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A REVIEW ON APPLICATIONS OF BIOSURFACTANTS PRODUCED FROM UNCONVENTIONAL INEXPENSIVE WASTES IN FOOD AND AGRICULTURE INDUSTRY

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ABSTRACT

Biosurfactants can serve as green alternative in different areas due to their ecological acceptance as they are biodegradable and nontoxic. Nowadays biosurfactants are predominantly used in pharmaceutical, oil industry, and for the bioremediation of pollutants. Apart from these, biosurfactants also show potential applications in many sectors of food industry and agriculture. Allied with emulsion forming and breaking, antiadhesive, functional ingredient, are some properties that can be exploited in agro-food biotechnology. Potential role of biosurfactants in food and agricultural sectors as well as present concern of lowering the production cost of biosurfactants by using the unconventional wastes as substrate is discussed in this article.

KEYWORDS: Biosurfactant, Unconventional wastes, Food applications, Emulsifiers, Antiadhesive.

INTRODUCTION

Surfactants are surface active compounds with both hydrophilic and hydrophobic domain. They are capable of reducing surface and interfacial tension at the surface and interface between liquids, solids and gases. These surfactants form micro-emulsion where hydrocarbons can solubilise in water or water can solubilise in hydrocarbon. For such characteristics surfactant confer an excellent detergency, emulsifying, foaming and dispersive traits as versatile process chemical.[1,2] The huge market demand for surfactants is currently met by synthetic derived from petroleum feedstock. These chemically derived surfactants are toxic and non-biodegradable to the environment. Tightening environmental regulation and increasing awareness for the need to protect bionetwork have effectively resulted in an increasing interest in biosurfactants as promising alternatives over synthetic surfactants.[1] Biosurfactants are amphiphlic molecule produced by a wide variety of plants, animals and microorganisms (bacteria, yeast and fungi) and the microbial derived surfactants are either adhere to cell surface or excreted extra-cellularly in the growth medium; contain both hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases thus reducing surface and interfacial tension at the surface and interface respectively.[3,4] Recently, biosurfactants attracted an attention in the past five decades as an improved alternative to chemical surfactants due to their low toxicity, higher biodegradability, and effectiveness at extremes of temperature, pH, and salinity. [3] Due to these traits, biosurfactants find widespread application in the field of bioremediation of pollutants,[5] oil, food, cosmetic, and pharmaceutical industry.[1,3] During the past few decades biosurfactant production from various microorganisms has been studied extensively. To the best of our knowledge, very few attempts have been made to describe the research and development strategies of making the biosurfactant production process cheaper and commercially attractive. Main aim of this review article is to emphasise the exploitation of cheap and easily available agro-industrial waste as substrate for commercial production of biosurfactants along with waste management and their potential applications in food and agriculture field.

BIOLOGICAL ORIGIN AND CLASSIFICATION OF BIOSURFACTANTS

Unlike chemically synthesized surfactants, which are usually classified according to the nature of their polar grouping, biosurfactants have been categorized mainly by their chemical composition and microbial origin.[2]

The biosurfactants have been classified into low molecular mass molecules which efficiently lower surface and interfacial tension; include glycolipids, lipopeptides and phospholipids and high molecular mass polymers, which are more effective as emulsion stabilizing agents include polymeric and particulate surfactants.[6] The major classes of biosurfactants include glycolipids, lipopeptides, and lipoprotein, fatty acid, phospholipids, neutral lipids, and polymeric surfactants (TableI).

Table I: Major classes of biosurfactants and microorganisms involved[6,66,67]

Class of Biosurfactant	Microorganism involved
Glycolipids 1. Rhamnolipids 2. Trehalose lipids 3. Sophorolipids 4. Mannosylerythritol lipids	 1. *Pseudomonas aeruginosa* 2. *Rhodococcus erythropolis, Arthobacter sp.* 3. *Candida bombicola , C. apicola* 4. *C. antartica*
Lipopeptides 1. Surfactin/iturin/fengycin 2. Viscosin 3. Lichenysin 4. Serrawettin 5. Phospholipids	 1. *Bacillus subtilis* 2. *P. fluorescens* *3. B. licheniformis* 4. *Serratia marcescens* *5. Acinetobacter sp., Corynebacterium lepus*
Surface-active antibiotics 1.Gramicidin 2. Polymyxin 3. Antibiotic TA	 1. *Brevibacterium brevis* 2. *B. polymyxa* 3. *Myxococcus xanthus*
Fatty acids/neutral lipids Corynomicolic acids	 1. *Corynebacterium insidibasseosum*
Polymeric surfactants 1. Emulsan 2. Alasan 3. Liposan 4. Lipomanan	 1.*Acinetobacter calcoaceticus* 2. *A. radioresistens* 3. *C. lipolytica* 4. *C. tropicalis*
Particulate biosurfactants 1.Vesicles 2. Whole microbial cells	 1. *A. calcoaceticus* 2. *Cyanobacteria*

PRODUCTION OF BIOSURFACTANTS USING UNCONVENTIONAL WASTES

Production economy is the major bottleneck in the biosurfactant production. The amount of raw material can contribute considerably to the production cost. Most of the work is based on glucose, sucrose, glycerol or ethanol as fermentation medium. It is estimated that raw materials account for 10-30% of the total production cost in most biotechnological processes. To reduce this production cost, research in the area of biosurfactants has expanded quite a lot in recent years due to their potential use in different areas, such as the food, agriculture, pharmaceutical and oil industry, and bioremediation of pollutants. The one possibility explored extensively can be the use cheap and agro-based raw material as substrate for biosurfactant production.[3,7] Biosurfactants could easily be produced from renewable resources via microbial fermentation, having an additional advantage over synthetic surfactants. The important challenges for the competitive production of biosurfactants include high yields; alternative low cost substrates and cost effective bioprocesses.[8] A variety of cheap raw materials, including plant derived oils, oil wastes, starchy substance, lactic whey, molasses, and distillery wastes have been reported to support biosurfactant production.[9,10] The uses of some inexpensive raw materials for the production of biosurfactants by various microbial strains are discussed in Table II.

Table II. Low-cost raw materials used for the production of biosurfactants by various microbial strains

Low cost raw material	Biosurfactant class	Producer microbial strain	Maximum yields (g l^{-1})
Molasses[68]	Rhamnolipids	*Pseudomonas putida B17*	0.52
Rapeseed oil[10]	Rhamnolipids	*Pseudomonas* sp. DSM 2874	45
Babassu oil[27]	Sophorolipids	*Candida lipolytica* IA 1055	11.72
Turkish corn oil[26]	Sophorolipids	*Candida bombicola* ATCC 22214	400
Sunflower and soybean oil[29]	Rhamnolipids	*Pseudomonas aeruginosa* DS10-129	4.31
Sunflower oil[28]	Lipopeptide	*Serratia marcescens*	2.98
Soybean oil[28]	Mannosylerythritol lipid	*Candida* sp. SY16	95
Curd whey and distillery waste[18]	Rhamnolipids	*Pseudomonas aeruginosa* BS2	0.92
Potato process effluents[23]	Lipopeptide	*Bacillus subtilis*	2.7
Cassava flour wastewater[41]	Lipopeptide	*B. subtilis* ATCC 21332, *B. subtilis* LB5a	2.2
Fruit waste (Orange peel)[33]	Rhamnolipids	*Pseudomonas aeruginosa* MTCC 2297	9.18

Molasses

Molasses, a co-product of sugar production is obtained from sugar cane as well as from sugar beet. Molasses generally consists of 48-56% total sugar (mainly sucrose), 9-12% non-sugar organic matter, 2-4% protein (N×6.25), 1.5-5% potassium, 0.4-0.8% calcium, 0.06% magnesium, 0.6-2.0% phophorus, 1.0-3.0 mg kg^{-1} biotin, 15-55 mg kg^{-1} pantothenic acid, 2500-6000 mg kg^{-1} inositol and 1.8 mg kg^{-1} thiamine. [11] Molasses has been used as the major raw material for production of xanthan gum [12], baker's yeast [13] citric acid [14] as well as fructo-oligosaccharides.[15]

Molasses and corn steep liquor have been used as the primary carbon and nitrogen source to produce rhamnolipid biosurfactant from *P. aeruginosa* GS3 and production reached maximum when 7% (v/v) molasses and 0.5% (v/v) of corn steep liquor were used. Maximal surfactant production (0.25 g L^{-1}) occurred after 96 hours of incubation, when cells reached the stationary phase of growth. [16] Similar, reports have also been reported with molasses as a carbon source after dilution without any additional supplements. [17] Molasses medium supplemented with soya-okra can be a suitable medium for biosurfactant production. *P. aeuroginosa* MTCC 2297 growing in a fermentation medium having molasses (4%) and soya-okra (0.15%) with pH 6.7-7.0 incubated at 35-37^{o}C yield maximum emulsification index (70%) after 120 hours of incubation period.[7]

Lactic Whey and Distillery Wastes

Whey is a liquid by-product of cheese production containing the water soluble components. The main constituents of whey are high level of lactose (75% of dry matter) and protein (12-14%). In addition organic acids, minerals, and vitamins are also present. Whey disposal represent a major pollution problem for countries depending on dairy economy. Due to the high BOD (Biochemical Oxygen Demand) cheese whey is regarded as a pollutant and only half of the whey produced annually is recycled as food ingredients and animal feed.[17]

It have been suggested that dairy waste liquor supports good microbial growth and was used as a cheap raw material for biosurfactant production.[18] *P. aeruginosa* BS2 cultivated on whey waste produced 0.92 g L^{-1} biosurfactant as the secondary metabolites and its maximal production occurred after the onset of nitrogen limiting conditions. The isolated biosurfactant possessed the potent surface-active properties, as it effectively reduced the surface tension of water from 72 to 27mN m^{-1} and formed 100% stable emulsion in variety of water insoluble compounds. [19] Similarly, *Lactobacillus pentosus* was growing on whey at 31^{o}C effectively lowering the surface tension of medium from 54mN m^{-1} to 45mN m^{-1}.[20]

Starchy Substrates

The processing of cassava or potato produces large amount of waste and regarded as pollutant. Due to the high amounts of starch or reducing sugars, these wastes are recognized as suitable feedstock for industrial fermentations such as production of pullulan [21] and volatile compounds.[22] Potato process effluent was used to produce biosurfactant by *B. subtilis*. [23] Similarly, potato process substrates have been used as carbon source for the surfactant production by *Bacillus subtilis* ATCC 21332. The surface tensions dropped from 71.3 mNm^{-1} to 28.3 mNm^{-1} and 27.5 mNm^{-1} when potato medium and mineral salt medium were used, respectively. Furthermore, high-solids (HS) and low-solids (LS) potato process effluents were used as substrates for surfactin production by *B. subtilis* ATCC 21332. Surfactin production from LS potato effluent gave the greater yield (0.39 g L^{-1}) than that from HS potato effluent (0.097 g L^{-1}). [24]

It has been reported that cassava flour-processing effluent used as substrate for biosurfactant production by *Bacillus subtilis* LB5a and *Bacillus subtilis* ATCC 21332. *B. subtilis* LB5a reduced the surface tension of the medium from 49.5 mNm^{-1} to 26.6 mNm^{-1} and produced crude biosurfactant concentration of 3.0g L^{-1}. While *B. subtilis* ATCC21332 reduced the surface tension of the medium from 49.5 mNm^{-1} to 25.9 mNm^{-1}, giving a crude biosurfactant concentration of 2.2 g L^{-1}. The FTIR spectra shown that the commercial surfactin and the semi purified surfactant produced by strain LB5a was surfactin-like surfactant.[26]

Vegetable Oil and Oil Wastes

Several studies have shown that the plant derived oils can act as effective cheap raw materials for biosurfactant production. Rapeseed oil was the good substrate for the production of rhamnolipids and l-(+)-rhamnose by *Pseudomonas* sp. DSM 2874. [10] Babassu oil and corn oil were used for the production of a new bioemulsifier and sophorolipids by exploiting *Candida lipolytica* and *C. bombicola* ATCC 22214 respectively. [26,27] Similarly, vegetable oils such as sunflower and soybean oil were used for the production of rhamnolipid, sophorolipids, and mannosylerythritol lipid biosurfactants by various microorganisms. [28,29] However various vegetable oils, oil wastes from vegetable oil refineries, soap industries and food industry were found to support microbial growth and good substrate for biosurfactant production.[30]

Fruit Wastes

The citrus fruits are the most important value added fruit crops in international trade. India is the fifth largest producer of oranges and more than 80% of its orange processing is for juice production. The increased demand and consumption of oranges generates large quantities of wastes. [31] This waste is often an economic liability to the processor and waste disposal is an emergent problem, which explains the increasing interest in the utilization of this waste for microbial transformation. [32] The exploitation of these types of wastes for biotechnological processes will mitigate the waste management problem and reduce biosurfactant production cost. It have been reported that *Pseudomonas aeruginosa* MTCC 2297 growing on various cost effective waste materials such as orange peelings, carrot peel waste, lime peelings, coconut oil cake, and banana wastes produce a surface-active compound rhamnolipid by submerged fermentation. The orange peel was found to be the best substrate generating 9.18 g L^{-1} of rhamnolipid with a surface tension reduction up to 31.3 mN m^{-1}.[33]

APPLICATIONS Of BIOSURFACTANTS IN FOOD INDUSTRY

Biosurfactants can be explored for several food processing applications. These are the agents that decrease surface and interfacial tension, thus promoting the formation and stabilization of emulsions. The various applications of biosurfactant in food industry are listed in Table III.

Table III. Major applications of biosurfactants in food and agricultural sectors[34,36, 45]

Industry	Application	Role of biosurfactant
Food	Emulsification and De-emulsification	1. Emulsifier, solubilizer, demulsifier, suspension, wetting, foaming, Defoaming,thickener, lubricating agent
	Functional ingredient	Interaction with lipids, proteins and carbohydrates, protecting agent
	Antiadhesive agent	Inhibit the colonization and biofilm formation
	Food processing plants	For cleaning sanitizing
	Fruits and vegetables	Improve removal of pesticides, and in wax Coating
	Bakery and ice cream	Solubilize flavour oils, control consistency, retard staling
	Crystallization of sugar	Improve washing, reduce processing time
	Cooking fat and oils	Prevent spattering due to super heat and water
Agriculture	Biocontrol and Pesticide formulation	Facilitation of biocontrol mechanisms of microbes such as parasitism, antibiosis, competition, Induced systemic resistance and hypovirulence, emulsifier
	Phosphate fertilizers	Prevent caking during storage

Functional Food Formulation Ingredients

The functional ingredients of the biosurfactant directly interact with lipids, proteins and carbohydrates.[34] For example, to control the agglomeration of fat globules, improve texture and shelf-life of starch containing products, modify rheological properties of wheat dough and improve consistency of fat based products. [35] In bakery and ice-cream formulations biosurfactants act by controlling consistency, retarding staling and solubilizing flavour oils. They are also utilized as fat stabilizers and antispattering agents during cooking of oil and fats.[36] Addition of rhamnolipids improved the dough stability, texture, volume, and conservation of bakery products, properties of butter cream, croissants, and frozen confectionery products.[37] L-rhamnose has considerable potential precursor for flavouring. It is already used as a precursor of high quality flavour components like furaneol (trademark of Firmenich SA, Geneva). Recently, a bioemulsifier isolated from marine bacteria *Enterobacter cloacae* was described as a potential viscosity enhancement agent in food industry especially due to the good viscosity observed at acidic pH allowing its use in products containing ascorbic acid.[38]

Food Bioemulsifier (Bio-additives)

Biosurfactants have also been applied in food industries as food additives (emulsifier). Lectin and its derivatives, fatty acids esters containing glycerol, sorbitan, or ethylene glycol, and ethoxylated derivative of monoglycerides and oligopeptide,[39] improve the flavour, taste, and quality of product with minimal health hazards,[40] evaluation of emulsifying ability of biosurfactants is in general related to hydrocarbons such as kerosene because of their potential environmental applications. Few attempts have been made to evaluate emulsion formation by biosurfactants with oils and fats used in food industry. A lipopeptide obtained from *B. subtilis* was able to form stable emulsions with soybean oil and coconut fat, suggesting its potential as emulsifying agent in foods.[41] A manoprotein from *Kluyveromyces marxianus* was able to form emulsions with corn oil that was stable for 3 months; the yeast was cultivated on whey based medium suggesting potential application as food bioemulsifier.[42] The extracellular carbohydrate rich compound from *Candida utilis* was successfully used as emulsifying agent in salad dressing formulations.[43] The use of yeast for production of biosurfactant is interesting because these organisms are generally recognized as safe and they are already present in many food manufacturing processes. In some cases, the emulsion, which is generated in one part of the process, may have to be destabilized in a subsequent operation to develop a certain functional property to the final product. De-emulsification can be of interest in food processing specially when related to fat and oil products as well as in waste treatment.[35]

Antiadhesive Agents

Biosurfactants are good antiadhesive agents that inhibit the biofilm formation thus inhibiting the contamination of food. A biofilm is described as a group of bacteria that have colonized a surface. The biofilm not only includes bacteria, but it also describes all the extracellular material produced at the surface and any material trapped within the resulting matrix. Bacterial biofilms present in the food industry surfaces are potential sources of contamination, which may lead to food spoilage and disease transmission, thus controlling the adherence of microorganisms to food-contact surfaces is an essential step in providing safe and quality products to the consumers. The involvement of biosurfactants in microbial adhesion and detachment from surfaces has been investigated. [44] A surfactant released by *Streptococcus thermophilus* has been used for fouling control of heat exchanger plates in pasteurizers, as it retards the colonization of other thermophilic strains of *Streptococcus* responsible for fouling,[45] the pre conditioning of stainless steel surfaces with a biosurfactant obtained from *Pseudomonas fluorescens* inhibits the adhesion of *L. monocytogenes* L028 strain that is classically used in food industry. The bioconditioning of surfaces through the use of microbial surfactants has been suggested as a new strategy to reduce microbial adhesion.[46]

Bio-preservation and Fortification of Food

It has been suggested that some species of *Lactobacilli* and *Streptococcus* are shown to produce biosurfactants as their secondary metabolite during the process of fermentation. These microorganisms are regarded as GRAS (Generally regarded as safe) and have direct food application. The use of biosurfactants released by *Lactobacilli* strains is very promising since these microorganisms are naturally present in human flora and have also a probiotic effect.[47] *Lactobacillus* species are often together with *Streptococcus* being used as acid and flavour producers in the dairy industry.[48] In addition to their occurrence in plant material and food products, *Lactobacilli* also inhabit the gastrointestinal tract of healthy mammals, and they are the most common members of indigenous microflora of the urogenital tract.[49] *Lactobacillus* and *Streptococcus* species have been shown to be able to displace adhering uropathogenic *Enterococcus faecalis* from hydrophobic and hydrophilic substrata in a parallel-plate flow chamber, possibly through biosurfactant production.[50] Lactic acid bacteria such as *Latococcus lactis* and *Streptococcus thermophilus* preserve the nutritive qualities of food material for an extended shelf life by inhibiting food spoilage and growth of pathogenic bacteria.[51,52] It have been reported that metabolites from lactic acid bacteria can be exploited as biological preservatives in food packaging materials. [53] Lactic acid bacteria and their food products confer a variety of important nutritional and therapeutic benefits in humans such as inhibition of pathogenic organism, improvement of microbial balance in the intestine, immune system modulation, alleviation of lactose intolerance and reduction of blood cholesterol, etc.[54] *Lactobacillus pentosus* a lactic acid bacteria cultivated on whey at 31^{o}C effectively lowering the surface tension of medium from 54 mN m^{-1} to 45 mN m^{-1}. [20]

Much more research is needed however, to understand the contribution of *Lactobacilli* surfactants in preventing pathogen colonization, the biochemical aspects of biosynthesis and their structural characterization.

POTENTIAL AGRICULTURAL APPLICATIONS OF BIOSURFACTANTS

The agriculture industry has also benefited from the production of biosurfactants (Table-3).

Biological Control Agent

It have been reported that rhamnolipids are highly effective against three representative genera of zoosporic plant pathogen; *Pythium aphanidermatum, Phytophthora capsici and*

Plasmopara lactucea-radicis. [55] The purified mono and di rhamnolipids with concentration ranging from 5-30 mg L^{-1} caused cessation of motility and lysis of the entire zoospore population in less than 1 minute. The mechanisms of surfactant action include facilitation of penetration or infection by the control agent or its products or coformulated components into the cells or tissues of the target organism.[56,57] The antifungal activity of several microbial surfactants against phytopathogenic fungi has been demonstrated such as for glycolipids or cellobiose lipids,[58,59] rhamnolipids,[60,61] and cyclic lipopeptides including surfactin, iturin and fengycin.[62,63]

Pesticide Formulation

Bioemulsifier are active compounds potentially used in formulation of several herbicides and pesticides as dispersing agent.[6,34] A glycolipopeptides bioemulsifier produced by *Bacillus* have been used for emulsifying immiscible organophosphorus pesticides.[64] Similarly, a biosurfactant produced by *P. aeruginosa*, solubilize toxic organic chemicals and increase the solubility and recovery of hexachlorobiphenyl from soil slurries by 31%.[65] It have been also reported that the addition of a biosurfactant (400 µg ml^{-1}) produced by *B. subtilis* MTCC 2423 enhanced the rate of biodegradation of the chlorinated pesticide α- and β-endosulfan by 30-40%, in both flask-coated and soil-bound conditions.[1]

CONCLUSIONS AND FUTURE PROSPECTIVES

Biosurfactants confer several properties which could be exploited in many fields of food processing industry and agriculture. The various properties *viz.*, emulsifying, antiadhesive and antimicrobial activities suggest potential application as versatile ingredients and biocontrol agent. High production cost seems to be the major cause for the limited use of biosurfactants in food and agriculture field. At present, the costs of microbial surfactants are not competitive with those of the synthetic surfactants due to their high production costs and low yields. Hence, they have not been commercialized extensively. The one promising alternative could be the use of low cost substrate i.e. agro-industrial wastes for commercial synthesis of biosurfactants to reduce the production cost. In the future we can foresee continuing research on biosurfactants in the following main direction such as, the use of agro industrial wastes as substrate for the commercial production of biosurfactant; process optimization coupled with novel and efficient multistep downstream processing methods to achieve high yield; the use of recombinant and mutant hyper producing microbial strains can make biosurfactant production economically feasible. Apart from these, the use of agro-industrial waste can reduce biosurfactant cost as well as the waste management expenditure, and also translates new opportunities for food and food related industries. Biosurfactants obtained from GRAS (generally regarded as safe) microorganisms like *Lactobacilli* and yeasts are of great promise for food and medicine applications though, much more research is needed. The future vision of the microbial derived surfactants for the detection of different molecules in terms of cost effective production, characterization, properties and the toxicological aspects of new and current biosurfactants should be emphasized so that the microbial surfactants contribute important role in food to be certified as safe for food utilization. With the emphasis on the building of a sustainable society in harmony with the environment, the introduction of green technology in all fields of industry is one of the most important challenges. Considering the technological and ethical backgrounds, utilization of biosurfactants, which are eco-friendly and highly functional, have become more and more important future aspect.

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