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REVIEW ARTICLE

PRODUCTION OF BIOSURFACTANTS FROM AGRO-INDUSTRIAL WASTES

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ABSTRACT

Biosurfactants are surface active compounds produced by microorganisms by growth on various substrates, including renewable resources such as vegetable oil, distillery and dairy wastes. Biosurfactants are attracting attention in recent years because they offer several advantages over chemical surfactants such as low toxicity, inherent good biodegradability and ecological acceptability. The majority of known biosurfactants are synthesized by microorganisms grown on water immiscible hydrocarbons, but some have been produced on such water- soluble substrates as glucose, glycerol and ethanol. Chemically-synthesized surfactants have been used in the oil industry to aid clean up of oil spills, as well as to enhance oil recovery from oil reservoirs. They are extensively used in industry as antifoaming agents. Challenges for production of biosurfactant production is the economic feasibility due to high production cost. This has been a limiting factor in the utility of several prospective biosurfactants. Material selection of raw materials particularly when working with bulk product market, greatly influences production cost. The amount to be used, form (solid or liquid), packaging, transportation purity all affect cost. Specifically, the carbon source is a major constituent of medium .A wide variety has been used, including, hydrocarbons, carbohydrates, and vegetable oil sources. Microorganisms use several substrates, together or independently. This review puts together materials like agro-industrial wastes that have successfully been used for biosurfactant production so as to facilitate technology development in this field.

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INTRODUCTION

Surfactants and biosurfactants

Surfactants are used by many Industries. There is almost no modern industrial operation where properties of surface active agents are not exploited. Surfactant properties enable emulsifying, foaming, detergency and dispersing properties (Makkar, Cameotra and Banat, 2011; Diaz *et al*., 2012). Biosurfactants (BS) are environmentally friendly options produced by microbial fermentation processes or by enzymatic syntheses (Syldatk and Hausmann, 2010; Diaz *et al.,* 2012; Vaz *et al.,* 2012). The fact that only small amounts of BS are needed to reduce surface tension coupled with their known biodegradability makes them environmentally friendly comparing with chemical surfactants. Additionally they are stable at extremes of environmental conditions have, little or no toxicity and higher biodegradability. Their variety in structural and chemical composition makes them versatile in terms of applicability (Banat *et al.*, 2000; Diaz *et al*., 2012).

Market trends in demand for surfactants

Surfactants and emulsifiers and are widely used in the pharmaceutical sector, cosmetics industry, soap and detergent Industry, textile Industry, oilfield chemicals, agrochemicals, food Industry, and others. About 54% of the total surfactant output is utilized in household/laundry detergents, with only 32% for industrial use (Bannat *et al.*, 2000; Diaz *et al*., 2012). According to market research sources, surfactants market is projected to reach 24,037.3 KT (Kilotons), in terms of volumetric demand, and $42,120.4 Million in terms of value, by 2020, estimated to grow at a 5.5%, by value, during the forecast period of 2015 to 2020. The soap/detergent sector contributes maximally to the global surfactants market. The elastomers and plastics application segment and the oilfield chemicals surfactants segment in the North American region is expected to lose its dominance to Asia-Pacific, owing to the increased off-shore exploration activities and expected commercial production of shale gas by China, which has the largest shale gas reserves. (http://www.prnewswire.com/news-releases/ surfactants-market-worth-421204-million-by-2020-506134181, html, http://www.transparencymarketresearch.com/pressrelease/specialty-surfactants-market.htm, 2015,

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http://www.freedoniagroup.com/industry-study/3247/surfactants.htm)

Challenges for production of biosurfactants

Economic feasibility due to high production cost, has been a limiting factor in the utility of several prospective biosurfactant. However, the search for bacteria with high biosurfactant yields is an ongoing process. Studies have shown that it is possible to influence production yields, compositions, structures, and properties of bacterial biosurfactant by modifying culture conditions, such as temperature (Leito *et al*., 1992; Martins and Correia, 1994), dissolved oxygen tension (Leito and Correia 1993; Leito and Correia 1997a), and growth medium composition (i.e., the concentration of cations (Leito and Correia, 1997b, Martins *et al*., 1990) and the carbon source used (Bryan *et al.*1986,Corning *et al*., 1994, Novak *et al*., 1992). Since bacterial biosurfactants show great diversity in structure and function and its production is not limited by taxa , it is suitable for human usage provided they meet GRAS (generally regarded as safe) status or at least have a cost effective means of neutralizing toxic constituents in cases of environmental applications such as in water (waste and municipal) treatment. Manipulation of fermentation conditions as well as the exploration of cheap fermentation substrates for their production are suggested tools for improving the chances of commercial scale production and field application of these compounds. Biosurfactant production has been shown to be strongly influenced by environmental factors such as pH, temperature, oxygen and agitation through their effects on cellular growth or activity. All microorganisms need a source of carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus for their growth. These materials are offered in several forms as media constituents. Material selection of raw materials particularly when working with bulk product market, greatly influences production cost. The amount to be used, form (solid or liquid), packaging, transportation purity all affect cost. Specifically, the carbon source is a major constituent of medium .A wide variety has been used, including, hydrocarbons, carbohydrates, and vegetable oil sources. Microorganisms use several substrates, together or independently.

Biosurfactant production using oils and oil wastes

World production of oils and fats is about 2.5-3 million tons, being 75% derived from plants (Haba *et al*., 2000). Several researchers have reported the use of plant derived oils as effective and cheap raw materials, namely rapeseed oil, Babassu oil, Canola oil and corn oil (Trummler *et al*., 2003; Vance *et al*., 2003, Pekin *et al*., 2005 and Sarrubbo *et al*., 2007). Likewise, vegetable oils such as sunflower and soybean oils (Ferraz *et al*., 2002; Kim *et al*., 2006) have been used for the production of rhamnolipids, sophorolipid and mannosylerythritol by a variety of microorganisms. Oil wastes from vegetable oil refineries and the food industry were also reported as good substrates. In addition, industrial oil wastes such as tallow, soap stock, marine oils, lard and free fatty acids have also shown the potential to induce microbial growth and metabolite production owing to their typical fatty acid composition. These oils and oil wastes are readily available in good amounts throughout the world. Additionally, as waste disposal is a growing problem, an increasing interest in its use in microbial transformation has been observed over the years. Incorporation of a range of less expensive plant-derived oils and oil wastes not suitable for human consumption, such as jatropha oil, mesua oil, castor oils, ramtil oil and jojoba oil, in the industrial production of biosurfactants represent a significant reduction of the overall production costs Mukherjee *et al*., 2006). The use of domestic vegetable oils for the production of biosurfactants by *Tsukamurella spec* DSM 44370 was reported Elke *et al*. (1999). The glycolipids produced by *Tsukamurella* were found to be as effective as commercially available surfactants. Another example of the use of oils as raw materials for biosurfactants production was described by Sarubbo and co-workers (Sarubbo *et al*., 1997) In their work they evaluated the production of bioemulsifiers by two strains of *Candida lipolytica* using media supplemented with 5% Babassu oil and 1% glucose as carbon source. The same authors also studied the co-utilization of Canola oil and glucose on the production of the bioemulsifiers by the same yeast strain .Olive oil mill effluent (OOME) as a new substrate for production (Rhamnolipids) by *Pseudomonas sp.*JAMM was also studied (Mercarde *et al*., 1993). This oil effluent is a pollutant of the agricultural industry especially in the Mediterranean countries. It is a black liquor containing the water-soluble fraction of ripe olives and water that is used in the process of olive oil extraction. Several strains were screened for growth in this medium and it was found that strains belonging to the genus *Pseudomonas* were good candidates. Additionally, the same authors studied production by *Pseudomonas* strain 42A2 using a sub-product from the distillation of non-specific mixtures of vegetable oils with a high content of oleic acid (98% w/w) as raw material. Moreover, Abalos and co-workers (2001) reported the use of soybean oil refinery wastes for the production of new Rhamnolipids by *P. aeruginosa* AT110. Furthermore, *Candida antarctica* and *Candida apicola* were found to produce glycolipids when grown in a cultivation medium supplemented with oil refinery waste, either with soap stock (5-12% v/v) or post-refinery fatty acids (from 2 to 5% v/v). The efficiency of glycolipids synthesis was from 7.3 to 13.4 g/L and from 6.6 to 10.5 g/L in the medium supplemented with soap stock and post-refinery fatty acids, respectively (Bednarski *et al*., 2004). Nitschke *et al*. (2005) also evaluated edible oil soap stocks as alternative low-cost substrates for the production of Rhamnolipids by *P. aeruginosa* LBI strain. Wastes obtained from soybean, cottonseed, babassu, palm and corn oil refinery were tested. The soybean soap stock waste was the best substrate, generating 11.7 g/L of rhamnolipids and a production yield of 75%. Vegetable oils and residues from vegetable oil refinery are among the most used low-cost substrates for rhamnolipids production (Nitschke *et al*., 2005). Frying oil is produced in large quantities for use both in the food industry and at the domestic scale. After being used, cooking oil changes its composition and contains more than 30% of polar compounds (Kock *et al*., 1996) depending on the variety of food, the type of frying and the number of times it has been used. Haba and co-workers Haba *et al*. (2000) compared the composition of used olive and sunflower oils with the standard unused oils in their study and found that the most important difference in the used oil is the presence of 22.52% of fatty acids of low chain length (<C10), myristic acid and lauric acid. In their study they screened 36 microorganisms for production of biosurfactant in submerged culture with 2% waste olive or sunflower oil as carbon source using the lowering of surface tension (below 40 mN/m) as the selection criteria. After 72 h of growth most of the *Pseudomonas* strains tested showed satisfactory growth when cultivated on used olive or used sunflower oil. Used olive oil was found to be a better substrate for cell growth, as well as for production. Results achieved for the production of lipopeptides by various *Bacillus* strains were not as good as the ones achieved with *Pseudomonas*. In addition, the other strains tested (*Rhodococcus* sp., *Acinetobacter calcoaceticus* and *Candida sp.*) did not compare favorably with the previous ones.

Waste or used lubricating (lube) oils have become a serious environmental problem. In the environment, the waste oil can bind to organic matter, mineral particles and organisms (Shale *et al*., 1989). Mercade and co-workers (1996) reported the screening and selection of microorganisms capable of utilizing waste lube oil for producing biosurfactant. In their study they isolated 44 different microorganisms from contaminated soil samples but only 10% of these strains produced biosurfactant. Further characterization of these strains showed production of trehalose glycolipids (from *Rhodococcus sp.*) and lipopeptides (from *Bacillus sp.*)

Production using cheese whey

A good substrate for production is whey, as it is composed of high levels of lactose, protein, organic acids and vitamins. Whey is a waste product from cheese production that represents a major pollution problem for countries depending on dairy economics and is normally used as animal feed. To use the lactose effectively, a chosen organism must be able to consume the lactose and its breakdown products, glucose and galactose. Koch and co-workers (Koch *et al*., 1988) have developed a strain of *P. aeruginosa* to use whey for the production of rhamnolipids .*B. subtilis* is also known to produce surfactin using this substrate (Makkar and Cameotra 1999; Rodrigues[a] *et al*., 2006). In a study on the optimization of the media composition for the production of biosurfactants by lactic acid bacteria (*L. lactis* 53 and *S. thermophilus* A), Rodrigues[b] and co-workers (2006) achieved an increase of about two times in the mass of produced cell-bound biosurfactant (milligram) per gram cell dry weight. However, it was interesting to notice that the change in the carbon source (from glucose to lactose) induced the cells to produce more biosurfactant. Lactic acid bacteria ferment sugars via different pathways and are also capable of forming other products, e.g. flavors such as diacetyl and acetoin, bacteriocins or biosurfactants. The different carbon sources give varying amounts of by-products (Rodrigues[c] *et al*., 2006). Hence, it was speculated that the use of lactose as carbon source instead of glucose induced the cells to use another metabolic pathway, and therefore the amount of mass of cell-bound biosurfactant produced milligram per gram cell dry weight varied. Based on these results, the authors evaluated the use of cheese whey as an alternative media and compared with the conventional synthetic one. Several combinations of media supplementation were studied and, despite a higher biosurfactant production yield from *L. lactis* 53 was achieved with a medium composed of whey (50 g/L lactose) supplemented with 5.8 g/L yeast extract and 44.8 g/L peptone, an increase of 40% in the medium preparation costs comparing with the synthetic MRS medium was estimated. This was due to the high amounts of peptonethat was supplemented in the whey medium. Thus, a compromise situation must be established to obtain higher biosurfactant production yields with lower medium preparation costs. With another medium composed of whey (50 g/L lactose) supplemented with 3 g/L yeast extract and 5 g/L peptone, the mass of produced biosurfactant per gram cell dry weight increased 1.2 times with an estimated 60% decrease in the medium preparation costs comparing with the synthetic MRS medium. Similar conclusions were established for *S. thermophilus* A, where the use of a medium composed of whey (50 g/L lactose) supplemented with 3 g/L yeast extract and 5 g/L peptone, resulted in a biosurfactant production yield 1.5 times higher with an estimated 60% reduction in the medium preparation costs comparing with the synthetic M17 medium. In sum, the results achieved using the cheese whey as a substrate showed that the fermentations were carried out effectively with high yields and productivities of biosurfactant. Furthermore, other *Lactobacillus* strains have been screened for their ability to produce biosurfactants using lactose as carbon source (Fo *et al*., 2000). The results achieved showed that *Lactobacillus pentosus* CECT-4023 was a strong biosurfactant producer strain. Therefore, the biosurfactant production from this strain using cheese whey as substrate was studied.

Biosurfactant production using agro- industrial wastes

Several agro-industrial wastes are also potential alternative raw materials for the production of biosurfactants. Potato process effluents (wastes from potato processing industries) have been used by several workers using *B. subtilis* (Fox and Bala 2000, Thompson *et al*., 2001, Noah *et al*., 2005, Nitschke and Pastore 2003). Potatoes are composed of 80% water, 17% carbohydrates, 2% protein, 0.1% fat and 0.9% vitamins, inorganic minerals and trace elements. Other carbohydrate-rich residues, such as cassava wastewater, have also been used for the production of surfactin by *B. subtillis* (Nitschke and Pastore 2004, 2006, Makkar and Cameotra 2002). Another interesting alternative is the use of molasses, as this by-product of the sugar cane industry has many applications due to its low price as compared to other sources of sugar and the presence of several other compounds besides sucrose. These include minerals, organic compounds and vitamins, which are valuable for the fermentation process (Rodrigues[c] *et al*., 2006; Bogaert 2007; Joshi *et al*., 2008; Makkar and Cameotra 1997; Patel and Desai 1997; Solaiman *et al*., 2004). Rodrigues[c] *et al*. (2006) studied different combinations of medium supplemented with molasses as alternative substrates for production by *Lactococcus lactis* 53 and *Streptococcus thermophilus* A. The production yields achieved with supplemented molasses medium were higher than the obtained whether with conventional or supplemented cheese whey medium. Although higher amounts were produced with a medium composed of molasses (20 g/L sucrose) supplemented with 2.3 g/L yeast extract and 18 g/L peptone and with a medium composed of molasses (20 g/L sucrose) supplemented with 8.8 g/L yeast extract, 17.5 g/L peptone and 92.6 g/L sodium glycerophosphate for *L. lactis* 53 and *S. thermophilus* A, respectively; a better compromise between good yields and low costs is achievable with a medium where peptone and yeast extract amounts are lower (molasses (20 g/L sucrose) supplemented with 3 g/L yeast extract and 5 g/L peptone). Thus, an increase about 1.2 to 1.4 times in the mass of produced biosurfactant per gram cell dry weight and a 80% medium preparation costs reduction comparing with the synthetic MRS or M17 medium were achieved, for both strains. Solaiman and collaborators (Moldes *et al*., 2007) used soy molasses and oleic acid as co-substrates for the production of sophorolipids by *C. bombicola*.

Most agricultural wastes are made up mainly of cellulose, hemicelluloses, and lignin, and before fermentation, they have to be fractionated upon chemical and/or enzymatic stages to obtain sugar solutions, which (after nutrients supplementation) can be used as fermentative media for the production of biosurfactants. In their work, Moldes and co-workers (2007) tested barley bran, trimmed vine shoots, corn cobs, distilled grape marc and *Eucalyptus globules* chips and found that all of them except barley bran allowed high production yields (Rivera *et al*., 2007; Dubey and Juwarker 2001). Comparative studies on the kinetic parameters of rhamnolipid production by *P. aeruginosa* using distillery and whey wastes as substrates were also reported (Sudhakar-Babu *et al*., 1996). The results indicated that the kinetic parameters (specific growth rates and specific product formation rates) from both types of waste were comparatively better than the synthetic medium, revealing that both these industrial wastes (distillery and whey) could be successfully utilized as substrates for production. These wastes are obtained at low cost from the respective processing industries and are as potential low-cost substrates for industrial level production. Several other starchy waste substrates, such as rice water (effluent from rice processing industry and domestic cooking), corn steep liquor, and wastewater from the processing of cereals, pulses and molasses, have tremendous potential to support microbial growth and biosurfactant production.

Conclusion

Microbial production of biosurfactant can only be commercialized if the production process is economically cheaper. The success of development of cheaper processes depends , both, on the use of low cost raw materials and development of downstream processing methods. Future research should, then, be more focused on the economics of biosurfactant production processes, particularly through the use of alternative ---low-cost fermentative media. Development of cost-efficient and sustainable fermentation processes to obtain biosurfactants from agricultural and agro-industrial streams proposals should address

- Improvement of the strategies for metabolic engineering of the production strain
- Scale-up and improvement of fermentation and downstream processing with view to reducing processing time and costs.
- Life-cycle assessment to evaluate the environmental and socio-economic performance of the developed technologies.

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