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CRITICAL REVIEW OF BIOSURFACTANTS AND THEIR ROLE IN BIOREMEDIATION OF PETROLEUM HYDROCARBON CONTAMINANTS.

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ABSTRACT

Biotechnological advances for the past decades have provided new surfactant production technologies. Surface active substances (biosurfactants) produced by fermentative processes have proven to be a safer and sustainable alternative to many synthetic molecules. Biosurfactants are a promising substitute due to their synthesis potential by a wide variety of microorganisms. They are a highly diverse group of structures, such as glycolipids, lipopeptides, polysaccharide-protein complexes, phospholipids, fatty acids, and neutral lipids. This diversity promotes many advantages compared to synthetic surfactants, thereby making biosurfactants a suitable choice for technological advances associated with sustainable development. Such advantages include fermentative production viability using renewable resources, effectiveness in small concentrations even under extreme conditions, selective and specific potential for several applications, lower toxicity, higher biodegradability, and better stability to physicochemical variations. Despite these benefits enumerated, they are not widely used because of the high production costs. Hence, finding cost-effective substrates is imperative to making biosurfactants an economically competitive product against synthetic surfactants. In this review, inexpensive and renewable substrates that can be used for biosurfactants production are discussed. Also, biosurfactants and their potential use in bioremediation of hydrocarbon contaminants, and effectiveness in bioremediation of hydrocarbon contaminants compared to synthetic surfactants are reviewed.

Keywords: Biosurfactants, Hydrocarbon degradation, Bioremediation, Environment.

INTRODUCTION

Surfactants are amphiphilic molecules consisting of both hydrophilic (polar) and hydrophobic (non-polar) moieties (Santos et.al. 2016). These molecules can decrease the surface tension and the interfacial tension between aqueous and other immiscible solutions when they accumulate at the interface. Surfactants are synthesized chemically from petrochemical and oleochemical resources. Nowadays, due to global environmental awareness, the utilization of biological surface-active agents produced from living organisms has attracted scholarly attention. Biosurfactants are surface-active compounds synthesized by a broad range of microorganisms (Khan & Butt, 2016). They have acquired increased attention due to their diverse applicability, biodegradability, low toxicity, effectiveness at extreme conditions of pH, temperature, and ability to be produced from inexpensive substrates (Ghasemi et.al. 2019). They have both hydrophobic and hydrophilic domains and can decrease the surface tension and the interfacial tension of growth medium; they can be produced on microbial cell surfaces and can be secreted extracellularly. Biosurfactants are commonly classified based on the biochemical nature of the microbial producer species. Bioremediation is a branch of biotechnology employing the use of living organisms like microbes and their metabolic products as in biosurfactants to remove contaminants, pollutants, and toxins from soil and water. Bioremediation enhances the ability of microorganisms to metabolize petroleum hydrocarbons into biomass, carbon dioxide, water, and innocuous oxygenated end products. The microbes essentially treat oil as food (Wahab, 2015). Bioremediation is a promising option for remediation since it is effective and economic in removing oil with less environmental damage (Sheppard, 2018.) Besides cost-effectiveness, it is a permanent solution, which may lead to the complete mineralization of the pollutant. Furthermore, it is a non-invasive technique, leaving the ecosystem intact (Perelo, 2010).

Hydrocarbon compounds, ranging from crude oil to its derivative products, have the potential to cause environmental problems on a global scale. The emergence of these hydrocarbon compounds in the ecosystem makes it a concern to the general health of public and environmental aspects, owing to their toxic nature, low to less biodegradability, and potentially accumulated in the food chain (Effendi et al. 2018). Hydrocarbon compounds including petroleum oil derivation products and petrochemical products as well as oil sludge as a by-product have the potential to form contaminants on a large scale. The existence of these compounds in the environment is considered both dangerous both environmentally, and to the public health because these compounds accumulate, persist, and have high toxicity value (Taha et al. as cited in Effendi et al. 2018).

The productive use of biodegradative processes to remove or detoxify pollutants that have found their way into the environment and threaten public health, usually as contaminants of soil, water, or sediments is bioremediation (Thapa et al. 2012). Environmental pollution has been on the rise in the past few decades owing to increased human activities on energy reservoirs, unsafe agricultural practices and rapid industrialization. Amongst the pollutants that are of environmental and public health concerns due to their toxicities are: heavy metals, nuclear wastes, pesticides, greenhouse gases, and hydrocarbons. The remediation of polluted sites using microbial process (bioremediation) has proven effective and reliable due to its eco-friendly features. Bioremediation can either be carried out ex situ or in situ, depending on several factors, which include but not limited to cost, site characteristics, type and concentration of pollutants. Therefore, choosing an appropriate bioremediation technique, which will effectively reduce pollutant concentrations to an innocuous state, is crucial for a successful bioremediation project (Azubuike et al. 2016).

Crude oil is a composite mixture of thousands of different chemical compounds. As the composition of each type of oil is unique, there are different ways to deal with them through microbes. Bioremediation can occur naturally or can be encouraged with the addition of microbes (Thapa et al. 2012). The microbes present in the soil first recognize the oil and its constituent by biosurfactants and bio emulsifiers, and then they attach themselves and use the hydrocarbon present in the petroleum as a source of energy and carbon. The low solubility and adsorption of high molecular weight hydrocarbons limit their availability to microorganisms. The addition of biosurfactants enhances the solubility and removal of these contaminants, improving oil biodegradation rates (Thapa et al. 2012).

The biodegradation of different petroleum compounds occurs simultaneously but at different rates because different species of microbes preferentially attacks different compounds. This leads to the successive disappearance of individual components of petroleum over time.

Unlike synthetic surfactants, Biosurfactants are generally classified by their molecular weight, chemical composition, or microbes that produce them (Ron and Rosenberg as cited in Effandi et al. 2018 stated that biosurfactants or bioemulsifiers generated by numerous microbes based on their molecular weight are basically split into two major class, i.e., low molecular weight biosurfactants and high molecular weight biosurfactants. The biosurfactants having low molecular weight are glycolipids such as rhamnolipid and sophorolipid or lipopeptides such as polymyxin and surfactin, while high molecular weight biosurfactants include lipoprotein, lipopolysaccharide, and amphipathic polysaccharides. Biosurfactants having low molecular weight can serve to decrease surface tension, while biosurfactants having higher molecular weight are highly efficient for oil emulsion stabilization in aquatic conditions. Desai and Banat (1997) classify biosurfactants based on the composition of their chemical structures. Biosurfactants comprise a varied group of surface-active compounds and occur in nature with diversity of biochemical structure, such as lipopeptides, glycolipid, phospholipids, neutral lipids, fatty acids, particulate, and polymeric structures (Huszcza and Burczyk as seen in Effandi et al. 2018). The naturally produced biosurfactants cause no harm to environment, as compared to synthetically manufactured surfactants. The most common type of biosurfactant is glycolipid. Glycolipids are a combination of carbohydrates having long chain of aliphatic acids or hydroxy aliphatic acid. Glycolipids are divided into rhamnolipid, trehalolipid, and sophorolipid. The numerous findings and studies by several scientists with regard to biosurfactant, which improved biological degradation of contaminants, have shown the ability of biosurfactants to degrade a number of pollutants including petroleum hydrocarbons, nitroaromatic compounds and pesticides amongst others.

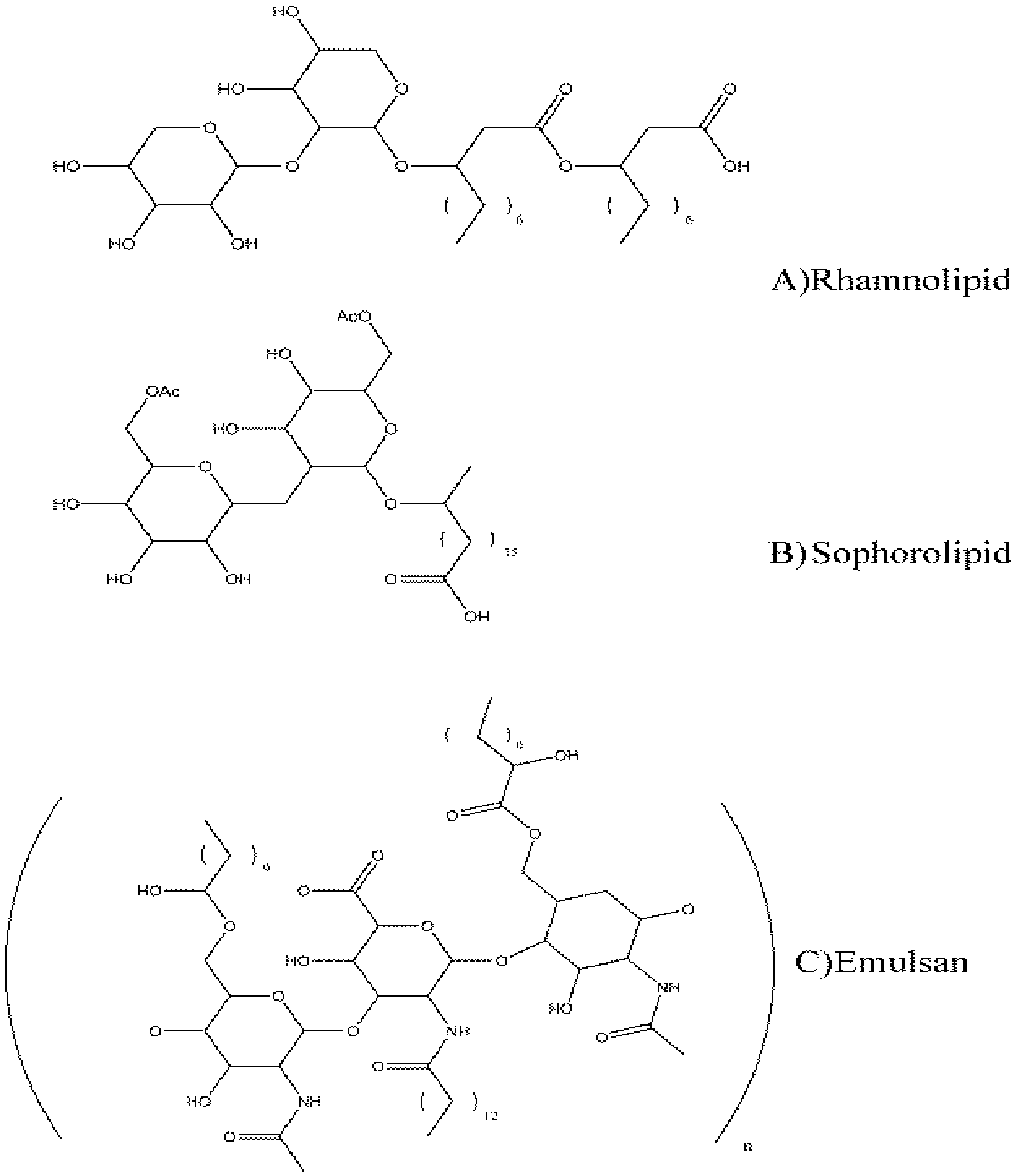

Fig1 Chemical structures of some of the most common biosurfactants

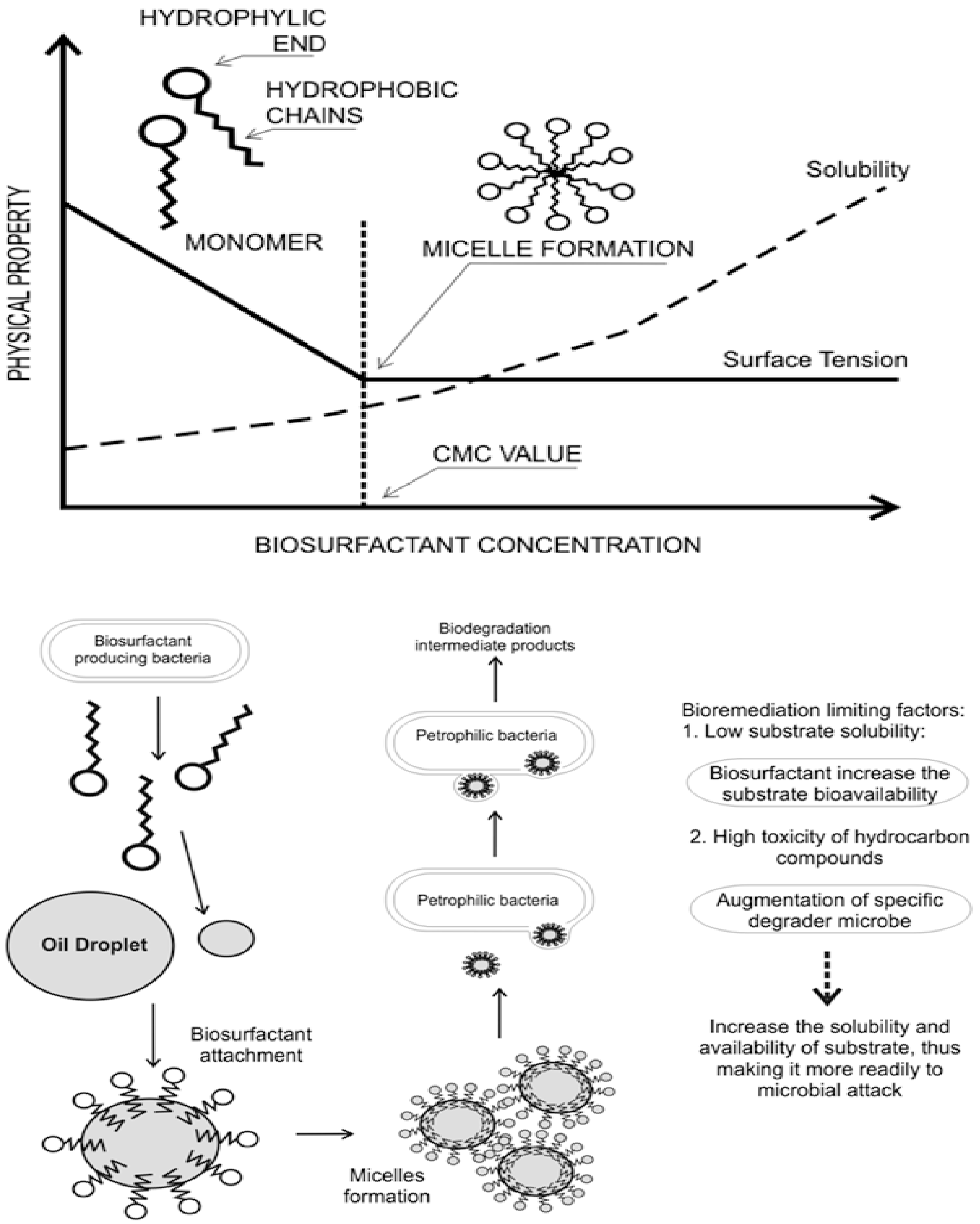

Fig 2 surface tension value as a function of biosurfactant concentration (critical micelle concentration)

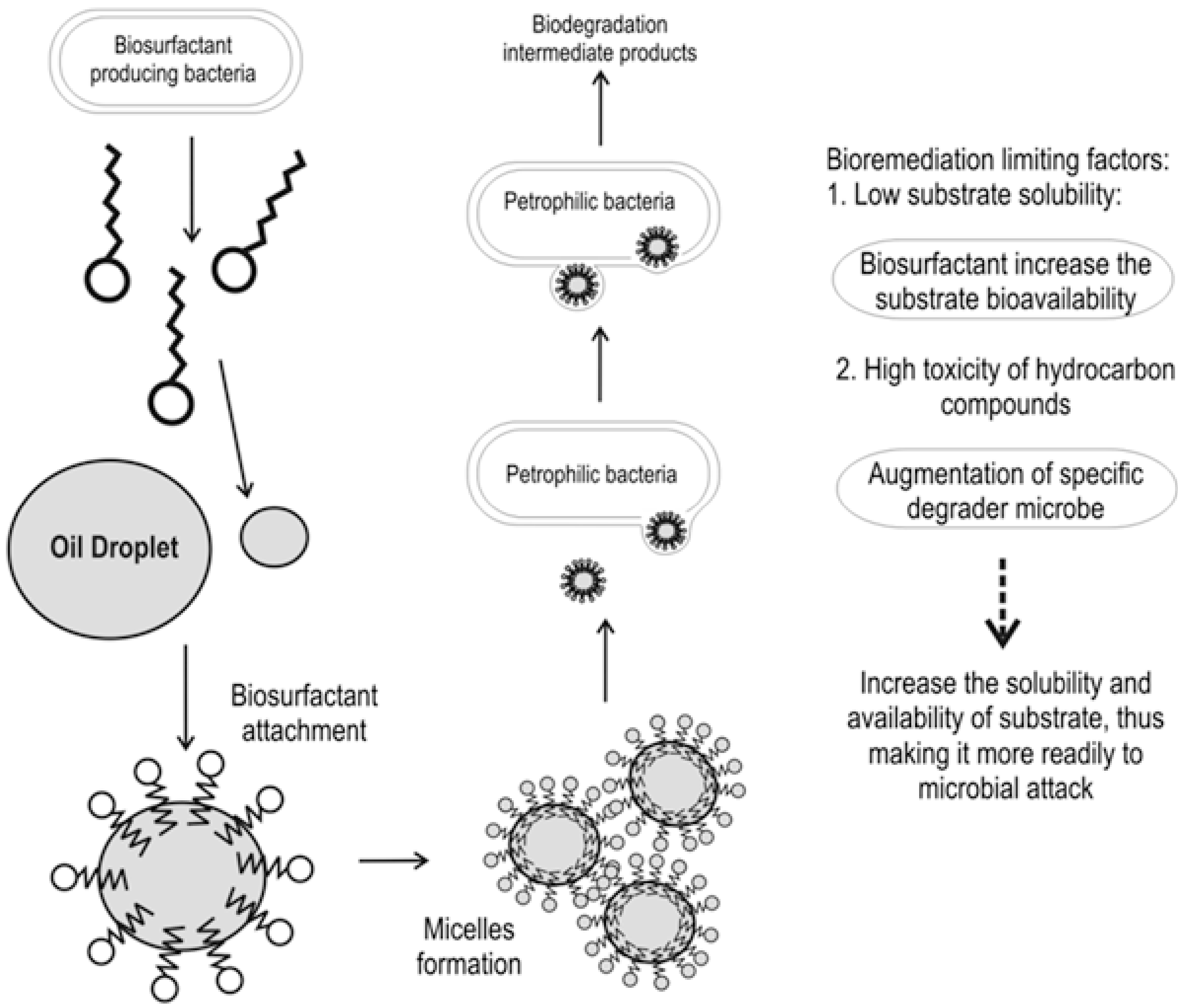

Fig. 3 mechanism of microbial degradation of hydrophobic compounds by Biosurfactants

2.0 : BIOSURFACTANT-PRODUCING MICROORGANISMS

Microorganisms producing biosurfactants as reported by Desai and Desai (1997) consist of various types of bacteria and fungi and yeast though fewer studies have been conducted on them. Some of these include Pseudomonas sp., Pseudomonas aeruginosa, Pseudomonas fluorescens, Arthrobacter sp., Azotobacterchroococcum, Azotobactervinelandii, Bacillus licheniformis, and Bacillus subtilis. Several types of biosurfactants can also be produced from fungi such as sophorolipid produced by Torulopsisbombicola, Torulopsisapicola, Candida lipolytica, Candida tropicalis, Candida antarctica, and Candida glabrata.

Organism	Type of biosurfactant	Potential Applications
Rhodococcuserythropolis 3C-9	Glycolipid and trehalose lipid	Oil spill cleanup operations
Pseudomonas aeruginosa S2	Rhamnolipid	Bioremediation of oil contaminated sites
Pseudozymasiamensis CBS 9960	Mannosylerythritol lipid	Promising yeast biosurfactant
Pseudozymagraminicola CBS 10092	Mannosylerythritol Lipid	Washing detergents
Pseudomonas libanensis M9-3	Lipopeptide	Environmental and biomedical applications
Bacillus subtilis strain ZW-3	Lipopeptide	Potential in pharmaceutics, environm protection, cosmetic, oil recovery
Rhodococcus sp. TW53	Lipopeptide	Bioremediation of marine oil pollution.
Pseudozymahubeiensis	Glycolipid	Bioremediation of marine oil pollution
R. wratislaviensis BN38	Glycolipid	Bioremediation applications
Bacillus subtilis BS5	Lipopeptide	Bioremediation of hydrocarbon- contami sites

METHODS AND MATERIALS

3.1: BIOSURFACTANTS - A BETTER ALTERNATIVE TO SYNTHETIC SURFACTANTS

When compared to chemical or synthetic surfactants, biosurfactants gained several advantages including their biodegradability, biocompatibility, low toxicity, and digestibility (Vijayakuma and Saravanan, 2015). The unique and distinct properties of biosurfactants when compared to their chemically synthesized counterparts have led to an increased research on the use of biosurfactants for different application. According to Patowary et al. (2016) on conduction of the biodegradation study, using soil samples A and B, it was observed that the total petroleum hydrocarbon (TPH) concentration in soil samples decreased gradually in case of the biosurfactant and the surfactant sodium dodecyl sulfate (SDS) treated sets, where as in the untreated soil used as control, the TPH concentration did not change markedly. In soil amended with rhamnolipid biosurfactant, degradation percentage of TPH was found to be 86.1 and 80.5% in the sample A and B respectively, at the end of six months. However, in SDS treated soil, the degradation percentage of TPH was found to be 70.8 and 68.9% in sample A and B respectively, which was lesser than the degradation occurred in biosurfactant treated soil. This provides an inference that the biosurfactants have better efficiency than their synthetic counterpart. Furthermore, synthetic surfactant can impose toxic effect on the environment and may be non-biodegradable in nature. Thus, the application of the biosurfactant for the enhancement of biodegradation of crude oil contaminated soil is preferable over synthetic detergent (Patowary et al. 2016)

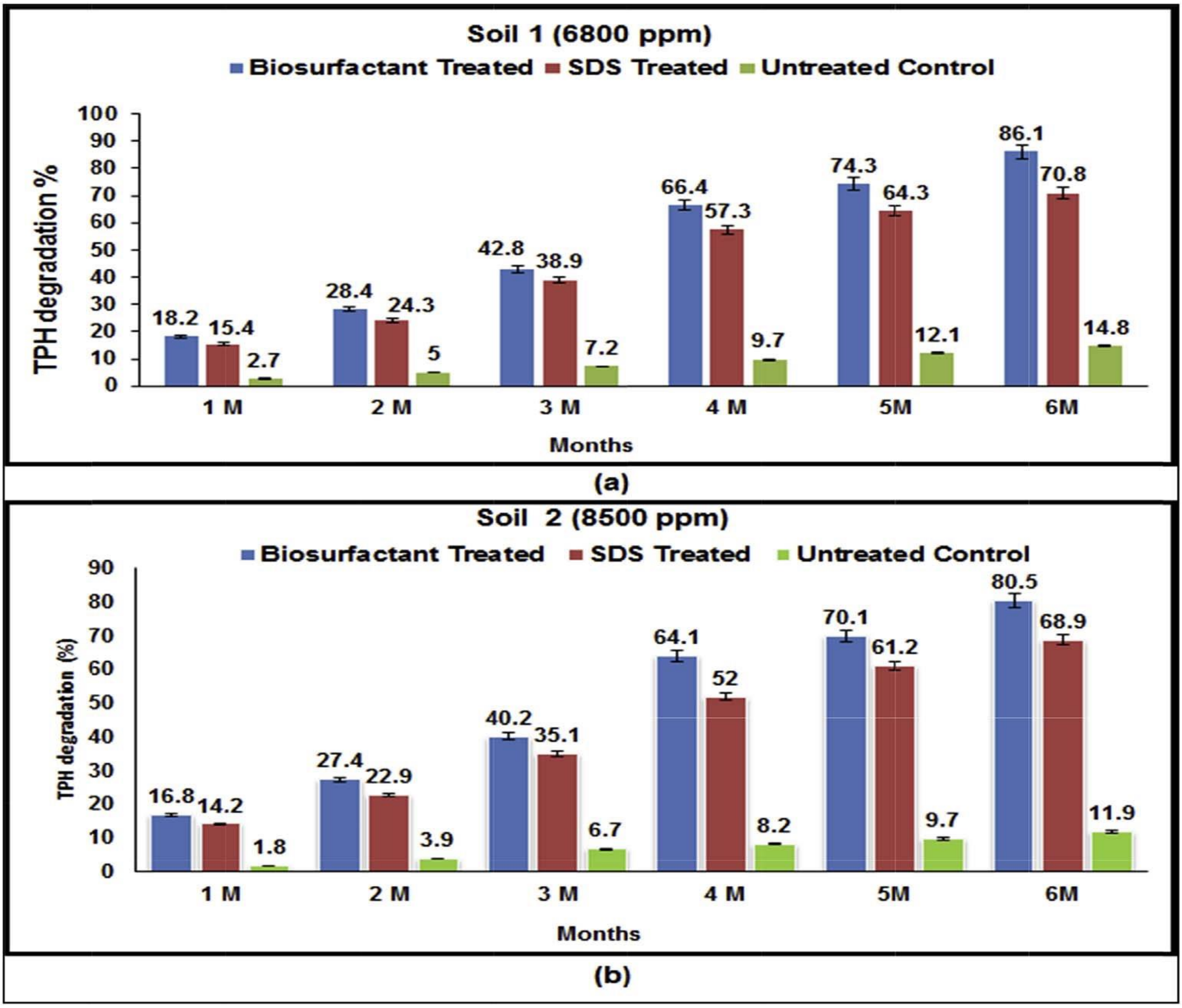

Fig 4: TPH degradation of two soil samples (A) and (B) on treatment with biosurfactants and synthetic surfactant, compared with the untreated soil. Here, fig (a) denotes the TPH degradation of soil sample (A) and fig (b) denotes the TPH degradation of soil sample (B) respectively.

The activity of the biosurfactant in enhancing the bioremediation of hydrocarbon contaminated soil compared to the widely used synthetic surfactant sodium dodecyl sulfate (SDS) was carried out and it was found that the biosurfactant possess higher efficiency. The natural degradation of oil contaminants was very slow, as too little degradation was observed in the untreated soil. The degradation of the complex hydrocarbon also led to betterment of certain vital physical and chemical properties of soil, which are important for sustaining cultivation. Thus, the biosurfactants can be utilized as an eco-friendly means for enhancement of bioremediation of oil components including PAHs in natural environment.

Similarly, a research conducted by Lai et al. (2009) shows the performance of the two biosurfactants, rhamnolipids and surfactin with that of two commonly used synthetic surfactants (i.e., Tween 80 and Triton X-100). After adding 0.2 mass% of (bio) surfactants on low contaminated soil (LTC) and high contaminated soil (HTC) soils for 1 day, the TPH removal efficiency appeared to decrease in the order of rhamnolipids>surfactin> Triton X-100 > Tween 80, regardless of the type of soil used. For LTC soil, addition of 0.2 mass% of rhamnolipids, surfactin, Triton X-100 and Tween 80 resulted in a TPH removal of 23, 14, 6 and 4, respectively, while for HTC soil a significantly higher TPH removal efficiency of 63%, 62%, 40% and 35%, respectively, was obtained. These results indicate the superior performance of biosurfactants over synthetic surfactants in terms of mobilization of oil pollutants from the contaminated soil and thus the two biosurfactants (especially, rhamnolipids) examined in this work have the potential to be used as biostimulation agents for bioremediation of oil-polluted soils. Among four biosurfactants tested, rhamnolipids and surfactin showed superior performance on TPH removal from both slightly and high TPH-contaminated soils. Moreover, the effectiveness of biosurfactant-stimulated mobilization of petroleum hydrocarbons from contaminated soil was better than synthetic ones. Rhamnolipids possessed the highest TPH removal efficiency of 23% and 63% for LTC and HTC soil, respectively, and is considered as a good candidate for assisting oil pollutant remediation in practice. In addition, the results from this work also provide a useful assessment tool for rapid selection of biosurfactants for their effectiveness of removing petroleum hydrocarbons from contaminated soil. It can also be concluded from literature above that rhamnolipids are the best type of biosurfactants for use in bioremediation of hydrocarbon contaminants. It has been shown that rhamnolipids exhibit excellent effect for enhancing biodegradation of hydrocarbons by microorganisms. Rhamnolipids help degradation by solubilizing or emulsifying hydrocarbons (Zeng, et al. as cited in Liu 2018), increasing the interfacial uptake of hydrocarbons by degrading bacteria (Zhong et al. 2014 as cited in Liu et al. 2018).

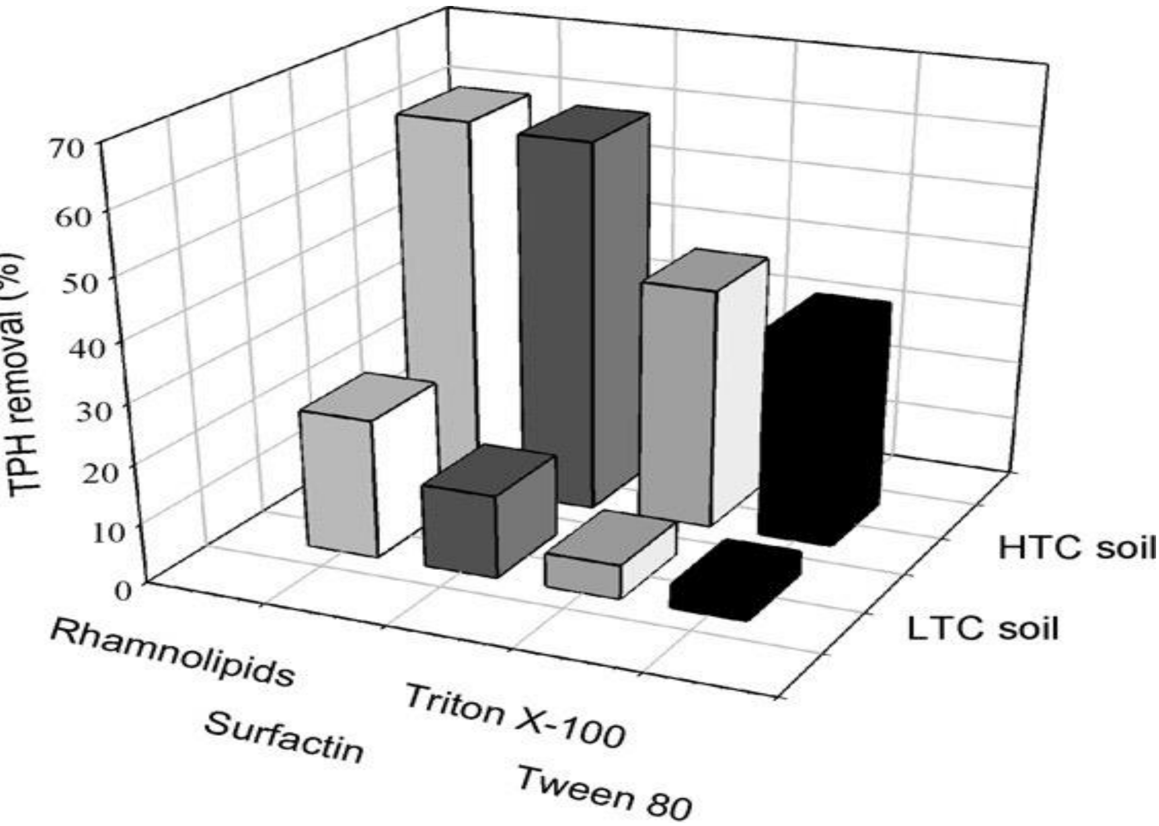

Fig 5: Comparison of TPH removal efficiency of biosurfactants with LTC and HTC.

3.2. BIOSURFACTANTS AS POTENTIALS FOR BIOREMEDIATION OF HYDROCARBON CARBON CONTAMINANTS.

Bioremediation studies begin with the isolation and identification of microorganisms from soil and water that can degrade hydrocarbon contaminants. The hydrocarbon degrading microbes can produce biosurfactants. Biosurfactants produced utilizes the hydrocarbon as carbon sources, either by changing its cell surface so that the contaminant can be absorbed or by making available the hydrocarbon by releasing the biosurfactant into the environment.

A research conducted by Joy et al. (2017), shows potent biosurfactant producers and hydrocarbon degraders, obtained from their investigation provides an insight for the productive competence of different types of biosurfactants with *Achromobacter*sp. and *Bacillus* sp. producing glycolipids and *Ochrobactrum*sp. and *Bacillus* sp. producing lipopeptides as confirmed by TLC, FT-IR and GC-MS results. All the biosurfactants exhibited high emulsification activity with low surface tension values and efficiently degraded crude oil revealing their promising applicability in bioremediation processes. Moreover, the utilization of cheap glucose as the carbon source in the case of *Achromobacter*sp. and *Bacillus* sp. further directs towards the use of agro-waste residues for sustainable cost effective biosurfactant production with high yields. Kang et al. (2009) evaluated the effectiveness of a microbial biosurfactant, sophorolipid, in washing and biodegradation of model hydrocarbons and crude oil in soil. Thirty percent of 2-methylnaphthalene was effectively washed and solubilized with 10 g/L of sophorolipid with similar or higher efficiency than that of commercial surfactants. Addition of sophorolipid in soil increased biodegradation of model compounds: 2-methylnaphthalene (95% degradation in 2 days), hexadecane (97%, 6 days), and pristane (85%, 6 days). Also, effective biodegradation method of crude oil in soil was observed by the addition of sophorolipid, resulting in 80% biodegradation of saturates and 72% aromatics in 8 weeks. These results showed the potentials of the microbial biosurfactant, sophorolipid, as an effective surfactant for soil washing and as an in-situ biodegradation enhancer.

A study conducted by Obayori et al. (2009) on *Pseudomonas sp. strain LP1*, an organism isolated on the basis of its ability to grow on pyrene, was assayed for its degradative and biosurfactant production potentials when growing on crude, diesel and engine oils. The biodegradation of hydrocarbons in polluted environment is mainly through the activities of bacteria and fungi. Typically, individual organisms degrade only a limited range of hydrocarbons. *Pseudomonas sp.* represents one of the most versatile groups of organisms involved in the degradation of hydrocarbons (Wackett and Hershberger as seen in Obayori et al. 2009). *Pseudomonas sp. LP1,* the organism used in the study conducted by Obayori et al. (2009), has been reported to have specificity for a range of hydrocarbon compounds including biphenyl, PAHs and petroleum products commonly used in the Nigerian environment (Obayori et al. 2009). The ability of LP1 to remarkably degrade diesel is of interest because diesel is an excellent model for studying hydrocarbon biodegradation since it consists of a variety of molecules such as paraffin, olefins, naphtha and aromatic compounds (Ilori et al. as seen in Obayori et al. 2009). The strain also exhibited remarkable growth rate on engine oil.

Recently a study by Sharma and Pandey (2020) aimed to unveil the effect of biosurfactant as stimulant in crude oil (hydrocarbon) bioremediation. Isolated oil-degrading strain, *Bacillus subtilis RSL 2* was optimized for the maximum oil degradation and biosurfactant production. The biosurfactants produced was characterized and investigated for its effect on microbial oil degradation in two modes (a) sequential and (b) simultaneous. The strain produced 3.5 g/ L of biosurfactant at pH 4.0, 25 °C, using 1 g/L crude oil as the only C-source in 7 days, which was characterized as lipopeptide with a critical micelle concentration (CMC) of 0.5 g/L. The simultaneous feed of biosurfactant at 0.5 CMC enhanced oil biodegradation (72%) and biosurfactant production (5.2 g/L) by about 1.6 times than the sequential mode due to improvement in mobilization of oil thus making it more bioavailable.

4.0: RESULTS AND DISCUSSIONS

4.1: ROLE OF SUBSTRATE IN THE NATURE OF BIOSURFACTANT PRODUCED

At the beginning of scientific interest in biosurfactants around 1980 (Syldatk and Wagner as seen in Lopes et al. 2019), only pure hydrocarbons were used as carbon sources for their production (Fish et al.; Hisatsuka et al.; Itoh and Suzuki; Syldatk et al. as seen in Lopes et al. 2019). There are many alternative substrates currently proposed for the production of biosurfactants: These compounds are known to exhibit high levels of carbohydrates and lipid, both required for the growth of microorganisms and the biosynthesis of biosurfactants (Diaz et al. 2018). This consequently raised the biosurfactant market value to an unfeasible acceptance scenario. Despite its many advantages over synthetic chemical surfactants, the biosurfactants still have economic obstacles to overcome in the large-scale process, including a drastic reduction in production costs. In fact, there are efforts in the recent decades that focused on minimizing biosurfactant production costs to promote commercial acceptance (Heryani and Putra 2017).

Currently, biosurfactants commercialized in the USA are more expensive than synthetic surfactants (Makkar et al. 2011). In this context, alternative strategies have been adopted to establish a competitive price. The use of more cost-effective substrates for biosurfactant production, as we will be discussing in this review, and the development of economically viable production processes on a large scale (Makkar et al. 2011) are strategies to be adopted for cost effective production of biosurfactants. The use of agro-industrial waste becomes an economically interesting strategy since the raw material accounts for about 10–30% of the total cost in this biotechnological process (Makkar et al. 2011). Agricultural residues result in lower production costs and a much smaller volume of compounds released into the environment. By successfully developing effective ways of producing surfactants, the environmental impact of surfactant industry may become smaller. Moreover, there is a sustainable gain by recycling waste (Accorsini et al. 2012). Molasses are concentrated syrups by-products of sugar cane and beet, grains and pulses processing industries. This cheap substrate contains 75% dry matter, 9–12% non-sugar organic matter, 2.5% protein, 1.5–5.0% potassium and ≈1% calcium, magnesium, and phosphorus. Other components like biotin, pantothenic acid, inositol, and thiamine at 1–3% are also present giving it a thick, dark brown colored appearance. The high sugar content ranging approximately between 48 and 56% represents a good substrate for growth as well as production of microbial bioactive compounds for various microorganisms (Banat et al. 2014)

Soy molasses is a product cogenerated during soybean processing that has high production and low commercial value. Its use has great potential in fermentative processes due to the high concentration of carbohydrates, lipids and proteins. This study by Rodrigues et al. (2017) investigated the use of *Pseudomonas aeruginosa*to produce biosurfactants in a soy molasses-based fermentation medium. A central composite design (CCD) was prepared with two variables and three replicates at the central point to optimize the production of biosurfactant. The bands found in Fourier transform infrared spectroscopy analysis had characteristic glycolipids as reported in the literature (Dwivedi et al. as reported by Rodrigues et al. 2017). Thus, it is possible to state that the biosurfactant produced belongs to the group of glycolipids and, possibly, to the class of rhamnolipids which are surface-active molecules used widely in bioremediation. These values show a great potential for biosurfactant production using soy molasses as a substrate and bacteria of the species *P. aeruginosa*. Another interesting work contributed by Benincasa (2007) suggests that the rhamnolipid produced from agro-industrial wastes has an important role for hydrocarbon biodegradation in contaminated soil. Such studies have proved the importance of agro-industrial wastes in bioremediation processes.

Saravanan and Subramaniyan (2014) isolated *P. aeruginosa PB3A* strain from oil contaminated soil and examined BS production on various substrates namely, castor oil, coconut oil, rapeseed oil corn oil, motor oil, sunflower oil, olive oil, olein, barley bran, cassava flour waste, rice bran peanut cake, potato waste, and wheat bran instead of routine carbon sources. Corn oil and cassava waste flour were found to be highly effective. Once again, these studies have confirmed the potential role of agro-industrial wastes for BS production in place of synthetic media. Production of biosurfactants by fermentation of fats, oils, and their co-products has also been reported. (Nitschke et al. as seen in Banat et al. 2014) carried out BS production by using soybean oil waste along with molasses, whey and cassava flour, as substrates. Their observation suggests that cassava flour wastewater as a promising source of nutrients for BS production. In this study by Sharma et al. (2019), *Pseudomonas aeruginosa 7815*strain was explored for its ability to produce biosurfactant using waste cooking oil (WCO) exclusively as C source. This study has highlighted that the produced biosurfactant is a mixture of mono and di-rhamnolipids demonstrating an unprecedented low critical micelle concentration (CMC value of 8.8 mgL-1. These results demonstrated an outstanding and potential candidate for the biosurfactant production at commercial scale. The strain also showed about 90% utilization in overall WCO amount within 5 days of incubation. Altogether, this work takes a plunge to explore and optimize the production of a novel biosurfactant with excellent surface activity along with biodegradation. These results suggest the suitability of *Pseudomonas aeruginosa* for the biosurfactant production at commercial scale along with remediation in an economic way. The study by Jimoh and Lin (2020) supports *Paenibacillus sp*. biosurfactant economies by the utilization of possible low-cost materials. These outcomes demonstrated the use of waste frying oils (coconut and sunflower) to be utilized as viable substrates for the economic production of *Paenibacillus sp.* biosurfactant. The biosurfactants was successful in dispersing engine oil that shows its potential to be used for bioremediation of hydrocarbon contaminants (Jimoh and Lin, 2020). The study highlighted the biosurfactants yield using different low-cost substrates using the same organism *paenibacillus sp.* as shown in figure 3.3. This current study showed the enormous prospect of these wasted frying oils for enhanced BS production yield, thus reducing the high cost of production, and hence the significance of this research to literature.

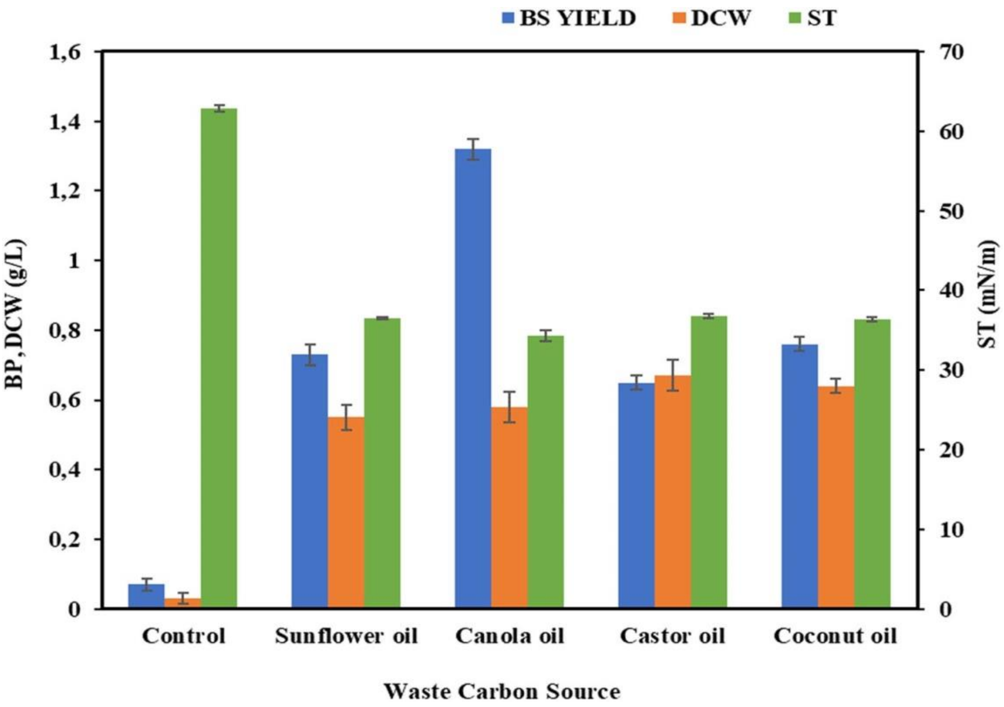

Fig 3.3. Varying biosurfactant yield using different low cost substrate.BS-Biosurfactant; BP-Biosurfactant production; DCW-Dry cell weight; ST- Surface tension

Mostagh et al. (2018) demonstrated that the brewery waste, a sustainable and inexpensive carbon source could be used for biosurfactant production by local *Bacillus subtilis N3-1P* effectively. Biosurfactant production using brewery stream was compared with a commercial carbon source. This experiment conducted by Mostagh et al. (2018) using the brewery waste as the sole carbon source for biosurfactant production reduces costs associated with biosurfactant production and helps to generate an environmentally friendly way for waste treatment and disposal. Given the potential for biotechnological application of these compounds in the oil industry, the optimization of the use of two industrial wastes, corn steep liquor and ground–nut oil refinery residue as low cost nutrients for the production of a biosurfactant by *Candida sphaerica* (UCP 0995) was studied by luna et al. (2015) Then the properties of the biosurfactant was described, and its isolation, preliminary chemical characterization. They used an optimized medium with distilled water supplemented with 9 % ground nut oil refinery residue and 9 % corn steep liquor as substrates to produce biosurfactants by Candida sphaerica, at 28°C during 144h under 200rpm. The isolated biosurfactant was formed with a yield of 9g/L. The biosurfactant was characterized as glycolipid and recovered 95 % of motor oil adsorbed in a sand sample, showing great potential to be used in bioremediation processes, especially in the petroleum industry. Biosurfactants can emulsify hydrocarbons enhancing their water solubility, decreasing surface tension and increasing the displacement of oil substances from soil particles. The results obtained for the removal of motor oil adsorbed in the samples of sand with distilled water (control) showed the removal of 95 and 10% of the oil, respectively. Once the tests were conducted with the containing biosurfactants cell-free broth (crude biosurfactants), the results were considered satisfactory.

5.0: CONCLUSIONS

This review's purpose is to analyze the suitable substrates for biosurfactants production used in bioremediation of hydrocarbon contaminants. This is significant because finding low cost and renewable substrate in biosurfactant production is key to reducing the cost associated with the production of biosurfactants.

Naturally, there are immense possibilities of resources (substrates) for production of biosurfactants that can be utilized in different fields. This work primarily reviewed the use of different low cost and renewable substrates for biosurfactants production and their effectiveness in bioremediation of hydrocarbon-contaminated environments. The use of the low cost and inexpensive substrates for biosurfactants production reduces the cost associated with production and makes the use of biosurfactants feasible for bioremediation of hydrocarbon-contaminated environments that has been proved to be a promising technique. This work further highlighted the adverse effect of chemical surfactants thereby making biosurfactants the best choice in bioremediation of the hydrocarbon pollutants. Information provided in this study gives an insight into the different uses of renewable and cost-effective substrates used in biosurfactants production and the types of biosurfactants they can produce. This will aid researchers in choosing the right substrates to be used for their production. Recently, the use of economically cheaper and renewable substrate is paving the way for cost effective production. However, there are still other factors such as optimization of the fermentation process that can further make the production a more economically viable process.

There has been intense research and discussion conducted over the past decades but there is still more to be done in mitigating the issues associated with large-scale biosurfactant production. Most of the research has focused on lab scale bioremediation of hydrocarbon contaminants, I recommend researchers work on large-scale bioremediation, for proper optimization of the process.Another limitation from most studiesconducted is they focus mostly on the use of cheap and low cost substrate for production, which is a key area but I will recommend more research be conducted towards optimizing the processes involved in the fermentation that can be achieved by using alternative low cost and effective production media and recovery process. Coproduction of the biosurfactants along with other compounds of commercial importance such as enzyme would make the entire fermentation process highly economical.

Another strategy that can be implemented is in the field of genetic engineering whereby the desired trait (gene) will be inserted in an organism. The desired can be one that is responsible for production of a certain type of biosurfactant so that the microorganisms are able to produce that biosurfactant and at a higher yield .The organism can also be engineered to require less amount of substrate ultimately reducing the amount of substrate needed for production. These strategies will open up new avenues of research on production of biosurfactants and will go a long way in ensuring biosurfactants become a successful commercialized, profitable and economically feasible product.

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