

A DISSERTATION ON
“Production of Biosurfactant from Cooking Oil by Strain *Bacillus sp.*
(HUR-IU16) For the Removal of Heavy Metal”

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
FACULTY OF ENGINEERING
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IN PARTIAL FULLFILMENT
FOR THE
DEGREE OF MASTER OF TECHNOLOGY
IN
BIOTECHNOLOGY
BY

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(M. Tech Biotechnology) (IV Semester)

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UNDER THE SUPERVISION OF

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DECLARATION FORM

I, **Shekh Rasid**, a student of (**M. Tech Biotechnology**) (IIYear/ IV Semester), Integral University have completed my six months dissertation work entitled “**Production of Biosurfactant from cooking oil by Strain *Bacillus sp.* (HUR-IU16) for the removal of heavy metal**” successfully from **Integral University** under the able guidance of **Dr. Ashish**.

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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CERTIFICATE

This is to certify that Ms. **Shekh Rasid** (Enrollment Number 1700100281) has carried out the research work presented in this thesis entitled “**Production of Biosurfactant from cooking oil by Strain *Bacillus Sp. (HUR-IU16)* for the removal of heavy metal**” for the award of **M.Tech Biotechnology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of his **M.Tech Biotechnology** degree.

I wish him good luck and bright future.

Dr. Ashish

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This is to certify that **Shekh Rasid**, a student of **M.Tech Biotechnology** (II Year/IV Semester), Integral University has completed his six months dissertation work entitled “**Production of Biosurfactant from cooking oil by Strain *Bacillus Sp. (HUR-IU16)* for the removal of heavy metal**” successfully. He has completed this work from Integral University under the guidance of Dr. Ashish, Assistant Professor, Integral University, Lucknow. The dissertation was a compulsory part of his **M.Tech Biotechnology** degree.

I wish him good luck and bright future.

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TO WHOM IT MAY CONCERN

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I wish him good luck and bright future.

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| • BS – Biosurfactant |
| • EI – Emulsification Index |
| • ST – Surface Tension |
| • IFT – Interfacial Tension |
| • OVAT – One variable at a time |
| • CMC – Critical micelle concentration |
| • LMW – Low-molecular-weight |
| • HMW - High-molecular-weight |
| • OS – Oil Spreading |
| • AAS- Atomic Absorption Spectrometry |
| • μl – Micro liter |

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1. INTRODUCTION

Surfactants (surface-active a broad collection of compounds composed of a polar, water-soluble head group and a non-polar, water- agents) are insoluble tail group(Ying, 2006).Hence, surfactants are molecules that mix hydrophobic and hydrophilic characteristics. Synthetic surfactants are important substances in the economy. Basically Surfactants are well known for being soluble and having cleaning properties, which have allowed them to find a position in detergents and other cleaning products.A wide range of products employ surfactants extensively, including detergents for home cleaning, personal care products, textiles, paints, polymers, pesticide formulations, medicines, mining, oil recovery, pulp and paper, and textiles, paints, and polymers. A large number of these surfactants are spread throughout numerous environmental compartments. (soil, water, sediment) (Di Corcia, 1998).

The capacity of surfactant to produce micelles in solution is a crucial characteristic. Each surfactant molecule has both hydrophobic and hydrophilic groups, which contribute to this feature. Surfactants have the detergency and solubilization qualities that are caused by the creation of micelles in solution. Surfactant molecules exist as monomers in water when dissolved at low concentrations. Higher concentrations of the surfactant molecules can aggregate form clusters , which have the hydrophilic center groups towards the center of the cluster and the hydrophobic head groups facing the solvent. This lowers the free energy of the system.CMC stands for critical micelle concentration, the concentration at which this happens(Haigh, 1996).

In comparison to chemical surfactants, biosurfactants have a number of benefits, including the ability to degrade naturally due to their simple chemical structure, compatibility with the environment, low toxicity, which allows for use in the cosmetic, pharmaceutical, and food industries, high selectivity due to the presence of specific functional groups, which enables specificity in the detoxification of specific pollutants, and activity in conditions of extreme heat, pH, and salinity. The usefulness of biosurfactants in various sectors is influenced by these characteristics.

The term "biosurfactants" refers to a class of modern surfactants generated from microorganisms. These are the substances that are surface-active and are produced by both microorganisms and plants, though they are primarily produced by filamentous fungus, yeast, and bacteria.

Pseudomonas, *Bacillus*, *Rhodococcus*, and *Candida* are the most commonly employed genera for the synthesis of biosurfactants. (Müller et al., 2012).

Biosurfactants are amphiphilic surface active chemicals generated by bacteria. These amphiphilic compounds mix at the interfaces of fluids with different polarities, such as water and hydrocarbons, because they contain both hydrophilic and hydrophobic areas. reduces interfacial surface tension as a result(Jennings & Tanner, 2000).

Biosurfactants have different chemical compositions depending on the generating microbe, raw materials, and process conditions, but they all contain fatty acids, glycolipids, lipopeptides, lipopolysaccharides, and lipoproteins. It is an amphiphilic molecule that contains both hydrophilic and hydrophobic (typically hydrocarbon) moieties and preferentially partitions at the interface of fluid phases with various degrees of polarity and hydrogen bonding, such as oil/water or air/water interfaces. Biosurfactants have many properties that make them excellent for use in physicochemical and biological remediation techniques for both organic and metal pollutants, including foaming, dispersion, wetting, emulsification/de-emulsification, and coating (Pacwa-Płociniczak et al., 2011).

The property of a microbial surfactant to chelate toxic heavy metals and form an insoluble precipitate was seen as the perfect solution to the treatment of heavy metals in wastewater due to its greater environmental compatibility, lower toxicity, and higher biodegradability than synthetic surfactants (Mulligan, 2005).

Bio-surfactants are in general classified into lipopeptides, lipoproteins, glycolipids, polymeric, fatty acids, phospholipids and neutral lipids(Peters, 1999). The most known bio-surfactants are glycolipids. Long-chain aliphatic acids or hydroxyaliphatic acids are coupled with carbohydrates to form glycolipid molecules..

Rhamnolipids, trehalolipids, and sophorolipids are the three different forms of glycolipid. Rhamnolipids are one of the many bio-surfactants made by microorganisms, and they have been used to improve oil recovery, clean up contaminated soil, pharmaceutical industries, food & beverage industries etc (Aşçı et al., 2008)..The bacterium *Pseudomonas* species produces rhamnolipids, which are anionic bio-surfactants. It is made up of one or two rhamnose sugar molecules connected to one or two β -hydroxydecanoic acid molecules. The efficiency of these molecules for environmental applications is determined by the formation of films at the air-water

interface (Banat, 1995). It's interesting to note that while it is generally known that microbial cells can complex metals from solutions, there is little information in the literature about the use of bio-surfactants to complex with heavy metals (Salehizadeh & Shojaosadati, 2003).

Even while biosurfactants have demonstrated significant promise for wider application, industrial-scale manufacture and commercialisation have not yet fully materialised. Before large-scale industrial production can begin, problems including high production costs and challenges with recovering the pure products must be resolved, giving their synthetic equivalents a competitive advantage (Helmy et al., 2011). The recovery of biosurfactants during subsequent procedures is also influenced by the mixture of microorganisms and culture media employed for synthesis.

Due to their high production costs, biosurfactants are only used in a restricted number of applications. These costs can be reduced by process optimisation, strategies for downstream processing, and fermentation of agro-industrial waste. The selection of adequate medium components and ideal growing conditions to increase biosurfactant productivity is one technique that achieved the aforementioned goal. The implementation of a single factor optimization approach (leaving other parameters constant) can be used to get around the drawbacks of the traditional way of media optimization (Perfumo et al., 2010).

This work is an initial attempt to systematically screen, isolate, and characterize biosurfactant-producing bacteria capable of utilizing used cooking oil as the sole carbon source.

This study also describes the production, characteristics, and surface properties of the extracted biosurfactant of *Bacillus sp.* HUR-19(PET1), which contributes to the attempts to reduce the biosurfactants' production cost by using cheap substrates as well as evaluating the efficiency of biosurfactants from used cooking oil, as a substrate by locally isolated bacteria, for heavy metals removal.

Objectives:

- ▶ **Production of Bio-surfactant from waste kitchen oil.**
- ▶ **Estimation of Bio-surfactant activity.**
- ▶ **Estimation of Biosurfactant potential for the bioremediation of heavy metals (Pb, Cr and Cd).**

2. REVIEW LITERATURE

Microorganisms can produce a wide range of surface-active chemicals known as bio-surfactants. These compounds are prospective candidates for improving oil recovery and de-emulsification processes, as they lower surface and interfacial tensions in both aqueous solutions and hydrocarbon mixtures.(Sarkar et al., 1989)(Shepherd et al., 1995)

In comparison to chemical surfactants, bio-surfactants have a number of properties, including lower toxicity, higher biodegradability , better compatibility with the environment , increased foaming , high selectivity and specific activity at extreme temperatures, pH, and salinity , and the ability to be synthesized from renewable feed-stocks (Desai & Banat, 1997).

Bio-surfactants are microbial products that are beneficial to the environment and have been shown to be effective at removing petroleum hydrocarbon pollutants from drill cuttings and hydrocarbon-contaminated waste streams (such soil and wastewater from refineries). They have many advantages, including a wide range of industrial applications, high biodegradability, minimal environmental effect, low toxicity, high specificity, high stability and activity under harsh conditions, and structural diversity.(R. Makkar & Cameotra, 2002)

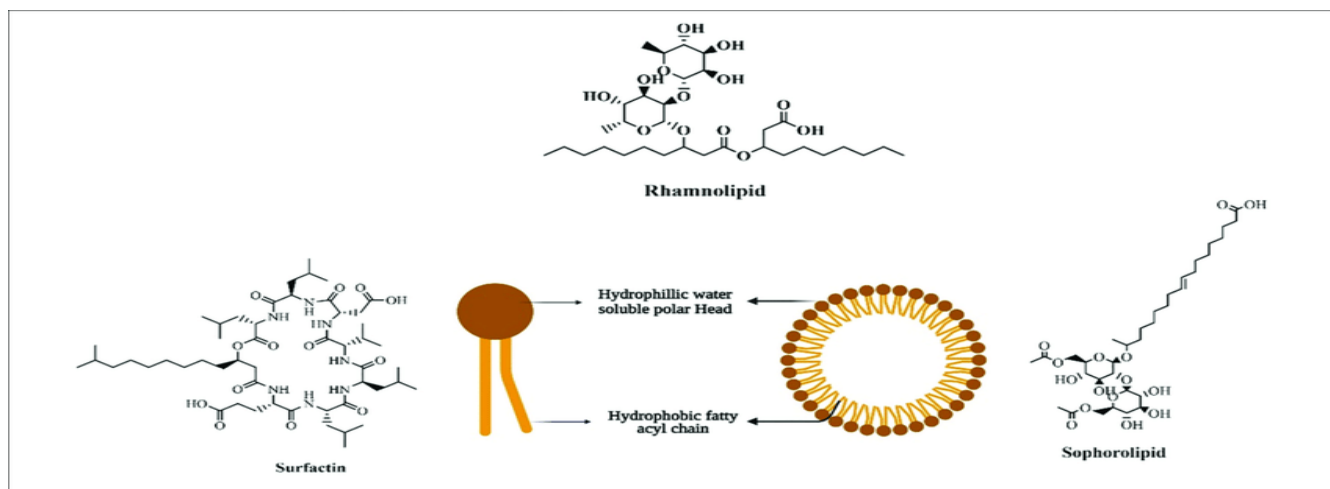


Figure1 . Amphiphilic properties of surfactant

Bio-surfactants are used for several important roles—namely, emulsification, dispersal, solubilization, mobilization, wetting, surface tension reduction, formation of micelles and foam

formation—due to their ability to partition into different interfaces: liquid/solid, liquid/gas, liquid/liquid. Since of their amphiphilic qualities, biosurfactants (as shown in figure1) can be used in these tasks since they contain both hydrophobic and hydrophilic molecules. Biosurfactants have thus been recognised as one of the most promising and adaptable process chemicals, with exceptional potential applications in environmental restoration (Das & Mukherjee, 2007a).

Biosurfactant are considered environmentally beneficial. Biosurfactant are correctly and properly described as "multifunctional biomolecules/materials of the twenty-first century". As the relevance of sustainable manufacturing processes and sustainability activities in general is becoming more recognised and climbing to the top of many firms' agendas, attention to biosurfactants has increased . The rising focus on biosurfactant is also related to the ongoing emphasis on sustainable and environmentally friendly societies , as well as the necessity for environmental protection (Freitas et al., 2016).

Biosurfactant are a diverse group of microbial chemicals synthesized by bacteria, yeasts, and fungi . Bacterial biosurfactants rhamnolipid and surfactin, generated by the *Pseudomonas* and *Bacillus* species, have received the greatest attention (Brumano et al., 2016).

Biosurfactants are classified into six types:

- (i) Glycolipids
- (ii) Phospholipids
- (iii) Lipopeptide
- (iv) Lipoproteins
- (v) Fatty acids
- (vi) Neutral acids
- (vii) Polymeric and particulate surfactants.

Because of their functional and structural diversity, biosurfactants are an interesting class of molecules with the potential to be used in a wide range of biotechnological and industrial applications (Varjani & Upasani, 2017).

2.1 Types of biosurfactant

Biosurfactant are primarily categorised by their chemical makeup and their microbiological origin, as opposed to chemically synthesised surfactants, which are grouped according to the kind of polar grouping they include. A hydrophilic moiety made up of amino acids or peptides, anions or cations, mono-, di-, or polysaccharides, and a hydrophobic moiety made up of unsaturated, saturated, or fatty acids make up the majority of their structure. Accordingly, glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants, and particle surfactants are the main types of biosurfactants.

Some biosurfactants have been reported to be formed on water-soluble substances like glucose, sucrose, glycerol, or ethanol, despite the fact that there are several studies on the synthesis of biosurfactants by hydrocarbon-degrading microbes.

Glycolipids

Most known biosurfactants are glycolipids. They are long-chain aliphatic acids or hydroxyaliphatic acids combined with carbohydrates. A fatty acid molecule is joined to a lipid molecule by a glycosidic bond to form the structure of Glycolipids. The overall molecule changes depending on how the lipid and carbohydrate portions are made up. Rhamnolipid, trehalose lipids, mannosylerythritol lipids (MELs), cellobiose lipids, or Sophorolipids are some examples of glycolipids, depending on the kind of carbohydrate. Different microorganisms produce different glycolipids. Among the glycolipids, the best known are Rhamnolipid, trehalolipids, and sophorolipids.(Ribeiro et al., 2020)(Williams & Trindade, 2017)

Rhamnolipids: Rhamnolipid, which are among the most researched glycolipids to date, are made up of one or two L-rhamnose molecules joined to a monomer or dimer of β -hydroxy fatty acids . They have a variety of novel uses, such as in the treatment of soil or the management of pests . *Pseudomonas aeruginosa*, the leading producer with titers > 100 g/L, is one of many bacteria from the *Pseudomonas* and *Burkholderia* genera that manufacture Rhamnolipid. (Hörmann et al., 2010)

Two pathways are required to provide the essential chemicals for the production of rhamnolipids. The first one is the rmlABCD operon's synthesis of dTDP-L-rhamnose; glucose-1-phosphate is transformed into activated rhamnose, which acts as a donor for the hydrophilic component of rhamnolipids. The hydrophobic part originates from β -hydroxy fatty acids coupled to an acyl carrier protein. (Geys et al., 2014).

Trehalolipids :- Trehalolipids are a subclass of glycolipid biosurfactants that have a variety of potential industrial uses because of their unique characteristics. These include of usage as emulsifiers and solubilizers of hydrocarbons in bioremediation, MEOR (microbial enhanced oil recovery), cosmetic, and food applications. Additionally, they have anticancer and immunomodulatory properties that can be exploited in biomedicine.. (Bages-Estopa et al., 2018)

The genus *Rhodococcus* produces trehalolipids, which are primarily linked to growth on hydrocarbons and are necessary for substrate absorption and surface adherence. These biosurfactants make it easier for cells to access water-insoluble substrates by allowing hydrophobic cells to directly interact with oil droplets (cell bound biosurfactant) or by adhering to the emulsified oil (extracellular biosurfactant) (Weber & Zeiner, 2014)

Sophorolipids: - Sophorolipids are mostly produced by yeasts like *Torulopsis bombicola*, *T. petrophilum*, and *T. apicola*, which attach a dimeric carbohydrate termed sophorose to a long-chain hydroxy fatty acid to make sophorolipids. These biosurfactants are synthesised by the combination of at least six to nine different hydrophobic sophorosides. Additionally, it has been discovered that different yeasts create comparable mixtures of water-soluble sophorolipids. Sophorolipids are inefficient emulsifiers, although they have the capacity to lower surface and interfacial tension. Both lactonic and acidic sophorolipids were able to lower the interfacial tension between n-hexadecane and water from 40 to 5 mN/m while also demonstrating outstanding resistance to temperature and pH fluctuations (Van Bogaert et al., 2011).

Lipopeptide biosurfactant:- The most popular interesting group of biosurfactants are Lipopeptide biosurfactant. Two primary portions of lipopeptide molecules include an acyl tail or tails and a brief linear oligopeptide sequence with an amide bond. The lipopeptide biosurfactant has a hydrophobic tail with a hydrocarbon chain and a hydrophilic head with a peptide sequence.

The peptide component contains cationic and anionic residues, as well as non proteinaceous amino acids on occasion (Kumar & Ngueagni, 2021).

The industrial, environmental protection, and medical domains are where cyclic lipopeptides showed their finest performance. According to certain research lipopeptides have promise as antibacterial and anticancer agents. Because of their unique structural makeup and functional capabilities, lipopeptides may be used in a variety of contexts . Different forms of lipopeptide surfactants are produced by a variety of microorganisms. Non-ribosomal peptide synthetase (NRPS) is the enzyme that helps to synthesize cyclic lipopeptide (Janek et al., 2016).

Surfactin:-

This is a standout amongst the most potential biosurfactant is incorporated by *Bacillus subtilis*. It is made up of an unsaturated fat chain attached to a seven-ring structure with seven amino corrosive atoms. At a fixation as low as 0.005%, it reduces surface strain from 72 to 27.9 mN/m.

Lichenysin:-

Several biosurfactants incorporated by *Bacillus licheniformis* go about as synergistically and display extraordinary temperature, salt and pH security. They are comparable to surfactin in terms of their auxiliary and physio-synthetic capabilities. The surface tension of water can be reduced to 27 mN/m and the interfacial pressure between water and n-hexadecane to 0.36 mN/m by the surfactants provided by *B. licheniformis*.

Fatty acids, phospholipids and neutral lipids

During the course of their development on n-alkanes, certain bacteria and yeast produce enormous quantities of unsaturated fats and phospholipid surfactants. Phosphatidyl ethanolamine-rich vesicles are transported to *Acinetobacter* spp. 1-N where they form optically transparent smaller scale emulsions of alkanes in water. For therapeutic applications, these biosurfactants are essential. According to Gautam and Tyagi, the lack of phospholipid protein complex is thought to be the primary cause of the premature birth of children's breath disappointment. In addition, they have suggested that the fermentative synthesis of such

surfactants can make use of the detachment and cloning of the attributes responsible for such surfactant.

2.2 Advantages of biosurfactant

Biodegradability:- Microbial derived compounds can be easily degraded when compared to synthetic surfactants and are suitable for environmental applications such as bioremediation. The increasing environmental concern forces us to search for alternative products such as biosurfactants. Synthetic chemical surfactants impose environmental problems and hence, biodegradable biosurfactants from marine microorganisms were concerned for the biosorption of poorly soluble polycyclic aromatic hydrocarbon, phenanthrene contaminated in aquatic surfaces controlled the blooms of marine algae, *Cochlodinium* using the biodegradable biosurfactant sophorolipid with the removal efficiency of 90% in 30 min treatment. (Mohan & Pittman Jr, 2006)

Low toxicity:- Biosurfactants show greater toxicity than surfactants made from chemicals. Additionally, it was shown that biosurfactants displayed greater effective concentration than synthetic dispersants (effective concentration to reduce 50% of test population).

Availability of raw materials: - Biosurfactant can be made from readily accessible, extremely affordable raw sources. Hydrocarbons, carbohydrates, and lipids are possible sources of carbon and can be used alone or in combination.

Physical factors:- Environmental parameters like temperature, pH, and ionic strength tolerances do not impact many biosurfactants. Temperatures up to 50 °C, a pH range of 5 to 9.0, a NaCl concentration of 50 g/l, and a Ca concentration of 25 g/l had no effect on the lichenysin generated by the *Bacillus licheniformis* strain (Muthusamy et al., 2008).

Surface and interface activity:- According to Mulligan, an effective surfactant may reduce the interfacial tension between water and hexadecane from 40 to 1mN/M and the surface tension of water from 75 to 35mN/m. Surfactin can lower the interfacial tension of water and hexadecane to less than 1mN/M and the surface tension of water to 25mN/M (Mulligan, 2005).

2.3 Factors affecting biosurfactant production

The nature of the carbon source, the nitrogen source, as well as the C:N ratio, nutritional requirements, chemical and physical parameters such as temperature, aeration, divalent cations, and pH influence not only the amount of biosurfactant produced but also the type of polymer produced. The composition and emulsifying activity of the biosurfactant depends not only on the producer strain but also on the culture conditions (Salihu et al., 2009).

Carbon sources:- The type of the carbon substrate affects and influences the quantity and quality of biosurfactant synthesis [13]. For the manufacture of biosurfactants, it has been suggested that diesel, crude oil, glucose, sucrose, and glycerol are good sources of carbon substrate (Rahman & Gakpe, 2008).

Nitrogen sources:- Nitrogen is important in the biosurfactant production medium because it is essential for microbial growth as protein and enzyme syntheses depend on it. The creation of biosurfactants has utilised a variety of nitrogen compounds, including urea peptone, yeast extract, ammonium sulphate, ammonium nitrate, sodium nitrate, meat extract, and malt extracts. Although yeast extract is the most popular nitrogen source for making biosurfactants, its use in terms of concentration depends on the organism and the culture medium. *Arthro bacter paraffineus* prefers ammonium salts and urea as nitrogen sources, whereas *P. aeruginosa* uses nitrate to support maximal surfactant synthesis (Adamczak & Bednarski, 2000).

Physical factors:- These have a crucial role in the yield and properties of the produced biosurfactant. It is always vital to optimise the bioprocess in order to produce significant amounts of biosurfactants because the product may be impacted by variations in temperature, pH, aeration, or agitation speed. According to reports, most biosurfactant productions are reportedly carried out at a temperature range of 25-30 C. Zinjarde and Pant investigated how pH affected the amount of biosurfactant generated and found that the best production happened at a pH of 8.0, which is the pH of sea water in its natural state. (Zinjarde & Pant, 2002)

Both aeration and agitation have a significant role in the transfer of oxygen from the gas phase to the aqueous phase, which has an impact on the synthesis of biosurfactants. It has been proposed

that the synthesis of bioemulsifiers can improve the solubilization of water-insoluble substrates and subsequently ease nutrient delivery to bacteria, suggesting that it may also be connected to the physiological function of microbial emulsifier.

Salt concentration:-Although salt concentration affects the cellular activity of microorganisms, salt concentration had a proportional influence on biosurfactant formation. Nevertheless, contrary observations were noticed for some biosurfactant products which were not affected by concentrations up to 10% (weight/volume) although slight reductions in the CMC were detected (Md, 2012).

2.4 Physiological role of biosurfactant in microorganisms

A number of microorganisms create biosurfactants, which are mostly released extracellularly or linked to cell components during development on water-impermeable substrates. By lowering the surface tension at the phase boundary, biosurfactants' primary physiological function is to enable microorganisms to grow on water-immiscible substrates. This makes the substrate more readily available for uptake and metabolism, though the molecular mechanisms underlying this uptake are still unclear and incompletely understood. Biosurfactants' antibacterial properties towards diverse microorganisms are another physiological function of these substances. As a rule, different surfactant inhibits different taxonomy. Additionally, it has been demonstrated that biosurfactants have a role in cell adhesion, which confers the highest stability under adverse environmental circumstances, pathogenicity, and cell desorption when organisms need to find new homes to survive (Desai & Banat, 1997).

2.5 Applications of biosurfactant-;

Commercial Applications of Biosurfactants:- The global output of surfactants reached 22 million metric tonnes (including soaps), with 3-4% annual growth rates predicted worldwide. These chemically created surfactants are mostly petroleum-based and often not biodegradable, therefore they continue to be detrimental to the environment in which they are used.(Rahman & Gakpe, 2008)

Environmental scientists have been tightening environmental regulations as a result of growing awareness of the need to protect ecosystems, which has led to an increase in interest in surfactants of microbial origin as potential substitutes for chemically synthesised ones. These compounds may also bio-accumulate, and their production processes and byproducts may be environmentally hazardous.

Application of Biosurfactants in Agriculture:- Utilising surfactants as mobilising agents is one method for increasing the solubility of bio-hazardous chemical compounds like PAH. The apparent solubility of hydrophobic organic contaminants (HOC) is increased as a result. The diffusion route length between the site of absorption and the site of biouptake by the microorganisms is also stated to be reduced by surfactants, which aid bacteria in adhering to soil particles containing contaminants. Surfactants are also used in agriculture to hydrophilize heavy soils to get high wettability and promote uniform fertiliser distribution in the soil. Additionally, they facilitate the distribution and penetration of the toxicants in pesticides and stop some fertiliser from caking during storage. The rhamnolipid biosurfactant, mostly produced by the genus *Pseudomonas* is known to possess potent antimicrobial activity.

Furthermore, cumulative exposure to rhamnolipid biosurfactants is not expected to have any negative impacts on people or the environment. Fengycins may be used in the biocontrol of plant diseases because it has been found that they have antifungal action (R. S. Makkar & Rockne, 2003).

Applications of biosurfactants in commercial Laundry detergents:- Biosurfactants like Cyclic Lipopeptide (CLP) can withstand a wide pH range (7.0–12.0) without losing any of their surface-active properties when heated at high temperatures. They revealed great compatibility and stability with conventional laundry detergents and demonstrated good emulsion forming capabilities with vegetable oils, both of which supported their inclusion in the formulation of laundry detergents. (Das & Mukherjee, 2007b)

Biosurfactants as Biopesticide:- The traditional approach to controlling arachnids requires the use of pesticides and chemicals with a wide range of potential side effects. Additionally, the rise of insect populations that are resistant to pesticides and the growing cost of new chemical

pesticides have sparked a hunt for alternative environmentally benign vector control methods. It is possible to employ lipopeptide biosurfactants as a biopesticide since they have insecticidal action against the fruit fly *Drosophila melanogaster* (Mulligan, 2005).

Application of bio-surfactants in medicine:-

Antimicrobial activity:

Biosurfactants can function in a number of ways due to the diversity of their architectural structures. Biosurfactants, like detergents, have a negative effect on the permeability of cell membranes due to their structural makeup. Gharaei-Fathabad discovered that some biosurfactants exhibited strong antibacterial, antifungal, and antiviral activity. These surfactants work as anti adhesive agents to infections, making them advantageous for treating a range of ailments as well as its use as a therapeutic and probiotic agent. A great example is the marine bacterium *B.circulans*' biosurfactant, which has proven to have potent antibacterial activities against both Gram-positive and Gram-negative diseases as well as semi-pathogenic microbial strains like MDR strain.(Gharaei-Fathabad, 2011)

Anti-cancer activity:- Sophorolipids (SLP) display anticancer activity against several kinds of tumoral cells and might have a potential use for cancer treatment. SLP synthesized by *Wickerhamiella domercqiae* show cytotoxic effects in several cancerous cell lines. SLP exhibited significant inhibition of cell proliferation on cancer cells of H7402 (liver cancer line).

Some microbial extracellular glycolipids promote cell differentiation rather than proliferation in the human promyelocytic leukaemia cell line, and exposure of PC 12 cells to MEL increased the activity of acetylcholine esterase and disrupted the G1 phase of the cell cycle, leading to overgrowth of neurites and partial cellular differentiation. These findings suggest that MEL promotes neuronal differentiation in PC 12 cells and lay the foundation for the use of microbiome-derived growth factors(Muthusamy et al., 2008).

Anti-adhesive agents:- The adhesion of pathogenic organisms to solid surfaces or infection sites has been shown to be inhibited by biosurfactants. (Fathabad, 2011) showed that pre-coating vinyl urethral catheters with a surfactin solution before inoculating them with media reduced the amount of biofilm formed by *Salmonella typhimurium*, *Salmonella enterica*, *E. coli*, and *Proteus*

mirabilis. According to (Muthusamy et al., 2008) *S. thermophilus* surfactant pretreated silicone rubber suppressed *C. albicans* adherence by 85%, and surfactants from *L. fermentum* and *L. acidophilus* adsorbed on glass decreased the number of adhering uropathogenic cells of *Enterococcus faecalis* by 77%.

Immunological adjuvants:- When combined with traditional antigens, bacterial lipopeptides serve as powerful non-toxic, nonpyrogenic immune adjuvants. Low molecular mass antigens Iturin AL and herbicolin A have been shown to enhance the humoral human response (Md, 2012).

Antiviral activity:- Biosurfactants have been mentioned in the literature as having antibiotic properties and preventing the proliferation of the human immunodeficiency virus in leucocytes . Additionally, Muthusamy et al. indicated that a female regulated, effective, and safe vaginal topical microbicide was required due to the rise in the prevalence of HIV in women. The most effective spermicidal and virucidal agent is sophorolipids surfactants from *C. bombicola* and its structural analogues, such as the sophorolipid diacetate ethyl ester; it has also been noted that this chemical has virucidal action against human semen that is comparable to nonoxynol-9.(Muthusamy et al., 2008)

Genetic Manipulation:- For fundamental sciences and clinical applications like gene therapy, it is essential to develop a reliable and secure technique for delivering exogenous nucleotides into mammalian cells. Lipofection using cationic liposomes is considered to be a save way to deliver foreign gene to the target cells without side-effects among various known methods of gene transfection (Gharaei-Fathabad, 2011). According to research cited by Kitamoto et al., biosurfactant-based liposomes exhibit greater gene transfection efficacy than commercially available cationic liposomes. Some approaches and procedures for liposome-based gene transfection have been developed in the previous ten years. By adding biosurfactants in this area, Ueno et al. in 2007 tested MEL-A-containing liposome for gene transfection.

Application of biosurfactant in food processing industry:- Biosurfactants have been used for a variety of food processing applications, but they typically play a role as food formulation ingredients and anti-adhesive agents. As food formulation ingredients, they encourage the formation and stabilisation of emulsion due to their capacity to reduce surface and interfacial

tension. It is also used to adjust the rheological qualities of wheat dough, stabilise aerated systems, regulate the aggregation of fat globules, enhance the texture and shelf life of starch-containing goods, stabilise aerated systems, and stabilise products with a fat foundation.(Muthusamy et al., 2008).

Application of biosurfactant in petroleum:- In areas like bio-corrosion and biofouling degradation of hydrocarbons within oil reservoirs, enzymes and biocatalysts for petroleum upgrading, biosurfactant and bio emulsifiers are among the most potent and versatile by-products that modern microbial technology can offer. Biosurfactants also play a significant part in the production of petrochemicals as well as the upgrading, transportation, and refinement of petroleum.(Perfumo et al., 2013)

Application of biosurfactant in microbial enhanced oil recovery:- The recovery of residual oil via microbially enhanced oil recovery (MEOR), which makes use of microorganisms and the products of their metabolism, is a significant technological advancement. It is generally acknowledged that current Enhanced Oil Recovery (EOR) technology can recover around 30% of the oil that is available in a reservoir. Poor recovery in oil producing wells may be brought on by the oil's high viscosity, which causes poor mobility, or the limited permeability of specific reservoirs. Because most of the oil is left in the reservoir when primary and secondary recovery methods are used, interest has grown in tertiary recovery techniques. It is clear that using chemical surfactants is not an appropriate choice for cleaning up oil reservoirs since they are risky, expensive, and leave behind undesired residues that are difficult to get rid of without harming the environment. The presence of several species of microorganisms with diverse growth characteristics and metabolite synthesis determines the distinct recovery impacts on the reservoir environment. There are several methods for using biosurfactants in MEOR including injecting biosurfactant-producing microorganisms into a reservoir through a well and then propagating them in situ through the reservoir rock injecting selected nutrients into a reservoir to encourage the growth of native biosurfactant-producing microorganisms; and another method that involves producing biosurfactants (Shoeb et al., 2013).

Application of biosurfactant in cosmetic industry:- Biosurfactants have been suggested as a replacement for chemically produced surfactants in the cosmetics sector because of their effects on viscosity and product consistency through emulsification, foaming, water binding ability,

spreading, and wetting. Multifunctional biosurfactants have several cosmetic applications because of their exceptional surface properties such as detergency, wetting, emulsifying, solubilizing, dispersing and foaming effects. Biosurfactants are known to have advantages over synthetic surfactants such as low irritancy or anti-irritating effects, better moisturizing properties and compatibility with skin, therefore, highly on demand. The most widely used biosurfactant glycolipids in cosmetics are sophorolipids, rhamnolipids and mannosylerythritol lipids. Sophorolipids have good skin compatibility and excellent moisturizing properties, rhamnolipids are natural surfactants and emulsifiers that can replace petrochemical based surfactants used in most of the cosmetic products. They also have been used in acne pads anti-dandruff, anti-wrinkle and anti-ageing products, deodorants, nail care products and toothpastes in several different formulations, because of its high surface and emulsifying activities (Piljac & Piljac, 1999). Mannosylerythritol lipids are generally used in skin care formulations as the active ingredient to prevent skin roughness (Masaru et al., 2007).

3. Materials and methods

The *Bacillus sp.* strain HUR-IU (PET-1) was collected from the Bioprocessing laboratory of Department of bioengineering in Integral university, Lucknow, India. Production of Biosurfactant and estimate the efficiency of produced biosurfactant for the bioremediation of heavy metals.

Strain: *Bacillus sp.* HUR-IU (PET-1)

3.1. Media for growth of bacterial strain (Nutrient broth): The bacterial strain was grown in nutrient broth medium with distilled water. 13 gm of nutrient broth was suspended in 1000ml of distilled water and the medium was dissolved completely. Media was sterilized by autoclaving at 15 lbs. pressure at 121°C for 15 minutes and after inoculation with bacterial strain kept in orbital shaking incubator for 2 days (48 hours) at 37°C. After growth, it was stored in refrigerator for seed culturing and inoculation.

50 ml of growth media (Nutrient broth) (g/L) is prepared containing:

- Peptone [5]
- Sodium chloride [5]
- Meat extract [1.5]
- Yeast extract [1.5]

3.2. Production of Biosurfactant from Used Cooking Oil as Carbon Source

The HUR-IU (PET-1) bacterial strains were added to 100 ml of nutrient broth and incubated until they reached the exponential phase, as measured by optical density at 600 nm, as a seed culture, at 30C and 150 rpm of agitation. A 250 ml Erlenmeyer flask containing 50 ml of mineral salt medium (MSM) and 2% (v/v) of UCO, respectively, was infected with the bacterial suspensions (4%, v/v). Before the medium was autoclave sterilized, the pH was initially adjusted to 7.0 with the help of HCl and NaOH. It was then incubated for 72 hrs at 37C in a rotary incubator shaker at 120 rpm.

3.3. Production of biosurfactant:

The bacterial strain was grown in minimal salt medium(MSM) with hydrocarbon (HC) source as kitchen waste oil (2%v/v) and nitrogen source as urea (2%w/v) for 3 days (72 hours) in an orbital shaker incubator at 37°C. The cell-free broth obtained after centrifugation (10000 rpm for 20

minutes) obtained supernatant was used for qualitative assay *i.e.*, spread assay, drop collapse method emulsification test.

250ml of Production media (g/L) composition:

- NH_4CL [2]
- Na_2HPO_4 [2.2]
- KH_2PO_4 [0.14]
- $\text{MgSO}_4.7\text{H}_2\text{O}$ [0.6]
- $\text{FeSO}_4.7\text{H}_2\text{O}$ [0.02]
- NaCl [0.01]
- Yeast extract [0.02]
- CaCl_2 [0.04]

Trace elements (0.1 ml/L)

- $\text{ZnSO}_4.7\text{H}_2\text{O}$ [1.7]
- $\text{MnSO}_4.4\text{H}_2\text{O}$ [0.85]
- H_3BO_3 [0.56]
- $\text{CuSO}_4.5\text{H}_2\text{O}$ [0.8]
- $\text{Na}_2\text{MoO}_4.2\text{H}_2\text{O}$ [0.16]
- $\text{CoCl}_2.6\text{H}_2\text{O}$ [0.45]
- EDTA [1.0]
- $\text{NiCl}_2.6\text{H}_2\text{O}$ [0.005]
- KI [0.6]

3.4. Recovery of biosurfactant

The microbial cells were harvested from the culture broth by centrifugation at 12,000 g for 20 min. The supernatant was acidified with 6N hydrochloric acid solute on to pH 2.0. The precipitated biosurfactant was recovered by centrifuging it at 12,000 g for 20 minutes, and the acidified biosurfactant was neutralized with 1N NaOH (pH 7) solution. An equal volume of ethyl acetate was added to the supernatant, and the organic phase was dried on a rotary vacuum evaporator.

3.5. Qualification and Quantification analysis

Measurement of oil spreading activity: (Morikawa et al., 2000) described the Oil spreading experiment in a Petri dish, 20 ml of distilled water was first poured, followed by the addition of 15 ml of oil (mobile oil from a motorcycle), creating a thin layer on the water's surface. The oil surface was then topped off with 10 µl of cell-free culture broth. The diameter of the oil-free clearing zone, which forms when biosurfactant is present in the cell-free culture broth, is a good indicator of the activity of the surfactant.

Drop collapse method: Approximately 100 µl of culture filtrate (supernatant) was placed on the hydrophobic surface of crude oil to observe the shape of the drop. The presence of surfactant leads to a reduction on the interfacial tension between the hydrophobic film and hydrophilic drop which cause the appearance of flat/ collapsed drops on the film.

Emulsification test: The production of biosurfactant was estimated in terms of the Emulsification index value i.e., %E₂₄ at different time intervals. The % E₂₄ is the height of the emulsion layer, divided by the total height of the liquid, multiplied by 100. Emulsification index (E₂₄) was measured after 24 h. 2ml of culture supernatant or biosurfactant crude extract, 2 ml of carbon source, was added and vortexed at high speed for 2 minutes. The mixture was allowed to stand for 24 hours prior to measurement. High percentage of emulsification index indicates production of biosurfactant. *Bacillus sp.* HUR-IU (PET-1) favored kitchen oil as carbon source for the production of biosurfactant.

$$EI = (\text{Height of emulsion layer} / \text{Height of the total mixture}) \times 100$$

3.6. Preparation of heavy metal solutions

The protocol of samples was prepared by dissolving specified weight of metal salts in deionized water to produce metal solution. The pH of each solution was measured using pH meter. There are 4 types of heavy metals are used in their salt form as follow;

Cadmium

Chromium

Lead

Arsenic

Each heavy metals (2.5mg) were dissolved in (250ml) distilled water and kept in rotatory shaker for 24 hours at room temperature. The biosurfactant was recovered after 72 hours in the cell free culture broth using a solvent extraction method were then added into heavy metal solution of lead, chromium, and cadmium respectively with different dilution rate. The medium without biosurfactants are served as the control. The samples were then analyzed for the concentration of metals present after treatment according to standard procedure using atomic absorption spectroscopy. All experiments were performed in triplicate, and the average of the results is presented.

Experimental design

Aliquots of 10 ml samples (500 mg/L) of the metal solution of each heavy metals were transferred into four test tubes as control. . The concentration of the heavy metals in the solution with biosurfactant supernatant are 2:8,4:6,6:4,8:2 in ml.. Samples were then incubated at room temperature for one hour, and the average value was calculated and presented as a percentage of metal removed. All experiments were made in triplicate, and the average value was calculated and presented as a percentage of metal removed.

3.7. Heavy metal estimation:

In this study, a simple, reliable, sensitive and convenient AAS method has been developed for quantitative estimation of heavy metals. The most widely techniques to analyze heavy metals are atomic absorption spectrometry (AAS).

Atomic absorption spectrophotometer equipped with a deuterium lamp for background correction was used for determination of trace elements and heavy metals. The hollow-cathode lamps for, Cd, Pb Cr were employed as radiation source. Hydride generator was used for hydride formation in As and Pb analysis. The flames used were air/acetylene and N₂O/acetylene. Nitrogen was used as carrier gas.(Frentiu et al., 2013).

4. Result & Discussion:

Biosurfactant considered as an efficient biological *surface-active agent* may provide an alternative solution for the removal of heavy metals from industrial wastes. Biosurfactant exhibit the properties of reducing surface and interfacial tension, stabilizing emulsions, promoting foaming, high selectivity, and specific activity at extreme temperatures, pH, and salinity, and the ability to be synthesized from renewable resources.

This study aimed to produce bio-surfactant from renewable feedstock, which was used cooking oil (UCO), through a newly isolated strain, *Bacillus sp.* HUR-IU for heavy metals removal (Cd, Cr, Pb). *Bacillus sp.* HUR-IU is a Gram-positive isolate that gave the oil displacement area with the lowest surface tension, after 4 days of culturing in mineral salt medium and 2% (v/v) UCO at a temperature of 36°C and agitation at 120 rpm. The bio-surfactant was capable of removing of Chromium, Lead and Cadmium, respectively, from artificially contaminated water, highlighting its potential activity used for bioremediation.

There is a little information about the effect of bio-surfactant-metal interactions on metal structure. Our earlier work has concluded that the isolated bacteria strain *Bacillus sp.* HUR-IU16 was found to have the ability of producing the biosurfactant (Rhamnolipid) in the form of biological molecules.

In this study presents experimental results that evaluate the capability of Biosurfactant and their ability on enhancing removal of heavy metals, in the water systems contaminated with heavy metals.

The goal of the addition of a biosurfactant may promote desorption of heavy metals from contaminated water through complexation of the free form of the metal residing in solution. This decreases the solution-phase activity of the metal resulting in direct contact between the biosurfactant and the sorbed metal and, therefore, promotes desorption. Clearly, biosurfactant structure size and charge will affect movement of bio-surfactant-metal complexes(Elouzi et al., 2012).

4.1. Oil spreading technique (OS):

This technique measures the diameter of clear zones caused when a drop of a bio-surfactant containing solution is placed on an oil water surface. When the bio-surfactant containing solution was added drop-wise with the help of pipette on the oil water surface, then there was formation

of clear zone. This indicates that bio-surfactant production was maximum when kitchen oil was used as carbon substrate. Figure 1 (a) show the control that when water was used over waste mobile oil no clear zone observed simultaneously in Figure1 (b) produced biosurfactant added drop-wise clear zone was formed as shown in figure 1. This activity of produced biosurfactant reveals that it have high potential for remediation of sea spills.

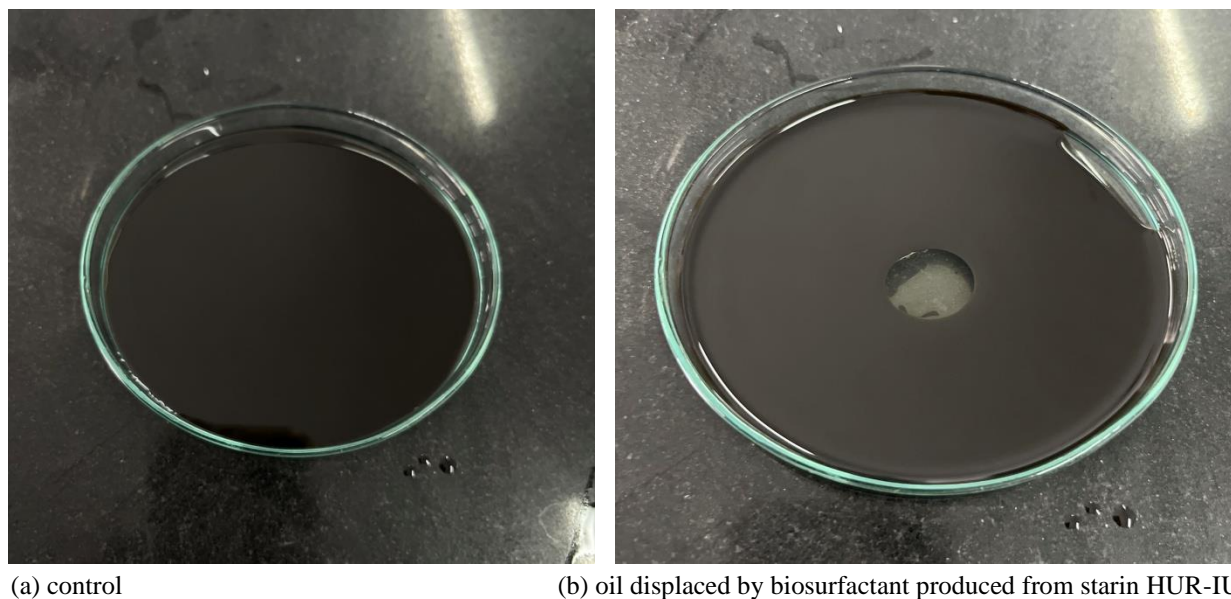


Fig 1:-Oil spreading analysis

4.2. Drop collapse method:

A sensitive rapid method was devised for screening bacterial colonies that produce surfactants. Drops of cell suspensions of surfactant-producing HUR-IU collapsed on an oil-coated surface. When the drop of cell suspension was added on an oil coated surface, the oil surface collapsed. This was the indication of presence of biosurfactant, drop collapse method was considered as qualitative estimation method for biosurfactant activity determination. Figure 2 show that biosurfactant produced from strain HUR-IU having potential to reduce the surface tension and so oil droplet collapsed when biosurfactant was added.



Figure 2: Drop collapse assay using automobile waste oil.

4.3.Emulsification test:

2ml of culture supernatant or biosurfactant crude extract, 2 ml of Petrol, diesel, kerosene and Terpene, was added and vortexed at high speed for 2 minutes. The mixture was allowed to stand for 24 hours prior to measurement. High percentage of emulsification index indicates production of biosurfactant. *Bacillus sp.* HUR-IU (PET-1) favored kitchen oil as carbon source for the production of biosurfactant. Table 1 shows that petrol showed highest emulsification activity.

Table 1. Emulsification index of different oils with produced Biosurfactant

| Oils | EI% |
|----------|-------|
| Petrol | 45.9% |
| Diesel | 40.6% |
| Kerosene | 23.4% |
| Terpene | 19.2% |

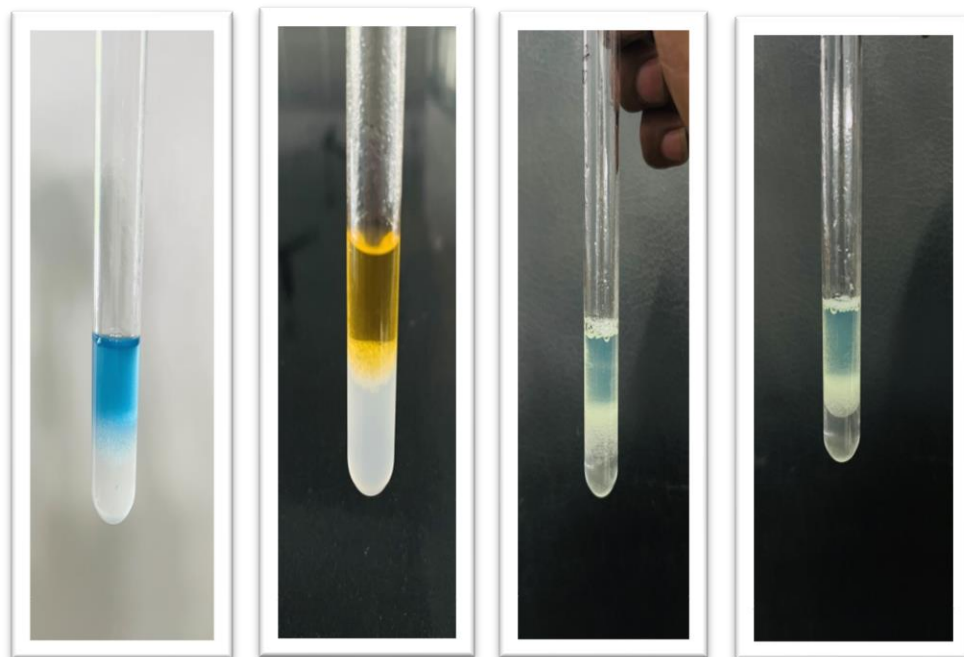


Figure 3. Kerosene Terpene Diesel Petrol

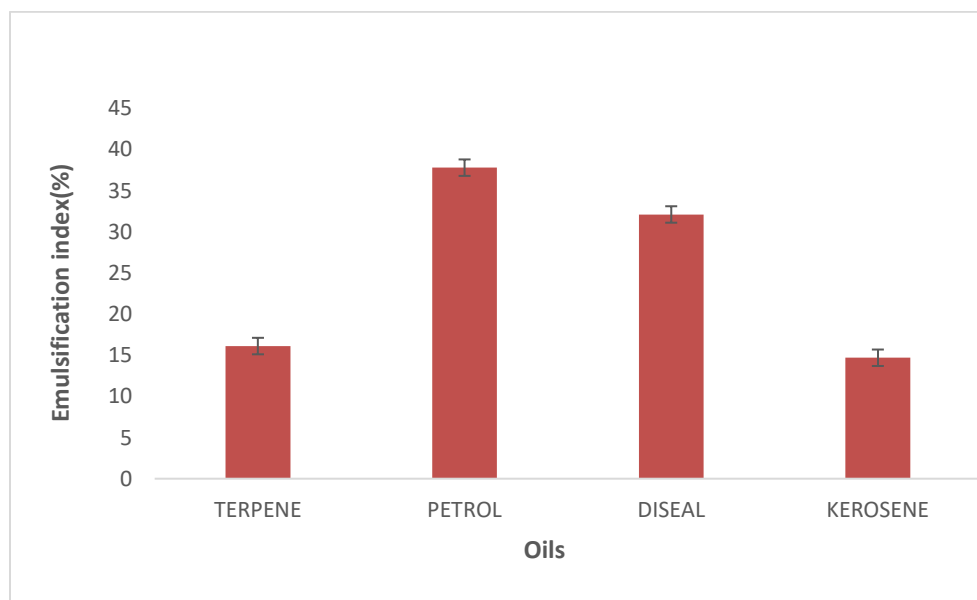


Figure 4. Emulsification index of different oils with produced Biosurfactant

4.4. Effect of Biosurfactant on Heavy Metal Remediation

The heavy metal solution of lead, chromium, and cadmium was mixed with Biosurfactant supernatant with different dilution rate i.e 2:8, 4:6, 6:4, 8:2. The medium without bio-surfactants

are served as the control. The samples were then analyzed for the concentration of metals present after treatment according to standard procedure using atomic absorption spectroscopy (AAS). All experiments were performed in triplicate, and the average of the results is presented.

Biosurfactant-mediated heavy metal remediation was observed by AAS. The results revealed that heavy metals were reduced when different concentrations of the biosurfactant were used. A whitish co-precipitate of the metal-biosurfactant complex was produced after the heavy metals were treated with the biosurfactant. The result Obtained in AAS showed that the removal of heavy metals was increased when the concentration of biosurfactant were increased.

The obtained AAS result revealed that the biosurfactant have potential to remediate approximately 70% of heavy metals (Cd,Pb,Cr) from the solution.

4.5. Calculation:

The percentage of metal removed (η) was calculated based on the initial metal content (control) in the aqueous solution using the following equation:

$$\eta = \frac{C_i - C_f}{C_i} \times 100$$

Where; C_i is the initial concentration of heavy metals (control, i.e. without treatment) and C_f is the final concentration of heavy metals after treatment with biosurfactant.

Compared to control, a significant reduction in the concentration of metal was observed after the addition of biosurfactant. A significant decrease in Cadmium, chromium & Lead concentration was high with biosurfactant at 8% concentration compared to 2% and 4% concentrations. Biosurfactant at 8% was found to be a superior adsorbent in removing cadmium, chromium and lead from aqueous solution.

Table 2 and figure... showed the removal efficiency of biosurfactant produced from strain HUR-IU16 when waste kitchen oil used as a carbon source. This result reveals that produced biosurfactant having high potential to remove heavy metals present in water bodies or environment.

Table 2 : The effect of bio-surfactants concentration on heavy metals removal from aqueous solution.

| Concentration (v/v) | Chromium removed | Lead removed | Cadmium removed |
|---------------------|------------------|--------------|-----------------|
| Blank | 37.6% | 63.1% | 33.6% |
| 2% | 20.7% | 15.8% | 7.1% |
| 4% | 33.5% | 37.4% | 26.7% |
| 6% | 94.6% | 53.4% | 53.5% |
| 8% | 77.1% | 73.2% | 77.6% |

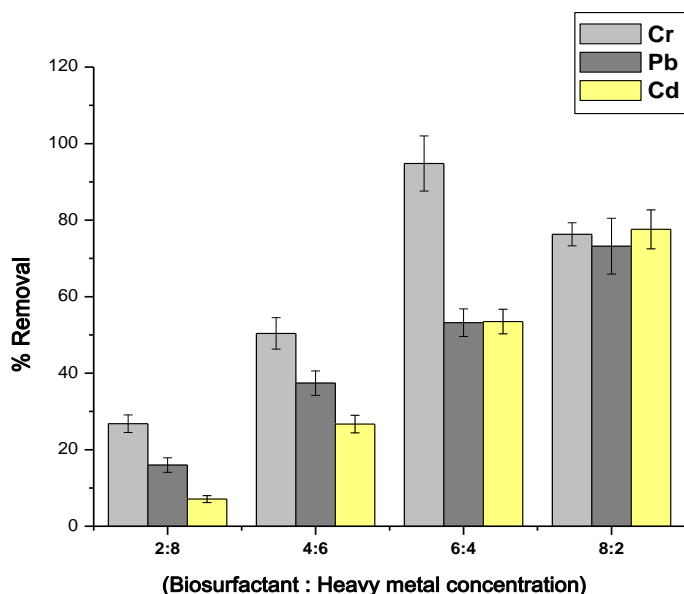


Figure 5. Percentage removal efficiency of Cd, Pb, Cr by biosurfactant.

As we increase the concentration of biosurfactant with heavy metal solution, the concentration of heavy metal reduce simultaneously. Among different ratio of biosurfactant and heavy metal concentration 8:2 shows the highest removal of Pb and Cd and the 6:4 ratio show the highest remediation of Cr.

5. Conclusion

The conclusions drawn from this study that; Biosurfactant produced from strain *Bacillus sp.* HUR-IU when waste kitchen oil used as a carbon source having potential to remove heavy metals. Produced biosurfactant showing good emulsification activity reduce surface tension which was confirmed by Oil spreading method, Emulsification index and drop collapse method. Oil spreading method also confirms that produced biosurfactant having potential to be used as bioremediation of sea spills. This study is more focused to remove heavy metals, Using inductively Atomic absorption spectrometer established that the biosurfactant can removed heavy metal up to 94.6% of cadmium, 73% of lead, and 73.2% of cadmium respectively. The effect of biosurfactant on Cadmium, Lead and Chromium suggest further research need to be conducted. Hence, the evolution of Biosurfactant effective concentration in Cadmium, Lead and Chromium, the removal is a matter of investigation.

6. REFERENCES

- Adamczak, M., & Bednarski, W. odzimierz. (2000). Influence of medium composition and aeration on the synthesis of biosurfactants produced by *Candida antarctica*. *Biotechnology Letters*, 22(4), 313–316.
- Aşçı, Y., Nurbaş, M., & Açikel, Y. S. (2008). Removal of zinc ions from a soil component Na-feldspar by a rhamnolipid biosurfactant. *Desalination*, 223(1–3), 361–365.
- Bages-Estopa, S., White, D. A., Winterburn, J. B., Webb, C., & Martin, P. J. (2018). Production and separation of a trehalolipid biosurfactant. *Biochemical Engineering Journal*, 139, 85–94.
- Banat, I. M. (1995). Characterization of biosurfactants and their use in pollution removal—State of the Art. *Acta Biotechnologica*, 15(3), 251–267.
- Brumano, L. P., Soler, M. F., & da Silva, S. S. (2016). Recent advances in sustainable production and application of biosurfactants in Brazil and Latin America. *Industrial Biotechnology*, 12(1), 31–39.
- Das, K., & Mukherjee, A. K. (2007a). Comparison of lipopeptide biosurfactants production by *Bacillus subtilis* strains in submerged and solid state fermentation systems using a cheap carbon source: some industrial applications of biosurfactants. *Process Biochemistry*, 42(8), 1191–1199.
- Das, K., & Mukherjee, A. K. (2007b). Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. *Bioresource Technology*, 98(7), 1339–1345.
- Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews*, 61(1), 47–64.
- Di Corcia, A. (1998). Characterization of surfactants and their biointermediates by liquid chromatography–mass spectrometry. *Journal of Chromatography A*, 794(1–2), 165–185.
- Fathabad, E. G. (2011). Biosurfactants in pharmaceutical industry. *Am J Drug Discov Dev*, 1, 58–69.
- Freitas, B. G., Brito, J. G. M., Brasileiro, P. P. F., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2016). Formulation of a commercial biosurfactant for application as a dispersant of petroleum and by-products spilled in oceans. *Frontiers in Microbiology*, 7, 1646.

- Geys, R., Soetaert, W., & Van Bogaert, I. (2014). Biotechnological opportunities in biosurfactant production. *Current Opinion in Biotechnology*, *30*, 66–72.
- Gharaei-Fathabad, E. (2011). Biosurfactants in pharmaceutical industry: a mini-review. *American Journal of Drug Discovery and Development*, *1*(1), 58–69.
- Haigh, S. D. (1996). A review of the interaction of surfactants with organic contaminants in soil. *Science of the Total Environment*, *185*(1–3), 161–170.
- Helmy, Q., Kardena, E., Funamizu, N., & Wisjnuprpto. (2011). Strategies toward commercial scale of biosurfactant production as potential substitute for its chemically counterparts. *International Journal of Biotechnology*, *12*(1–2), 66–86.
- Hörmann, B., Müller, M. M., Syldatk, C., & Hausmann, R. (2010). Rhamnolipid production by *Burkholderia plantarii* DSM 9509T. *European Journal of Lipid Science and Technology*, *112*(6), 674–680.
- Janek, T., Rodrigues, L. R., Gudiña, E. J., & Czyżnikowska, Ż. (2016). Structure and mode of action of cyclic lipopeptide pseudofactin II with divalent metal ions. *Colloids and Surfaces B: Biointerfaces*, *146*, 498–506.
- Jennings, E. M., & Tanner, R. S. (2000). Biosurfactant-producing bacteria found in contaminated and uncontaminated soils. *Proceedings of the 2000 Conference on Hazardous Waste Research*, *306*, 299–306.
- Kumar, P. S., & Ngueagni, P. T. (2021). A review on new aspects of lipopeptide biosurfactant: Types, production, properties and its application in the bioremediation process. *Journal of Hazardous Materials*, *407*, 124827.
- Makkar, R., & Cameotra, S. (2002). An update on the use of unconventional substrates for biosurfactant production and their new applications. *Applied Microbiology and Biotechnology*, *58*, 428–434.
- Makkar, R. S., & Rockne, K. J. (2003). Comparison of synthetic surfactants and biosurfactants in enhancing biodegradation of polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry: An International Journal*, *22*(10), 2280–2292.
- Masaru, K., Michiko, S., & Shuhei, Y. (2007). Skin care cosmetic and skin and agent for preventing skin roughness containing biosurfactants. *World Patent WO2007060956 A, 1*.
- Md, F. (2012). Biosurfactant: Production and Application. *Journal of Petroleum & Environmental Biotechnology*, *03*(04). <https://doi.org/10.4172/2157-7463.1000124>

- Mohan, D., & Pittman Jr, C. U. (2006). Activated carbons and low cost adsorbents for remediation of tri- and hexavalent chromium from water. *Journal of Hazardous Materials*, 137(2), 762–811.
- Müller, M. M., Kügler, J. H., Henkel, M., Gerlitzki, M., Hörmann, B., Pöhnlein, M., Syldatk, C., & Hausmann, R. (2012). Rhamnolipids—next generation surfactants? *Journal of Biotechnology*, 162(4), 366–380.
- Mulligan, C. N. (2005). Environmental applications for biosurfactants. *Environmental Pollution*, 133(2), 183–198.
- Muthusamy, K., Gopalakrishnan, S., Ravi, T. K., & Sivachidambaram, P. (2008). Biosurfactants: properties, commercial production and application. *Current Science*, 736–747.
- Pacwa-Płociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., & Cameotra, S. S. (2011). Environmental applications of biosurfactants: recent advances. *International Journal of Molecular Sciences*, 12(1), 633–654.
- Perfumo, A., Rudden, M., Smyth, T. J. P., Marchant, R., Stevenson, P. S., Parry, N. J., & Banat, I. M. (2013). Rhamnolipids are conserved biosurfactants molecules: implications for their biotechnological potential. *Applied Microbiology and Biotechnology*, 97, 7297–7306.
- Perfumo, A., Smyth, T., Marchant, R., & Banat, I. (2010). Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates. In *Handbook of hydrocarbon and lipid microbiology* (pp. 1501–1512). Springer.
- Peters, R. W. (1999). Chelant extraction of heavy metals from contaminated soils. *Journal of Hazardous Materials*, 66(1–2), 151–210.
- Piljac, T., & Piljac, G. (1999). Use of rhamnolipids in wound healing, treating burn shock, atherosclerosis, organ transplants, depression, schizophrenia and cosmetics (European Patent 1 889 623). *Paradigm Biomedical Inc New York*.
- Rahman, P. K. S. M., & Gakpe, E. (2008). Production, characterisation and applications of biosurfactants-Review. *Biotechnology*.
- Ribeiro, B. G., Guerra, J. M. C., & Sarubbo, L. A. (2020). Biosurfactants: Production and application prospects in the food industry. *Biotechnology Progress*, 36(5), e3030.
- Salehizadeh, H., & Shojaosadati, S. A. (2003). Removal of metal ions from aqueous solution by polysaccharide produced from *Bacillus firmus*. *Water Research*, 37(17), 4231–4235.
- Salihu, A., Abdulkadir, I., & Almustapha, M. N. (2009). An investigation for potential

- development on biosurfactants. *Biotechnol Mol Biol Rev*, 3(5), 111–117.
- Sarkar, A. K., Goursaud, J. C., Sharma, M. M., & Georgiou, G. (1989). A critical evaluation of MEOR processes. *Situ*, 13(4), 207–238.
- Shepherd, R., Rockey, J., Sutherland, I. W., & Roller, S. (1995). Novel bioemulsifiers from microorganisms for use in foods. *Journal of Biotechnology*, 40(3), 207–217.
- Shoeb, E., Akhlaq, F., Badar, U., Akhter, J., & Imtiaz, S. (2013). Classification and industrial applications of biosurfactants. *Academic Research International*, 4(3), 243.
- Van Bogaert, I. N. A., Zhang, J., & Soetaert, W. (2011). Microbial synthesis of sophorolipids. *Process Biochemistry*, 46(4), 821–833.
- Varjani, S. J., & Upasani, V. N. (2017). Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. *Bioresource Technology*, 232, 389–397.
- Weber, A., & Zeiner, T. (2014). Purification of biosurfactants. *Biosurfactants: Production and Utilization—Processes, Technologies, and Economics*, 159, 129.
- Williams, W., & Trindade, M. (2017). Metagenomics for the discovery of novel biosurfactants. *Functional Metagenomics: Tools and Applications*, 95–117.
- Ying, G.-G. (2006). Fate, behavior and effects of surfactants and their degradation products in the environment. *Environment International*, 32(3), 417–431.
- 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.