Phosphate buffer, EDTA, Sodium carbonate and all other chemicals procured were of analytical grade.

Biochemical Characterization of Microorganisms:

The isolated colony from Nutrient agar was characterized. Colony morphology, shape, size, arrangements, motility, opacity, texture and pigmentation of the stains were studied.

Acid Precipitation:

The isolated colony from nutrient agar was inoculated into MR broth, incubated at 37^0C for 48 h. Later the broth was centrifuged at 10,956 X g for 15 min, the supernatant was collected and subjected to acid precipitation. 6M HCL was added to the supernatant and kept overnight at pH of 2.0. After acid precipitation the samples were centrifuged at 10,956 X g for 15min. The steps were repeated until the pellet was obtained. Finally it was washed with sodium hydroxide (pH 11.0) and made to freeze dried overnight, later extracted using methanol. Then the semisolid substance was poured into a petri plate and air dried at room temperature for 24 h and stored at room temperature.

Screening Of Biosurfactant Activity:

Hemolytic Efficacy:

Hemolytic activity was determined by infusing 2 L of bacterial culture overnight onto blood agar plates containing 5% sheep blood and BBL tm Trypticase tm Soy Agar (TSA II). At 28°C, plates were incubated for 72 h. During the period of 12-72 h of incubation, the creation of hollow zones has been observed. The formation of hollow zones demonstrated that a bacterial strain has bio-surfactant characteristics (Hagwell *et al*., 1992).

Oil Distribution Activity:

In a petri dish containing 25 L of crude oil and 25 ml of distilled water, the added supernatant of a bacterial culture was cultivated in LB medium for 72 h at 28 °C. If the oil zones begin to vanish as clearing zones, the cell-free supernatant exhibits bio-surfactant activity, and distilled water was

employed for the negative control activity (Hassan *et al*. ,2014).

Emulsification Process:

Bacterial strains were cultivated in LB broth at 28± 2℃ for 72 h before being incubated at 121℃ for 20 min and centrifuged at 5000 rpm to extract cell-free culture broth for bio-surfactant activity testing. In a test tube, 1 ml of cell-free culture broth and 10 mg of hydrocarbon were mixed for 2 min with 5 ml of 50 mM Tris buffer (pH 8.0). Using a spectrophotometer, the OD was measured at 610 nm after a 40 min incubation of the emulsion at room temperature. Positive control was Triton X-100, while the negative control was the buffer solution mixed with crude oil (Chowdhury *et al*., 2015).

Reduced Collapse Test:

The drop collapse test was conducted using the Maier technique. The wells of a 96-well microplate were filled with crude oil and left to equilibrate for 24 h. Five microliters of cell-free culture broth were poured to the oil-coated wells, and the drop size was measured after 1 min using a microscope. Biosurfactant activity was measured for diameters greater than 1 mm, with 1 mg/ml Triton X-100 serving as a control (Aleti *et al*., 2016).

Biofilm Development:

100 µl of the material was placed overnight in 96 microwell plate-titer plates. The cells were stained with 1% crystal violet for 45 min and then washed with 200 µl of 100% ethanol to evaluate biofilm development (Aleti *et al*., 2015).

Partial Purification of Enzyme:

Bacillus sp. Bio-surfactant was partly purified using typical purification techniques, including ammonium sulphate precipitation, followed by dialysis, and all processes were performed at 40℃.

Precipitation of Ammonium Sulfate:

On ice, 50% and 70% ammonium sulphate precipitation was performed on the crude material. After adding all of the salts, the mixture was refrigerated at 40 ℃for about half an hour. The mixture was centrifuged for 20 min at about 10,000 rpm. The supernatant was discarded, and the precipitates were suspended in phosphate buffer, dialyzed, and kept at 40 ℃until further use (Rosenberg *et al*., 1980).

Thin layer Chromatography:

The dried product (0.1 mg) was dissolved in methanol (0.1 ml), and 10 µl of this solution was spotted on a pre-coated silica gel 60 F254 (Merck, Darmstadt, Germany) and developed with the mobile phase consisting of chloroform/ methanol/water (65:25:5, v/v/v). The plates were exposed to 0.2% ninhydrin, air-dried, and viewed under ultraviolet light.

FTIR Analysis:

Using Fourier Transform Infrared (FTIR) spectroscopy, the chemical components and structures of molecules in the partly purified bio-surfactant were determined.

Deteriorating Property:

The partly purified Bio-surfactant was used to assess the removal of oil stains from oil-stained cotton garments (73 cm).

Results and Discussion

Isolation and Biochemical characterization:

Two different bacterial strains were isolated from the soil sample obtained from the petrol bunk which was inoculated in nutrient agar. The isolated two different types of the bacterial strains were characterized by its shape, margin elevation, pigmentation and Grams staining (Table 1; Fig. 1). Biochemical tests such as catalase, lactose fermentation and citrate utilization tests were positive for isolate 1 which is *Staphylococcus* sp. Starch hydrolysis, Methyl red test, Catalase, Lactose Fermentation, Citrate utilization tests and Nitrate reduction was positive which showed that the isolates 2 is *Bacillus* sp. (Table 2). The bacterial strains were further screened for bio-surfactant production on the MR broth.

Table 1: Morphological characteristics of Strain 1 and Strain 2 on Nutrient Agar

S. No.	Appearance	Strain 1	Strain 2
1	Shape	Clustered Cocci	Rod
2	Margin	Circular	Translucent
3	Elevation	Smooth	Large
4	Pigmentation	Golden yellow	White
5	Gram staining	Gram Positive	Gram Negative

(A) **(B)**

Fig. 1: *Staphylococcus* sp. (A) and *Bacillus* sp. (B).

Table 2: Biochemical Characterizations of *Staphylococcus sp*. and *Bacillus sp.*

Parameters	***Staphylococcus* sp.**	***Bacillus* sp.**
Lactose Fermentation	Positive	Negative
Starch Hydrolysis	Negative	Positive
Methyl red	Negative	Positive
Citrate utilization	Positive	Positive
Nitrate reduction	Positive	Positive
Motility	Non-motile	Motile
Catalase	Positive	Positive
Urease	Positive	Negative

Haemolytic Activity:

Staphylococcus sp. and *Bacillus* sp. both showed Haemolytic activity by forming halo zones of 1cm around the plates (Fig. 2).

Oil Spreading Activity:

The polluted engine oil used for the oil spreading activity has shown the positive result for *Bacillus* sp. and negative for the *Staphylococcus* sp. The oil spreading activity of *Bacillus sp*. has shown positive (Fig. 3).

Emulsification activity:

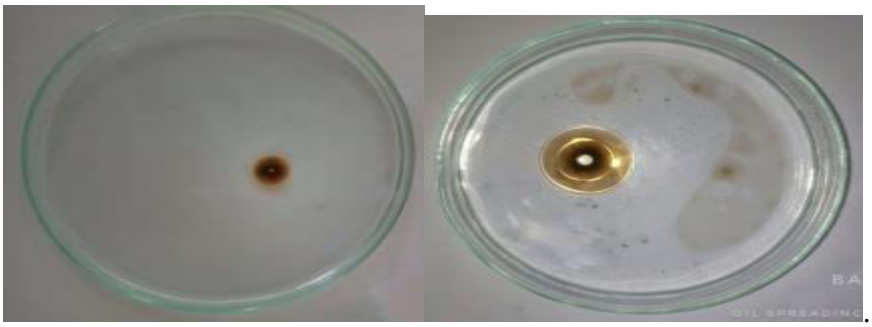
Emulsification assay showed positive for *Bacillus* sp. and moderate effect was found in *Staphylococcus* sp**.** (Table 3).

a. *Staphylococcus* species b. *Bacillus* species

Fig. 2: Haemolytic Activity of *Staphylococcus sp.& Bacillus sp*.

Table 3: Emulsification activity of *Staphylococcus* sp. and *Bacillus* sp.

Sample	Emulsion	Positive Control (OD)	Negative Control (OD)
A	0.5	2.0	0.3
B	1.3	2.0	0.3



a. *Staphylococcus* species b. *Bacillus* species

Fig. 3: Oil spreading activity of *Staphylococcus* sp. and *Bacillus* sp.

Drop collapse assay:

Drop collapse assay contains 5 µl of culture supernatant which was transferred to 96 well for 1 min. The *Bacillus* sp. showed the best results as compared to *Staphylococcus* sp. (Monteiro *et al*., 2002) (Fig. 4).

Biofilm formation:

Biofilm formation was almost similar in both the samples of *Staphylococcus* sp. and *Bacillus* sp. The Bio-film obtained for the samples is shown in Figure 5.

Partial Purification:

Ammonium Sulphate Precipitation and Dialysis:

The crude enzyme of *Bacillus* sp. is partially purified by saturating with 50% and70% ammonium sulphate precipitation and dialysed. The samples were further used for TLC (Fig. 6).

Thin layer chromatography:

The dialysed content was mixed with equal volume of ethyl acetate, methanol and ethanol and loaded in the TLC plate and chromatography was

Fig. 4: Drop collapse assay of *Staphylococcus* sp. and *Bacillus* sp.

Tube 1	Distilled water
Tube 2	*Staphylococcus* sp. (broth) and crystal violet
Tube 3	*Bacillus* sp. (broth) and crystal violet

Fig. 5: Bio-Film formation *Staphylococcus* sp. and *Bacillus* sp.

Fig. 6: Dialysed sample after Ammonium Sulphate precipitation.

performed. Titron x 100 was a positive control. Solvent system was Chloroform: methanol: water in ratio of 65: 25: 5. Chromatogram was sprayed with distilled water and 0.2% Ninhydrin, air dried at 40^{o}C and visualized under UV transilluminator. Results obtained showed the presence of bio-surfactant compounds as that of positive control (Fig. 7).

FTIR Spectroscopy:

The functional group detected in the FTIR are presented in Table 4 and Figure 8. FTIR analysis was carried out for the Biosurfactant from *Bacillus sp.* The results obtained for FTIR ranged from 569 to 3500 cm^{-1} wave length.

Destaining Property:

Oil stained clothes were made to be specimen for the run trial of the extracted bio-surfactant. From the acid precipitation method a dried product is extracted and applied on clothes to remove oil stains. The bio-surfactant were applied on the oil stained cloth and made it dry. After sometimes the cloth is washed in water flow. Titron x 100 was

A – Dialysed sample with Methanol
B – Dialysed sample with Ethanol
C – Dialysed sample with Ethyl acetate
D – Titron x 100

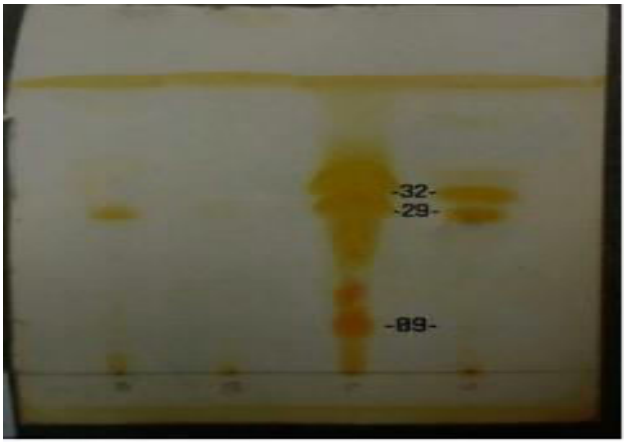


Fig.7: Thin layer chromatography of partially purified bio-surfactant.

Table 4: Functional groups detected using FTIR Analysis of bio-surfactant from B*acillus* sp.

BOND	**TYPE**	**FREQUENCY RANGE cm^{-1}**	**NATURE**
O-H	Hydrogen bonding Alcohol's, Phenols	3200-3600	Variable, sometimes broad
C=C	Alkenes	1610-1680	Variable
C-H	Alkanes	1340-1470	Strong
C-O	Alcohols,ethers, carboxylic acids, esters	1050-1300	Strong
C-H	Alkenes	675-995	Strong
-C≡C-H	Alkynes	2100-2270	Variable
C-H	Alkanes	2850-3000	Stretching
N-O	Nitro compound	1556	Stretching
O-H	Alcohol	3584-3700	Stretching
C-O-C	Alkenes	871.82-671.233	Weak

used as a positive control and normal distilled water as negative control. The isolated biosurfactant acts as an best emulsifier in removing stains from the cloth stained with oil. They act efficiently to remove dirt and more economic (Fig. 9).

The *Bacillus and Staphylococcus* strains show the maximum haemolytic activity by creating hallow zones around the plate (Somasegaran and Hoben, 1994). *Pseudomonas aeruginase* produces rhamnolipid Bio-surfactants (Kaeppeli *et al*., 1986). Biodegradation of oil, heavy metals, Polycyclic aromatic hydrocarbons are cleaned up with the help of bio-surfactant (Haritash and Kaushik, 2009; Liu *et al*., 2017; Bharathi *et al*., 2019). Lipopeptides such as Surfactin, iturin and fengucin are highly active bio-surfactants (Zhang *et al*., 2016). *Bacillus subtilis* B5-37 strains produces surfactant which has efficient oil recovery (Liu *et al*., 2015). Bio-surfactants are emulsifiers, foaming agent and detergents. It play important role in oil recovery since it has the ability to reduce surface tension of molecules (Volkering *et al*., 1998). Simple method for measuring cell hydrophobicity and to derive surfactant quality was the emulsification activity (Djordjevic *et al*., 2002). Bacterial strains isolated from the marine Caspian sea CpA1 and CpA2

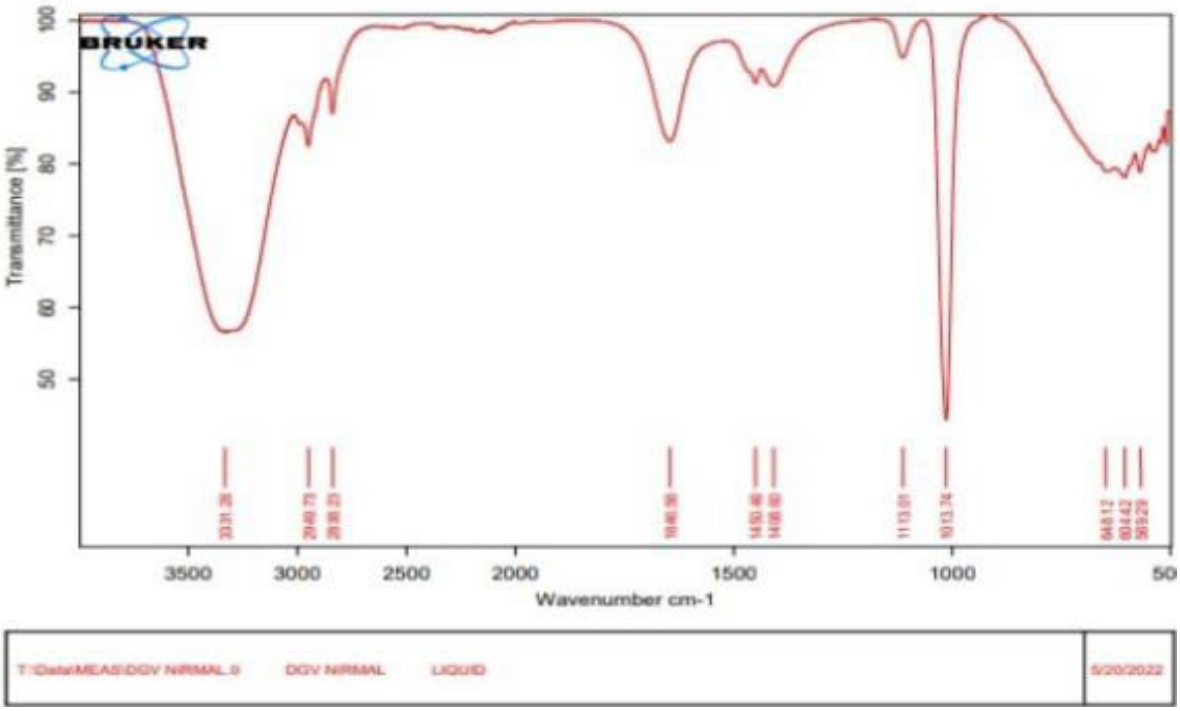
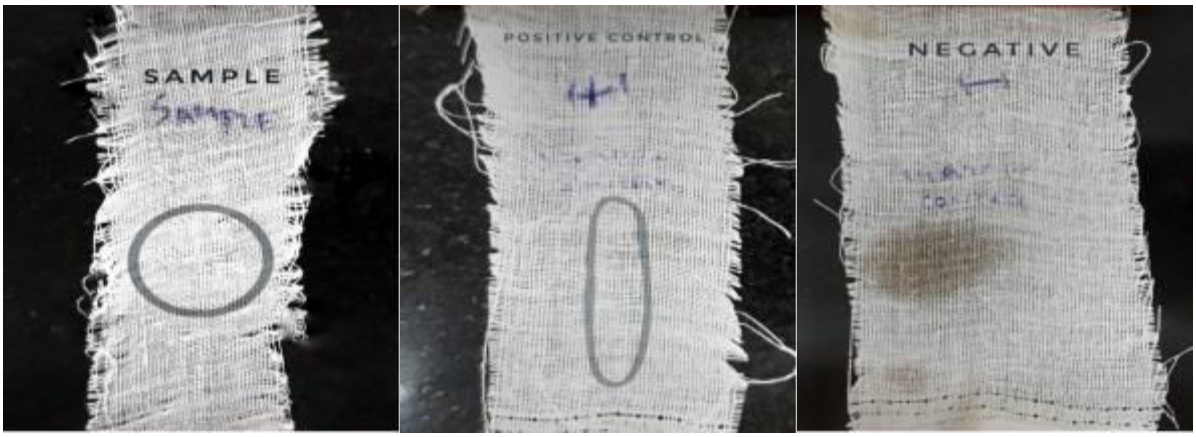


Fig. 8: FTIR analysis of *Bacillus* sp.



a. Cloth treated with *Bacillus* sp. b. Cloth treated with Triton X-100 c. Cloth treated with distilled water.

Fig. 9: Destaining property of partially purified bio-surfactant of *Bacillus* sp.

emulsifies crude oil within 48 h and 96 h, respectively and results shows marine bio-surfactant has the ability to degrades crude oil (Safary *et al*., 2010). The maximum drop collapse activity was observed for strains F2B42 a NH-100 whereas strains of 176's, 168 and Cc125 shows negative for drop collapse (Madi and Henis, 1998). The surfactants are used to control the aggregation of fat globules, stabilization of aerated systems, and improvement of texture and shelf-life of products (Kachholz and Schlingmann, 1987). In food industry Bio-surfactants increase the dough volume, texture and preservation of Bakery products (Haesendonck and Vanzeveren, 2004). Bio-surfactants which is used in bioremediation process has the property to increase the surface

area of hydrophobic water-insoluble substrates and increasing the bioavailability of hydrophobic compounds (Vijayakumar and Saravana, 2015). Biosurfactant produced by *Bacillus* sp. is Fungycin. Lipopeptide has antifungal activity against filamentous fungi. The Rf value of ethyl acetate biosurfactant shows similar Rf value of standard surfactant (Titron x100) (Morikawa *et al*., 2000). Bio-surfactant have hemolytic activity (Bicca *et al*., 1999; Tabatabaee *et al*., 2005). Bio-surfactant produced by the microorganisms isolated from Iranian oil reservoirs shows that 39 bacterial strains have beta haemolytic, emulsifying activity and surface tension reduction to confirm bio-surfactant (Amiriyan *et al*., 2004).

Conclusion

Bio-surfactants are widely used in industries, agriculture and they are more economical. Micro-organisms are rich in our Ecosystem and are more potent in removing the harmful components from the environment which is needed for unique human life. We can convert the waste materials obtained from the house-hold into useful component by using Eco-friendly microbes. They are cost effective and easy to eradicate harmful waste from the environment.

References

Aleti G, Lehner S, Bacher M, Compant S, Nikolic B, Plesko M, Schuhmacher R, Sessitsch A and Brader G. (2016) Surfactin variants mediate species-specific biofilm formation and root colonization in Bacillus. Environ Microbiol. 18(8): 2634-45.

Aleti G, Sessitsch A and Brader G. (2015) Genome mining: Prediction of lipopeptides and polyketides from *Bacillus* and related Firmicutes. Comput struct Biotechnol J. 13: 192-203.

Amiriyan A, Assadi MM, Saggadian VA and Noohi A. (2004) Bioemulsan production by Iranian oil reservoirs microorganisms. Iranian J Env Health Sci Eng, 1(2): 28-35.

Arun J, Mohanasundaram S, Pennarasi A, Alagulakshmi, Monica S, Narendhiran SL, Dineshkumar A and Akshaya (2014) Determination of in vitro antioxidant potency, antibacterial efficacy, antifungal and phytochemical activity of Withania somnifera. Int J Innov Pharm Sci Res. 2(2): 610- 619.

Besson F, Peypoux F, Michel G and Delcambe L. (1978) Identification of antibiotics of iturin group in various strains of *Bacillus subtilis*. J Antibiot. 31: 284-288. PMID: 96084.

Bharati P and Pennarasi M. (2016) Production of lipids from municipal sewage sludge by two stage extraction process. Asian J Chemical Engg. 16(1): 38-44.

Bharati P and Pennarasi M. (2019) Production of biodiesel from municipal sewage sludge by transesterification process. In: Biomass Valorization to Bioenergy. Energy, Environment, and Sustainability, (eds.) Praveen Kumar R., Bharathiraja B., Kataki R. and Moholkar V., Springer, Singapore.

Bicca FC, Fleck LC and Ayub MAZ. (1999) Production of biosurfactant by hydrocarbon degrading *Rhodococcus ruber* and *Rhodococcus erythropolis*. Rev Microbiol. 30: 231-236.

Chowdhury SP, Hartmann A, Gao X and Borriss R. (2015) Biocontrol mechanism by root-associated Bacillus amyloliquefaciens FZB42 - a review. Front Microbiol. 28(6): 780.

Djordjevic D, Wiedmann M and McLandsborough LA. (2002) Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. Appl Environ Microbiol. 68: 2950-2958.

Hagwell IS, Delfino LM and Ras JJ. (1992) Partitioning of polycyclic aromatic Hydrocarbon from oil into water. Environ Sci Technol. 26: 2104-2110.

Haritash AK and Kaushik CP. (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater. 169: 1-15.

Kaeppeli O and Guerra-Santos L. (1986) Process for the Production of Rhamnolipids. 1986; Patent no. US 4628030 A.

Katz E and Demain AL. (1977) The peptide antibiotics of *Bacillus*: chemistry, biogenesis, and possible functions. Bacteriol Rev. 41(2): 449-474.

Lamichhane S, Bal Krishna KC and Sarukkalige R. (2017) Surfactant-enhanced remediation of polycyclic aromatic hydrocarbons: a review. J Environ Manag. 199: 46-61.

Liu Q, Lin J, Wang W, Huang He and Li S. (2015) Production of surfactin isomers by *Bacillus subtilis* BS-37and its applicability to enhanced oil recovery under laboratory conditions. Biochem Engineer J. 93: 31-37.

Liu Z, Li Z, Zhong H, Zeng G, Liang Y, Chen M, Wu Z, Zhou Y, Yu M and Shao B. (2017) Recent advances in the environmental applications of biosurfactant saponins: a review. J Environ Chem Eng. 5(6): 6030-6038.

Madi L and Henis Y. (1989) Aggregation in *Azospirillum brasilense* Cd: conditions and factors involved in cell-to-cell adhesion. Plant Soil. 115: 89-98.

Mannanov RN and Sattarova RK. (2001) Antibiotics produced by *Bacillus* bacteria. Chem Nat Compd. 37:117-123.

Mohanasundaram S, Arun J, Thirumalai S, Pennarasi M, Prasanna M, Gowthamraj S, Vimalraj A, Sasirekha B, Alagulakshmi G and Monika S. (2012) Production and partial characterization of extracellular glucose isomerase using thermophilic *Bacillus* sp. isolated from agricultural land. Biocat Agri Biotech. 2: 45-49.

Mohanasundaram S, Doss VA, Maddisetty P, Magesh R, Sivakumar K and Subathra M. (2019) Pharmacological analysis of hydroethanolic extract of *Senna alata* (L.) for *in vitro* free radical scavenging and cytotoxic activities against HepG2 cancer cell line. Pak J Pharm Sci. 32(3): 931-934.

Monteiro L. (2002) Produc¸ão de substaˆncias bioativas de Bacillus spp. contra Xanthomonas campestris pvcampestris. MSc Thesis. Universidade Federal de Pernambuco, Brazil.

Morikawa M, Hirata Y and Imanaka TA. (2000) A study on the structure-function relationship of lipopeptide Bio-surfactants. Biochim Biophys Acta. 1488: 211-218.

Olivera NL, Commendatore MG, Delgado O and Esteves JL. (2003) Microbial characterization and hydrocarbon biodegradation potential of natural bilge waste microflora. J Ind Microbiol Biotechnol. 30(9): 542-548.

Razafindralambo H, Popineau Y, Deleu M, Hbid C, Jacques P, Thonart P and Paquot M. (1998) Foaming properties of lipopeptides produced by *Bacillus subtilis*: Effect of lipid and peptide structural attributes. J Agric Food Chem. 46: 911-916.

Rosenberg M, Gutnick D and Rosenberg E. (1980) Adherence to bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. FEMS Microbiol Lett. 9: 29-33.

Safary A, Ardakani MR, Suraki AA, Khiavi MA and Motamedi H. (2010) Isolation and characterization of biosurfactant producing bacteria from Caspian sea. Biotechnol. 9: 378-382.

Shah N, Nikam R, Gaikwad S, Sapre V and Kaur J. (2016) Biosurfactant: types, detection methods, importance and applications. Indian J Microbiol Res. 3(1): 5-10.

Singh S, Kang SH, Mulchandani A and Chen W. (2008) Bioremediation: environmental clean-up through pathway engineering. Curr Opin Biotechnol. 19(5): 437-444.

Somasegaran P and Hoben HJ. (1994) Handbook for Rhizobia, Methods in Legume-Rhizobium Technology, Heidelberg, NY: Springer. doi.org/ 10.1007/978-1-4613-8375-8.

Tabatabaee A, Assadi MM, Noohi AA and Sajadian VA. (2005) Isolation of biosurfactant producing bacteria from oil reservoirs. Iran J Environ Health Sci Eng. 2: 6-12.

Van Haesendonck IPH and Vanzeveren ECA. (2004) Rhamnolipids in bakery products. W. O. 2004/040984, International Application Patent (PCT), Washington, DC., USA.

Vanittanakom N, Loeffler W, Koch U, Jung G. (1986) Fengycin-a novel antifungal lipopeptide antibiotic produced by *Bacillus subtilis* F-29-3. J Antibiot (Tokyo) 39: 888-901.

Vijayakumar and Saravanan V. (2015) Bio-surfactants-types, sources and applications. Res J Microbiol. 20110(5): 181-192.

Volkering F, Breure AM and Rulkens WH. (1998) Microbiological aspects of surfactant use for biological soil remediation. Biodegradation 8: 401-417.

Wang Q, Sivakumar K and Mohanasundaram, S. (2022) Impacts of extrusion processing on food nutritional components. Int J Syst Assur Eng Manag. 13: 364-374.

Youssef NH, Duncan KE, Nagle DP, Savage KN, Knapp RM and McInerney MJ. (2004) Comparison of methods to detect biosurfactant production by diverse microorganisms. J Microbiol Methods 56: 339-347.

Zhang J, Xue Q, Gao H and Lai PH. (2016) Production of lipopeptide Bio-surfactants by *Bacillus atrophaeus* 5-2a and their potential use in microbial enhanced oil recovery. Microb Cell Fact. 15(1): 168.