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Standard Review

An investigation for potential development on biosurfactants

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Biosurfactants are surface-active metabolites produced by microorganisms. The applications of these biological compounds in the field of enhanced oil recovery and bioremediation proved effective. Besides their environmental applications; biosurfactants have shown interesting properties in several processes. Thus, this article attempts to organize this Information for ease of reference and further stimulates those that have interests in the area to explore further especially in biodegradation of recalcitrant compounds.

Key words: Biosurfactant, bioremediation, biodegradation.

INTRODUCTION

Biosurfactants have received more and more attention in recent years as surface-active compounds released by microorganisms that have some influence on interfaces, most notably on the surface tension of liquid - vapor interfaces. They are interesting amphiphilic molecules of microbial origin; whose hydrophobic and hydrophilic domains depend on the carbon substrate and the organism strain. They have various biological functions/ properties and have potentials in commercial applications in the food, microbiological, pharmaceutical and therapeutical agents in biological industries, as a bio-control agent in agricultural applications and in health and beauty products for the cosmetic industries (Nayak et al., 2009; Mukherjee et al., 2009; Tugrul and Cansunar, 2005; Benincasa et al., 2004; Volkering et al., 1998; Fiechter, 1992). Many microorganisms have ability to produce a wide range of biosurfactants, as such initial classification was made into two; based on molecular weights, properties and cellular localizations. The low molecular weight biosurfactants e.g. glycolipids, lipope-ptides, flavolipids, corynomycolic acids and phospholipids lower the surface and interfacial tensions at the air/water interfaces while the high molecular weights are called bioemulsans, (such as emulsan, alasan, liposan, polysaccharides and protein complexes) and are more effective in stabilizing oil-in-water emulsions (Neu, 1996; Franzetti et al., 2009). These high molecular weight biosurfactants are highly efficient emulsifiers that work at low concentrations and exhibit considerable substrate specificity (Dastgheib et al., 2008).

However, general classification based on parent chemical structure and surface properties are represented in the following groups:

1. The glycolipids, in which carbohydrates such as sophorose, trehalose or rhamnose are incorporated to a long-chain aliphatic acid or lipopeptide. This is seen in rhamnolipids produced by *Pseudomonas aeruginosa* which constitute one or two sugar moieties linked to caprilic acid group by a glycosidic bond (Rosenberg and Ron, 1999).
2. Biosurfactant with amino-acid moieties such as surfactin produced by *Bacillus subtilis* composed of several amino-acid structures linked to a molecule of 3-hydroxy-13-methyl tetradecanoic acid.
3. The emulsan synthesized by *Acinetobacter calcoaceticus* RAG-1 is an extracellular polysaccharide - lipid complex (Rosenberg et al., 1979).
4. Protein-like substances (e.g. liposan) constitute small amount of carbohydrates as produced by *Candida lipoltica* (Calvo et al., 2008).

Biosurfactants have unique advantages which include: structural diversity that may lead to unique properties

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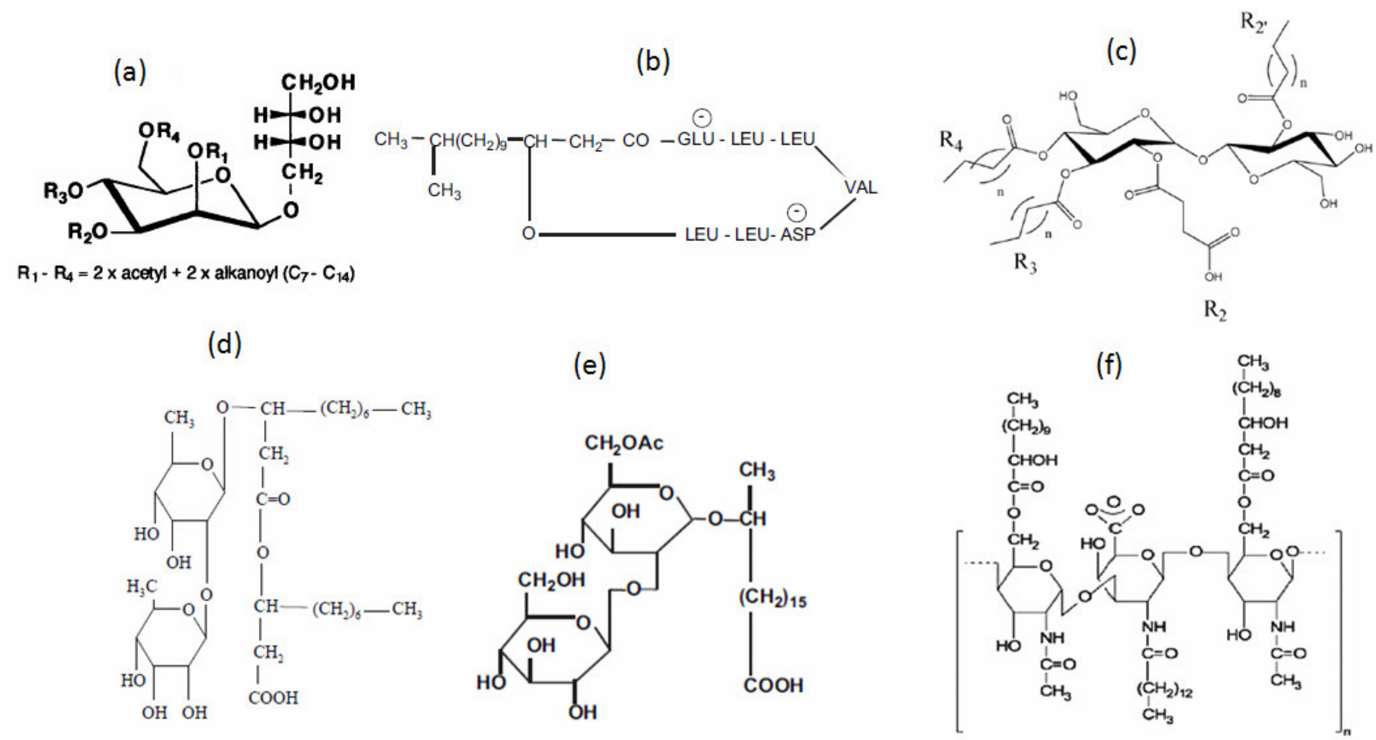

Figure 1. Chemical structures of some common biosurfactants (a) Mannosylerythritol lipid (b) Surfactin (c) trehalose lipid (d) Sophorolipid (e) Rhamnolipid (f) Emulsan.

(chemical structures of commonly studied biosurfactant are shown in Figure 1); the possibility of cost effective production and their biodegradability. These properties make them a promising choice for applications in enhancing hydrocarbon bioremediation (Whang et al., 2008). Biosurfactants are able to retain their properties even under extreme conditions of pH, temperature, salinity (Ron and Rosenberg, 2002) and have low irritancy and compatibility with human skin (Pornsunthorntawee et al., 2009).

Other important advantages, such as bioavailability, activity under a variety of conditions, ecological acceptability, low toxicity, their capacity to be modified by biotechnology and genetic engineering and their capability of increasing the bioavailability of poorly soluble organic compounds, such as polycyclic aromatics are among the unique properties of these agents (Tugrul and Cansunar, 2005). Thus, their use could offer some solutions in bioremediation of contaminated soil and subsurface environments (Lai et al., 2009). Also, biosurfactants could easily be produced from renewable resources via microbial fermentation, making them have an additional advantage over chemically synthetic surfactants. The important challenges for the competitive production of biosurfactants include high yields, alternative low-cost substrates and cost-effective bioprocesses (Pornsunthorntawee et al., 2009). Some of the biosurfactants that have been studied using alternative low-cost substrates, such as molasses (Makkar and Cameotra, 1997) agro-industrial wastes,(Makkar and Cameotra, 1999), soapstock and a by-product of the vegetable oil refining processes (Benincasa et al., 2004), are surfactin produced by *B. subtilis* and rhamnolipids produced by *P. aeruginosa*. Others produced by different species of microorganisms are presented in Table 1. The foci for reduction of biosurfactant production costs are the microbes (selected, adapted, or engineered for high yields of product), the process (selected, designed and engineered for low capital and operating costs), the microbial growth substrate and/or the process by-products (minimized or managed as saleable products rather than treated and discarded as wastes).

Synthetic surfactants

Conventional chemical surfactants have been extensively used and their derivatives are costly and of serious environmental concern; they are potential threats to the environment due to their recalcitrant and persistent nature. Therefore, with current advances in biotechnology, attentions have been paid to the alternative environmental friendly processes for production of different types of biosurfactants from microorganisms (Lotfabad et al., 2009).

Also, as part of the problems; most manufacturers of

Table 1. Some of the common biosurfactants with their producing microorganisms.

Organisms	Biosurfactants	Sources	References
Pseudomonas aeruginosa	Rhamnolipids	Petrochemical wastewater, soap stock, Petroleum contaminated soil, etc.	Whang, et al., 2008; Haba, et al., 2003; Wei, et al., 2008.
Pseudoxanthomonas spp PNK-04			Nayak, et al., 2009.
Pseudomonas alcaligenes			Oliveira, et al., 2009.
Rhodococcus erythropolis 51T7 *Micrococcus luteus* BN56	Trehalose lipids	Oil contaminated soil, Hydrocarbon gas station soil, etc.	Marquez, et al., 2009; Tuleva, et al., 2009.
Bacillus subtilis ATCC 21332	Surfactin	Petroleum sludge, Waste soybean oil, etc.	Pornsunthorntawee, et al., 2008; Lee, et al., 2008.
Candida antarctica	Mannosylerythritol lipids	Vegetable oil and soybean oil waste	Kim, et al., 2002; Kitamoto, et al., 2001.
Gordonia spp BS29	Bioemulsan	Diesel contaminated soil	Franzetti, et al., 2009.

chemical surfactants set the recommended dispersal ratios for their products on the basis of the economics and effectiveness of the dispersant with minimal consideration for the potential harm that can be caused in the receiving ecosystem (Laux et al., 2000; Putheti and Patil, 2009). However, concern still exists on the possible toxic effects of these surfactants on aquatic organisms, especially if they are used in near shore waters (Otitoloju and Popoola, 2009; Venosa and Holder, 2007).

The effects of chemical surfactants on biostimulation of indigenous microorganisms in enhancing the removal of organic pollutants yielded inconsistent results. Decrease in the rate of biodegradation of organic pollutants especially at higher concentrations as suggested by Sun et al. (2008) may be linked to the interaction of surfactant with the lipid membrane and their effects on enzymes and other cellular proteins necessary for basic functions of the microorganisms. Thus, the use of biosurfactants in place of chemical surfactants can minimize all sets of threats posed by the latter.

Despite all these effects; chemical surfactants find applications in different operations, such as drilling, cement production, slurries, fracturing, acidization, demulsification, corrosion inhibition, transportation, cleaning, water flooding, chemical, foam and steam flooding and environment protection as oil spill dispersants (Atta et al., 2006).

Factors affecting biosurfactant production

Composition and emulsifying activity of the biosurfactant depend not only on the producer strain but also on the culture conditions. Thus, the nature of the carbon source, the nitrogen source as well as the C: N ratio, nutritional limitations and chemical and physical parameters such as temperature, aeration, divalent cations and pH influence not only the amount but also the type of polymer produced (Toledo et al., 2008; Lotfabad et al., 2009). The commonly used carbon sources include carbohydrates, hydrocarbons and vegetable oils. It has been concluded from a number of studies that different carbon sources can influence the composition of biosurfactant formation. *Arthrobacter* produces 75% extracellular biosurfactant when grown on acetate or ethanol but it is totally extracellular when grown on hydrocarbon (Mulligan and Gibbs, 1993; Putheti and Patil, 2009). The nitrogen source in the medium plays a significant role in production and contributes to pH control. Several organic and inorganic sources proved effective. Whang et al. (2009) showed that ammonium concentration and pH enhanced biosurfactant efficiency and increase emulsification ability on diesel oil, thus establishing the optimum pH for the production to be towards neutrality. Environmental factors such as temperature, pH, agitation and oxygen availability also affect the production of biosurfactant (Banat, 1995).

METHODS USED FOR IDENTIFICATION

Several methods for screening and estimation of biosurfactants have been developed.

Drop-collapse method is one of the qualitative methods used to determine the presence of biosurfactant. Tugrul and Cansunar (2005) conducted experiments to confirm the reliability of the method using polystyrene microwell plate; oil-coated wells collapse was observed when the

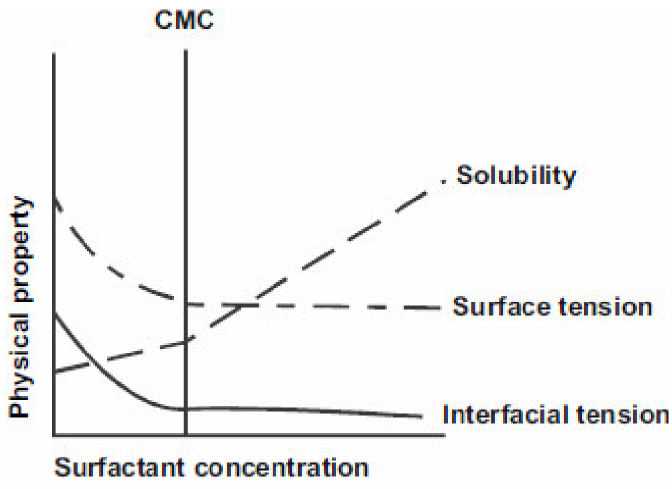

Figure 2. Relationship of surface tension, interfacial tension and the CMC with surfactant concentration (Mulligan, 2005).

culture broth contained biosurfactant and there was no change in the shape of the droplets in the absence of biosurfactant.

Thin layer chromatography (TLC) is also used in preliminary characterization of the biosurfactant where the cell free extract containing biosurfactant is separated on a silica gel plate using chloroform: methanol: water (70:10:0.5, v/v/v); this is then followed by using colour developing reagents. Lipopeptide biosurfactant showed red spots in the presence of ninhydrin reagent, while glycolipid biosurfactant is detected as yellow spots when anthrone is used as the colour reagent (Yin et al., 2009).

Additionally, blood agar hemolysis tests is another method used; where the organisms with biosurfactant ability are streaked on blood agar plates and incubated at 40°C. The plates are visually monitored for the presence of clearing zone around the colonies which is indicative of surfactant biosynthesis. The diameter of the clear zones depends on the concentration of the biosurfactant produced (Youssef et al., 2004; Ghojavand et al., 2008).

Surface tension measurement by a du Nöuy ring-type tensiometer (Krüss, K10T) is one of the simplest techniques used. The surface tension measurement is carried out at room temperature after dipping the platinum ring in the solution for a while in order to attain equilibrium conditions. A higher biosurfactant concentration in the test sample provides a lower surface tension until the critical micelle concentration (CMC) is reached. The CMC is obtained where the surface tension remains steady despite the changes in concentration as represented in Figure 2 (Desai and Banat, 1997; Crosman et al., 2002). This is widely used because of its accuracy, eases to use and provides a fairly rapid measurement of surface and interfacial tension. In general, biosurfactants produced by *P. aeruginosa* strains have the ability to reduce the surface tension of water, when measured with a tensionmetre from 72 to 30 mN/m (Pornsunthorntawee et al., 2008).

In contrast to the methods described so far, high performance liquid chromatography (HPLC) is not only appropriate for the complete separation of different biosurfactants, but can also be coupled with various detection devices (UV, MS, evaporative light scattering detection, ELSD) for identification and quantification of biosurfactants (Heyd et al., 2008).

Das and Mukherjee (2005, 2007) described the acidification of the solution containing biosurfactant in the presence of HCl and equal volume of ethyl acetate in a separatory funnel. Organic layer is collected following phase separation and further dehydrated in rotatory evaporator. The crude sample is then dissolved in sodium Bicarbonate and filtered; adjusting the pH with HCl; precipitation occurs overnight at 4°C. The pure product of biosurfactant is quantitatively obtained by centrifugation and freeze-drying.

Biosurfactants enhanced biodegradation of hydrocarbon compounds

Itoh and Suzuki (1972) were the first to show that hydrocarbon culture media stimulated the growth of a rhamnolipid producing strain of *P. aeruginosa*. Recent researches confirmed the biosurfactant effects on hydrocarbon biodegradation by increasing microbial accessibility to insoluble substrates and thus enhance their biodegradation (Zhang and Miller, 1992; Hunt et al., 1994).

Various experiments have been conducted that showed the effects of biosurfactants on hydrocarbons; enhancing their water solubility and increasing the displacement of oily substances from soil particles. Thus, biosurfactants increase the apparent solubility of these organic compounds at concentrations above the critical micelle concentration (CMC), which enhance their availability for microbial uptake (Chang et al., 2008). For these reasons, inclusion of biosurfactants in a bioremediation treatment of a hydrocarbon polluted environment could be really promising, facilitating their assimilation by microorganisms (Calvo et al., 2008).

Many of the biosurfactants known today have been studied to examine their possible technical applications (Nayak et al., 2009). Most of these applications involve their efficiency in bioremediation, dispersion of oil spills and enhanced oil recovery. *Alcanivorax* and *Cycloclasticus* genera are highly specialized hydrocarbon degraders in marine environments. *Alcanivorax borkumensis* utilizes aliphatic hydrocarbons as its main carbon source for growth and produces an anionic glucose lipid biosurfactant and thus potentials of *Alcanivorax* strains during bioremediation of hydrocarbon pollution in marine habitats have been studied (Olivera et al., 2009); thus, this property needs to be studied extensively in soil to ensure its effectiveness.

Several species of *P. aeruginosa and B. subtilis* produce rhamnolipid, a commonly isolated glycolipid biosurfactant and surfactin, a lipoprotein type biosur-factant respectively; these two biosurfactants have been shown by Whang et al. (2008), to increase solubility and bioavailability of a petrochemical mixture and also stimulate indigenous microorganisms for enhanced biodegradation of diesel contaminated soil. *Gordonia* species BS29 grows on aliphatic hydrocarbons as sole carbon source has been found to produce Bioemulsan, which effectively degrade crude oil, PAHs and other recalcitrant branched hydrocarbons from contaminated soils. The rate of biodegradation is dependent on the chemico-physical properties of the biosurfactants and not by the effects on microbial metabolism (Franzetti et al., 2008; Franzetti et al., 2009).

Corynebacterium alkanolyticum produces a phosphorlipid biosurfactant with a relatively low yield; however, the use of self-cycling fermentation processes resulted in three fold increase in the biosufactant production. Also, the yield could be further increased to five fold by addition high amount of the limiting substrates (Crosman et al., 2002)

Persistent organic pollutants found in oil containing wastewater and sediments, such as PAHs (phenanthrene, crysene), are also hydrophobic in nature and thus water solubility of PAHs normally decrease with the increasing number of rings in molecular structure. This property induces the low bioavailability of these organic compounds that is a crucial factor in the biodegradation of PAHs. The water solubility of some PAHs can be improved by addition of biosurfactants 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, additions of solubilization agents, such as biosurfactants to the system enhance the bioavailability of low solubility and highly sorptive compounds (Shin et al., 2004).

Conclusion

The biosurfactant family constitutes an interesting group of microbial secondary products. Production of biosurfactant is related to the utilization of available hydrophobic substrates by the producing microbes from their natural habitat. Selection of suitable alternative substrates and the design of feasible processes for cost-effective production which involves media and process optimization are the main research focus. The potential use of some hyper producing microbial strains in addition to novel cost-effective bioprocesses throws real challenges and offers tremendous opportunities for making industrial production of biosurfactants a success story.

However, on the basis of their biological and physico-chemical properties, biosurfactants can be used as an antimicrobial, emulsifier and conservative agent in agro-chemical, food industries, as well as in bioremediation applications.

These surface-active compounds enhanced the bio-availability of recalcitrant and hydrophobic organic pollutants through different mechanisms as suggested by Volkering et al. (1998):

1. Decrease in interfacial tension between an aqueous and a non-aqueous phase resulting in formation of emulsions that lead to improved mass transfer of pollutants to the aqueous phase.
2. Formation of micelles results in solubilization of hydrophobic organic compounds; thus the hydrophobic compounds remain in the core of the micelles whereas the hydrophilic molecules interact with the exterior part (Volkering et al., 1995); however, opinion is divided whether the solubilized hydrocarbons are directly available to the degrading micro-organisms.
3. Surfactants' interaction with hydrocarbons in the soil through mobilization lowers the surface tension of the pore water and hydrocarbons in soil particles (Deitsch and Smith, 1995).

Finally, more insight is required for large scale use of biosurfactants, this involves laboratory and field experiments, especially *in situ* studies with proper control processes.

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