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REVIEW: BIOSURFACTANT AND HYDROCARBON DEGRADATION

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ABSTRACT: Oil pollution is one of the major environmental problems today. Extraction, processing, use of petroleum product, and spills and accidental release of petroleum product introduce various hydrocarbons in environment. Environmental damage from such a released product is of major concern, as most of them are toxic and belong to carcinogenic family. It cause harm to both aquatic and terrestrial life. Biosurfactant producing microorganisms have shown promising effect on degradation of hydrocarbon. Biosurfactant solubilise and increases bioavailability of hydrocarbon to microorganism. Microorganism converts these hydrocarbons into simpler organic compound. Both mechanical and physical methods for removal of hydrocarbons were expensive and associated with release of secondary pollutants. Bioremediation technology has shown to perform well than mechanical and chemical methods for removal of hydrocarbons from contaminated sites.

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1.INTRODUCTION

Biosurfactant are extracellular or membrane associated, low molecular weight surface active compounds produce by different microorganism such as bacteria, fungi and yeast. On the basis of chemical composition they are categorized into glycolipids, lipopeptides, phospholipids, neutral peptides, fatty acids (Wei *et al.*, 2007),(Sambhaji B. Thakar, Kailas D. Sonawane 2013). They mainly composed of hydrophilic and hydrophobic moiety. Hydrophilic moiety consists of acids, peptides and mono, di, or polysaccharides. While hydrophobic moiety consist of saturated or unsaturated fatty acids. They have great potential to reduce surface and interfacial tension of liquids, solid and gases, and enhance their solubilisation in liquid solution. They have shown good stability at extreme temperature, pH and salt concentration Because of their unique characteristic and better performance than synthetic surfactant they have gained attention and importance in various fields such as enhanced oil recovery, environmental bioremediation, food processing and pharmaceuticals (Mullican *et al.*, 2005). Among all biosurfactant, rhamnolipid has shown better performance in bioremediation of hydrocarbons (Wei *et al.*, 2008).

Production of Biosurfactant

Though Biosurfactant have shown better performance than synthetic surfactant, they are not widely used for commercial purpose, mainly because of high production cost, expensive raw materials, high downstream processing cost and low production yield (Mukherjee *et al.*, 2006). Efforts are being made by the researcher all over the world to overcome these obstacles. Use of immobilized microbial cells is more effective than by using free cells for production of Biosurfactant. In former, microbial cells can be protected from extreme environmental conditions such as change in pH, temperature. They can also be protected from organic solvents and toxic material. Immobilized system is easy to handle, easy to recover from bioreactor and can be reused. Final product can be recovered easily during downstream processing without any difficulties.(Onwosiet *et al.*, 2013) Conducted experiment to study production of Rhamnolipid Biosurfactant from immobilized *Pseudomonas nitroreducens*. *Pseudomonas nitroreducens* was immobilized on calcium alginate beds. Optimum yield of rhamnolipid was reported i.e 5.6 g/lit. (Jeon *et al.*, 2004) conducted experiment to study continuous production of Biosurfactant Rhamnolipid using immobilized *Pseudomonas aeruginosa*. Microorganisms were immobilized in polyvinyl alcohol beads. 6 g/lit of Rhamnolipid was obtained.(Abouseoundet *et al.*, 2008) studied continuous production of

Biosurfactant from free and alginate entrapped cells of *Pseudomonas fluorescens* using olive oil as sole source of carbon. Biosurfactant produced was rhamnolipid type in nature and, has shown good stability at high temperature (up to 120 °C for 15 min), high salt concentration (up to 10% NaCl) and wide range of pH. (Heydet *et al.*, 2011) carried out studies on continuous production of rhamnolipid (RL) using *Pseudomonas aeruginosa* DSM 2874. Biosurfactant was continuously removed in situ by foam fractionation. One of the major drawback of foam fractionation is the loss of biocatalyst. i.e. cells. This problem was overcome by immobilization of cells in magnetic alginate beads.

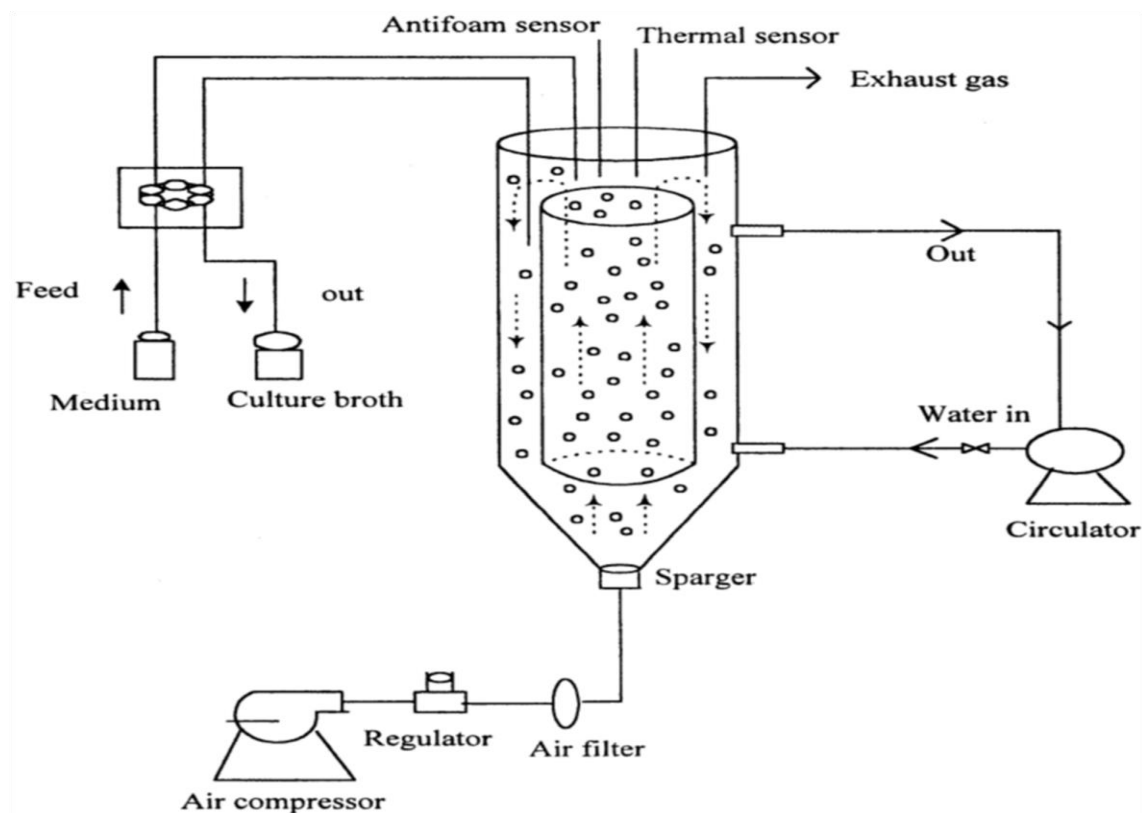


Fig 1: Schematic diagram of an airlift bioreactor for production of Rhamnolipid (Jeonget *et al.*, 2004)

Cheap Substrate:

(Daverey *et al.*, 2009) have reported work on production of biosurfactant sophorolipid from yeast *Candida bombicola* using low cost fermentative medium containing sugarcane molasses, yeast extract, urea, and soybean oil. 63.7 g/l of Sophorolipid yield was reported in this study. (Daniel *et al.*, 1998) have used dairy waste (cheese whey) as a carbon source for production of biosurfactant

sophorolipid from *Candida bombicola* ATCC22214. (Solaiman *et al.*, 2004) carried out production of biosurfactant sophorolipid using soy molasses as carbon source. 21 g/l of sophorolipid yield was reported from *Candida bombicola*. (Deshpande *et al.*, 1995) produced sophorolipid from *Candida bombicola* using inexpensive substrate- animal fat. Optimum production was obtained, about 120 g/l of Sophorolipid in 68 hours. While restaurant oil waste was used as substrate for Sophorolipid production by (Shah *et al.*, 2008). (Thavasiet *et al.*, 2007) produced Biosurfactant glycolipids using low cost carbon source like Crude oil, Waste lubricant oil and Peanut oil from *Bacillus megaterium*. Among all of them, use of peanut oil has shown high glycolipids production.

Substrate	Microorganism	Biosurfactant	Yield (g/lit)	References
Turkish corn oil	<i>Candida bombicola</i> ATCC 22214	Sophorolipids	400	Pekinet <i>et al.</i> , 2005
Rapeseed oil	<i>Pseudomonas</i> species DSM 2874	Rhamnolipids	45	Trummler <i>et al.</i> , 2003
Sunflower oil soapstock waste	<i>Pseudomonas aeruginosa</i> LBI	Rhamnolipid	16	(Benincasa <i>et al.</i> , 2002) and (Benincasa, <i>et al.</i> , 2004)
Cassava flour wastewater	<i>Bacillus subtilis</i> ATCC 21332 and <i>Bacillus subtilis</i> LB5a	Lipopeptide	2.2– 3.0	Nitschke and Pastore, 2006, Nitschke and Pastore, 2003, Nitschke and Pastore, 2004

Media optimization

Extensive research is carried out on fermentative production of Biosurfactant. Efforts are being made to obtain maximum yield of Biosurfactant. Studies reported have shown that nutrient elements, media components and precursors affect production of Biosurfactant. While carrying out studies on production of Biosurfactant from *Pseudomonas aeruginosa* strain BS-2, limitation of nitrogen source in media component have shown to enhance the production of Biosurfactant (Dubey and Juwarkar, 2002). (Hewald *et al.*, 2004) also reported that when *Ustilagomaydis* is

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grown on media having limited Nitrogen content, increases production of biosurfactant was observed. Presence of high concentration of iron in cultural media has shown to enhance the growth of *Bacillus subtilis* ATCC 21332 and also significant increase in production of surfactin. (Wei *et al.*, 2003). (Amezcua-Vega *et al.*, 2002) reported C/P, C/Ninorganic, C/Fe, C/Mg ratios and yeast extract concentration affect production of Biosurfactant. High C/Fe and C/P ratio have shown to increase in production of Biosurfactant. Studies on optimization of cultural condition for Biosurfactant production from *Pseudomonas aeruginosa* OCD1 and mutant strain of *Bacillus* sp(m28) were reported by (Sahoo *et al.*, 2011) and (Ray *et al.*, 2012) respectively. Use of classical method of medium optimization was associated with time consuming and laborious. This problem was overcome by using response surface methodology (RSM), which has been used by many researchers for media optimization for Biosurfactant production. (Abalose *et al.*, 2002) have reported media optimization by RMS for production of Biosurfactant rhamnolipid from *Pseudomonas aeruginosa* AT 10. Maximum yield of Rhamnolipid was obtained, about 18.7 gm/dm³. (Rodrigues *et al.*, 2005) also used similar strategy for media optimization to obtain maximum yield of Biosurfactant from *Lactococcus lactis* 53 and *Streptococcus thermophilus* A. (Eswari *et al.*, 2012) reported use of an artificial neural network-based response surface model (ANN RSM) for media optimization for Rhamnolipid production from *Pseudomonas aeruginosa* AT10. Use of media optimization strategy have resulted increase in production of Biosurfactant and lower the production cost, make the process economical.

Recovery of Biosurfactant:

Downstream processing cost contribute majorly (approximately 60%) to the production cost of Biosurfactant. Precipitations, centrifugation and solvent extraction technique were commonly used for the recovery of the Biosurfactant from fermentation media (Desai and Desai, 1993). Methanol, chloroform, acetone and dichloromethane are the common used solvents. These techniques are widely used in batch process. Solvent used are expensive, toxic and harmful to nature, which limit their use in Biosurfactant recovery. (Kuyukina *et al.*, 2001) used methyl tertiary-butyl ether (MTBE), for the recovery of biosurfactant glycolipid. As compare to other solvent MTBE is cheap, easily available, less toxic, low flammability and explosion safety. Leonard and Lemlich in 1965 proposed Foam fractionation technique for the recovery of surface active molecules. This technique is cost effective, yield highly concentrated product, require less space and eco-friendly.

Microorganism	Biosurfactant	Reference
<i>Pseudomonas aeruginosa</i> SP4	Rhamnolipid	Sarachatet <i>et al.</i> , 2010
<i>Pseudomonas aeruginosa</i>	Rhamnolipid	Heydet <i>et al.</i> , 2011
<i>B. subtilis</i> ATCC 21332	Surfactin	Daviset <i>et al.</i> , 2001
<i>Bacillus subtilis</i> BBK006	Surfactin	Chen <i>et al.</i> , 2006
<i>Bacillus subtilis</i>	surfactin	Noahet <i>et al.</i> , 2002

Table 1: Recovery of Biosurfactant by Foam fractionation.

All above mentioned technique fail to recover Biosurfactant from *Pseudomonas aeruginosa* strain BS2, when distillery wastewater (DW) is used as nutrient medium. This problem was overcome by using wood-based activated carbon (WAC) in downstream processing (Dubey *et al.*, 2005). It can be used for continuous recovery of Biosurfactant from fermenter. Wood-based activated carbon (WAC) can be regenerated and reused and, thus help in minimize downstream processing cost. (Reilinget *al.* 1986) used ion exchange chromatography for the recovery of Biosurfactant Rhamnolipid from *Pseudomonas aeruginosa*. 90% of the product was recovered by this technique. Main advantage of this technique was chromatography column can be regenerated and reused. He has also reported use of polystyrene resins for recovery of Biosurfactant. It has shown fast and high product recovery.

Recombinant strain

Advances in recombinant technology have made possible to develop a high Biosurfactant yielding strains. such as bacterial strain have shown maximum yield of Biosurfactant than natural strains.

Recombinant strain	Reference
Recombinant <i>Pseudomonas aeruginosa</i> strains	Yakimovet <i>et al.</i> , 2000
Recombinant <i>Gordoniaamarae</i>	Doganet <i>et al.</i> , 2006
Recombinant <i>Bacillus subtilis</i>	Koch <i>et al.</i> , 1998

Hydrocarbons

Petroleum is a fossil fuel, composed a mixture of various hydrocarbon (alkanes, aromatics, resins and asphaltenes) and liquid organic compounds. It is most widely used energy source in industries and transport sectors. During extraction, processing and use of petroleum, various hydrocarbons are released in environment. Released hydrocarbons get accumulated in soil and in aqueous environment such as underground water, marine, and cause extensive damage to local system (Plants, animals, humans) (Lin *et al.*, 2005; Liang *et al.*, 2009a; Liang *et al.*, 2009b). These hydrocarbons have poor solubility in water, which make it difficult to remove from ecosystem. Bioremediation is a process in which microorganism are used to remove or detoxify the pollutant. Microorganism involve in petroleum bioremediation have shown great potential to utilize various types of hydrocarbon such as short-chain, long-chain hydrocarbons and aromatic hydrocarbons compounds, including polycyclic aromatic hydrocarbons, as source of carbon and energy (Reisfeld *et al.*, 1972; Rosenberg *et al.*, 1998). Bioremediation of petroleum has shown to be cost effective, versatile and environmental friendly. While physical and chemical methods for hydrocarbon remediation were expensive, laborious and require use of toxic solvent. (Thomassin *et al.*, 2002; Vinaset *et al.*, 2002). Polycyclic aromatic hydrocarbons (PAHs) are the hydrocarbons consist of two or more aromatic ring. These are environmental pollutants and widely distributed in environment. They are found in air, water, soil and food products (Johnsen *et al.*, 2005). PAHs are toxic to plants and human. They have been associated in development of various cancers like lung, intestinal, liver, pancreas and skin cancers in humans (Samanta *et al.*, 2002). Polycyclic aromatic hydrocarbon, released in environment from incomplete combustion of coal, crude oil and also from petrochemical industries and oil refinery activities. Studies have reported the use of Biosurfactant producing microorganism for degradation of polycyclic aromatic hydrocarbons (PAHs). Inadequate bioavailability of hydrocarbons to microorganisms is main problem in bioremediation, as almost all petroleum hydrocarbons are insoluble in water (Bognolo 1999; Rahman *et al.*, 2002; Seiet *et al.*, 2003). (Desai and Banat 1997; Rosenberg *et al.*, 1999) have reported that most of the hydrocarbon degrading bacteria have shown to produce surfactive compound known as biosurfactant. It allows bacteria to utilize or to grow on hydrocarbon substrate. Biosurfactant has shown to solubilize hydrocarbons by reducing its surface tension (Mulligan *et al.*, 2001). It has

also shown to affect surface hydrophobicity of bacterial cells and thus improve affinity between bacterial cell and hydrophobic compound (Zhang and Miller, 1994).

Hydrocarbon degradation

Hexadecane: Hexadecane is alkyl hydrocarbon, a major component of diesel (Chénieret *et al.*, 2003). This hydrocarbon has been used as model molecule for hydrocarbon biodegradation studies (Graham *et al.*, 1999), (Beal *et al.*, 2001) investigated role of rhamnolipid Biosurfactant in uptake and degradation of hydrocarbon hexadecane in *Pseudomonas aeruginosa*. Two strains of *Pseudomonas aeruginosa* were used for these studies, rhamnolipid producing strain (PG201) and rhamnolipid deficient strain (U2099). PG201 strain has shown high uptake of hexadecane than U2099 strain. However study shown that difference in uptake of hexadecane in both of this strain is less and thus rhamnolipid has minor role in mineralization of hydrocarbon, but it has shown to enhance this process. They have observed change in surface hydrophobicity of microorganism when grown on media containing hexadecane. Surface hydrophobicity change was observed more in PG201 than in U2099 strain. They have reported that uptake of hexadecane is energy dependent process, as addition of Carbonyl cyanide m-chlorophenyldimethylsulfoniurea (CCCP) has shown to reduce uptake of hexadecane. CCCP inhibit activity of cytochrome oxidase enzyme, and thus affect oxidative phosphorylation pathway in mitochondria. (Liu *et al.*, 2012) carried out studies on biodegradation of n-hexadecane which is major component of diesel and crude oil. During extraction and processing of petroleum hydrocarbons are released and get accumulated in water and soil, which become difficult to remove them from such ecosystem because of their insoluble characteristic. They isolated two bacterial strains (name B1 & B2) from petroleum contaminated soil in Tianjin (China), which were able to degrade hydrocarbon n-hexadecane. Identification of two bacterial strains was done by 16S rDNA sequencing. Bacterial strain B1 and B2 were identifies as *Pseudomonas aeruginosa* and *Acinetobacter junii* respectively. Among all the bacteria *Pseudomonas* is well known to produce Biosurfactant and utilize hydrocarbons as energy source (Beal & Betts, 2000; Rahman *et al.*, 2002). Both of this strain has shown to utilize n-hexadecane as a carbon sources. Both of them produce biosurfactant. (Bai *et al.*, 1997) conducted experiment to investigate the potential of monorhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* to remove hexadecane hydrocarbon from sand column. In further studies performance of rhamnolipid biosurfactant was then compared with synthetic surfactant like Sodium dodecyl

sulfate and polyoxyethylene. Sand column saturated with hexadecane were used for the experiment. Rhamnolipid of various concentration ranges from 40 to 1500mg/lit were used. Rhamnolipid was effective at 500mg/lit in removal of hexadecane and have shown better performance in removal of hydrocarbon than synthetic surfactant used in experiment (Noordman *et al.*, 2002) carried out studies on degradation of hexadecane by using *Pseudomonas aeruginosa*UG2, *Acinetobacter calcoaceticus*RAG1, *Rhodococcuserythropolis* DSM 43066, *R. erythropolis*ATCC 19558, and strain BCG112. Biosurfactant rhamnolipid produced by *Pseudomonas aeruginosa* UG2 stimulate biodegradation of hexadecane hydrocarbon. However, this rhamnolipid does not shown same result in remaining above mentioned organisms. This shows that *Pseudomonas aeruginosa* UG2 has different mechanism of uptake and degradation of hexadecane. They have stated that energy dependent system in *Pseudomonas aeruginosa* UG2 has a major role in uptake of hydrocarbon compounds. Mixture of Biosurfactant rhamnolipid and synthetic surfactant, have shown better performance in reduction of the interfacial tension (IFT) than rhamnolipid used alone (Nguyen *et al.*, 2008. Propoxylated sulphate synthetic surfactants were used for studies.

Benzene, Toulene and Xylene

(Martino *et al.*, 2012) isolated bacterial strain from water contaminated by oil. 16S rRNA gene sequencing shown that isolated bacterial strain belong to genus *Pseudomonas*. Isolated strains were able to degrade Benzene, Toulene and xylene. Both of this strain presence of *xylA*&*xylB* gene, which code for xylene monooxygenase (TOL Pathway) and catechol extradioldioxygenase. One of the isolate also has shown presence of *todC1*, which code for aromatic dioxygenase (TOD Pathway). Presence of these both pathways in *Pseudomonas* very rare and can be useful in bioremediation of hydrocarbon. (Pirollo *et al.*, 2008) isolated *Pseudomonas aeruginosa* LBI (Industrial Biotechnology Laboratory) from soil contaminated with hydrocarbon. The isolate was examined for production of Biosurfactant and degradation of hydrocarbon. Isolate has shown effective growth on diesel oil, kerosene, crude oil, oil sludge except Toulene and benzene. *Pseudomonas aeruginosa* LBI shows maximum production of Biosurfactant i.e.9.9gm/lit when grown on diesel oil. (Plaza *et al.*, 2008) carried out studies on performance of Biosurfactant producing microorganism *Ralstoniapicketti*SRS and *Alcaligenespiechaudii*SRS in degradation of crude oil and distillation product. Both of these strains were able to degrade crude oil after 20 days

of incubation period. Toulene, Benzene, xylene, hexadecane and cyclodecane were also degraded. Effective degradation was observed when both of these strains were used in form of mixture.

Anthracene

(Santos *et al.*, 2007) carried out studies to investigate effect of iron in biodegradation of polycyclic aromatic hydrocarbon (PAH) anthracene and biosurfactant production by *Pseudomonas* spp. *Pseudomonas citronellolis* isolate 222A, *Pseudomonas aeruginosa* isolate 332C and *P. aeruginosa* isolate 312A were the bacterial strain selected for this studies. Iron at concentration of 0.1mM has shown to stimulate production of biosurfactant in *Pseudomonas citronellolis* isolate 222A. They were first to report that iron enhances biodegradation of anthracene and stimulate the production of Biosurfactant. (Rodrigo *et al.*, 2005) isolated *Pseudomonas* spp. as well as from petrochemical sludge land farming site. *Pseudomonas citronellolis* 222A strain has shown to reduce surface tension of polycyclic aromatic hydrocarbon (PAH) anthracene and therefore enhances its degradation. They were first to report biodegradation of anthracene by the surfactant produced by *Pseudomonas citronellolis*.

Phenanthrene

(Zhang *et al.*, 1997) investigated performance of biosurfactant monorhamnolipid and dirhamnolipid on dissolution, bioavailability and biodegradation of phenanthrene. Both biosurfactant has shown to solubilise and degrade phenanthrene. Performance of monorhamnolipid was effective insolubilisation of phenanthrene. While in case of bioavailability of phenanthrene, dirhamnolipid was more effective. (Prabhu and Phale, 2002) isolated *Pseudomonas* spp. Strain P22, which has shown to degrade phenanthrene, benzoate and hydroxybenzoate fail to degrade naphthalene. They studied metabolic pathway and enzyme involve in degradation. They have stated that production of biosurfactant and increase in hydrophobicity of the cell surface has an essential role in degradation of hydrocarbon in *Pseudomonas* spp. Strain P22. (Schippers *et al.*, 2000) conducted experiment to study effect of biosurfactant sopholipid on biodegradation of phenanthrene and its toxicity on bacteria *Sphingomonasyanoikuyae*. No toxicity was reported up to 1 g l⁻¹ sophorolipids, whereas slight growth hindrance of *Sphingomonasyanoikuyae* was observed at a lower concentration of sopholipid i.e at 250 mg/lit. Sopholipid has shown rise in degradation of phenanthrene and decrease in consequence of residual pollutant. Previous studies have reported the effect of synthetic surfactant and biosurfactant on solubilisation and

degradation of hydrocarbons under mesophilic conditions. These studies under thermophilic condition were carried out by (Wong *et al.*, 2004). Performance of biosurfactant produced by *Pseudomonas aeruginosa* has shown effective solubilisation of polycyclic aromatic hydrocarbons (PAHs) phenanthrene than synthetic surfactant like Tween 80 and Triton X-100. (An *et al.*, 2011) conducted experiment to study effect of organic acid (acetic acid, oxalic acid, tartaric acid and citric acid) on solubilisation of phenanthrene in presence of biosurfactant rhamnolipid. Rhamnolipid with organic acid has shown enhance in solubilization of phenanthrene as compared with rhamnolipid used alone. Rhamnolipid with citric acid has shown to be more effective as compare to other organic acid used. (Zhoet *al.*, 2011) has shown that thermophilic temperature has synergetic effect on biodegradation of phenanthrene carried out by biosurfactant produced by *Acinetobacter calcoaceticus BU03*. Significant increase in biodegradation was observed at 55° C. (Zhoet *al.*, 2011) conducted experiment to investigate effect of biosurfactant rhamnolipid produced by *Pseudomonas aeruginosa ATCC9027*, on biodegradation on Phenanthrene by two thermophilic bacteria, *Bacillus subtilis BUM* and *P. aeruginosa P-CG3*. Presence of rhamnolipid has shown increase in cell surface hydrophobicity of *P. aeruginosa PCG3* and therefor rises in biodegradation to 92.7%. While in absence of rhamnolipid no increase in cell surface hydrophobicity was observed, in this case *Bacillus subtilis BUM* was major contributor in degradation of Phenanthrene. (Hong *et al.*, 2010) conducted experiment to study effect of rhamnolipid and synthetic surfactant like tween 80 on degradation of phenanthrene (PHE) carried out by *Sphingomonas sp. GF2B*. Addition of biosurfactant rhamnolipid has shown enhance in biodegradation of PHE than by synthetic surfactant, Tween 80.

Napthalene

(Dezielet *al.*, 1996) had shown that Biosurfactant production has been observed in polycyclic aromatic hydrocarbon (PAH) utilising bacteria. Isolated bacteria strain has shown growth on media containing naphthalene and phenanthrene as sole carbon source. Production of biosurfactant was detected by reduction in surface tension and emulsifying activities. (Tileva *et al.*, 2005) conducted experiment to study biosurfactant activity and degradation of naphthlene by *Bacillus cereus 28BN*. This bacterial has shown effective growth on media (containing nalkanes, naphthalene, crude oil and vegetable oils) and production of biosurfactant. They were first to revel degradation of naphthalene by biosurfactant rhamnolipid produced by *Bacillus cereus 28BN*.

N-alkane

(Throne-Holst *et al.*, 2007) identified novel gene *almain* *Acinetobacter* sp. Strain DSM 17874 which encode for flavin binding monooxygenase. This gene has shown involvement in degradation of long chain n-alkane. Earlier studies have reported involvement of monooxygenase/hydroxylase enzyme in degradation of long chain n-alkane. (Chaillan *et al.*, 2004) isolated bacteria, fungi and yeast from petroleum contaminated soil, which have shown great potential to degrade hydrocarbons. Bacteria isolated belong to genus *Gordonia*, *Brevibacterium*, *Aeromicrobium*, *Dietzia*, *Burkholderia* and *Mycobacterium*. Fungi isolated were belongs to genus *Aspergillus*, *Penicillium*, *Fusarium*, *Amorphoteca*, *Neosartorya*, *Paecilomyces*, *Talaromyces* and *Graphium*. And yeast with genus *Candida*, *Yarrowia* and *Pichia*. (Obonet *et al.*, 2006) have reported growth of bacteria on Kerosene, diesel and naphthalene, which were isolated from Nigerian Bitumen. These bacteria have shown great potential to degrade hydrocarbon's. (Ojo, 2006) isolated bacteria from wastewater canal from south west Nigeria, which have shown major effect on degradation of hydrocarbons. Bacteria isolated were belonging to genus *Bacillus*, *Pseudomonas*, *Micrococcus* and *Enterobacter*.

Effect of Biosurfactant in Soil Bioremediation

During extraction and processing of petroleum, hydrocarbons are released and get accumulated in water and soil. Biosurfactant produced by microorganism have shown to enhance the degradation of hydrocarbons. Study conducted by (Kosarić *et al.*, 1987) have reported that addition of sophorose lipid has shown to enhance degradation of compound 2,4-dichlorophenol (2,4-DCP), Naphthalene and some polycyclic aromatic hydrocarbons (PAH) from contaminated soil. (Jain *et al.*, 1992) conducted experiment to study degradation of hydrocarbon in Soil. Biosurfactant producing bacteria, *Pseudomonas aeruginosa* UG2 have shown to degrade hydrocarbon like tetradecane, hexadecane, and pristane except 2-methyl-naphthalene after incubation period of 40 days. (Van Dyke *et al.*, 1993) conducted experiment and have shown that effective recovery of hydrocarbon was observed when the biosurfactant Rhamnolipid produced by *Pseudomonas aeruginosa* were added at a concentration of 5g/L to hydrocarbon contaminated silt-loam soil and sandy-loam soil. By using biosurfactant and indigenous microbes successful bioremediation of machine-oil-contaminated soil and water was observed (Fry *et al.*, 1992). (Harvey *et al.*, 1990) have reported removal of the oil from water by using biosurfactant from *Pseudomonas aeruginosa* SB30. (Bragg

et al., 1994) have carried out studies on bioremediation of oil, *in situ*. (Al-Awadhi *et al.*, 1994) reported removal of oil from desert sand in Kuwait. Reusing of immobilised cells has shown no decline in hydrocarbon degradation. Thus immobilisation of cells has shown promising effect in degradation of hydrocarbon contaminated soil. (Cunningham *et al.*, 2004) conducted experiment to study potential of hydrocarbon degrading microorganism immobilised in polyvinyl alcohol to remove or to clean Diesel-contaminated soil. Performance of immobilised cells was compared with cells in liquid culture and bio stimulation in laboratory biopiles. Immobilised system has shown better performance in clean up of Biodiesel from soil. (Daizet *et al.*, 2002) conducted experiment to study biodegradation of crude oil by halotolerant bacteria Consortium MPD-M immobilised on polypropylene fiber. Immobilised system has shown enhance in biodegradation of crude oil as compare to free living cells. Biodegradation was observed over wide range of salinity condition ranging from 0 to 180 gm/lit.

2. CONCLUSION

Removal of hydrocarbon from environment is a major problem. Biosurfactant producing microorganisms have shown great potential to utilize and degrade hydrocarbons. Microorganisms belong to genera *Pseudomonas*, *Bacillus* and *Candida* have shown maximum yield of biosurfactant. Biosurfactant are nontoxic, biodegradable, do not cause any harm to environment and can be produced by utilizing cheaper substrate. These unique characteristics of biosurfactant make them better candidate than chemical and physical methods used for removal of hydrocarbons, and have gained more importance for industrial and environmental application.

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