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Bioemulsifires: An overview

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Abstract: **Bioemulsifiers are considered as multifunctional biomolecules of 21st century because of their functional abilities and eco-friendly properties. Currently, they are considered as "green molecules" because of their wide applications in bioremediation of soil. They can also be applied for removal of heavy metal and chlorinated solvent compounds, in drugs industry, food industry, cosmetic industry, metallurgy, agrochemicals, fertilisers and many others. Their importance has been increasing day by day in the global market as they are the natural resources with high-aggregate value.**

Keywords: Emulsion, biphasic system, emulsifier, bioemulsifier, bioremediation, interfacial tension

I. INTRODUCTION

An emulsion (Khan et al., 2011) may be defined as a biophasic system consisting of two immiscible liquids, one of which (the dispersed phase) is finely and uniformly dispersed as globules throughout the second phase (the continuous phase).In other words, an emulsion is an insoluble mixture composed of two different types of liquid in which one liquid i.e. dispersed phase, is spreading in the other liquid, which is a continuous phase. The two common types are o/w emulsion and w/o emulsion .The o/w (Martinez-Palou et al., 2013) system appears as a heterogeneous emulsion in which oil is dispersed in a form of bubbles of different sizes in the continuous water phase. In contrary, in w/o emulsions oil forms the primary (continuous) phase in which water droplets disperse with different sizes.

Emulsifiers or emulsifying agents are substances that help stabilize an emulsion to allow it to stay in this form for a significant amount of time. The mechanism by which they proceed -1) reducing surface tension and ii) increasing viscosity of the medium. They increase the uniformity of nutrients, such as fatty acids, fat soluble vitamins and amino acids and is closely related to their chemical structure (Calvo et al.2004).Physiologically, in animals digestive system, the presence of bile salts, benefits fat absorption.

Bioemulsifiers (BE)are amphipathic molecules with hydrophobic and hydrophilic moieties that act between fluids of different polarities (oil/water and water/oil), allowing access to hydrophobic substrates and increasing the hydrocarbon contact area, mobility, bioavailability, and biodegradation of such compounds(Jagtap et al.2010, Fracchia et al. 2012, Sharma et al. 2015, Rahman et al.2018).They have a tendency to change the physical and chemical properties of fluids, causing the ability to detergency, lubrication , foaming and solubilisation , emulsification , and reduction in liquid viscosity (Santos et al.2016, Gaur et al. 2019).They are classified according to their hydrophile- lipophile balance (HLB); those having a a low HBL are strong lipophiles and used as water-in-oil emulsifiers, whereas those having a high HLB are strong hydrophiles and used as oil-in –water emulsifiers.

They may be classified into the following main groups:

1. The glycolipids, in which carbohydrates such as sophoroser, trehalose or rhamnose are attached to a long chain aliphatic acid or lipopeptide.
2. Aminoacid containing bioemulsifiers like surfactin produced by Bacillus subtilis composed of seven amino-acid ring structure coupled to one molecule of 3-hydroxy-13 methyl tetredecanoic acid.
3. Polysaccharide-lipid complexes-The emulsan synthesized by Acinetobactor Calcoaceticus RAG-1 is an extracellular heteropolysaccharide polyanionic complex.
4. Protein-like substances such as liposan produced by Candida lypolytica composed of protein and carbohydrates.

The activities of bioemulsifier depend on the surface-active compounds until CMC is reached. Above the CMC, micells, bilayers and vesicles are formed by bioemulsifiers. The formation of micelles allows bioemulsifiers to reduce surface and interfacial tension and increase the solubility. When the value of CMC is low, it means that less bioemulsifier is needed for surface tension reduction.

II. MATERIALS

Now-a-days, due to profound emulsifier effect on human health and limited resources as well as expensivity, researchers have produced emulsifiers using natural sources. Especially microorganisms (Torabizadeh et al. 1996). Microbial bioemulsifiers are produced by a wide variety of diverse microorganisms including bacteria, yeast and fungi and have very different chemical structures and surface properties.

Table 1: List of bioemulsifiers producing yeast

Microoganism's	Bioemulsifiers	Ref
Torulopsis petrophilum	Sophorolipids	Cooper et al.1983
Torulopsis apicola	Sophorolipids	Rau et al., 2001
Saccharomyces cerevisiae	Mannoprotien	Cameron et al., 1988
Kluyveromyces marxianus	Mannoprotien	Lukondeh et al., 2003
Rhodotorula glutinis	Polymeric bioemulsifier	Johnson et al., 1992

Table 2: List of bioemulsifiers producing fungi

Microoganism's	Bioemulsifiers	Ref
Candida tropicalis	Mannan-fatty acid	Miura et al. 2017
Candida utilis	NDA	Sekhar et al. 2015
Candila tropicalis	Liposan	Sarubbo et al. 1999
Candida bombicola	Sorhorolipids	Hommel et al.1994
Penicillium chrysogenum	Polyketide derivative	Gao et al.2010
Pseudomonas aeruginosa	Rhamnolipids	Gusmao et al. 2010

Table 3: List of bioemulsifiers producing bacteria

Microoganism's	Bioemulsifiers	Ref
Pseudomonas fluorescens	Viscosin	Banat et al., 2010
Pseudomonas aruginosa	Rhamnolipids	Jadhav et al., 2011
Bacillus amyloliquefaciens	Surfactin/Iturin	Arguellers et al.2009
Bacillus subtilis	Subtilism	Abriouel et al. 2010
Bacillus subtilis	Lichenysin	Yakimov et al., 1997
Acinetobactor calcoaceticus	Emulsan	Rosenberg et al., 1999
Acinetobactor radioresistens	Alasan	Shrekhar et al., 2015
Leuconostoc mesenteriods	Dextran	Sarwat et al., 2008
Serratia Marcescens	Serrated rubidea Serrawettin	Wei et al, 20054
Pseudomonascepacia CCT6659	Rhamnolipids	Silva et al., 2013

III. DISCUSSION

Applications of bioemulsifiers

Bioemulsifier can be applied in a variety of areas such as bioremediation of hydrocarbon polluted environment, microbial enhanced oil recovery, removal of heavy metal and chlorinated solvent compounds, food processing industry, and cosmetic industry.

Natural roles: Bioemulsifiers are produced by a large number of microorganisms and are significant in many aspects of growth. Due to their very diverse chemical structures and surface properties, different groups of bioemulsifires may have different roles in the growth of the producing microorganisms and provide advantages in a particular ecological niche. Some are essential for the mobility of the microorganisms (gliding and swarming). They also play an important role in regulating the attachment detachment of microorganisms to and from surfaces.

They are involved in cell-to-cell interaction such as bacterial pathogenesis, quorum sensing and biofilm formation, maintenance and maturation. The most widespread role however is believed to be the interaction between microbes and insoluble substrates such as hydrocarbons.

Biomedical applications: Use of bioemulsifier in the medical field have increased during the past decade. Their antibacterial, antifungal and antiviral activities make them relevant molecules for applications in combating many diseases and as therapeutic agents.

Removal of oil and petroleum contamination: Biodegradation is one of the primary mechanisms for elimination of petroleum and other hydrocarbon pollutants from the environment. It is considered as an environmentally acceptable way of eliminating oils and fuel because of majority of hydrocarbons in crude oils and refined products are biodegradable. Petroleum hydrocarbon compounds bind to soil components and are difficult to remove and degrade. Bioemulsifiers can emulsify hydrocarbons by enhancing the apparent solubility of hydrocarbon compounds at concentration above critical micelle concentration which also enhance their availability for microbial uptake (Chang et al., 2008).Inclusion of bioemulsifiers in a bioremediation treatment of hydrocarbon polluted environment could be really promising , facilitating their assimilation by microorganisms (Calvo et al., 2009).

Alcanivorax and Cycloclasticus genera are highly specialized hydrocarbon degraders in marine environment. Alcanivorax borkumensis utilizes aliphatic hydrocarbons as its main carbon source for growth and produces an anionic glucose lipid bioemulsifier and thus potentials of Alcanivorax strains during bioremediation of hydrocarbon pollution in marine habitats have been studied (Olivera et al., 2009).

Several species of P.aeruginosa and B. subtilis produce rhamnolipid, a commonly isolated glycilipid bioemulsifier and surfactin, a lipoprotein type bioemulsifier, respectively. These two (Whang et al. 2008), helps to increase solubility and bioavailability of petrochemical mixture and also stimulates indigenous microorganism for enhanced biodegradation of diesel contaminated soil. The rate of biodegradation is dependent on physicochemical properties of bioemulsifires and not by the effects on microbial metabolism (Franzetti et al., 2008).

Bioremediation of toxic pollutants:

Bioremediation involves the acceleration of natural biodegradation processes in contaminated environments by improving the availability of materials and prevailing microorganisms. Bioremediation usually consists of the application of nitrogenous and phosphorous fertilizers, adjusting the pH and water context, if necessary, supplying air and often adding bacteria, the addition of emulsifiers is advantageous when bacterial growth is slow or when the pollutants consist of compounds that are difficult to degrade, such as PAHs. Bioemulsifier can be applied as an additive to stimulate the bioremediation process and increase in bioemulsifier concentration during bioremediation would be achieved by the addition of bacteria that overproduce bioemulsifiers. This technique is used in the cleaning of oil pipes.

Persistent organic pollutants found in oil containing wastewater and sediments such as PAHs (Phenanthrene, crysene) are also hydrophobic and water solubility decrease with increasing number of rings in molecular structure. This property induces the low bioavailability of these organic compounds which is a crucial factor in the biodegradation of PAHs. The water solubility of some PAHs can be improved by addition of bioemulsifiers owing to their amphipathic structure by several folds(Yin et al., 2009).In addition, most hydrocarbons exist in strongly adsorbed forms when they are introduced into soils. Thus, their removal efficiency can be limited in low mass transfer phases. However, addition of bioemulsifiers to the system enhance the bioavailability of low solubility and highly sorptive compounds (Shin et al., 2004).

Other uses:

They are used in various industries such as food, pharmaceutical and cosmetic, as they are biodegradable and less toxic than the synthetic surfactants recently used (Tabatabaee et al., 2005). The most important surface-active properties evaluated in screening microorganisms for bioemulsifiers with potential industrial applications are surface tension reduction, emulsion forming and stabilizing capacity.The criterion used for selection of bioemulsifier produces is the ability to reduce surface tension below 40 mNm^{-1}(Banat et al.,2000) . Sophorose lipids produced by certain strains of yeast have been formulated for anti-dandruff solutions, hair gels, deodorant sticks, after-shave lotions and hair and body shampoo.

Apart from reducing surface and interfacial tension, they facilitate the formation and stabilization of emulsion. Their functions in food industry include:

Starch reaction: Most emulsifiers have a lean fatty acid layer in their molecule which form an amylose mixture. It is very important in delaying bread and bakery products staling and reducing their adhesion to staple products such as potato and pasta (Shepherd et al., 1995; Nitschke et al., 2007; Campos et al., 2014; Mnif et al., 2012)

Generating interaction with proteins: Emulsifiers have ionic structure which react with proteins in food products and produce a modifiable structure. They respond to gluten present in wheat and increase protein elasticity that leads to increase the volume of bakery products (Shepherd et al., 1995; Zajic et al., 1976; Torabizadeh et al. 1996).

Adhesion correction: Some emulsifiers are added to food products containing sugar crystals that are scattered in fat and, by coating on glucose crystals reduce adhesion. This affects the fluidity of molten chips and prevent fat appearance on the surface of chocolate(Nitschke et al., 2007; Mnif et al., 2016; Kasaric et al., 2010; Gutierrezet al., 2014).

Creating foam: Emulsifiers with saturated fatty acids stabilize the bottommost surface of aqueous solutions which is an important factor factor in raw instant deserts (Krog et al., 1997; Nitschke et al., 2007; Lukondeh et al., 2003)

Modifying the dispersion of liquids in another liquid to formulate clear solution: many greed and colors require emulsifiers for solving (Nitschke et al., 2007; Zajic et al. , 1976).

IV. CONCLUSION

Bioemulsifiers are complex mixtures of heteropolysaccharides, lipoproteins and proteins. They are involved in solubilisation of poorly –soluble substrates, thus increasing their access and availability for biodegradation. Reports have shown that the efficient emulsifying activity of bioemulsifiers is a function of their chemical composition (Calvo et al., 2009). The combination of polysaccharide, fatty acid and protein components in bioemulsifiers confers upon them better emulsifying potential and ability to stabilize emulsions. Salinity, pH, temperature are very important factors that affect the synthesis of emulsifiers. Effect of salinity on the emulsifier activity is tested when bioemulsifier solution (1g/l) were supplemented with different NaCl concentrations, ranging grom 10 to 300 g/l. The addition of NaCl, even in amounts above the limit of saturation (300g/l), did not have a negative effect on emulsifying activity, which remained almost constant. The common chemical lsurfactants SDS, Triton X-100 or Tween 80 do not show emulsifying activity at NaCl concentration of 100-120 g/l (De Sousa et al. 2012, Monterio et al. 2010). Bioemulsifiers produced by some yeasts are stable in a broad spectrum of pH values (from 2 to 10). Alasan, produced by Acinetobacter radioresistens KA53 increases activity approximately 30% after incubation at 100 ^{0} C for 10 minute, which is accompanied by changes in conformation of the polysaccharide-protein complex (Toren A. et al.).

Bioemulsifiers have gained great attention due to their safety and biodegradable properties. They have certain advantages in comparison to synthetic components, such as, biodegradability, low toxicity, availability of raw materials, surface and interface activity, biocompatibility and digestibility. Careful and controlled use of bioemulsifiers will help to clean up the toxic pollutants and render the environment clean. Bioemulsifiers generally obtained from Generally Regarded as Safe (GRAS) microorganisms like lactobacilli and yeasts are of great promise for food and medicine though much more research is already required on this field. In spite of their immense applications, large scale production of bioemulsifier has been limited due to low yields and high production costs. Alternative approach to ameliorate the aforementioned challenges include the selection of efficient strains for optimum bioemulsifier production. Extrapolation of indigenous strains could be of greater significance for bioemulsifier production since these strains can be assumed to perform better in their native environments than toxic strains. There is minimal research on the discovery and characterization of new emulsifiers from microorganisms due to lack of a clear picture of the distinguishing features between biosurfactants and bioemulsifiers. Biosurfactants and bioemulsifiers are both unique microbial products showing advantageous features and may become future substitutes for chemically produced ones. Bioemulsifiers have often been erroneously eliminated or mis-identified in the past, but, since they have great potential for green technology, carefully designed screening methods will be an essential step toward the discovery of novel microbial emulsifiers.

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