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Influence of Biosurfactants on Bioremediation of Hydrocarbons

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ABSTRACT: Petroleum-contaminated soil contains various hazardous materials such as aromatic hydrocarbons and polycyclic aromatic hydrocarbons. This study has been focused on application of Biosurfactants in enhancing the Biodegradation Rate of Total Petroleum Hydrocarbon (TPH) in the petroleum contaminated soil. There are many advantages of biosurfactants as compared to chemically synthesized surfactants.. Some of them being Biodegradability, low Toxicity. Biocompatability and Digestibility, Hence facilitates their application in cosmetics, pharmaceuticals and as a functional food additive. The TPH reduction with varying concentration of boisurfactant was studied in 10 reactors & a control reactor under optimum conditions The results exhibits their biodegradation rate of **0.0122** day^{-1} and TPH removal efficiency of **84.8 %** under the optimal conditions.

Key words: Total Petroleum Hydrocarbons, Biosurfactants, Bioremediation

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I. INTRODUCTION

Biosurfactants have become an important product of biotechnology in recent years for industrial, Environmental and medical applications, Due to their popularity as high value microbial products due to their specific action, low toxicity, relative ease of preparation and widespread applicability. These are being used as emulsifiers, de-emulsifiers, wetting agents, spreading agents, foaming agents, functional food ingredients and detergents in various industrial sectors such as Petroleum and Petrochemicals, Organic Chemicals, Foods and Beverages, Cosmetics and Pharmaceuticals, Mining and Metallurgy, Agrochemicals and Fertilizers, Environmental Control and Management, and many others(1,2).

The biosurfactants behaviour in soil–water, water–oil and oil–soil systems with respect to foaming, solubilization, sorption to soil, emulsification, surface and inter-facial tension is found to be better results when compared to a well-known chemical surfactant. The biosurfactants were able to remove significant amount of crude oil from the contaminated soil at varied solution concentrations. Due to the reduction in surface, interfacial tensions & Volatilization while emulsification effects in oil removal were negligible due to the low crude oil volatilization. Thus, these studies suggest that knowledge of surfactants, behavior across different systems is of most important, before they are put to use.(3,4)

Biosurfactants are biologically synthesized surface-active agents. They are extra cellular macromolecules produced as metabolic byproducts of microbial transformation of organic substrate. Biosurfactant, have the advantages of being readily biodegradable and posses properties comparable to chemical produced surfactants, can be used for various applications. Thus, producing biosurfactants from used vegetable oil and used motor oil is a sound strategy of waste management for food and auto industries in reducing generation of wastes at source(1,5)

Surfactants are amphiphilic molecules that consist of a hydrophilic head and a hydrophobic tail. They are the active ingredients found in soaps and detergents with the ability to concentrate at the air–water interface and are commonly used in separating oily materials from a particular media. Surfactants are classified as ionic and non ionic with varying chemical structures according to their hydrophilic group. For industrial applications, the ionic surfactants are classified based on the charge they carry when dissociated in water at their neutral pH. Surfactants increase the aqueous solubility of non-aqueous phase liquids (NAPLs) by reducing their surface/interfacial tension at air–water and water–oil interfaces. As the interfacial tension is reduced and the aqueous surfactant concentration increased, the monomers aggregate to form micelles. The concentration at which micelles first begin to form is known as the Critical Micelle

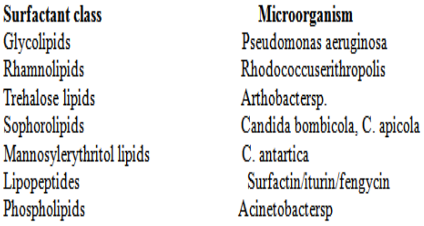
Concentration (CMC) corresponds to the point where the surfactant first shows the lowest surface tension. Many physical properties used to characterize surfactants depend on the CMC such as emulsion formation, oil solubilization, foaming and detergency, interfacial and surface tensions. These properties may be used to assess surfactant suitability for environmental remediation such as soil washing. Holmberg (2002) observes that as the hydrocarbon alkyl group increases, surfactant CMC increases depending on the alkyl length. The CMC of non-ionic surfactants are apparently two fold less than that of the ionic surfactants. Generally, with increase in temperature the CMC of some non-ionic surfactants decreases and increases solubility of ionic surfactants. Salt addition reduces the CMC of ionic surfactant while those of non-ionic are less affected. Surfactants produced from chemically based materials are known as Synthetic Surfactants and those from biologically based materials are Biosurfactants. Biosurfactants can be synthesized by many different microorganisms and are grouped into six major classes based on the producer microorganism. These classes are glycolipids, phospholipids, polysaccharide–lipid complexes, lipoproteins lipopetides, hydroxylated and cross-linked fatty acids. The complete cell surface & the combined behaviour of two immiscible liquids such as oil and water that result in the formation of the emulsion is important in the application of surfactants in oil-contaminated soil treatment.(3,4,6)

The behaviour of the emulsion is related to the equilibrium phase behavior of the oil-water -surfactant system from which it is made. The complex fluid dispersions generally formed and stabilized by adsorbing surfactants onto air-liquid interface are known as Foams. These dispersed fluids contain small bubbles with large surface areas, which can be stabilized using surfactants. Deshpande studied surfactant foaming and relates Foaming stability and formation to surfactant effectiveness in soil washing. Heavy formation of foams indicates good detergency which shows surfactants effectiveness in separating oily materials from soil. However, the formation of large amounts of foam makes handling the surfactant solution difficult and may not be acceptable in soil washing due to regulatory restrictions(6,7).

Classification Of Biosurfactants

Unlike chemically synthesized surfactants, which are usually classified according to the nature of their polar grouping, biosurfactants are generally categorized mainly by their chemical composition and microbial origin. Rosenberg and Ron suggested that biosurfactants can be divided into low-molecular-mass molecules which efficiently lower surface and interfacial tension, while high molecular mass polymers are more effective as emulsion-stabilizing agents. The major classes of low-mass surfactants include glycolipids, lipopeptides and phospholipids, whereas high-mass surfactants include polymeric and particulate surfactants. Most of the Biosurfactants are either anionic or neutral and the hydrophobic moiety is based on long-chain fatty acids or fatty acid derivatives, whereas the hydrophilic portion can be a carbohydrate, amino acid, phosphate or cyclic peptide.(1,4)

Table 1.Major biosurfactant classes and microorganisms involved(1,4)



Surfactant class	Microorganism
Glycolipids	Pseudomonas aeruginosa
Rhamnolipids	Rhodococcuserithropolis
Trehalose lipids	Arthobactersp.
Sophorolipids	Candida bombicola, C. apicola
Mannosylerythritol lipids	C. antartica
Lipopeptides	Surfactin/iturin/fengycin
Phospholipids	Acinetobactersp

Glycolipids :

Most known biosurfactants are glycolipids. They are carbohydrates in combination with long-chain aliphatic acids or hydroxyaliphatic acids.

Rhamnolipids :

These glycolipids, in which one or two molecules of rhamnose are linked to one or two molecules of β-hydroxydecanoicacid are the best studied. While the OH group of one of the acids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide, the OH group of the second acid is involved in ester formation

Trehalose lipids:

Several structural types of microbial trehalolipidbiosurfactants have been reported Disaccharide trehalose linked at C-6 and C-6′ to mycolic acid is associated with most species of Mycobacterium, Nocardiaand Corynebacterium. Mycolic acids are long chain, α-branched-β-hydroxy fatty acids. Trehalolipids from different organisms differ in the size and structure of mycolic acid,

Sophorolipids:

These glycolipids, which are produced mainly by yeast such as Torulopsisbombicola, T. petrophilumand T. apicolaconsist of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid by glycosidic linkage. Generally, sophorolipids occur as a mixture of

macrolactones and free acid form. It has been shown that the lactone form of the sophorolipid is necessary or at least preferable for many applications.
These biosurfactants are mixture of at least six to nine different hydrophobic sophorolipids.

Lipopeptides and lipoproteins :

A large number of cyclic lipopetides including decapeptide antibiotics (gramicidins) and lipopeptide antibiotics (polymyxins) are produced. These consist of a lipid attached to a polypeptide chain.Surfactin. The cyclic lipopeptidesurfactants is produced by Bacillus subtilisATCC 21332 is one of the most powerful biosurfactants. It is composed of a seven amino-acid ring structure coupled to a fatty-acid chain via lactone linkage. It lowers the surface tension from 72 to 27.9 mN/m Bacilluslicheniformis produces several biosurfacants which act synergistically and exhibit excellent temperature, pH and salt stability. These are also similar in structural and physio-chemical properties to the surfactin26

Properties Of Biosurfactants (3,7)

Biosurfactants are of increasing interest for commercial use because of the continually growing spectrum of available substances. There are many advantages of biosurfactants compared to their chemically synthesized surfactants. The main distinctive features of biosurfactants and a brief description of each property are discussed in the following sections.

Surface and Interface Activity

A good surfactant can lower surface tension of water from 72 to 35 mN/m and the interfacial tension of water from 40 to 1 mN/m Surfactant from B. Subtiliscan reduce the surface tension of water to 25 mN/m and interfacial tension of water <1 mN/m. Rhamnolipids from P. aeruginosadecrease the surface tension of water to 26 mN/m and the interfacial tension of water to <1 mN/m. The sophorolipids from T. bombicolahave been reported to reduce the surface tension to 33 mN/m and the interfacial tension to 5 mN/m. In general, biosurfactants are more effective and efficient and their CMC is about 10–40 times lower than that of chemical surfactants, i.e. less surfactant is necessary to get a maximum decrease in surface tension.

Temperature, pH and Ionic Strength Tolerance

Many biosurfactants and their surface activities are not affected by environmental conditions such as temperature and pH.McInerneyet al.(2006) reported that lichenysin from B. licheniformisJF-2 was not affected by temperature (up to 50°C), pH (4.5–9.0) and by NaCl and Ca concentrations up to 50 and 25 g/l respectively.

Biodegradability

Compared to synthetic surfactants, microbially produced surfactants can easily degrade and particularly suited for environmental applications such as bioremediation.

Low toxicity

Biosurfactants are generally considered as low or non-toxic products and therefore,appropriate for pharmaceutical, cosmetic and food uses. A report suggested that a synthetic anionic surfactant displayed an LC50 (concentration lethal to 50% of test species) against Photobacterium phosphoreum ten times lower than rhamnolipids, demonstrating the higher toxicity of the chemically derived surfactant. While comparing the toxicity of six biosurfactants, four synthetic surfactants and two commercial dispersants. It was found that most biosurfactants degraded faster, except for a synthetic sucrose-stearate that showed structure homology to glycolipids and was degraded more rapidly than the biogenic glycolipids. It was also reported that biosurfactants showed higher EC50 (effective concentration to decrease 50% of test population) values than synthetic dispersants.

Biosurfactants in Petroleum Processing Industries (2,5,7)

Emphasizing the use of biosurfactants in petroleum related industries is primarily for Enhanced Oil Recovery (EOR). for this utilization, biosurfactants can be used ex situ' and/or in situ. In both cases, biosurfactants are applied (as a replacement or addition to synthetic surfactants) to enhance oil recovery The presence of surfactant (or biosurfactant!) lowers the surface and interfacial tensions of the oil in the reservoir, which facilitates oil flow and percolates through pores in the reservoir during water, steam or fire flooding recovery operations, as practices in EOR (or Microbial EOR, MEOR). For production of the biosurfactant, the selected culture is grown in bioreactors. The biosurfactant recovered or simply concentrated in the broth is then pumped into the reservoir. This external production of the biosurfactant is used for producing more quantity under optimum fermentation conditions by supplying sufficient aeration to the culture in the bioreactor. Use of cheap and waste substrates for this application would be particularly advantageous. A logical and more attractive approach is to produce the biosurfactant in the reservoir itself (in situ) by providing nutrients to indigenous microbial population in the reservoir or to a seed culture mixed with the medium and pumped into the well. However, microorganisms introduced in this way into the reservoir are subjected to a number of stresses which may affect their growth or even inhibit both growth and biosurfactant production. Microbial growth and biosurfactant production are also affected by temperature,pH, salinity, heavy metals which may considerably differ from those for optimum biosurfactant production. One of the major difficulties in maintaining microbial growth for biosurfactant production in the reservoir is the lack of oxygen and proper mixing in the reservoir for ensuring good mass transfer and nutrient metabolism by the organisms. Further biosurfactants produced in situ must exert their action at the targeted sites, which cannot be easily controlled. Some crude oil in the reservoir could also be metabolized. However, oxygen is needed for metabolism of hydrocarbons.

Applications of Biosurfactants in Pollution Control(1)

Microbial enhanced oil recovery

An area of considerable potential for BS application is microbial enhanced oil recovery (MEOR). In MEOR, microorganisms in the reservoir are stimulated to produce polymers and surfactants which aid MEOR by lowering interfacial tension at the oil–rock interface. To produce MS in situ, microorganisms in the reservoir are usually provided with low-cost substrates, such as molasses and inorganic nutrients to enhance growth and surfactant production. The MEOR in situ, bacteria must be able to grow under extreme conditions encountered in oil reservoirs such as high temperature, pressure, salinity, and low oxygen level.

Hydrocarbon degradation(6,7)

Hydrocarbon-utilizing microorganisms excrete a variety of biosurfactants. BS being natural products are biodegradable and environmentally safe. An important group of BS is mycolic acids which are the a -alkyl, b -hydroxyls very long chain fatty acids contributing to some characteristic properties of a cell such as acid fastness, hydrophobicity, adherability, Enriching waters and soils with long- and short-chain mycolic acids may be potentially hazardous.

Hydrocarbon degradation in the soil environment

CxHy degradation in soil is dependent on presence of hydrocarbon-degrading species of microorganisms, hydrocarbon composition, oxygen availability, water, temperature, pH, and inorganic nutrients. The physical state of CxHy can also affect biodegradation. Addition of synthetic surfactants or MS results in increased mobility and solubility of CxHy is essential for effective microbial degradation. Use of MS in CxHy degradation exhibited produced variable results. As per Lindley and Heydeman, the fungus Cladosporiumresiuae grown on alkane mixtures produced extracellular fatty acids and phospholipids mainly dodecanoic acid and phosphatidylcholine. Supplement of the growth medium with phosphatidylcholine enhanced the alkane degradation rate by 30%.

Hydrocarbon degradation in aquatic environment

When oil is spilled in aquatic environment, the lighter hydrocarbon components volatilize while the polar hydrocarbon components dissolve in water. However, because of low solubility (< 1 ppm) of oil, most of the oil components will remain on the water surface. The primary means of hydrocarbon removal is due to photo oxidation, evaporation, and microbial degradation. since CxHy, degrading organisms are present in seawater. Biodegradation is one of the most efficient method in removal of pollutants. Surfactants enhance degradation by dispersing and emulsifying hydrocarbons. Microorganisms that are able to degrade CxHy can be isolated from aquatic environment. These microorganisms which exhibit emulsifying activity as well as the soil microorganisms that produced surfactants may be useful in aquatic environment. Chakrabarty reported that an emulsifier produced by P. aeruginosaSB30 was able to quickly disperse oil into fine droplets, therefore it may be useful in removing oil from contaminated soils. BS produced by oil-degrading bacteria may be useful in cleaning oil tanks.

II. METHODOLOGY

Biosurfactants are eco-friendly, due to their biodegradability and have many applications such as emulsifiers, wetting agents, spreading agents, foaming agents, detergents including Bioremediation of contaminated environment. In the present study, the biosurfactants were produced from Pseudomonas aeruginosa which was isolated from petroleum contaminated soil collected from VRL Logistics, Mysore Road, Bangalore. Thus selected micro-organisms were propogated on selective cetrimide agar petriplates. The cultures of Pseudomonas aeruginosa were transferred to an aerobic reactor to produce the biosurfactant using rice bran as the substrate by the submerged fermentation technique. From Biochemical assays, it was confirmed that the Biosurfactant produced by Pseudomonas aeruginosa belongs to the class of

Rhamnolipids which can be efficiently used for bioremediation of hydrocarbons.

III. RESULTS & DISCUSSION

The TPH reduction with varying concentration of Boisurfactant was studied in 10 reactors & a control reactor under optimum conditions, the same is summarized in the following sections.

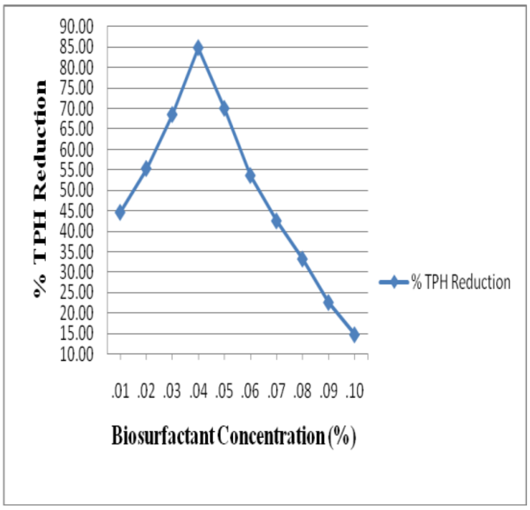


Figure 1: Graph showing percentage TPH reduction under various Biosurfactant concentrations in percentage.

The maximum reduction in TPH was observed in B4 having 84.8% reduction during the seventeen weeks of study period. The Fig.2 shows the variation in the percentage reduction of TPH in the 10 Bioreactors.

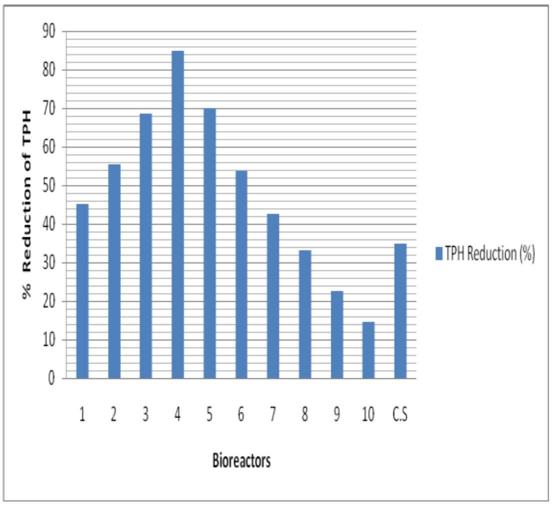


Figure 2 : TPH reduction in Bioreactors B1 to B10

From the above results, For 8% TPH (80gm/Kg), 4gm/Kg of Biosurfactant yielded highest degradation of 84.8% in 17 weeks study period.. Hence, Ratio of TPH: BIOSURFACTANT addition is 10:0.5 to get optimum degradation, i.e., if there's 1% TPH (10gm/Kg) in soil then 0.5gm of Biosurfactant should be added to yield maximum degradation of TPH.

After Bioreactor B4 it is observed that there is reduged rate of Biodegradation of TPH due to use of single Biosurfactant like rhamnolipid which binds and stores all TPH specifically to be degraded by Pseudomonas spp. of micro-organisms and the availability of TPH to other micro-organisms will be reduced which inturn hinders rate of biodegradation. Hence, usage of mixture of two or more biosurfactants is necessary to achieve and enhance the biodegradation process of TPH

Table 1: Degradation rate constants in Bioreactors B1 to B10

Sl No	Bioreactor	Degradation Rate Constant (k)d^{-1}
1	B1	0.0046
2	B2	0.0061
3	B3	0.0083
4	B4	0.0122
5	B5	0.0086
6	B6	0.0058
7	B7	0.0043
8	B8	0.0031
9	B9	0.0020
10	B10	0.0012

The highest rate of degradation occurred in B4, where the value of k was obtained as 0.0122 d^{-1} · This corresponds with the fact that the growth in biomass was also maximum in reactor B4.This indicates that there is a relationship between degradation rate and biosurfactant addition which facilitates bioremediation.

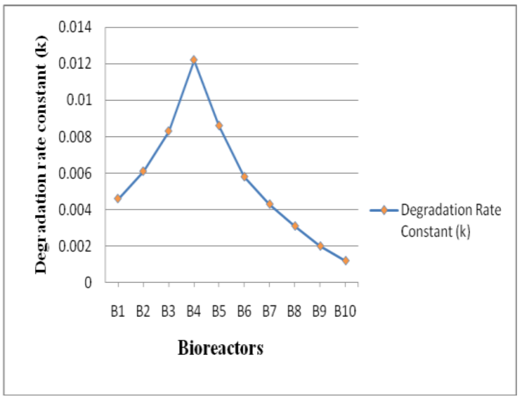


Figure 3 :Graph showing degradation rate constant (k) for Bioreactors B1 to B10

IV. CONCLUSION

From the study it is concluded that the optimal concentration of Biosurfactant to be added for better degradation of TPH was found to be **4gms/kg** of petroleum contaminated soil which gives the **TPH:Biosurfactant** ratio of addition as **10:0.5** (by w/w) resulting in biodegradation rate of **0.0122** day^{-1} and TPH removal efficiency of **84.8 %** under the optimal conditions.

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