



Advances on research in the use of agro-industrial waste in biosurfactant production

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Abstract

Biosurfactants are amphiphilic molecules produced by a variety of microorganisms, including bacteria, yeast and filamentous fungi. Unlike chemically synthesized surfactants, biosurfactants present advantages, such as biodegradability, low toxicity, high selectivity and activity under extreme temperature, pH and salinity conditions, as well as a low critical micelle concentration. Moreover, they can be produced from agro-industrial waste and renewable sources. Their structural diversity and functional properties mean that they have potential applications in various industrial processes as wetting agents, dispersants, emulsifiers, foaming agents, food additives and detergents, as well as in the field of environmental biotechnology. However, opportunities for their commercialization have been limited due to the low yields obtained in the fermentation processes involved in their production as well as the use of refined raw materials, which means higher cost in production. In an attempt to solve these limitations on the commercialization of biosurfactants, various research groups have focused on testing the use of inexpensive alternative sources, such as agro-industrial waste, as substrates for the production of different biosurfactants. In addition to enabling the economical production of biosurfactants, the use of such waste aims to reduce the accumulation of compounds that cause environmental damage. This review shows advances in biosurfactant production carried out using different waste materials or by-products from agro-industrial activities.

Keywords Agro-industrial by-products · Biosurfactants · Critical micelle concentration · Functional properties · Surface tension

Introduction

Biosurfactants are active surface molecules with both hydrophilic and hydrophobic moieties that enable them to accumulate at the interface between polar and non-polar media, thereby modifying surface and interface properties and increasing the solubility of polar molecules in non-polar substances, and vice versa (Khaled and Aboul-Enein 2015). Biosurfactants are produced by bacteria, yeasts and filamentous fungi and are classified into: glycolipids, phospholipids and fatty acids, lipopeptides and lipoproteins, polymeric

surfactants; and, particulate surfactants (Chen et al. 2015). The advantages of biosurfactants over synthetic surfactants are their biodegradability, low toxicity, high selectivity, activity under extreme temperature, pH and salinity conditions, and low critical micellar concentration (CMC), meaning that they require a lower amount of surfactant to reduce surface tension (ST).

The effectiveness of a biosurfactant is estimated by its ability to reduce ST, where a good biosurfactant is able to reduce the ST of water from 72 to 35 mN/m and the interfacial tension (IT) of *n*-hexadecane from 40 to 1 mN/m (Pacwa-Plociniczak et al. 2011; Santos et al. 2016). CMC is also a parameter commonly used to determine biosurfactant effectiveness, where the CMC value of several potent and effective biosurfactants is typically 10 to 40 times lower than of synthetic surfactants (Sharma 2016). For example, surfactin, a lipopeptide biosurfactant, lowers the ST of water from 72 to 27 mN/m, with a CMC between 13 and 25 mg/L (Rosenberg and Ron 1999).

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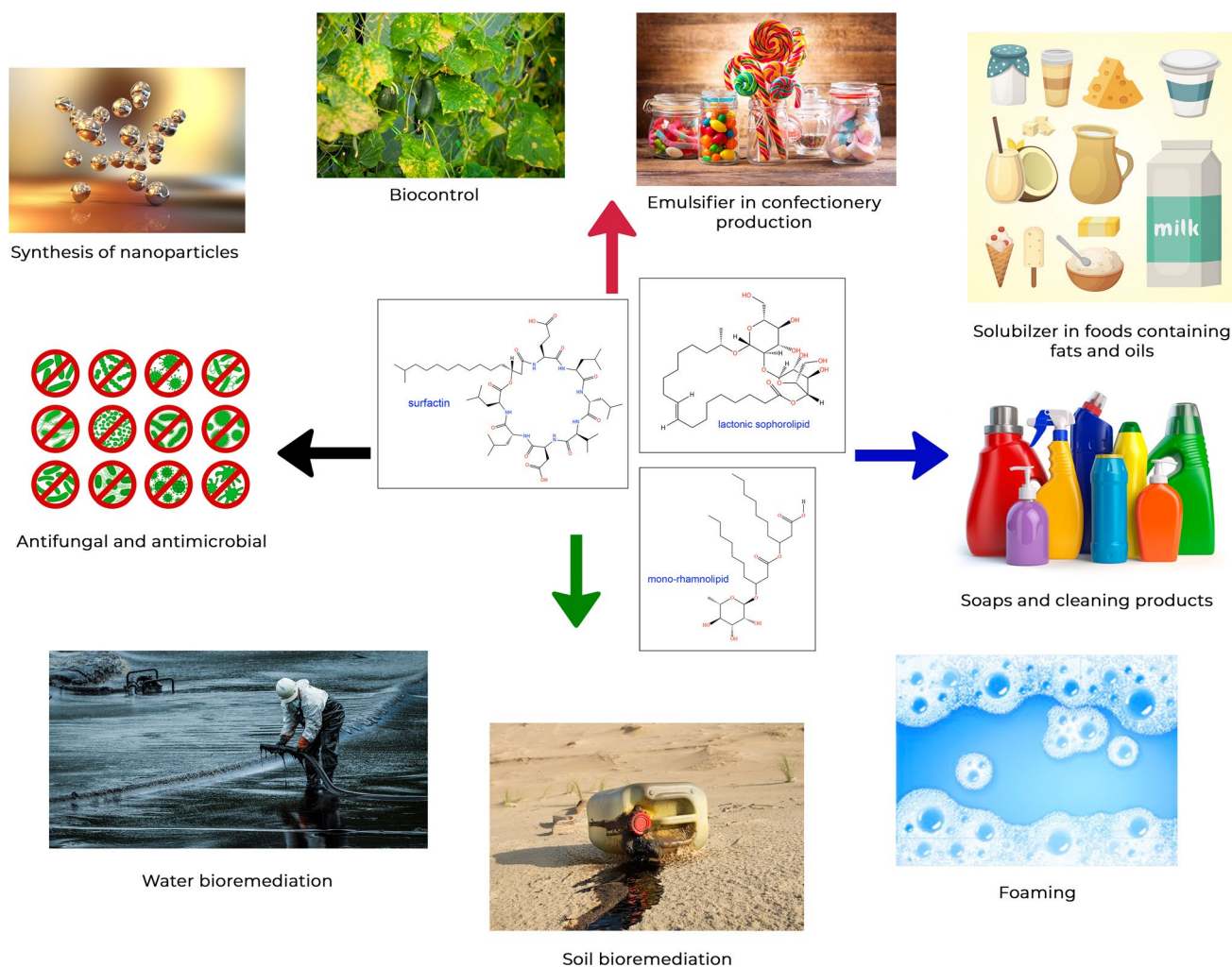


Fig. 1 Some industrial applications for biosurfactants

The biosurfactants properties enable them to overcome some of the problems involved in ocean oil spills and enhance the biodegradation of polycyclic aromatic hydrocarbon compounds (Makkar and Rockne 2003). In agricultural applications, they help to improve plant growth by removing phytopathogens (Sachdev and Cameotra 2013), while, in the pharmaceutical industry, they are used to ease the introduction of foreign genes into target cells during gene therapy (Gharaei-Fathabad 2011). Biosurfactants are used in the food industry as an emulsifier in the confectionery production or as a solubilizer in foods containing fats and oils (Ranasalva et al. 2014) and other varied fields including usage in detergent and cleaning solutions (Kourtmentza et al. 2017) (Fig. 1).

Owing to the great diversity of applications, the revenue generated by the biosurfactant market was over \$1.85 Billion USD in 2017 and is expected to reach \$2.6 Billion by 2023 (Global Markets Insights 2018). However, these amounts are low compared to the global surfactant market, which,

in 2016 represented USD \$30.64 Billion and was predicted to grow to USD \$39.86 Billion by 2021 (Markets and Markets 2017). This market difference must be seen as an area of opportunity for increasing biosurfactant production in order to satisfy the industrial needs, wherein prices should be equal to or lower than chemical surfactants (Dhanarajan and Sen 2014). Different factors contribute to the costly nature of biosurfactants production, among which are the use of refined raw materials, the generally low concentration of biosurfactant obtained, the formation of by-products, and the formation of foam in the fermentation process (Nurfarahin et al. 2018).

The literature describes various forms of waste and by-products, derived from agro-industrial processes, that are used for biosurfactants production, such as: oil processing waste, starch waste, sugar industry waste, fruit and vegetable waste, distillery waste; and, animal fat (Table 1). While the use of petroleum by-products has also been described in the literature, this review does not focus on this waste.

Table 1 Examples of the use of different waste types for biosurfactant production

Substrate	Microorganism	Type of biosurfactant	Cultivation mode	Production (g/L)	References
By-products and vegetable oil processing waste	<i>P. aeurogenosa</i> LBI	Rhamnolipids	Batch shake flask	11.7	Nitschke et al. (2005)
	<i>C. sphaerica</i> UCP 0995	Glycolipids	Batch shake flask	4.5	Sobrinho et al. (2008)
				Batch bioreactor	21
	<i>P. aeruginosa</i> AB4	Rhamnolipids	Batch shake flask	40	Hazra et al. (2011)
	<i>B. subtilis</i> 3–10	Iturin A	Batch bioreactor	0.60	Jin et al. (2014)
	<i>B. subtilis</i> SPB1	Lipopeptides	SSF	30.67 (mg/g)	Zouari et al. (2014)
	<i>P. aeruginosa</i> PAO1	Rhamnolipids	Batch shake flask	0.1914	Moya-Ramírez et al. (2015)
				Batch shake flask	0.43
	<i>S. bombicola</i> ATCC 22,214	Sophorolipids	SSF	179 (mg/g)	Jiménez-Peñalver et al. (2016)
	<i>B. subtilis</i> KB1	Surfactin	SSF	0.011	Jajor et al. (2016)
<i>T. versicolor</i>	Biosurfactants	SSF	373.6 (mg/100 g)	Lourenço et al. (2018)	
Frying oil waste	<i>S. bombicola</i> MTCC1910	Sophorolipids	Batch bioreactor	51.5	Jadhav et al. (2019)
	<i>P. aeruginosa</i> 47T2	Rhamnolipids	Batch shake flask	2.7	Haba et al. (2000)
	<i>C. bombicola</i> ATCC22214	Sophorolipids	Batch bioreactor	10	Fleurackers (2006)
				Batch bioreactor	34
	<i>P. aeruginosa</i> zju.ul M	Rhamnolipids	Batch bioreactor	20	Zhu et al. (2007)
	<i>P. aeruginosa</i> PACL	Rhamnolipids	Batch bioreactor	3.3	de Lima et al. (2009)
	<i>P. aeruginosa</i> DG30	Rhamnolipids	Batch bioreactor	15.6	Zheng et al. (2011)
	<i>M. circinelloides</i>	Glycolipids	Batch shake flask	12.3	Zadeh et al. (2017)
	<i>P. aeruginosa</i> OG1	Rhamnolipids	Batch shake flask	13.3	Ozidal et al. (2017)
	<i>B. thailandensis</i> E264	Rhamnolipids	Batch bioreactor	2.2	Kourmentza et al. (2018)
<i>Streptomyces</i> sp. DPUA1559		Glycoproteins	Batch shake flask	1.74	Santos et al. (2018)
		Lipoproteins	Batch shake flask	1.9	Santos et al. (2019)
<i>B. stratosphericus</i> FLU5		Lipopeptides	Batch shake flask	0.05	Hentati et al. (2019)
<i>P. cepacia</i> CCT6659		Biosurfactants	Batch bioreactor	40	Soares da Silva et al. (2019)
Cashew apple juice	<i>P. aeurogenosa</i> ATCC 10145	Rhamnolipids	Batch shake flask	3.8	Rocha et al. (2007)
	<i>B. subtilis</i> LAMI008	Surfactin	Batch shake flask	0.0035	Rocha et al. (2009)
	<i>B. subtilis</i> LAMI005	Surfactin	Batch bioreactor	0.123	Giro et al. (2009)
		Surfactin	Batch shake flask	0.319	de Oliveira et al. (2013)
<i>Y. lipolytica</i>	Biosurfactants	Batch shake flask	6.9	Fontes et al. (2012)	
Banana peel	<i>H. archaeon</i> AS65	Lipopeptides	Batch shake flask	5.3	Chooklin et al. (2014)
Orange peel	<i>P. aeurogenosa</i> MTCC 2297	Rhamnolipids	Batch shake flask	9.2	George and Jayachandran (2009)
	<i>B. licheniformis</i> KC710973	Lipopeptides	Batch shake flask	1.8	Kumar et al. (2016)
Carrot peel	<i>B. subtilis</i> I'1a	Iturin	Batch shake flask	0.428	Paraszkiwicz et al. (2018)
Potato processing effluent	<i>B. subtilis</i> 21332	Surfactin	Batch shake flask	0.44	Thompson et al. (2000)
		Surfactin	Chemostat	0.9	Noah et al. (2005)
Potato peel powder	<i>Klebsiella</i> sp RJ-03	Biosurfactants	Batch shake flask	15.4	Jain et al. (2013)

Table 1 (continued)

Substrate	Microorganism	Type of biosurfactant	Cultivation mode	Production (g/L)	References		
Cassava flour wastewater	<i>B. subtilis</i> LB5a	Surfactin	Batch shake flask	3.0	Nitschke and Pastore (2006)		
			Batch bioreactor	2.4	Barros et al. (2008)		
			Batch bioreactor	0.027	de Andrade et al. (2016)		
	<i>P. aeruginosa</i>	Rhamnolipids	Batch shake flask	0.660	Costa et al. (2009)		
	<i>P. tsukubaensis</i>	Mannosynthritol lipids-B	Batch bioreactor	1.26	de Andrade et al. (2017)		
Rice husk	<i>M. indicus</i>	Glycolipids	Batch shake flask	7.8	Oje et al. (2016)		
Lignocellulose hydrolysates	<i>L. pentosus</i>	Biosurfactants	Batch shake flask	0.0048	Portilla-Rivera et al. (2007)		
			<i>B. tequilensis</i> ZSB10	Biosurfactants	Batch shake flask	1.52	Cortés-Camargo et al. (2016)
	<i>S. bombycolia</i> NBRC 10243	Sophorolipids	Batch bioreactor	49.2	Konishi et al. (2015)		
	<i>C. bombycolia</i> ATCC 22214	Sophorolipids	Batch shake flask	3.6–84.6	Samad et al. (2015)		
			Batch bioreactor	52.1	Samad et al. (2017)		
<i>C. mucoides</i> UFMG-CM-Y6148	Sophorolipids	Batch shake flask	12.5	Marcelino et al. (2019)			
Animal fat, grease and animal waste	<i>C. bombycolia</i> ATCC 22214	Sophorolipids	Batch shake flask	120	Deshpande and Daniels (1995)		
			<i>P. aeruginosa</i> 101045	Rhamnolipids	Batch bioreactor	3.84	Borges et al. (2012)
			<i>C. lipolytica</i> UCP0988	Glycolipids	Batch bioreactor	8	Santos et al. (2014)
	<i>C. bombycolia</i> ATCC 22214	Sophorolipids	Batch shake flask	39.8	Minucelli et al. (2017)		

SSF solid state fermentation

The use of industrial waste has advantages, such as: reducing production costs; greater availability of many cheaper/renewable substrates; the production of substrates in large quantities; the basic functional properties of the product do not change; the product is not harmful to microorganisms; and, all components of the product are eco-friendly and safe (Banat et al. 2014). The present review describes studies undertaken on biosurfactant production which have used different waste materials or industrial by-products of animal and vegetable origin.

Classification of biosurfactants

Chemically, biosurfactants are classified into glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants, and particulate surfactants. Glycolipids contain different sugars linked by an ester group to linear or branched alkyl groups (Otzen 2017), with rhamnolipids, trehalolipids and sophorolipids the most well-known (Fig. 2).

Lipopeptides are classified as cyclic or linear compounds and consist of fatty acids combined with peptide residues (Mnif and Ghribi 2015), with surfactin, iturin and fengycin the most well-known lipopeptide families (Fig. 3). Certain

microorganisms capable of growing in hydrophobic nutrients, such as alkanes, produce fatty acids, phospholipids and neutral lipids which enable the absorption and consumption of nutrients (Sharma 2016). Polymeric biosurfactants are biopolymers with a high molecular weight, which can be constituted by lipoproteins, proteins, polysaccharides, lipopolysaccharides, or complex mixtures of these compounds. Particulate biosurfactants are formed as extracellular membrane vesicles, creating a microemulsion that exerts an influence on the absorption of alkanes in microbial cells (Vijayakumar and Saravanan 2015).

Biosurfactant production with by-products and vegetable oil processing waste

Vegetable oil processing generates large amounts of waste and by-products with a high content of fats, oils and other compounds, including soap stocks, oil seed cakes, fatty acid residues, semisolid effluents and water-soluble effluents (Dumont and Narine 2007). These residues are an important source of water and soil contamination, due to the low degradability of the lipid compounds they contain (Cammarota and Freire 2006). The use of this type of waste has been reported for biosurfactant production. Mercadé

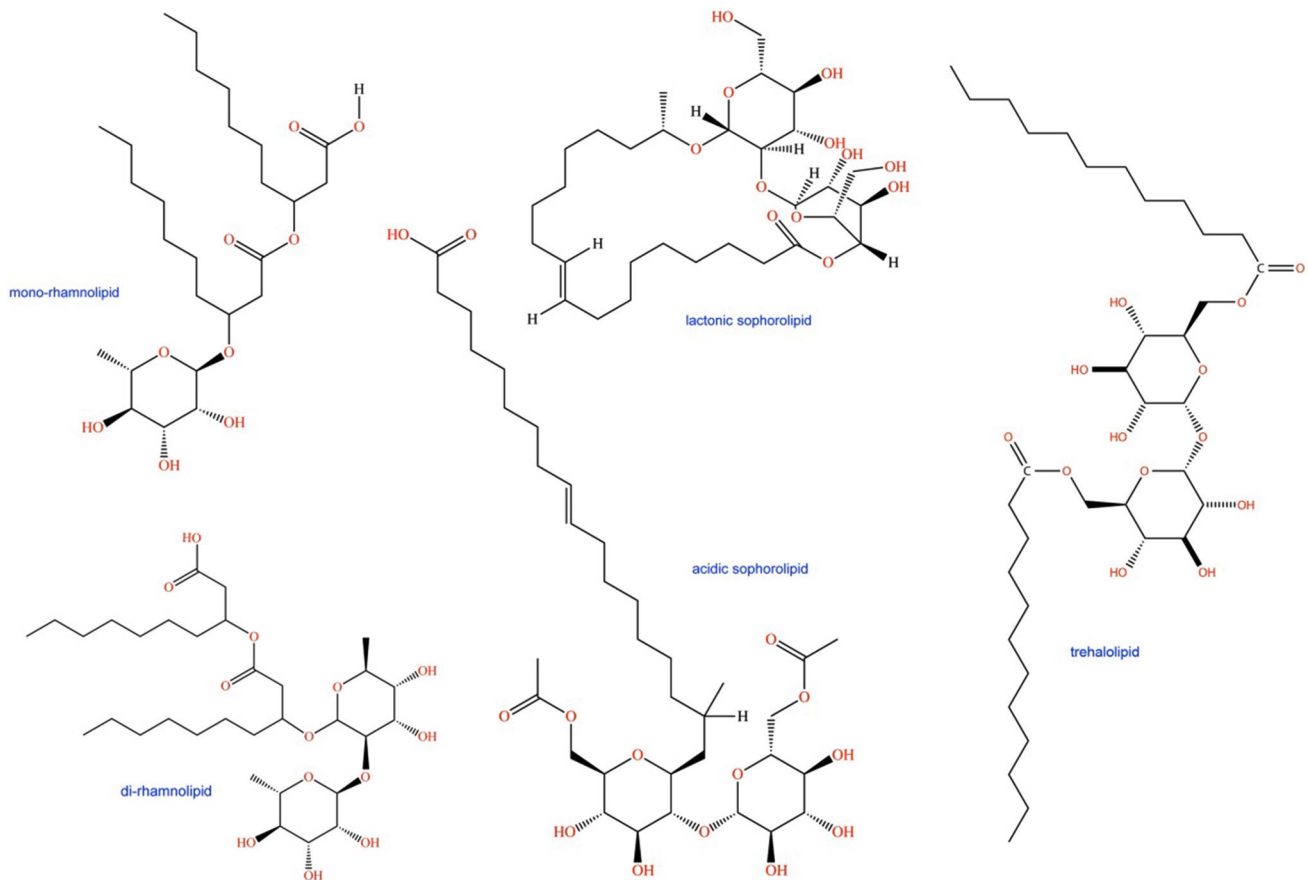


Fig. 2 Characteristic structure of mono-rhamnolipid, di-rhamnolipid, trehalolipid, acidic sophorolipid and lactonic sophorolipid (structures produced based on the PubChem Compound database: <https://www.ncbi.nlm.nih.gov/pccompound/>)

et al. (1993) first reported the use of olive oil mill effluents (OOME) as a substrate for the production of rhamnolipids by *Pseudomonas* sp. JAMM, producing 0.058 g/g of substrate with the use of 100 g/L of OOME and 2.5 g/L of NaNO_3 . The glycolipid obtained was able to decrease the ST of the medium from 40 to approximately 30 mN/m.

Abalos et al. (2001) used the waste from a soybean oil refinery to produce rhamnolipids with *Pseudomonas aeruginosa* AT10, reporting a glycolipid production of 9.5 g/L, an ST of 26.8 mN/m and a CMC of 122 mg/L. Bednarski et al. (2004) found that *Candida antarctica* ATCC 20509 and *Candida apicola* ATCC 96134 synthesize glycolipids when yeasts grown in a medium supplemented with two oil refinery waste residues (undefined oil types). Using soap stock at a level of 5 to 12% v/v in the media, they obtained glycolipid content of 7.3 to 13.4 g/L, respectively, while the use of post-refinery fatty acids at a level of 2 to 5% v/v obtained 6.6 and 10.5 g/L glycolipid, respectively. The authors concluded that the addition of soap stock had a positive effect on the efficiency of glycolipid synthesis. Nitschke et al. (2005) evaluated the application of oil refinery waste from soybean, cottonseed, babassu, palm, and corn oil for the production

of rhamnolipids using *P. aeruginosa* LBI, finding that soybean soap stock (2% w/v) was the best substrate, generating 11.7 g/L of rhamnolipids with an ST of 26.9 mN/m and a CMC of 51.5 mg/L.

Rufino et al. (2008) applied a sequential factorial design to optimize the production of *Candida lipolytica*, using soybean oil refinery waste as a substrate and evaluating the impact of refinery residue waste, glutamic acid and yeast extract on biosurfactant production. The biosurfactant produced under optimal conditions of 6% oil residues and 1% glutamic acid, showed an ST of 25.3 mN/m and emulsifying capacity and was stable in a wide range of pH (2–12), temperature (0–120 °C) and salinity (2–10% NaCl) conditions.

Coimbra et al. (2009) studied the cell surface properties and their relationship with the production of biosurfactants for environmental applications, cultivating six *Candida* strains in soluble and insoluble substrates, including *n*-hexadecane, soybean oil, ground nut oil refinery residue, corn steep liquor, and glucose. Their results showed the potential application of yeast for the removal of hydrophobic compounds, with the surfactant able to remove 90% of hydrophobic contaminants from sand samples.

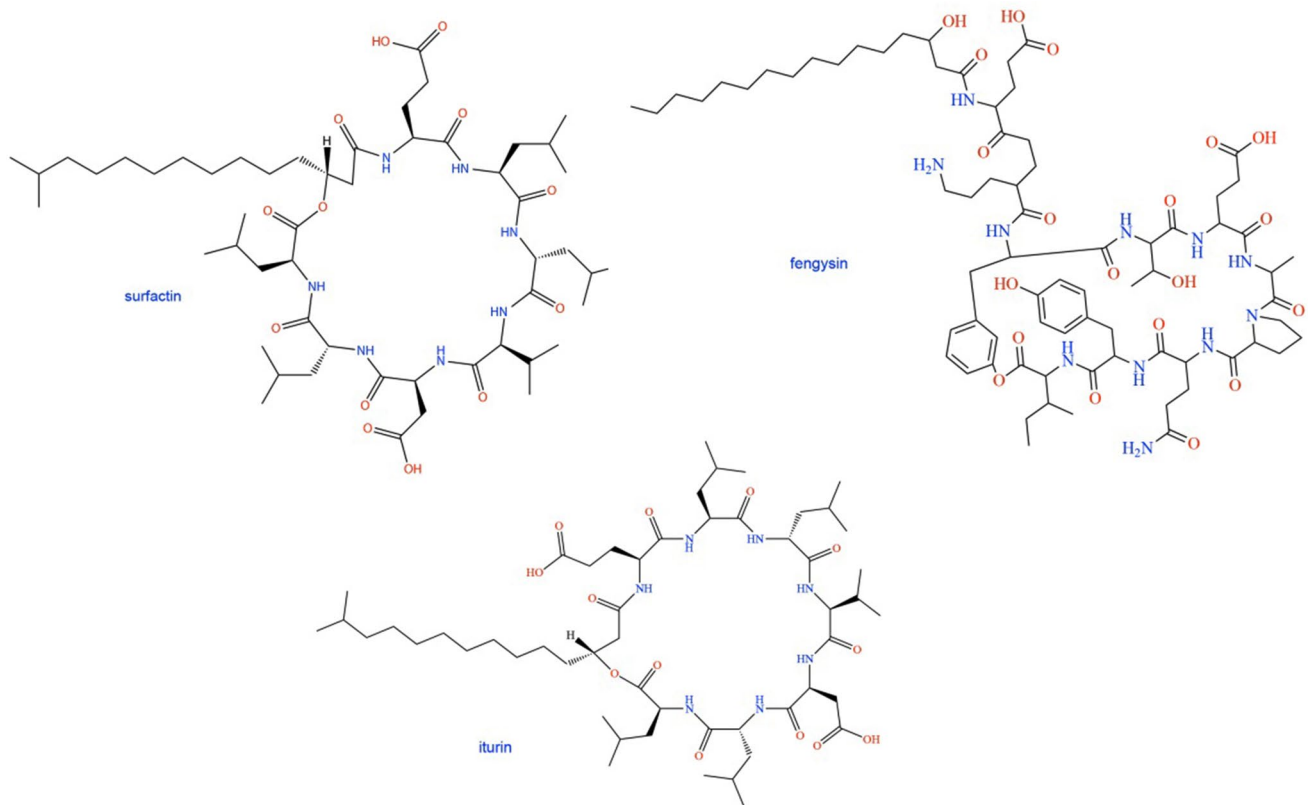


Fig. 3 Characteristic structure of surfactin, iturin and fengycin (structures produced based on the PubChem Compound database: <https://www.ncbi.nlm.nih.gov/pccompound/>)

Sobrinho et al. (2008) described a culture medium for the production of biosurfactants, using *Candida sphaerica* UCP 0995 with 5.0% ground nut oil refinery waste and 2.5% corn steep liquor in distilled water. They obtained biosurfactant production of 4.5 g/L, with an ST of 26 mN/m and a CMC value of 0.8 mg/L, demonstrating a potential use in oil recovery, removing 65% of oil from sand. Subsequently, Luna et al. (2013) reported a biosurfactant production of 9 g/L, using the same strain, *C. sphaerica* UCP 0995, in an optimized medium containing 9.0% ground nut oil refinery waste and 9.0% corn steep liquor. The biosurfactant they obtained reduced the ST of medium from 70 to 25 mN/m with a CMC of 0.25 mg/L, and recovered 95% of motor oil adsorbed to a sand sample. With this same medium and the same strain, Luna et al. (2015) produced biosurfactants in a 50 L bioreactor, obtaining 21 g/L after 144 h and reporting that the biosurfactant obtained reduced ST to 27 mN/m and was capable of dispersing approximately 90% of oil droplets in seawater. They found that the biosurfactant was both non-toxic to indigenous marine microbiota and solubilized motor oil in seawater.

Hazra et al. (2011) evaluated the rhamnolipid production by *P. aeruginosa* AB4 using de-oiled cakes of mahua (*Madhuca indica*), karanj (*Pongamia pinnata*),

jatropha (*Jatropha curcas*) and neem (*Azadiracta indica*). Achieving optimal production at 40 °C, pH 8.5 and a carbon:nitrogen (C:N) ratio of 10:0.6, they reported a maximum rhamnolipid production of 40 g/L and an ST of 40 mN/m when the strain was cultivated in basal mineral medium supplemented with 50 g/L of mahua oil waste. The biosurfactant presented Cd and Pb sequestration, suggesting its potential application in bioremediation.

Jin et al. (2014) studied the feasibility of using untreated rapeseed meal as a nitrogen source for producing cyclic lipopeptide iturin A via *B. subtilis* 3–10 in submerged fermentation in a 7 L bench-scaled bioreactor. They observed that rapeseed meal had a significant promoting effect on iturin A production, attaining a maximum concentration of 0.60 g/L with 70 g/L of glucose and 20 g/L of rapeseed meal, which was higher than the 0.06 g/L produced from peptone and the 0.48 g/L produced with ammonium nitrate media. Rapeseed meal was shown to have a positive induction effect on protease secretion, contributing to the release of soluble protein from solid rapeseed meal with low water solubility, providing an effective supply of available nitrogen during fermentation. Zouari et al. (2014) optimized the biosurfactant production by *B. subtilis* SPB1 under solid-state fermentation (SSF) using olive

mill by-products, employing a statistical experimental design and response surface methodology to optimize agro-industrial residue concentration, inoculum size and humidity. The best level of production was approximately 30.67 mg/g of dry solid material, obtained using a mixture of 6 g olive leaf residue flour (6.3% sugar and 8.05% fat content) and 4 g olive cake flour (3.4% sugar and 7.4% fat content).

Nalini and Parthasarathi (2014) explored the production of biosurfactants via *Serratia rubidaea* SNAU02 under SSF conditions using mahua oil cake as a substrate. The characterization of the biosurfactant obtained revealed the presence of rhamnolipids, which exhibited antifungal activity and showed no toxicity against *Brassica oleracea* and *Artemia salina* seeds. The antifungal activity of the rhamnolipids were shown to have a potential application as a biocontrol agent against plant pathogens.

Moya-Ramírez et al. (2015) reported the use of olive mill waste (OMW) as the sole carbon source in the production of biosurfactants from *B. subtilis* N1 and *P. aeruginosa* PAO1, with OMW at 2% w/v, the *B. subtilis* produced surfactin at a maximum concentration of 3.1 mg/L, while 10% OMW obtained a 0.57 mg/L concentration. In contrast, *P. aeruginosa* produced 8.78 mg/L of rhamnolipids with 2% w/v OMW, which increased to 191.4 mg/L with 10% OMW. Working with these same strains, this research group submitted the OMW to a hydrolysis pretreatment prior to using it as a carbon source for biosurfactant production, reporting rhamnolipid production of 299 mg/L at 2% OMW and 26.5 mg/L surfactin production at 5% OMW. The authors concluded that enzymatic hydrolysis effectively increases the production of these biosurfactants (Moya-Ramírez et al. 2016). Jiménez-Peñalver et al. (2016) produced sophorolipids with SSF, using *S. bombicola* ATCC 22214, obtained from winterization oil cake (WOC) (a residual oil cake produced by the oil refining industry) as a substrate and sugar beet molasses (MOL) as a co-substrate. Fermentation was performed on a 100 g scale and was optimized in terms of both the substrate ratio and the aeration rate, using response surface methodology. Carried out under static conditions, the optimized SSF process (1:4 MOL:WOC mass ratio and 0.30 kgL/min aeration rate) obtained a maximum production of 179 mg/g dry matter.

Jajor et al. (2016) used rapeseed cake to study the biosynthesis of structural analogues of surfactin in response to culture conditions (SSF, liquid culture and concentrations of oxygen) using two *B. subtilis* strains, KB1 and #309. The effect of the oxygen level in the SSF on surfactin production was different for the two strains tested. For strain #309, decreased oxygen availability (9:1) resulted in a reduction in surfactin biosynthesis (≈ 1.8 mg/L), in contrast, for strain KB1, decreased oxygen availability (1:1), increased the biosynthesis of surfactin to ≈ 11 mg/L. The amount of

air influenced the relative ratios of the surfactin analogues, with a lower oxygen amount decreasing the amount of C15 analogues produced and increasing the amount of C12 analogues produced. Hence, the biosynthesis of a desired surfactin analogue may be controlled by both strain type and culture conditions. Lourenço et al. (2018) produced a biosurfactant using white-rot fungus *Trametes versicolor* grown on two-phase olive mill waste (TPOMW) in SSF, achieving the highest level of biosurfactant production, of 373.6 mg/100 g of culture medium, with a medium containing 35% (w/w) TPOMW, 10% wheat bran and 55% olive stones. The biosurfactant produced was able to reduce the ST of an aqueous extract taken from the culture medium by up to 34.5 mN/m.

Radzuan et al. (2017) used palm oil agricultural refinery waste as the carbon source for the production of rhamnolipids via *P. aeruginosa* PAO1. With an initial concentration of 100 g/L oil waste, they reported a rhamnolipid production of 0.43 g/L, an ST of 29 mN/m and a CMC of 420 mg/L. Although they highlight the use of palm oil agricultural refinery waste as a potential substrate, the rhamnolipid production attained was very low in comparison with the reports mentioned above, such as Hazra et al. (2011), who produced 40 g/L of rhamnolipids with 50 g/L of mahua oil waste. Moreover, the CMC observed by Radzuan et al. was higher than the 51.5 mg/L reported by Nitschke et al. (2005) and the 122 mg/L reported by Abalos et al. (2001).

Recently, Jadhav et al. (2019) evaluated the use of sunflower oil refinery waste as feedstock for the production of sophorolipids via *Starmerella bombicola* MTCC1910. The maximum sophorolipid production found was 41.6 g/L at shake flask level and 51.5 g/L at fermenter level in a medium with 10% w/v of waste oil and 10% glucose. They found that the biosurfactant reduced the water ST to 35.5 mN/m and the IT (water/*n*-heptane) to 0.92 mN/m.

Although vegetable oil and residue from vegetable oil refineries are among the most used low-cost substrates (Nitschke et al. 2005) not all these substrates offer good results. The main problem associated with these substrates is the selection of suitable waste material with the right balance of nutrients that would enable cell growth and product accumulation (Makkar et al. 2011). The highest concentrations of biosurfactants obtained from vegetable oil residues are 51.5 g/L by *S. bombicola* MTCC1910, using sunflower oil refinery waste (Jadhav et al. 2019), 40 g/L via *P. aeruginosa* AB4P using mahua oil waste (Hazra et al. 2011), and 21 g/L via *C. sphaerica* UCP 0995 using ground nut oil refinery waste and corn steep liquor (Luna et al. 2013). Despite SSF appearing to favor the use of fungi and yeast to produce biosurfactants, only 373 mg/100 g of substrate was produced using *T. versicolor* on two-phase olive mill waste (Lourenço et al. 2018).

Biosurfactant production using frying oil waste

Many agricultural products are grown mainly for the production of food commodities, with their use in subsequent industrial processes or activities producing derivative residues. Common residues produced by the food industry are waste or used vegetable cooking oil (Go et al. 2019). The nutritional value of frying oils varies depending upon the food products fried and number of times it has been reused, with, generally, used oil containing 30% more polar hydrocarbons than fresh oil (Marmesat et al. 2007). The major components of used frying oils are triglycerides and diglycerides, as well as monoglycerides and free fatty acids to a lesser proportion (6–8%) (Martino et al. 2014).

Haba et al. (2000) investigated the use of waste sunflower oil and olive oil used for frying in the production of rhamnolipids via *P. aeruginosa* 47T2, producing 2.7 g/L of rhamnolipids and a yield of 0.34 g/g with the use of 40 g/L of waste frying oil and 5 g/L of NaNO_3 . Fleurackers (2006) demonstrated that the *Candida bombicola* ATCC22214 strain was able to produce sophorolipids using frying oil waste, obtaining a production of 5 to 10 g/L from the cultivation undertaken in the bioreactor. Furthermore, Shah et al. (2007) investigated the ability of *C. bombicola* to transform restaurant oil waste into sophorolipids in batch and fed batch fermentations, obtaining 34 g/L sophorolipid production in batch fermentation using 40 g/L restaurant oil waste, 100 g/L glucose and 1 g/L urea. Zhu et al. (2007) studied the production of rhamnolipids using waste frying oil and *P. aeruginosa* zju.ul M, reporting a production of 20 g/L under optimum conditions of 35 g/L waste frying oil. Sadouk et al. (2008) screened the production of glycolipids using *Rhodococcus erythropolis* 16 LM. USTHB in batch cultures with sunflower frying oil, finding that the crude product decreased the ST of water to 31.9 mN/m with a CMC of 287 mg/L. Moreover, de Lima et al. (2009) investigated the efficiency of the *P. aeruginosa* PACL strain to produce biosurfactants using different waste soybean frying oils via submerged fermentation in a 10 L stirred tank reactor in a complete factorial experimental design applied in order to optimize the aeration rate conditions and agitation speed. At optimum levels of 4% (v/v) soybean frying oil, the authors reported a maximum rhamnolipid concentration of 3.3 g/L, an emulsification index of 100% and a minimum ST of 26 mN/m. Under ideal conditions, they established the kinetic behavior and modeling of rhamnolipid production, as well as, the nutrient consumption and cellular growth for this strain.

Vedaraman and Venkatesh (2011), produced surfactin via *B. subtilis* MTCC 2423 in submerged cultivation using 50 g/L waste frying oil, 5 g/L yeast extract and 1.7 g/L mineral salts. They found ST decreases of \approx 29, 32 and 34.5 mN/m with glucose and waste sunflower and rice bran

frying oils, respectively. Although the highest yield (2.1 g/L) was obtained with the use of glucose, they concluded that this process both presented a safe option for the disposal of waste frying oil and reduced surfactin production costs. Zheng et al. (2011) produced rhamnolipids from *P. aeruginosa* DG30 with a mineral medium and 5% (w/v) of used vegetable oil. Although these authors do not mention the type of oil, they report 15.6 g/L biosurfactant production. Sand package tests showed an approximately 20% increase in oil recovery via the treatment with bacterial broth culture. Furthermore, George and Jayachandran (2012) used waste coconut frying oil (2%) to produce rhamnolipids from *P. aeruginosa* D, reporting a production of 3.55 g/L and an emulsification index (EI) of 71%.

Zadeh et al. (2017), produced 12.3 g/L of glycolipids via the fungus *Mucor circinelloides* used with a culture medium with 5% of waste frying oil. The glycolipids obtained reduced the ST to 26 mN/m, generated a clear 12.9 cm-diameter zone in the oil spreading test, and demonstrated the ability to emulsify 65% of crude oil in seawater. In the report published by Ozdal et al. (2017), rhamnolipid production via *P. aeruginosa* OG1 was statistically optimized via response surface methodology and, under optimized cultivation conditions, rhamnolipid production reached up to 13.3 g/L, recording an EI of 80% with 52 g/L of waste sunflower frying oil, 9.2 g/L of chicken feather peptone and 4.5 g/L of KH_2PO_4 . Kourmentza et al. (2018) used 4% sunflower-derived cooking oil with 5 g/L of peptone and 3 g/L of meat extract to simultaneously produce rhamnolipids and polyhydroxyalkanoates via the *Burkholderia thailandensis* E264 strain. Rhamnolipid production was 2.2 g/L, while ST values of 37.7 mN/m and IT values against benzene and oleic acid of 4.2 and 1.5 mN/m, respectively, were obtained, while the polyhydroxyalkanoates were accumulated simultaneously and comprised up to 60% of the cell dry weight.

Santos et al. (2018) reported the production of biosurfactants via *Streptomyces* sp. DPUA1559 isolated from lichens from the Amazon region, with the strain cultivated in mineral medium containing 1% waste soybean frying oil as the carbon source. The authors obtained 1.74 g/L of glycoprotein when the strain was cultivated at pH 8.5 at 28 °C, while the ST was 25.3 mN/m and the CMC was 0.01 g/L. Additionally, the biosurfactant isolated showed no toxicity to either the micro-crustacean *Artemia salina* or lettuce (*Lactuca sativa*) and cabbage (*Brassica oleracea*) seeds. Subsequently, using this same strain, the research group applied a full 2^4 factorial design in order to study the effects of pH, percentage aeration, agitation and temperature on ST and EI. The maximum biosurfactant concentration was 1.9 g/L in conditions of 10 g/L waste soybean frying oil and 20 g/L corn steep liquor at pH 8.5, 150 rpm, 28 °C and 80% air saturation. The biosurfactant reduced the ST of water to 28 mN/m with a CMC of 0.8 mg/L. The biomolecule, which

was characterized as a lipoprotein and denominated bioelan, did not exhibit toxicity against vegetable seeds or brine shrimp (Santos et al. 2019). Similarly, Hentati et al. (2019) produced lipopeptides via *Bacillus stratosphericus* FLU5 in a basal medium with 1% waste frying oil, reporting a CMC for purified lipopeptide of 50 mg/L and an ST reduction of 72 to 28 mN/m. In addition, the use of the biosurfactant for oil recovery from hydrocarbon-contaminated soil showed greater effectiveness in the hydrocarbon remobilization than the synthetic surfactants tested. Soares da Silva et al. (2019) produced 40.5 g/L of biosurfactant via *P. cepacia* CCT6659, using a basal medium with 2% waste frying canola oil, 3% corn steep liquor and 0.2% NaNO₃. The ST was reduced to 29 mN/m.

According to the reports described above, a higher concentration of biosurfactant, without the addition of a refined substrate such as glucose, was obtained using *P. aeruginosa* zju.ul M (Zhu et al. 2007) and *P. cepacia* CCT6659 (Soares da Silva et al. 2019). That a cheap raw material can be used to support biosurfactant production without requiring the extra addition of a refined carbon source, such as glucose, is a notable finding.

Fruit and vegetable waste in the production of biosurfactants

The industrial processing of fruits and vegetables, such as cashew apple juice, and the peels from the banana, pineapple, orange, carrot and lime, generates large amounts of waste that can also be exploited for the production of biosurfactants.

The cashew apple is a tropical pseudo fruit, the real fruit of which is the cashew nut. Cashew nut production generates large amounts of cashew apples as a byproduct, only 12% of which is consumed as fresh fruit or processed industrially for the production of juices or desserts, with large amounts remaining in the soil and causing environmental pollution (Rocha et al. 2007). Its carbohydrate-rich composition, vitamin and mineral salt composition makes the cashew apple a valuable raw material for various potential applications (Rocha et al. 2007).

Rocha et al. (2006) evaluated the ability of *Acitenobacter calcoaceticus* RAG-1 to produce emulsan using cashew apple juice (CAJ), reducing ST by approximately 17% and achieving an EI of 59% for kerosene. In another study, Rocha et al. (2007), evaluated the ability of *P. aeruginosa* ATCC 10145 to produce rhamnolipids in a mineral medium supplemented with CAJ, which containing 90 to 97 g/L carbohydrate. The highest ST reduction was 29.5 mN/m, while the highest level of rhamnolipid production was 3.8 g/L, which was achieved via the supplementation of CAJ with 5 g/L of peptone. Later, Rocha et al. (2009) evaluated the surfactin production by *B. subtilis* LAMI008 in mineral medium

supplemented with clarified CAJ with 86.1 g/L carbohydrate content. The highest ST reduction of 38.1 mN/m was attained when the medium was supplemented with 5 g/L of yeast extract, while surfactin concentration was 3.5 mg/L, which was significantly lower than the 3.8 g/L rhamnolipid level reported by the same research group, using unclarified CAJ (Rocha et al. 2007). Subsequently, the same group undertook similar research with the *B. subtilis* LAMI005 strain, reporting that the highest level of surfactin production, 123 mg/L, was recorded after 48 h of fermentation using clarified CAJ supplemented with mineral medium. However, the production level obtained was almost two times lower than the amount produced using mineral medium supplemented with 10 g/L glucose and 8.7 g/L fructose. The CMC of the biosurfactant produced using clarified CAJ was 2.5 times lower than that produced using medium with glucose and fructose, thus suggesting a higher biosurfactant efficiency (Giro et al. 2009).

Using the strain LAMI005, de Oliveira et al. (2013) produced surfactin in a medium formulated with clarified CAJ, distilled water and (NH₄)₂SO₄. The crude biosurfactant obtained decreased the water ST to 30 mN/m with a CMC of 63 mg/L. The authors concluded that the production of surfactin was influenced by the amount of CAJ used in the media, reaching a maximum concentration of 319 mg/L. Fontes et al. (2012) produced a biosurfactant synthesized by *Yarrowia lipolytica* using a medium with 10 g/L (NH₄)₂SO₄, 0.5 g/L yeast extract and 3% v/v CAJ or crude glycerol, reporting a production of 6.9 and 7.9 g/L, an EI of 68 and 70% and ST values of 18 and 22 mN/m with CAJ and glycerol, respectively. Moreover, the production of biosurfactants from CAJ by *P. aeruginosa* MSIC02 was investigated by carrying out a 2⁴ full factorial experimental design. The authors reported a greater ST reduction of 27.5 mN/m, concluding that the increase in the temperature of the culture (37 °C) and the reduction of glucose present in CAJ (5 g/L), caused said ST reduction, thus indicating a higher level of biosurfactant production (Rocha et al. 2014).

Another waste product reported in biosurfactant production has been the banana peel, considered the main byproduct of industrial banana processing (Saisa-Ard et al. 2013). Chooklin et al. (2014) used the banana peel as the sole carbon source for the production of lipopeptides by *Halobacteriaceae archaeon* AS65, reporting a 5.3 g/L production level when the cells were incubated in mineral salt medium containing 35% (w/v) banana peel and 1 g/L commercial monosodium glutamate. The lipopeptide obtained presented an ST of 25.5 mN/m, a CMC value of 10 mg/L and a broad spectrum of antimicrobial activity, as well as increasing the solubility of polyaromatic hydrocarbons. George and Jayachandran (2009), in addition to banana waste, used orange fruit peelings, carrot peel waste, lime peelings and coconut oil cake to produce rhamnolipids via *P. aeruginosa* MTCC

2297. They found that orange peel (3% w/v) was the best substrate, generating 9.2 g/L of rhamnolipids, with an ST reduction of 31.3 mN/m. Similarly, Kumar et al. (2016) evaluated the potential of *B. licheniformis* KC710973 to produce lipopeptides using orange peel, banana peel, potato peel and two commercial extracts containing citrus peel. Their results showed that the orange peel (4%) was the best substrate for biosurfactant production, generating 1.8 g/L and an EI of 75% against diesel. The biosurfactant production achieved by this group was lower compared to the rhamnolipid levels obtained by George and Jayachandran (2009) with 1% less orange peel, suggesting that biosurfactant production is dependent on the types of waste and strain utilized.

Rane et al. (2017) investigated the potential of *B. subtilis* ANR 88 to grow in minimal medium with different carbon sources, such as extracts of potato peel, orange peel, banana peel and bagasse, as well as molasses and whey. After optimizing the conditions with 4% molasses and 0.25% ammonium ferric citrate, the production of lipopeptides increased from 0.241 to 0.746 g/L. The lipopeptide was effective in the synthesis of gold and silver nanoparticles in the total absence of conventional chemical reducing agents.

Paraszkiwicz et al. (2018) reported the production of lipopeptides by KP7 and I'-1a strains of *B. subtilis* grown on various media prepared from two different brewery wastewaters, beet molasses, apple peel extract and carrot peel extract, supplemented with yeast extract or peptone. The highest concentration of the lipopeptide iturin was the 428.7 mg/L obtained by *B. subtilis* I'1a, cultivated with 2% carrot peel extract and 0.25% peptone. The authors concluded that the level of production and structural diversity of the synthesized lipopeptides were dependent on the composition of medium.

Almeida et al. (2012) produced biosurfactants from a strain of *Pantoea* sp. isolated from effluent of ice cream industry waste. In a medium formulated with pineapple peel juice and corn oil, they obtained an ST reduction in distilled water of 36 mN/m and a CMC of approximately 10 mg/L. The biosurfactant was stable at a salinity of 2.5 to 5% of NaCl, a higher temperature (121 °C for 60 min) and a range of 6 to 8 pH.

Investigating the production of biosurfactants by *Candida glabrata* UCP1002 using vegetable fat waste, de Gusmão et al. (2010) applied a factorial design to study the effects on ST of, and interactions among, the waste, yeast extract and glucose. They found a CMC of 10 mg/L, obtaining a maximum ST reduction of 24 mN/m with 5% waste vegetable fat and 0.2% yeast extract. Using *C. bombicola* NRRL-Y 17069, Parekh et al. (2012) compared solid state fermentation and submerged fermentation in the production of sophorolipids from mango kernel fat waste (*Mangifera indica*). They reported a sophorolipid production of 17.48 g/100 g via SSF with 2 g glucose and 2 g of lipid substrate taken from

mango kernel fat, with 6 g wheat bran powder used as a solid support. In contrast, submerged fermentation produced 5.8 g/100 g substrate with 40 g/L glucose, 5 g/L yeast extract and 20 g/L lipid sources of mango fat. Although the authors reported high yields via SSF and considered that mango kernel fat is a cheap raw material, its use requires the downstream processing of the mango kernel in order to obtain an oleic acid-rich fraction, which translates into higher costs for industrial-level production. Jain et al. (2013), evaluated the ability of *Klebsiella* sp. RJ-03 to produce biosurfactants using unconventional carbon sources such as corn powder, potato peel powder, *Madhuca indica* and sugarcane bagasse. They produced 9.6 g/L and 6.2 g/L with potato peel powder (64% of sugars) and sugar cane bagasse (65% of sugars), respectively, achieving superior stability at high temperatures, a wide range of pH and salt stress. The biosurfactant obtained was highly effective in removing oil from both soil and clothing.

As can be seen, biosurfactant concentrations of 1.0 g/L or over were obtained using fruit and vegetable waste or residues as a substrate, such as the 9.6 g/L obtained via potato peel powder and the 6.2 g/L obtained via sugar cane bagasse, both of which were used with *Klebsiella* sp. RJ-03 (Jain et al. 2013). Other biosurfactants obtained with concentrations above 1.0 g/L are the 9.2 g/L of rhamnolipids produced with orange peel via *P. aeruginosa* MTCC 2297 (George and Jayachandran 2009), the 5.3 g/L of lipopeptides produced with banana peel via *H. archaeon* AS65 (Chooklin et al. 2014), and the 1.8 g/L of surfactin produced with orange peel via *B. licheniformis* KC710973 (Kumar et al. 2016).

Biosurfactant production from starch-rich waste

The industrial extraction of starch from crops such as corn, rice, cassava, wheat and the potato generate high amounts of wastewater, rich in starch and husks, that can serve as a substrate for the production of various products, including biosurfactants. For example, the potato is rich in starch (16–20%), protein (2–2.5%), fiber (1–1.8%) and fatty acids (0.15%). One raw potato, including peel, contains high levels of potassium, vitamins B and C, and minerals, such as phosphorus, magnesium and iron (Graeme and Sansonetti 2009). Fox and Bala (2000) evaluated potato substrates as an alternative carbon source for producing surfactants from *B. subtilis* ATCC 21332 in shake flasks. They compared the performance of an established potato medium, simulated liquid and solid potato waste media, and commercially prepared potato starch in mineral salts medium. The authors reported an ST reduction from 71.3 to 28.3 mN/m in solid medium and a CMC of 100 mg/L, when the strain was cultivated in 60 g/L of potato substrate only, without the addition of another nutrient. Fox and Bala argue for the replacement of the traditional carbon sources used for biosurfactant

production with potato substrates, stating that this could alleviate the waste management burden of the potato industry while addressing the economic issues related to surfactant production cost.

Thompson et al. (2000) evaluated the use of potato process effluent with high solid and low solid (16.2 and 6.5 g/L respectively) content for surfactin production by *B. subtilis* 21332. The potato effluent was diluted at 1:10, and were used both unmodified and modified via the addition of trace minerals or corn steep liquor. The performance of surfactin produced from low solids surpassed that of high solids in all cases, obtaining an ST reduction of 26.2 mN/m and a production level of 0.44 g/L in low solid medium. Additionally, the authors showed that corn steep liquor greatly lowered surfactin production. Subsequently, this research group produced surfactin using the same strain with low-solid potato effluent in a chemostat operated in batch mode, reporting a production of 0.9 g/L after 48 h (Noah et al. 2005).

Das and Mukherjee (2007) reported the efficiency of the *B. subtilis* strains DM-03 and DM-04 in the production of lipopeptides using 5 g potato peel as a substrate in SSF and 2% w/v of substrate in submerged fermentation. Lipopeptide production by *B. subtilis* DM-03 was 80 and 67 mg/g in submerged fermentation and SSF, respectively. Wang et al. (2008) co-produced fengycin and poly- γ -glutamic acid (γ -PGA) via the SSF of *B. subtilis* B6-1, using 5 g/L soybean curd and 5 g/L sweet potato waste. Lipopeptide concentration classified as biological activity reached a maximum level at 54 h, while the maximum yield of 3.63% γ -PGA was obtained at 42 h. The authors highlighted the ability of these lipopeptides to be used as both biological control agents and fertilizer synergists.

Another residue rich in carbohydrates that has also been used for biosurfactant production is cassava flour wastewater (CWW). One of the first studies to produce biosurfactants in a medium containing CWW was conducted by Nitschke and Pastore (2003), who produced biosurfactants from *Bacillus* sp. LB5a, with the substrate subjected to different treatments, including the removal of solids and dilutions. The results obtained showed that the bacteria were able to grow and produce biosurfactants in all media, with the best performance an ST of 26.6 mN/m, obtained from the media containing no solids, which was diluted 1:2 and had a total carbohydrate content of 30.2 g/L. In another study, the same group evaluated the biosurfactants produced by the *B. subtilis* strains ATCC 21332 and LB5a via the use of CWW. The ATCC 21332 strain produced 2.2 g/L of crude biosurfactant and reduced the ST of the medium to 25.9 mN/m, while *B. subtilis* LB5a reduced the ST of the medium to 26 mN/m, obtaining a crude biosurfactant concentration of 3.0 g/L (Nitschke and Pastore 2004). This same research group later reported the production and properties of the biosurfactants obtained from the *B. subtilis* LB5a strain

grown on CWW with 35.3 g/L total carbohydrate content. They reported that the microorganism was able to grow on CWW and produce lipopeptides, reducing the ST of the medium to 26.6 mN/m and providing a crude lipopeptide concentration of 3.0 g/L and a CMC of 33 mg/L. In addition, the biosurfactant was capable of forming stable emulsions with several hydrocarbons, maintaining its properties at high temperatures (100 °C), high salinity (20%) and a wide pH range (Nitschke and Pastore 2006). With the same strain and CWW with a 36.2 g/L total carbohydrate content, Barros et al. (2008) produced biosurfactants into a 40 L batch pilot bioreactor. The kinetic data corresponding to the process showed that both the microbial population and foam production coinciding with the highest level of biosurfactant production. The production of semi-purified biosurfactant in the foam was 2.4 g/L, while the ST of the medium was 27 mN/m and the CMC was 11 mg/L. The authors concluded that biosurfactant production using CWW on a pilot scale was a viable process. Moreover, they concluded that the biosurfactant produced in this medium presented high surface activity and low CMC values, thus characterizing it as an effective surfactant. Also using *B. subtilis* LB5a, this research group then produced surfactin from CWW in a 56 L volume bioreactor, reporting a crude biosurfactant concentration of 0.3 mg/mL, an ST reduction of 26 mN/mL, and a CMC of 28.3 mg/L. With surfactin obtained, they evaluated the anaerobic biodegradability of the effluent from a poultry slaughterhouse, achieving the elimination of oil and grease over 70% (Cosmann et al. 2017).

Using CWW, whey and activated carbon at different concentrations, de Andrade et al. (2016) optimized the simultaneous production of surfactin and 2,3-butanediol by *B. subtilis* LB5a. Their central composite design experiments indicated that the best substrate composition for both bio-products was 28 g/L whey, 25 g/L activated carbon and 74 g/L CWW. Bioprocessing at bench-top scale achieved the simultaneous production of ≈ 27.07 mg/L surfactin and ≈ 330 mg/L 2,3-butanediol. These results indicate an interesting strategy for simultaneous production using alternative substrates. Then, de Andrade et al. (2017) evaluated the production of mannosylerythritol lipids-B (MEL-B) by *Pseudozyma tsukubaensis* using 3 L CWW, reporting a production of 1.26 g/L and the purification of MEL-B in a single step, by ultrafiltration. The highest ST reduction obtained was 26 mN/m.

Costa et al. (2009) also evaluated CWW, along with glycerol and waste frying oil, to simultaneously produce rhamnolipids and polyhydroxyalkanoates (PHAs) via *P. aeruginosa*. The best overall production of rhamnolipids and PHAs was obtained with CWW (with a glucose content of 30 g/L) and 2% (w/v) waste frying oil, comprising a PHA production of 39% cell dry weight and 660 mg/L rhamnolipid production. Under these conditions, the ST fell to 30 mN/m while

the CMC was 26.5 mg/L. Recently, Araújo et al. (2019) obtained biosurfactants via *Serratia marcescens* UCP1549 and, applying a full-factorial design. They obtained the highest ST reduction of 25.9 mN/m in a medium containing 6% CWW, 0.2% lactose and 5% corn waste oil. The biosurfactant isolated exhibited a CMC of 15 mg/L, high stability under different temperatures, salinity levels and pH values, and was not toxic against cabbage seeds.

Another feedstock reported for biosurfactants production is rice mill polishing residue. Rice grain has a hard-outer coating (husk) covering the rice endosperm. Found between the grain and the husk there is a dark brown colored layer commonly known as rice bran and which contains 20% oil holding and more than 65% nutrients minerals such as Fe, Ca, Mg, Mn, K and Zn. Gurjar and Sengupta (2015) used rice mill polishing residue to produce surfactin via *B. subtilis* MTCC 2423 in a submerged fermentation process, with 4.8 g/L of carbohydrates present in the medium, reporting a yield of 4.17 g/kg waste. The recovered product in the foam accounted for 69% of the total yield, while the highest ST reduction achieved was 27 mN/m.

Zhu et al. (2012), produced lipopeptides via *Bacillus amyloliquefaciens* XZ-173 in SSF using soybean flour and rice straw as the substrate. Under optimal conditions of 1.8% starch, 1.9% yeast extract, 5.6 g soybean flour and 3.7 g rice straw, the results revealed a maximum lipopeptide production of 50 mg/g substrate. The lipopeptides extracted from fermented substrates showed strong antibiotic activity against *Rhizoctonia solani* and *Ralstonia solanacearum*. Freitas-Silva et al. (2012) investigated the potential of *Rhizopus arrhizus* to produce biomass and biosurfactants using rice bran husks and corn steep liquor. The highest biomass production of 9.1 g/L was obtained with 8% corn steep liquor and 3% rice bran husk, while and the highest water ST reduction of 26.5 mN/m was obtained with 6% corn steep liquor and 2% rice bran husk. Oje et al. (2016) evaluated the effect of acid and alkaline pretreatment on rice husks in the production of glycolipids, using the fungus *Mucor indicus*. Their results revealed a highest glycolipid yield of 0.78 g in 100 mL of culture with 50 g/L rice husk, 3 g/L NaNO₃ and 2 g/L mineral salts, with alkaline-pretreated rice husks. The highest EI recorded by the glycolipids produced was for automotive gas oil.

Cassava wastewater seems to favor lipopeptide production, where concentrations of 2.2 g/L have been obtained via *B. subtilis* ATCC 21332 (Thompson et al. 2000), and 3.0 g/L by *B. subtilis* LB5a (Nitschke and Pastore 2004, 2006). Cassava wastewater was also used to obtain 1.26 g/L of MEL-B via *P. tsukubaensis* (de Andrade et al 2017). It should also be noted that rice bran husk was used to produce 9.1 g/L of biosurfactant via *R. arrhizus* (Freitas-Silva et al. 2012).

Lignocellulosic waste in the production of biosurfactants

Lignocellulose is one of the most abundant sources of organic carbon, with primary cellulosic materials derived from plants grown specifically for their cellulose content. Portilla-Rivera et al. (2007) were the first researchers to analyze the capacity of *Lactobacillus pentosus*, used on hydrolyzed distilled grape marc (10.8% cellulose, 11.2% hemicellulose and 51% lignin) supplemented with corn steep liquor (10 g/L) and yeast extract (10 g/L), to produce both biosurfactants and lactic acid. They obtained a final lactic acid concentration of 5.5 g/L and an intracellular biosurfactant production of 4.8 mg/L, representing a 0.60 mg/g yield of sugars consumed. In a later study, Portilla-Rivera et al. (2008) evaluated the stability and emulsifying capacity of biosurfactants obtained from *L. pentosus* after growing cells on distilled grape marc hydrolysates and walnut and hazelnut shells. The biosurfactant obtained from distilled grape marc hydrolysates (with a 12.5 g/L hemicellulosic sugars content) produced relative emulsion volume values of close to 50% and was found to be stable after 72 h, with the use of gasoline or kerosene. The emulsion volume values obtained were higher than those achieved using commercial surfactin, at 14.1% for gasoline and 27.2% for kerosene.

Cortés-Camargo et al. (2016), used the *Bacillus tequilensis* ZSB10 strain, isolated from Mexican brines, to produce extracellular and cell-bound biosurfactants via culture broths formulated from hydrolysates obtained from cellulosic and hemicellulosic fractions taken from vine-trimming waste mixed with mineral medium. They obtained crude extracellular biosurfactant production of 1.52 g/L, an ST reduction of 38.6 mN/m, a CMC of 177 mg/L and an EI of 47%, with kerosene. The crude cell-bound biosurfactant produced only reached 0.078 g/L and presented a lower EI (41%) than the extracellular biosurfactant. Vecino et al. (2017) used cellulosic sugars extracted from vineyard pruning waste as a carbon source for biosurfactant production by *Lactobacillus paracasei* A20. Their results obtained showed that, when glucose from vineyard pruning waste was used, the biosurfactant obtained was a glycolipopeptide, whereas the biosurfactant produced was a glycoprotein when the waste was replaced by lactose. These authors highlighted the possibility of producing biosurfactants “a la carte”, with the same strain but changing the carbon source, thus increasing the potential for different industrial applications.

In order to reduce production costs and, consequently, make the process sustainable, Konishi et al. (2015) used a corncob hydrolysate medium to produce sophorolipids via *Starmerella bombicola* NBRC 10243. They obtained 49.2 g/L of sophorolipids, with a volumetric productivity of 12.3 g/Ld, using a medium with 50 g/L olive oil and a corncob hydrolysate containing 45 g/L glucose. Samad

et al. (2015), evaluated the capacity of *C. bombicola* ATCC 22214 to produce sophorolipids from lignocellulosic hydrolysates derived from sweet sorghum bagasse and corn fiber. The sophorolipid concentration produced was 3.6 and 1.0 g/L with bagasse and corn fiber, respectively; however, the addition of 100 g/L soybean oil to the culture medium increased sophorolipid concentration to 84.6 and 15.6 g/L, respectively. Similarly, Samad et al. (2017) investigated the production of sophorolipids from sweet sorghum bagasse and corn stover via *C. bombicola*. They obtained 52.1 g/L of sophorolipid when corn stover hydrolysate (87.3 g/L glucose, 59.4 g/L of xylose and 3.1 g/L of arabinose) and yellow grease (10 g/L) were used as substrate in a 3 L fermenter. The authors mentioned that lignocellulosic feedstocks for producing sophorolipids represents a potentially sustainable and renewable approach for generating these compounds. Recently, Marcelino et al. (2019) used sugarcane bagasse hemicellulose hydrolysate as a carbon source to produce sophorolipids from the yeast *Cutaneotrichosporon mucooides* UFMG-CM-Y6148. Biosurfactant production reached a maximum concentration of 12.5 g/L in 72 h and a volumetric biosurfactant productivity of 0.167 g/Lh with a mineral medium supplemented with 40 g/L of detoxified hydrolysate.

The latter two studies, discussed above, show that lignocellulosic residues could be good alternative low-cost carbon sources for producing sophorolipids, with 52 g/L of biosurfactant obtained by *C. bombicola* and 12.5 g/L of biosurfactant produced via *C. mucooides*. However, the pretreatment processes required to make lignocellulosic residues available for microorganisms, such as drying, the reduction of particle size, pre-hydrolysis, and chemical and/or enzymatic hydrolysis, may raise the global cost of biosurfactant production.

Animal waste in biosurfactant production

Meat processing industries generate large amounts of waste animal fat, tallow and lard, which have been used for the production of biosurfactants. Deshpande and Daniels (1995) produced sophorolipids via *C. bombicola* ATCC 22214 using 100 g/L fat, 100 g/L glucose, 4 g/L corn steep liquor and 100 g/L urea, reporting a production of 120 g/L at 27 °C in a 68 h timeframe.

Borges et al. (2012) examined the production of rhamnolipids by the *P. aeruginosa* strains ATCC 9027 and 101045 using waste obtained from a treatment station for floating grease waste at a poultry and pig slaughterhouse, residual brewery yeast and ammonium nitrate. Optimizing conditions via a central composite design, they obtained a rhamnolipid concentration of 3.84 g/L, an ST of 27.5 mN/m and an EI of 100% from the use of the *P. aeruginosa* 101045 strain with 12 g/L fat and 15 g/L yeast residues, and without ammonium nitrate.

Similarly, Santos et al. (2013) evaluated the ability of *C. lipolytica* UCP0988 to produce glycolipids in a medium with 5% bovine fat and 2.5% corn steep liquor, demonstrating that corn steep liquor favored growth, as growth was poor when fat alone was used as carbon source. The crude biosurfactant was effective in recovering up to 100% of the motor oil from the walls of the beakers and was able to reduce ST to 28 mN/m. Subsequently, this same research group maximized glycolipid production from the UCP0988 strain via cultivation with 5% animal fat and 2.5% corn steep liquor. The results showed the highest production of 8 g/L at 120 h and agitation of 200 rpm, with the biosurfactant demonstrating a strong potential application in the clean-up of oil spills both at sea and on shorelines. (Santos et al. 2014). After characterizing the optimal production conditions for the UCP0988 strain, they also investigated the potential application of the biosurfactant obtained from this strain in remediation processes for the hydrophobic pollutants and heavy metals generated by the oil industry. The yeast was cultivated in a submerged culture with 5% bovine fat and 2.5% corn steep liquor. The biosurfactant removed 70% of motor oil from contaminated cotton cloth in a detergency test, also removing 30–40% Cu and Pb from standard sand and ~30% of the heavy metals (Santos et al. 2017).

Bhange et al. (2016) simultaneously produced keratinolytic protease, amylase and biosurfactant via *B. subtilis* PF1. In an optimized medium with 12.5 g/L chicken feather meal, 12.5 g/L potato peel and 6 g/L rape seed cake, they increased protease production by 2.28%, amylase production by 0.85% and biosurfactant production by 1.2%. The stability of biosurfactants across a broad range of temperature and alkaline environments suggested its potential application in laundry detergents.

Minucelli et al. (2017) evaluated the production of sophorolipids by *C. bombicola* ATCC 22214 using chicken fat, sunflower oil, sugarcane molasses, sugarcane juice, sucrose or glucose. The production of sophorolipids was 39.8 g/L under optimal conditions of 75 g/L chicken fat, 77.5 g/L glucose and 2.5 g/L yeast extract, while the ST was 35 mN/m and the CMC was 65 mg/L.

Recently, Chaves-Martins and Guimarães-Martins (2018) produced biosurfactants from different industrial waste using *Corynebacterium aquaticum* and *Corynebacterium* sp. CCT 1968, with the waste materials used including sugarcane bagasse, fish waste (heads, bones, skin, scales, muscles and viscera), crude glycerol and petroleum sludge from storage tanks. The microorganism *C. aquaticum* showed efficient biosurfactant production with the use of 3% sugarcane bagasse, and 3% fish residue as a carbon source. The ST obtained from sugarcane bagasse treatment was 27.8 mN/m and 33.9 mN/m from the fish residue, with an EI of 87.6 and 61.6%, respectively. *Corynebacterium* sp., produced biosurfactants only in the medium containing 3% fish waste,

obtaining an ST value of 28.5 mN/m. The biosurfactant applied demonstrated a potential use in solubilization and paint removal.

Despite the low number of studies carried out with animal waste, its use in glycolipids production via *C. bombicola* yeast seems to be effective. Deshpande and Daniels (1995) reported obtaining 120 g/L using animal fat, while Minucelli et al. (2017) reported obtaining 39.8 g/L using chicken fat. However, in both studies, the culture media were supplemented with glucose. In contrast *P. aeruginosa* 101045 was used to produce 3.84 g/L of rhamnolipids from animal fat and brewery yeast residues without the extra addition of refined raw materials.

Biosurfactant by the numbers

Among the various microbial surfactants, glycolipids such as sophorolipids and rhamnolipids are still the most promising candidates for massive production and successful commercialization, owing to their superior physicochemical properties and, above all, their higher product titer (Dhanarajan and Sen 2014). In contrast, while lipopeptides are produced in lower quantities, they have great added value due to their exceptional biological properties (Dhanarajan and Sen 2014). The only report of a higher level of lipopeptide production is found in Yoneda et al. (2006), who mutated *B. subtilis* SD901 using the chemical mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and obtained a maximum 50 g/L surfactin production in a soybean flour medium. It should also be noted that rhamnolipids have been produced at a maximum concentration of 112 g/L via a mutation of *P. aeruginosa* DSM 7107, using soybean oil and olive oil as substrate (Giani et al. 1997).

In economic terms, biosurfactants will replace synthetic surfactants only when the costs of raw materials and processing become less expensive (Gong et al. 2015). Synthetic surfactants have an approximate market price of USD \$2/kg (Santos et al. 2016). According AGAE Technologies, LLC (USA), the current market price for rhamnolipids is within the range of USD \$1.5–\$1500/kg, depending on the level of purity and the manufacturer. AGAE Technologies sells rhamnolipids at 90% and 95% purity at a price of USD \$1250/kg and USD \$399.00/g, respectively (www.agaetech.com). Shandong Qilu Biotechnology Group Co. Ltd. sells sophorolipids at a price of approximately USD \$20.00–50.00/kg (www.qilugroup.com), while the surfactin, iturin and fengycin marketed by Sigma-Aldrich Co. LLC, USA cost USD \$206.00/10 mg, 527.00/5 mg and \$530.00/5 mg, respectively (www.sigmaaldrich.com). Given these prices, it seems logical that glycolipid type biosurfactants, such as sophorolipids and rhamnolipids, must be produced in large quantities to be economical, while, in

contrast, lipopeptides, such as surfactin, iturin and fengycin, are more expensive as they are produced in lower quantities.

Ashby et al. (2013) reported in large scale, 19,832 m³, sophorolipid production at a cost of USD \$2.95/kg, with sophorolipids produced using glucose and high oleic sunflower oil costing USD \$2.54/kg, while those produced using glucose and oleic acid cost USD \$2.54/kg. They state that refined substrates can be substituted for industrial by-products and agro-based low-cost raw materials. In comparison, Soares da Silva et al. (2018), estimated a price of USD \$20/kg for isolated glycolipid (40.5 g/L) produced in a 50 L fermenter via *P. cepacia* with canola frying oil, thus demonstrating that residues can be used for economical biosurfactant production without the extra addition of refined substrates.

The prices in USD of the cheap raw materials described above are as follows: corn steep liquor, \$0.46/kg; cane bagasse, \$0.04/kg; CAJ, \$0.30/kg; cashew apple, \$0.50/kg; and, rice husk \$0.08/kg (Rocha et al. 2007; Nurfarahin et al. 2018). The prices quoted above are one or two orders of magnitude lower than those for the refined raw materials that can be obtained from a local provider in México: glucose, USD \$0.70/kg; sucrose, USD \$0.49/kg; and, glycerol, USD \$0.43/kg.

Conclusion and future research perspective

To choose a good agro-industrial waste or residue for biosurfactant production, basic considerations must consider such as availability of material and cost of transportation, minimize or avoid pretreatment steps and avoiding extra addition of refined raw materials to production media. In the other hand, research efforts must be conducted to find robust microorganisms than can use agro-industrial waste or residues in such conditions and produce higher titers of biosurfactant. Depending on the type of biosurfactant, the producer strain, the process applied and the degree of purity, particular types of waste may be suitable for use as raw materials in the bioprocesses. As raw materials constitute about 50% of the overall biosurfactant production (Rufino et al. 2014), the use of cheaper agro-industrial waste and low-cost renewable substrates can lead to the significant reduction of the operating costs involved in the process. Therefore, the use of agro-industrial waste, of both animal and vegetal origin, for biosurfactants production is a potential approach for reducing production costs and would make biosurfactants economically viable and commercially competitive with synthetic surfactants.

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