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Removal of Heavy Metal (Cr) using Sulfolipid and it's Application

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***Abstract*—Sulfolipids are a type of biosurfactant that have recently gained attention as a potential alternative to chemical surfactants in various industrial applications. One potential application of Sulfolipids is in the removal of heavy metals from Contaminated soil. Chromium (Cr) is a toxic heavy metal that is commonly Found in polluted soil and poses a significant threat to the environment and Human health. Several studies have investigated the use of sulfolipids for the Removal of (Cr) from contaminated soil and water. These studies have shown That sulfolipids can effectively solubilize and mobilize Cr, making it easier to Remove from the contaminated site. Sulfolipids can also help to reduce the Toxicity of (Cr) by converting it to less toxic forms. The mechanism of Cr Removal by sulfolipids is based on the ability of the sulfolipids molecules to bind To the metal ions and form complexes. The resulting complexes are more water-Soluble and can be removed from the contaminated site using conventional Methods such as filtration or sedimentation. In this review have provided Valuable information on the chemical structure and properties of sulfolipids, Which can aid in their production and application in the field. Overall, the use of Sulfolipids for the removal of heavy metals such as Cr from contaminated soil And water shows promising results. However, further research is needed to Optimize the use of sulfolipids in terms of their production, purification, and Application in the field. Additionally, further characterization studies are needed To fully understand the properties of sulfolipids and their potential applications In various industries.**

***Keywords*—Sulfolipids; Chromium; biosurfactant.**

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I. Introduction

In later a long time, overwhelming metals contamination is found to be a genuine natural issue and different Innovations are being found for their clean up from environment. It was found that utilizing Biosurfactants for this reason is an ecologically generous strategy and a substitution for conventional Advanced clean up innovations. Biosurfactants are seen as a conceivable candidate for the natural Cleaning of contaminants due to their differing qualities. As a result, biosurfactants gotten more center, and their Imminent applications were recognized. Overwhelming metals defilement has been recognized as a serious Natural issue in later a long time, and various advances are being created to evacuate them From the environment. A overwhelming metal is any metallic chemical component with a moderately tall thickness that's dangerous or harmful at moo concentrations. Overwhelming metals are naturally show within the hull of the Soil. They can't be crushed or diminished to nothing. They Sporadically get into our frameworks through nourishment, water, and discuss. Certain overwhelming metals, such as copper, Selenium, and zinc, are vital as follow components to keep the human body's digestion system running Easily. All things considered, poisonous quality may result from higher measurements of these. For case, tall surrounding discuss concentrations near to outflow sources, ingestion through the nourishment chain, or defilement of drinking water due to lead channels can all cause overwhelming metal poisoning.Because they have a propensity to bioaccumulate, overwhelming metals are unsafe. When a chemical's concentration In a natural life form rises over time relative to its concentration within the environment, usually alluded to As bioaccumulation. When substances are ingested and kept in living life forms more rapidly than they are decomposed (metabolized) or disposed of, compounds accumulate.Heavy metal defilement in soil could be a critical natural concern that can have negative impacts on both the environment and human wellbeing. Conventional strategies for overwhelming metal expulsion from soil, such As physical evacuation or chemical medicines, can be expensive and may have confinements in viability. Be that as it may, an developing and promising approach for overwhelming metal evacuation from soil is the utilize of Biosurfactants.

A. *Biosurfactants*

Biosurfactants are surface dynamic particles created by microorganisms that have been highlighted as an Environmentally-friendly elective to their manufactured partner, chemical surfactants. In later a long time, Due to their properties like specificity, moo poisonous quality and relative ease of arrangement, these surface-active Biomolecules have pulled in wide intrigued. Due to their special utilitarian properties, biosurfactants were utilized in a few businesses counting natural chemicals, petroleum, petrochemicals, mining, metallurgy (basically bioleaching), agrochemicals, fertilizers, nourishments, refreshments, beauty care products, pharmaceuticals, and numerous others. Biosurfactants are amphiphilic compounds which are delivered by microbial cells on their surface or Emitted extracellularly. They contain hydrophobic and hydrophilic moieties that diminish the surface and Interfacial pressure between surfaces. Biosurfactants have Imperative characteristics that offer preferences over surfactants, which incorporate surface And interfacial action, resistance to temperature, pH, and ionic drive, moo harmfulness, accessibility, Specificity, biocompatibility, and biodegradability (Kiran et al., 2010a; Franzetti et al., 2014). Since of their promising benefits, they are broadly utilized for mechanical and therapeutic Purposes (Muthusamy et al., 2008). Overwhelming metals are the soil metals, an manufactured a few of those are imperative supplements for living creatures. Metals gather within the environment as the by-product of anthropogenic formative exercises such as tanneries, fabricating of arms and ammo, paint, metal channels, batteries, mining, etc. (Singh and Cameotra, 2004), Overwhelming metal gatherings within the environment are tireless to debasement, but can be changed over to less harmful shapes (Santona et al., 2006). Biosurfactants improve the solubility of hydrophobic compounds within the contaminated environment and makes a difference in bioremediation (Cameotra and Makkar, 2010).Among the lipopeptidebiosurfactants, surfactin and its analogs are the foremost broadly considered, and they comprise of a lipid chain associated to a brief straight or cyclic peptide (Peypouxet al., 1991)

Biosurfactants delivered by microorganisms (microbes, yeasts and filamentous parasites) are by and large classified agreeing to their chemical composition. For the generation of biosurfactants, carbon and nitrogen sources are utilized within the culture medium, where the most sources of carbon are carbohydrates and lipids (Marcelino, 2020; Alwaely et al., et al., 2019).

B. *Properties of Microbial Biosurfactants*

The foremost vital property for surfactant operators is surface pressure, which is the drive of fascination between fluid particles (Williams &Trindade, 2017; Santos et al., 2016). A surface is characterized as the boundary between a fluid and discuss, and an interface as the boundary between two fluids. Hence, the pressures between the air/water and oil/water stages are known as surfaces pressure and interfacial pressure, individually (Banat et al., 2014; Bezerra et al., 2018). Surface pressure is effortlessly quantitatively measured by a tensiometer. This estimation is the basis for most beginning evaluations to recognize the nearness of a surfactant within the medium. The air/water surface pressure for refined water is around 72 mN/m and the interfacial pressure for refined water versus n-hexadecane is around 40 mN/m. Regularly, surfactants can diminish these values to around 30-40 mN/m and 1 mN/m, individually (Marchant & Banat, 2012). The surface pressure diminishes when the concentration of surfactant within the aqueous medium increments, coming about within the arrangement of micelles, which are amphipathic particles totaled with the hydrophilic parcels situated exterior of the particle and the hydrophobic parcels to the interior. The concentration of these micelles shapes the Basic Micellar Concentration (CMC). This concentration compares to the least surfactant concentration vital for the surface pressure to decrease to a maximum, past which an increment of surfactant contains a non-significant impact. When the CMC is come to, a few micelles are shaped (Ribeiro et al., 2020) Over whelming METAL Overwhelming metals are considered primary toxins in soil, which can be exchanged to nourishment chain through crops. So distant, there have been a few strategies such as physical, chemical, biological and strategies to expel the overwhelming metals from contaminated soil. Overwhelming metals are common poisons within the soil environment, to be specific arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), copper (Cu), zinc (Zn), nickel (Ni). soil within the agrarian locales has been incompletely sullied by overwhelming metal, which lead to the diminishing accessibility of farmland. The over standard rate of soil contamination is 16.1%, among which the Cd, As, Hg, Pb, Cr over standard rates of overwhelming metals are as tall as 7.00%, 2.70%, 1.60%, 1.50%, 1.10%, respectively. In 2009, the worldwide emanation of heavy metal Cd come to 743.77 tons. Over the past 50 a long time, around 30,000 tons of Cr and 800,000 tons of Pb have been discharged into the environment around the world. Most of these overwhelming metals have as of now been gathered within the soil. Overwhelming metals, indeed at exceptionally moo concentrations, are by and large harmful and, as they are not biodegradable, stand up to ordinary transfer medications. In expansion to genuine natural issues, they are destructive to fauna and vegetation, and introduction to overwhelming metals can cause a few genuine human illnesses such as respiratory issues, kidney pathology, neurological clutters and cancer (Sall et al., 2020). Soil contamination by overwhelming metals has gotten to be a worldwide natural issue due to developing concern around the security of agrarian items. Universally, there are 5 million places with contaminated soils covering 500 million hectares of arrive. These soils are sullied by distinctive overwhelming metals or metalloids. Overwhelming metal contamination in soil has an evaluated worldwide financial affect of more than \$10 billion a year (Li et al., 2019) Remediation of overwhelming metals sullied soil can be done by the utilize of biosurfactants. Biosurfactants to evacuate overwhelming metals from an oil sullied soil with surfactin , a rhamnolipid and a sulfolipids

C. *Issues of Overwhelming Metals in Soil*

The bulk of overwhelming metals, which are characterized as components with a thickness more prominent than 4-5 g/cm3, are dangerous to human wellbeing . The foremost common heavy metals incorporate Pt, Pd, Ni, Hg, Cd, Zn, Pb, As, Ag, Cu, Fe, and Cr , which can be released into the environment through either anthropogenic or common sources, such as mining and production lines . Since overwhelming metals cannot biodegrade, they can collect within the environment and in the long run make their way into the nourishment chain.

D. *Sulfolipids*

Sulfolipids, a family of lipids that incorporate sulfur, are a imperative bunch of chemical substances that are show in microorganisms. It is pivotal to get it that the term "sulpholipid" fair signifies a certain chemical property of the atom, specifically the nearness of sulfur. It does not relate to any chemically partitioned groupings of particles, but or maybe a wide variety of chemical sorts. Sulpholipids are most plenteous within the brain and kidney of warm blooded creatures. Sulfoquinovosyl diacylglycerol, a glycoside of sulfoquinovose and diacylglycerol, is the foremost predominant sulfolipid. Sulfoquinovosyldiacylglycerides play a noteworthy part within the sulfur cycle in plants. Sulfatide and seminolipid, which are all sulfated glycolipids, are advance critical sulfolipids. Sulfolipids are a sort of biomolecule that are created by a few microorganisms, such as microbes and parasites. Later inquire about recommends that sulfolipids have the potential to evacuate heavy metals from sullied soil and water. Sulfolipids have a one of a kind chemical structure that permits them to tie to overwhelming metal particles such as lead, cadmium, and mercury. When sulfolipids come into contact with overwhelming metals within the environment, they can shape complexes that can be effectively evacuated from the sullied range, either through precipitation or filtration. One study published in the Journal of Environmental Science and Health demonstrated the effectiveness of sulfolipids in removing lead and cadmium from soil. The researchers found that sulfolipids extracted from the bacterium Rhodococcuserythropolis were able to reduce the concentration of lead and cadmium.

II. Material and Method

A. *Sample Collection*

The petroleum contaminated soil was collected from a local mechanical shed, Thanjavur which is highly polluted and contain heavy metals. Plant oil such as castor oil, frying waste oil, soya oil, coconut oil, sunflower oil were purchased in oil industry and dairy waste like milk was used for the production of yeast and bacteria. The chemicals were collected from the laboratory.

B. *Isolation of Microorganism*

1g of collected soil was serial diluted in laminar airflow chamber for upto concentration 106cells/mL followed by spread plate. Yeast Peptone Dextrose agar and Nutrient agar culture was prepared to isolate the microorganism and 0.15ml of sample was taken for spreading the culture plate. After the incubation at 210C for about 24-62 hours, the microbes were isolated. Later on, for the growth target bios it is then inoculated into stock media.

C. *Stock Media Preparation and Subculturing*

From the grown culture in order to obtain specific organism, streak plate technique was used. The isolated microbes were inoculated in the broth containing Yeast extract, Dextrose and Peptone which was sterilized and the broth culture was incubated at 310C in shaker at 200rpm for 48-72hours. For the growth and maintenance of culture, the microbes were sub-cultured using broth. The sub-culturing broth was formulated with Glucose, Peptone, and Yeast Extract which then sterilized. The sub-culturing formulated broth was incubated in shaker at 170rpm for 48hours aimed at reproducing of yeast and bacteria.

D. *Seed Fermentation Using Urea*

The seed ferment broth is prepared using glucose (50g/L), yeast extract (5g\L) and urea (0.05g\L). The requirements were dissolved in distilled water of about 200ml. The broth was sterilized, futhur the grown bacteria and yeast were inoculated in the seed fermentation broth in order to reach log phase for the replication of the microbes. The urea was added to provide nitrogen source for the microbes undergoing fermentation. The fermentation broth was incubated for 4 days at 310 C in fumigated incubator.

Arrangement of Generation MEDIA

The generation media were defined to supplement the focused on bios through giving improved carbon, and nitrogen source. At exponential stage of organisms, it was taken for generation by immunizing within the seed aging taken after by generation fermenter. The generation media was kept up at

pH 6. Particular source of supplement were given for accomplishing the sulfolipidcontainning organisms. The generation media avoids the cross defilement of pollutions and makes a difference the sulfolipid containing organisms duplicate quicker. The composition of nourishment source given underneath.

TABLE I
THE MEDIA FORMULATION I (PH 6)

Carbon source(2%w/v)	**Oil(10%v/v)**	**Nitrogen source(g/L)**	**Trace elements(g/L)**
Glucose	Frying waste oil	Yeast extract	$Na_3C_6H_5O_7$
Honey	Coconut oil	Peptone	Fe_2-$SO_4.7H_2O$
Potato	Sunflower oil		$CaCl_2$
Lactose	Soya oil		$MgSO_4$
Glycerol			$(NH_4)_2SO_4$
			NaCl

TABLE II
COMPOSITION OF PRODUCTION MEDIA FORMULATION I, THE MEDIA FORMULATION II (PH 6)

Carbon source(2%w/v)	**Oil(10%v/v)**	**Nitrogen source(g/L)**	**Trace elements(g/L)**
Glucose monohydrate	Rice bran oil	Yeast extract	KH_2PO_4
Honey	Olive oil	Peptone	$MnSO_4.7H_2O$
Lactose	Castor oil	Malt extract	$CoCl_2$
Sucrose	Plant oil		$MgSO_4.7H_2O$
Dairy waste H_2O	Oleic acid		$CaCl_2$

Composition of production media formulation II.

All the chosen source of supplement was taken in suitable sum and made it upto 500ml with refined water. The generation broth was sterilized and the matured microscopic organisms and yeast were immunized in to it. It was at that point taken in orbital shaker at 200rpm for 3-4 days of hatching. It is at that point put away for advance utilize.

E. Filteration of Rough Bios

Unrefined biosurfactant was gotten utilizing an corrosive precipitation and dissolvable extraction strategy. After 6 days of hatching, cells were evacuated from the culture broth by centrifugation at 500orpm for 10 min at 4°C. The cell free supernatant was fermented with ethyl acetaeto pH 2 and put away overnight at 4°C to upgrade the precipitation of biosurfactant. The come about accelerate was isolated by centrifugation at 3000 rpm, 15 min, 4°C and extricated a few times with ethyl acetic acid derivation at room temperature. The dissolvable was totally vanished beneath vacuum. The unrefined biosurfactantwas gotten as a gooey brown colored fabric. It was weighted and communicated as g/l. The 5ml unrefined bios were sifted utilizing watmen sifted. The bios were sifted along side heaxane(3ml) for division of sulfolpid from debasements.

F. Emulsification

Movement Emulsification stability(E24) of strains was measured by including 2 ml of oil to the same sum of each culture. The test was blended with a vortex for 2 min and cleared out for 24 hours. E24 file was calculated as rate of stature of emulsified layer (cm) isolated by stature of fluid column (cm). The emulsifying action measured as the emulsification list was decided concurring to Cooper and Golden-berg.

G. Formula:

Emulsifcation index (EI) = Height of emulsion layer X 100
Height of the total mixture

H. Surface Pressure Investigation

The cell free supernant was taken for deciding surface pressure utilizing stalagnometer. The clean and dry stalagmometerwas mounted on the vertical stand and weighed the mass of the purge weighing bottle (m0). The container was filled with refined water. The tubing with swell on the best conclusion of stalagmometer was mounted.Immersed the foot conclusion of stalagmometer into water and filled it up, such that the water level was over the wide portion of stalagmometer. The swell was evacuated and collected 20 water drops into the weighing bottle. At that point the mass of the weighing bottle with the drops (m0 + mwater = mtotal) was weighed and from this equation distermined the mass of 20 drops (mwater).Empted the weighing bottle and stalagmometer, dryed them and arranged for the following estimation. Over steps were rehashed for the other fluids with obscure surface pressure. Add up to mass was rise to (m0 + mLiquid = mtotal). Mass of the drops mLiquid was measured.Measured the research facility temperature and discover the comparing water surface pressure

Calculated the surface tensions of bios (g) according to the Drop Count Method Equation

$$\gamma = \gamma_{water} \times \frac{m}{m_{water}} \quad (\mathrm{N\ m^{-1}})$$

I. Fourier Transform Infra Red Assay

Measure FT-IR spectroscopy is regularly utilized to distinguish useful bunches inside a atom and can be utilized to quantitatively decide concentrations of particles inside a test. Infrared spectroscopy is based on the interaction between vitality from the occurrence IR light and the covalent bonds inside a atom. The dried powder biosurfactant was collected upto 1g and pressed for examination of sulfolipid.

III. RESULT AND DISCUSSION

A. Extraction of Biosurfactant Isolation and Culturing of Microorganism

Fig. 1 Isolated Microbes

The figure 1 shows the microorganism that was isolated from polluted soil using dilution and spread plate culturing. Figure 1.2 represents the culturing of microbes in the yeast peptone dextrose broth. The YPD broth was preferred for the nutrition of glucose, carbon and growth of yeast.

Fig. 2 Culturing of Microbes

B. Seed Fermentation Using Urea

The figure 3 shows the microorganism growth in the seed fermentation. The seed fermentation broth provided nitrogen source for the growth of sulfolipid. The urea added in the broth along with glucose and dextrose makes the microorganism to reach log phase. This obtained stage was reached which was confirmed by noticing the OD value of 1.126 using UV spectrometry.

Fig. 3 Seed Fermentation broth of microbes

C. Production Media of Sulfolipid

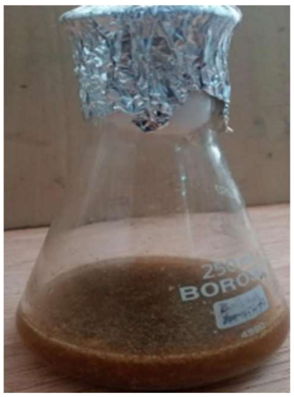


Fig. 4 Production Media of Sulfolipid

The figure 4 represents the sulfolipid reproduction in the production media. The production media provided dominant growth of sulfolipid over the other biosurfactant and impurities. The mass production of sulfolipid was stored for harvest of crude sulfolipid.

D. Analysis of Emulsification Activity

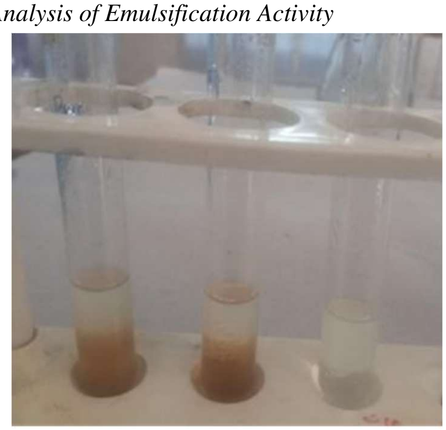


Fig. 5 Emulsification Stability observed in Sulfolipid

The figure 5 shows the emulsion stability of the sulfolipid with oil. The sulfolipid approved 52% of emulsifying stability with w/o emulsion. The key function of emulsifier surfactants is to accumulate between phases, lower the surface and interfacial tension of the emulsions, and increase solubility (Sandhya Mishra *et.al.,* 2021)

E. Surface Tension Assay For Sulfolipid

The surface tension was analysed using stagalmometer and the values were substituted in the formula

$$\gamma = \frac{m}{m_{water}} \times \gamma_{water} \quad (N\ m^{-1})$$

TABLE III
SURFACE TENSION DETERMINATION BY DROP WEIGHT METHOD

Liquid	m_o (g)	Mass of 20 drops of liquid	Mass of liquid m(liq)	δ (mN/m)
Water	19.68	22.58	0.145	72
Biosurfactant	19.68	20.93	0.0625	31

The table shows the calculation of surface tension by drop weight method. From the table 3 and the formula, the surface tension of biosurfactant was 31mN/m at 21^0 C. Emulsification Activity and Surface Tension are among the most important characteristics of biosurfactant producing microorganisms (Gautam and Tyagi 2005). These characteristics have been shown to increase the biodegradation rate of hydrocarbons, stimulate microbial growth, decrease crude oil viscosity and enhance oil recovery at oil fields (Ahmad F. Shahaby and Abdulla A. Alharthi 2015).

F. Effect of Contact Time

TABLE IV
EFFECT OF CONTACT TIME

Time (mins)	Initial Conc. of Cr(VI) 5 mg/L		10 mg/L		15mg/L		20 mg/L		25mg/L	
	Conc. Of Cr (mg/L)	Chromium Removal (%)	Conc. of Cr (mg/L)	Chromium Removal (%)	Conc. Of Cr (mg/L)	Chromium Removal (%)	Conc. of Cr (mg/L)	Chromium Removal (%)	Conc. of Cr (mg/L)	Chromium Removal (%)
0	5.000	0	10.000	0	15.000	0	20.000	0	25.000	0
15	3.450	31	6.700	33	9.180	38.8	11.890	40.5	14.710	41.16
30	3.060	38.8	5.650	43.5	8.480	43.4	11.190	44.05	13.850	44.6
45	2.584	48.32	5.060	49.4	8.060	46.2	10.650	46.75	13.180	47.2
60	2.090	58.2	4.100	59	7.520	49.8	9.160	54.2	12.410	50.36
120	1.476	70.4	3.750	62.5	6.600	56	8.540	57.3	10.680	57.28
180	1.376	72.48	3.070	69.3	4.210	71.9	6.330	68.35	9.280	62.8
240	1.050	79	2.340	76.6	3.880	74.13	5.340	73.3	7.600	69.6
270	0.910	81.8	1.880	81.2	3.120	79.2	4.640	76.8	6.220	75.12
300	0.670	86.6	1.650	83.5	2.900	80.6	4.330	78.35	6.600	75.36
330	0.550	89	1.500	85	2.700	82.6	4.000	80	6.000	76
360	0.550	89	1.500	85	2.700	80.6	4.000	80	6.000	76
390	0.550	89	1.500	85	2.700	80.6	4.000	80	6.000	76

Volume of Aqueous Metal Solution =100 ml, pH=2.0,
Initial Metal Ion Concentration (C0) = 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L;
Weight of Biosurfactant dosage = 1 g
Temperature = 370C.

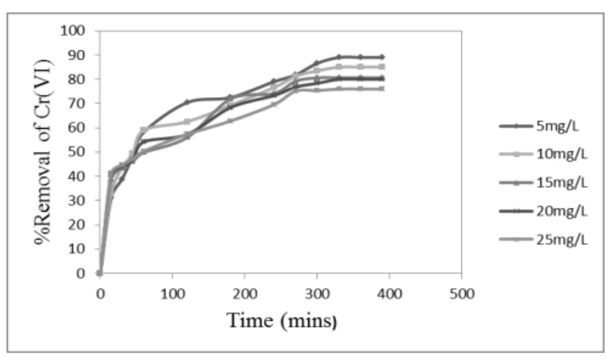


Fig. 6 Effect of contact time on removal of Cr (VI) using sulfolipid

The effect of contact time on Cr (VI) removal using biosurfactant was studied and the results are shown in Figure 1.6 and the values are tabulated in Table . It was found that the adsorption efficiency of Cr (VI) ion increases as the contact time is increased and equilibrium reached at 330 mins with a removal of 89%. Hence, in the present study, 330 mins was chosen as the equilibrium time. Initially the removal rate of the biosurfactant was rapid, but it gradually reached equilibrium and remained constant as time was increased above the equilibrium time. The rate in percent of metal removal was higher in the beginning due to the larger surface area of the adsorbent being available for the adsorption of the metals (Paresh *et.al.,* 2010). The adsorption of the Cr (VI) was greater at earlier stages due to the availability of more number of active sorption sites. As time passes the metal uptake by the sorbent surface slows down as the competition for the decreasing availability of active sites intensifies by the metal ions remaining in the solution.

G. *Effect of Initial Metal Ion Concentration*

TABLE V
EFFECT OF INITIAL METAL ION CONCENTRATION

Initial Metal Ion Concentration (mg/L)	Concentration of Cr (VI) (mg/L)	Chromium Removal (%)
5	0.55	89.00
10	1.50	87.02
15	2.70	86.66
20	4.00	83.34
25	6.00	76.00

Volume of Aqueous Metal Solution= 100 ml
Initial metal ion Concentration = 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L, 25 mg/L
Weight of biosurfactant dosage =1 g,
Temperature = 37^0C.

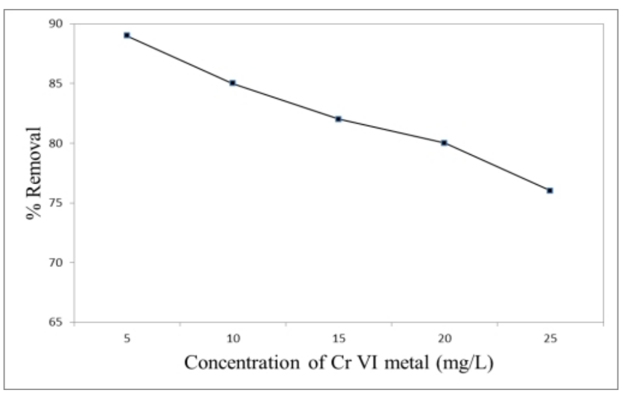


Fig. 7 Effect of initial metal ion concentration on removal of Cr (VI) using sulfolipid

The percentage removal of chromium decreases with increase in initial chromium concentration as shown in Figure 1.8 and the values are tabulated in Table 4.3. The percentage removal of chromium decreased from 89% at 5 mg/L to 76% at 25 mg/L. The decrease in the extent of removal percentage of chromium with increase in the initial metal ion concentration may be due to the reduction in immediate adsorption due to the lack of available active sites (Mabashir *et.al.,* 2007). For higher concentration of chromium the percentage removal decreases mainly due to the saturation of binding sites (Jianlong and Can, 2009).

H. *Effect of pH*

TABLE VI
EFFECT OF PH

pH	Concentration of Cr (VI) (mg/L)	Chromium Removal (%)
2	0.55	89
4	1.50	70
6	2.50	50

pH = 2.0, 4.0, and 6.0
Volume of Solution = 100 ml;
Initial Cr (VI) Concentration = 5 mg/L,
Weight of biosurfactant dosage= 1 g,
Temperature = 37^0 C

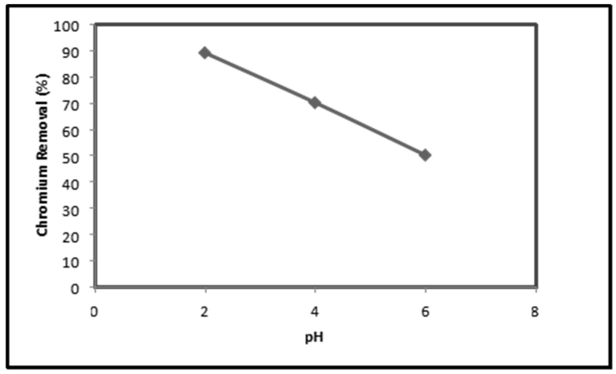


Fig. 8 Effect of pH on adsorption of chromium using sulfolipid

The effect of pH on the percentage removal of chromium at 5 mg/L chromium concentration was studied as a function of pH at constant fungal doses and the results are shown in Figure 1.8 and the values are tabulated in Table . It is clear from the figure that the percentage removal of chromium is 89 % at pH 2.0. The percentage removal increases with increase in pH from 1.0 to 2.0 and thereafter decreases with further increase in pH. This behavior can be explained by considering the nature of the biosurfactant at different pH in chromium removal. The cell wall of sulfolipid contains a large number of surface functional groups. The pH dependence of chromium biosorption can largely be related to the type and ionic state of these functional groups and also on the metal chemistry in solution (Mabashir *et.al.,* 2007). Maximum removal of chromium occurs at pH 2.0 which suggests that the negatively charged chromium species bind through electrostatic attraction to positively charged functional groups on the surface of fungal cell wall because at this pH more functional groups carrying positive charges would be exposed. But at pH above 3.0, it seems that fungal cell wall possess more functional groups carrying a net negative charge which tends to repulse the anions (Jianlong and Can, 2009).

I. *Effect of Biosurfactant Dosage*

TABLE VI
EFFECT OF BIOSURFACATANT DOSAGE

Biosurfactant Dosage (g)	Concentration of Cr (VI) (mg/L)	Chromium Removal (%)
0.1	0.550	89.0
0.5	0.525	89.5
1	0.450	91.0

Weight of biosurfactant = 0.1, 0.5 and 1.0g
Volume of Solution = 100 ml,
Initial Cr (VI) Concentration = 5 mg/L, pH = 2.0,
Temperature = 37^0C.

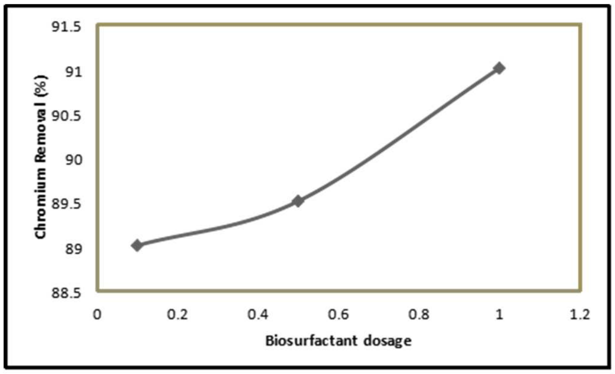
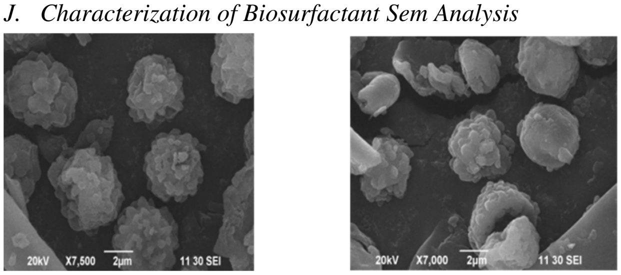


Fig. 9 Effect of biosurfactant loading on removal of chromium using sulfolipid

The effect of biomass loading on percentage removal of chromium was studied and is depicted in Figure 1.9 and the values are tabulated in Table . Metal uptake with variation in biosurfactant dosages has been reported (Paresh *et.al.,* 2010). It indicates that increase in the biosurfactant loading from 0.1g to 1g increases the rate of removal from 89% to 91%. The increase in Cr (VI) adsorption may possibly be due to the increased number of binding sites for chromium ions at higher cell loading (Jianlong and Can,2009).

J. Characterization of Biosurfactant Sem Analysis



(a) (b)

Fig. 10 Unadsorbed Fungus (a), Adsorbed Fungus (b)

Scanning electron microscopy analysis has been used for the characterization of the biosurfactant (Paresh *et.al.,* 2010). The biosurfactant sulfolipid was characterized before and after it was subjected to Cr (VI) metal ion adsorption is shown in Plate 5. The Scanning electron microscopy scans the surface of the biosurfactant and the change in morphology of the biosurfactant surface was studied. The surface of the fungal biomass showed that the biomass appeared scattered before adsorption to Cr (VI) and the biomass appeared complexed with Cr (VI) after removal.

K. Characterization Using Edax Analysis

EDAX (Energy Dispersive X-ray Spectroscopy) is an analytical technique which utilizes x- rays that are emitted from the specimen when bombarded by the electron beam to identify the elemental composition of the specimen. A resulting electron vacancy is filled by an electron from a higher shell, and an x-ray is emitted to balance the energy difference between the two electrons. The EDAX detector measures the number of emitted x-rays versus their energy. The energy of the x-ray is characteristic of the element from which the x-ray was emitted. A spectrum of the energy versus relative counts of the detected x-rays is obtained and evaluated for qualitative and quantitative determinations of the elements. Modern SEM/EDS instruments are operated using very sophisticated software. These software programs allow unattended feature analysis and “mapping” of the composition of the elements on the surface of the specimen. [Goldstein *et.al.,* 2003]

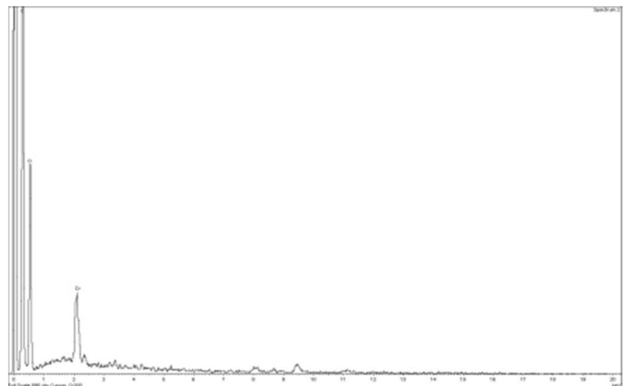


Fig. 11 Graph of EDAX analysis for sulfolipid after removal

L. Application of sulfolipids utilizing evacuation of overwhelming metals in soil

Sulfolipids, which are a sort of actually happening plant-derived lipid, have appeared potential for the evacuation of overwhelming metals from soil through a prepare called phytoremediation. Phytoremediation may be a plant-based approach that utilizes the capacity of plants construct up">to construct, up change, or detoxify poisons from the environment.The application of sulfolipids within the evacuation of overwhelming metals from soil includes a few components:

Chelation:Sulfolipids can frame solid complexes with heavy metals through chelation, which is the method of official metal particles with natural particles to create steady compounds. The sulfolipids can tie to overwhelming metals within the soil, decreasing their bioavailability and versatility, subsequently avoiding their take-up by plants or filtering into groundwater.

Phytoextraction:Sulfolipids can enhance the phytoextraction prepare, which is the take-up and aggregation of overwhelming metals by plants from the soil. Sulfolipids can increment the dissolvability and accessibility of heavy metals within the soil, making them more open for plant take-up. Once the metals are taken up by the plant roots, they can be translocated and sequestered within the plant's above-ground tissues, which can at that point be collected and evacuated from the location.

Rhizosphere alteration:Sulfolipids can alter the rhizosphere, which is the locale of soil encompassing plant roots, by advancing the development of advantageous microorganisms, such as bacteria and organisms. These microorganisms can shape advantageous affiliations with plant roots, forming a complex arrange of intuitive that can upgrade the take-up and change of heavy metals within the soil.

Soil accumulation:Sulfolipids can progress soil aggregation, which is the method of forming stable soil totals that can offer assistance to reduce soil erosion and increment water infiltration. This will offer assistance to immobilize overwhelming metals within the soil and anticipate their relocation to other ranges.

Generally, the application of sulfolipids within the expulsion of overwhelming metals from soil can be a promising approach for soil remediation. In any case, advance investigate is needed to get it their adequacy, natural impacts, and potential restrictions some time recently broad application in field settings. Furthermore, administrative contemplations and observing conventions ought to be taken after to ensure secure and feasible utilize of sulfolipids in soil remediation hones.

M. Applications of biosurfactant in expulsion of overwhelming metals

Among the different contaminations by overwhelming metals, the nearness of arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), mercury (Hg), lead (Pb), zinc (Zn) and nickel (Ni) within the environment is of awesome concern, since they are non-threshold poisons and are show in discuss, sea-going and terrestrial systems in concentrations numerous times over those set up by worldwide offices. Encourage, they can cause many health problems categorized as inorganic chemical dangers to people, creatures, and plants (Rahman & Singh, 2020; Akbari et al., 2018).

The overwhelming metals adsorbed on the surface of soil particles are isolated by the sorption of biosurfactant atoms at the interfaces between the damp soil and the metal in fluid arrangement. Hence, the metal is adsorbed by biosurfactants and caught inside the micelle through electrostatic intuitive. At long last, the biosurfactant can be recouped through the film partition handle.

The compelling evacuation of heavy metals from soil could be a major challenge. Biosurfactant leaching basically points to evacuate metals from soils through chelation with their particular work bunches and change of metal speciation divisions to extend their versatility (Yang et al., 2018).

A few microorganisms, such as Pseudomonas aeruginosa, Pseudomonas sp., Bacillus subtilis, Bacillus sp., Burkholderia sp., Citrobacterfreundii, Candida tropicalis, and Candida sp., have been separated as potential biosurfactants, illustrating huge overwhelming metal evacuation effectiveness.

IV. CONCLUSION

The biosurfactant - sulfolipid was extracted and produced using several nutrition sources. The sulfolipid showed 55% of emulsification stability and itacts as natural emulsifier in industrial applications. As the sulfolipid reduced the surface tension to 32mN/m, it help to emulsify oils and solubilize hydrophobic compunds, which is useful in the applications of agriculture, bioremediation and cleaning oil spills. The sulfolipid removed 89% of Cr (VI) by inoculating the biosurfactant in the heavy metal. The removal of heavy metal was fatser at early due to its large surface area and being available for the adsorption of the metals. The sulfolipid has several advantages include low toxicity and biodegradable which is safer to use in the environment and human health compared to synthetic surfactant. It is used in industries because of providing high stable under a wide range of conditions, including temperature, pH, and salinity. Sulfolipids have good emulsifying activity, that can disperse hydrophobic compounds in water and other aqueous solutions and makes it useful in the bioremediation and enhanced oil recovery. The researchers have been working on biosurfactant due to its unique properties and making a promising alternative to synthethic surfactants.

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