

Applications of Biosurfactant Obtained from *Pseudomonas Aeruginosa*

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Abstract

The ever-increasing urbanization and industrialization as a consequence of growing population are putting a major strain on the limited resources at our disposal. The treatment of industrial effluents, assuaging the growing use of chemicals in various fields and its grave repercussions are some of the urgent problems facing Governments and the concerned Industries alike. The increasing concern among intellectuals about the use of chemicals in Agriculture, Food processing and milk industries along with tangible ramifications of these chemicals has made it pertinent to look for alternatives to replace these chemicals. This is where biosurfactants with its diverse and unique functional properties come into the picture. This review focuses on the recent advances and enumerates various applications of rhamnolipids obtained from *Pseudomonas aeruginosa*. Biosurfactant promises immense potential in various disciplines and this biomolecule represents a new dawn for Man to jettison his chemical addiction.

Keywords: Biosurfactants, Application of Rhamnolipids, *Pseudomonas aeruginosa*, Surface activity, Biodegradability

1. Introduction

Biosurfactants are amphiphilic, surface-active compounds produced by several microorganisms including *Acinetobacter* sp., *Bacillus* sp., *Pseudomonas aeruginosa*. Biosurfactant possess unique properties such as specificity, low toxicity, biodegradability, surface and interfacial activity which imparts them an environmentally friendly nature. These properties have piqued the interest in using these amphiphilic compounds as a replacement for Chemical surfactants

Biosurfactant contains hydrophilic head and a hydrophobic tail, thus the name amphiphilic compounds. The ones produced using *Pseudomonas aeruginosa* are known as Rhamnolipids having a glycosyl head group, a

Rhamnose moiety and a fatty acid tail such as 3-hydroxydecanoic acid.

Biosurfactants possess unique advantage over others and has several below mentioned properties

1.1 Biodegradability

Probably the most important property of biosurfactant is its biodegradability which gives it an edge over its chemical counterparts, which is non-biodegradable and form secondary waste when used for degradation of hydrocarbons and other such applications.

1.2 Tolerance

Biosurfactant exhibits good resistance to relatively extreme temperatures, changes in pH, ionic strength and other concentration changes.

1.3 Surface activity

They possess surface and interfacial activities, they decrease the surface tension as well as the interfacial tension between two fluids. They act as a good emulsification/de-emulsification, dispersion and foaming agents. Biosurfactants also exhibits better selectivity which helps in its targeted applications.

2. Production

Biosurfactants are produced by several microorganisms which include *Acinetobacter* sp., *Bacillus* sp., *Candida antarctica*, *Pseudomonas aeruginosa*. Biosurfactant is more viable and economically feasible because of the fact that they can be produced by using really cheap raw materials. The low cost carbon sources can be clubbed with saw dust to make biosurfactant which is essentially a cheap starting material. Industrial waste such as motor oil can be used as substrate.

2.1 Using *P. aeruginosa*

Pseudomonas aeruginosa is a common Gram-negative, rod-shaped bacterium. It is found in soil, water, skin flora, and most man-made environments throughout the world and can easily be extracted from sea water sample for production of biosurfactants. Biosurfactants produced using *P. aeruginosa* are termed as Rhamnolipids.

Waste motor Oil and Peanut Oil Cake can be used as Substrate for Biosurfactant production.

Rhamnolipids are a class of glycolipid produced by *Pseudomonas aeruginosa*, amongst other organisms, and is thought to be the best characterized, among all the bacterial surfactants. They have a glycosyl head group, in this case a rhamnose moiety, and a fatty acid tail, such as 3-hydroxydecanoic acid.

3. Application

3.1 Wastewater treatment

Petroleum products are undisputed rulers of the present Energy scenario across the world and consequently chief contaminant present in various industries effluents which pose serious environmental concerns. Oil discharged from Oil and Gas Industries and various other heavy Industries is the principal contaminant in oily wastewater [1].

Traditional physicochemical processes such as flocculation, oxidation and membrane filtration used for the treatment of the hydrocarbon-containing oily wastewater displaces the hydrocarbons in concentrated wastewater instead of degrading, which adversely effects environment and the overall economics of the cost involved hampers the prospect of employing these methods [2]. Using biosurfactant in the aerobic active sludge system can degrade the petroleum hydrocarbons effectively and has thus been regarded as an efficient method to deal with the hydrocarbon-containing oily wastewater. It enhances the solubility of hydrophobic substrates and unlike their chemical counterparts, biosurfactant are biodegradable and do not foster secondary waste. The removal efficiency of crude oil in the laboratory is found to be over 80% with the presence of rhamnolipids(11.2 mg/L) compared with 22.3% in the absence of rhamnolipids [3].

3.2 Enhanced Oil Recovery

Use of biosurfactant has shown promising potential in the extraction of Oil located in difficult to access reservoirs, where the oil is trapped in pores by

capillary pressure. This process of using biosurfactant to extract remaining oil is done after the primary or mechanical process and secondary or physical process (Singh et al., 2007; Packwa P3ociniczak et al., 2011) and is usually known as the tertiary process of enhanced oil recovery. Biosurfactants are employed to reduce the oil-water and oil-rock interfacial tensions, which reduces the capillary forces that cripple the movement of oil through the pores of the rock. Biosurfactants can also strongly bond with the oil-water interface to form an emulsion, thus ensuring the stabilisation of the oil desorbed in the water and facilitates its removal and subsequent extraction through the injection of water (Marchant and Banat, 2012). Bordoloi and Konwar (2008) investigated the recovery of crude oil from a saturation column under laboratory conditions. Bordoloi and Konwar (2008) investigated the recovery of crude oil from a saturation column under laboratory conditions. Laboratory studies on Microbial-Enhanced Oil Recovery using biosurfactant made use of columns that contained the desired substrate (generally sand), which is essentially used to demonstrate the effectiveness of biosurfactants in the tertiary recovery of oil from reservoirs. For mirroring the conditions, a glass column packed with dry sand is saturated with crude oil, to which an aqueous biosurfactant solution is poured. The potential of biosurfactants in MEOR is estimated by measuring the amount of oil released from the column following the passage of the biosurfactant solution through the column. The experiment which can be carried out at room temperature as well as at high temperatures, in the range of 70-90°C, to assess the effect of temperature on biosurfactant-induced oil recovery. The biosurfactants used in the experiment by Bordoloi and Konwar (2008) were produced from strains of the bacterium *P. aeruginosa*, which assisted in recovering around 49 to 54% of the crude oil from the packed columns at temperatures in the range of 52-70°C and 58 to 62% at 90°C.

3.3 Food Processing Industries

The high antimicrobial activity and physicochemical properties of rhamnolipids can be exploited by food industry particularly in increasing of food shelf life without concern to consumer health, since the U.S. Environmental Protection agency has approved their use in food products, and other industrials applications [US Environmental Protection Agency Office of Pesticides] excluding the need of adding synthetics, which prove to be detrimental for environment and humans alike. Rhamnolipids used

in combination with niacin extend shelf life and inhibits hemophilic spores in UHT soymilk. Niacin along with rhamnolipids in salad has been found to be effective in extending its shelf life by inhibiting mould growth. Natamycin, nisin, and rhamnolipids when used together in cottage cheese, extend shelf life by inhibiting the growth of gram-positive and spore-forming bacteria. Succintly, they can be used to avoid food contamination directly, as food additive and as a detergent formulation to clean surfaces that come in contact with the food [4].

Rhamnolipids can be used in breads, rolls, cakes and the likes to improve their dough or batter stability volume and shape, structure, dough texture and microbiological conservation. Moreover, they can be used to improve the properties of butter cream and non-dairy cream filling for croissants and other fresh or frozen fine confectionery products [5].

Rhamnolipids not only perform their obvious role as agents that decrease surface and interfacial tension, but can also perform several other functions in food like improving texture and shelf-life of starch-containing products, controlling the agglomeration of fat globules, stabilizing aerated systems, modifying rheological properties of wheat dough and improving stability, consistency and texture of oils and fats based products. Biosurfactants help in the general mixing of ingredients and can also decrease the growth rate of mould and other bacteria in food [6]. Rhamnolipids also find application in ice cream and bakery formulations, where they are used to control consistency, retard staling and reduce spattering. Rhamnolipids can be explored to control the attachment and disruption of biofilms of individual and mixed cultures of the food-borne pathogens [7].

3.4 Agriculture

Biosurfactants also have a potential application as a pesticide, replacing chemicals which adversely affect humans, wildlife or bees. Chemical Pesticides meant to protect plants from pests have been found to be toxic to varying degrees, with developing countries reporting short-term very high level of exposure, also known as acute poisoning and developed countries more at risk of long-term low-level exposure of pesticides.

These Chemical pesticides can be readily replaced by their biological counterparts such as those obtained from biosurfactants which can efficaciously do the job of controlling pest and does not foster collateral damage.

BIOPESTICIDES REGISTRATION ACTION DOCUMENT by US EPA Office of Pesticide Programs identifies Zonix™ Biofungicide, as an effective means to prevent and control pathogenic fungi on horticultural and agricultural crops, including root and tuber vegetables, fruiting vegetables among others.

Zonix™ Biofungicide is produced by TGAI (technical grade active ingredient), a rhamnolipid biosurfactant with a transparent liquid like appearance, a light to dark amber tint and a mild, sweet soapy odour.

The TGAI essentially consists of two rhamnolipid molecules. The first molecule is identified as R1, containing a rhamnose ring and a fatty acid tail (decanoic acid, 3-[(6-deoxy-%-L-mannopyranosyl)oxy]-,1-(carboxymethyl) octyl ester (CAS No. 37134-61-5). The second, termed as R2, contains two rhamnose rings and a fatty acid tail (decanoic acid, 3-[[6-deoxy-2-O-(6-deoxy-%-L-mannopyranosyl)-%-L mannopyranosyl]oxy]-,1-(carboxymethyl) octyl ester (CAS No. 4348-76-9). The TGAI mixture of R1 and R2 is decanoic acid, 3-[[6-deoxy-2-O-(6-deoxy-%-L-mannopyranosyl)-%-L mannopyranosyl]oxy]-, 1-(carboxymethyl)octyl ester, mixture with 1-(carboxymethyl)octyl 3 [[6-deoxy-%-L-mannopyranosyl)oxy]decanoate (CAS No. 147858-26-2, PC code 110029).

The end-use product, Zonix™ Biofungicide, employed as a biopesticide contains 8.5% by weight rhamnolipid biosurfactant, composed of 3.34% R1 and 5.16% R2 [BIOPESTICIDES REGISTRATION ACTION DOCUMENT, US EPA Office of Pesticide Programs]

3.5 Medicine

Biosurfactant possess some unique functional properties which makes them apt, to be employed as an alternative to synthetic medicines and antimicrobial agents. Biosurfactant also exhibits the potential to become safe and efficacious therapeutic agents.

Different biosurfactants, obtained from variety of sources, provide various medicinal benefits. These include anti-microbial activity, anti-cancer activity, anti-fungal activity, anti-viral activity et cetera but the scope of our present discussion is limited to the application of rhamnolipids, produced using *Pseudomonas aeruginosa*.

Rhamnolipids obtained from *Pseudomonas aeruginosa* have been found to possess antimicrobial activity against *Mycobacterium tuberculosis* [8].

These Biosurfactants also exhibited appreciable anti-adhesive activity against several bacterial and yeast strains isolated from voice prostheses [9]. Antibacterial and antiphytoviral effects of various rhamnolipids have been described in the literature [10]. Seven different rhamnolipids were identified in cultures of *Pseudomonas aeruginosa* AT10 from soybean oil refinery wastes and these showed excellent antifungal properties against various fungi [11]. Although research in this field is still in its infancy as compared to its potential, efforts are being made to study the toxicity of these biomolecules produced by renewable resources on human system. We have to be study about the interaction and effects of these molecules on Human cell to be in a position to further enhance their use in biomedical and other related areas. Biosurfactant presents a great future potential and if fully and safely exploited, it could change the medicinal science and the way we treat diseases.

Conclusion

Biosurfactants have the potential to emerge as arguably the most important molecule for us in the 21st century to meet our requirements in various fields sustainably. It has demonstrated immense potential in the field of Medical, Agriculture and food processing Industries as enumerated above and many other fields such as Soil Washing Technology, Cosmetics et cetera. Biosurfactant increases the degradation of hydrocarbons in oily wastewater to over 80% from just 22%, has proven to be successful in replacing chemical pesticides in the agriculture industry and is potentially effective in extracting around 50% of intractable oil trapped in pores by capillary pressure. The major impediment now lies in its efficacious and remunerative production, extensive research on its toxicity to flora and fauna and promotion of its safe use in various tested disciplines among developing nations and Industrial conglomerates. Use of Biomolecules in various fields mentioned in this review is still in its infancy, therefore it needs extra prodding in research and a bit more investment commercially than their chemical counterparts.

Nevertheless, this should not deter us from further research on this environmentally friendly biomolecule and its widespread use in eliminating harmful chemicals, for our planet is showing signs, it simply can't afford business as usual.

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