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PRODUÇÃO DE BIOSURFACTANTES UTILIZANDO RESÍDUOS DA INDÚSTRIA DE ALIMENTOS E AGROINDUSTRIAIS: UMA BREVE REVISÃO

PRODUCIÓN DE BIOSURFACTANTES A PARTIR DE LOS RESIDUOS DE LA INDUSTRIA DE ALIMENTOS Y AGROINDUSTRIALES: UNA BREVE REVISIÓN

BIOSURFACTANT PRODUCTION FROM FOOD AND AGROINDUSTRIAL WASTE: A BRIEF REVIEW

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RESUMO

Os efeitos da degradação ambiental decorrentes das atividades industriais e urbanas vem atingindo níveis cada vez mais insustentáveis, surgindo a necessidade de desenvolvimento de uma consciência ambiental, através do reaproveitamento e reprocessamento de produtos e resíduos. Neste sentido, o reaproveitamento de resíduos alimentícios e agroindustriais vem adquirindo uma notória importância, visto que estes resíduos representam matérias-primas de baixo custo para a obtenção de produtos de elevado valor agregado. Aliado a isto, o estudo da produção de biomoléculas, como exemplo os biossurfactantes, principalmente glicolipídeos e lipopeptídeos, tem crescido nos últimos anos. Com utilização em diversos segmentos industriais, como de cosmético, de alimentos, de produtos de higiene pessoal e de limpeza, os biosurfactantes são agentes de superfície constituídas de uma porção hidrofóbica e uma porção hidrofílica. Apesar da ampla gama de aplicações e das diversas vantagens, a produção em grande escala de biossurfactantes é escassa, principalmente devido aos altos custos. Assim, a utilização de substratos residuais é uma das estratégias para viabilizar a produção destas biomoléculas. De acordo com os mecanismos de biossíntese verifica-se que substratos hidrofóbicos, como ácidos graxos, são indutores do metabolismo responsável pela síntese de biossurfactantes por diferentes microrganismos. Assim, o aproveitamento de resíduos oleosos, como resíduos de óleo de fritura, resíduos da extração de óleos vegetais, resíduos de oleaginosas e resíduos da indústria de laticínios, constituem-se em exemplos de substratos residuais com produtividades interessantes para a obtenção de biossurfactantes e cuja utilização representa um importante ganho ambiental. Nesta breve revisão são abordados alguns mecanismos de biossíntese de biossurfactantes, principais microrganismos produtores e resíduos de indústria de alimentos e agroindustriais que recentemente foram reportados na literatura como substratos potenciais.
Palavras-Chave: Proteção ambiental, Biomoléculas, Glicolipídeos, Lipopeptídeos, Biossíntese.

RESUMEN
Los efectos de la degradación ambiental derivada de las actividades industriales y urbanas han ido alcanzando niveles cada vez más insostenibles, con la necesidad de desarrollar una conciencia ambiental, por de la reutilización y reprocesamiento de productos y residuos. En este sentido, la

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reutilización de residuos alimentarios y agroindustriales ha adquirido una notoria importancia, una vez que estos residuos representan materias primas de bajo costo para la obtención de productos de alto valor agregado. Además de esto, el estudio de la producción de biomoléculas, como biosurfactantes, principalmente glicolípidos y lipopéptidos, ha crecido en los últimos años. Con uso en varios segmentos industriales, tales como cosméticos, alimentos, higiene personal y productos de limpieza, los biosurfactantes son agentes de superficie que consisten en una porción hidrófoba y una porción hidrófila. Apesar de la amplia gama de aplicaciones y las diversas ventajas, la producción a gran escala de biosurfactantes es escasa, principalmente debido a los altos costos. Así, el uso de sustratos residuales es una de las estrategias para posibilitar la producción de estas biomoléculas. Según los mecanismos de biosíntesis, se sugiere que los sustratos hidrófobos, como los ácidos grasos, son inductores del metabolismo responsables de la síntesis de biosurfactantes por diferentes microorganismos. Así, el uso de residuos grasos, como residuos de aceite de fritura, residuos de la extracción de aceites vegetales, residuos de oleaginosas y residuos de la industria láctea, son ejemplos de sustratos residuales con interesantes productividades para la obtención de biosurfactantes y cuya utilización representa un importante beneficio medioambiental. En esta breve revisión se abordan algunos mecanismos de biosíntesis de biosurfactantes, principales microorganismos productores y residuos de la industria alimentaria y agroindustrias que recientemente han sido reportados en la literatura como potenciales sustratos.
Palabras Clave: Protección ambiental, Biomoléculas, Glicolípidos, Lipopéptidos, Biosíntesis.

ABSTRACT

The effects of the environmental deterioration due to industrial and urban activities have been reaching increasingly unsustainable levels, resulting in the need to develop an environmental awareness by the recycling and reprocessing of products and waste. In this context, the recycling of food and agroindustrial residues has acquired notorious importance, since they represent low-cost raw materials for obtaining products with high added value. Also, the production of biomolecules such as biosurfactants, mainly glycolipids and lipopeptides, has grown in recent years. Biosurfactants are surface-active compounds made up of a hydrophobic and a hydrophilic portion and can be used in several industrial segments, such as cosmetics, food, personal hygiene, and cleaning products. Despite the wide range of applications and advantages, the large-scale production of biosurfactants is still low, mainly due to the high costs of the process. The use of residual substrates is one of the strategies to enable the production of biosurfactants. According to the mechanisms of biosynthesis, it appears that hydrophobic substrates, such as fatty acids, are inducers of the metabolism responsible for the synthesis of biosurfactants by different microorganisms. Thus, the use of oily residues, such as the residues from frying oil, residues from the extraction of vegetable oils, residues of oilseeds, and residues from the dairy industry, are examples of residual substrates with interesting productivities for obtaining biosurfactants and whose utilization represents an important environmental gain. In this brief review, some biosynthesis mechanisms of biosurfactants, main microorganisms producing, and residues from the food and agroindustrial that have recently been reported in the literature as potential substrates are addressed.
Keywords: Environmental protection, Biomolecules, Glycolipids, Lipopeptides, Biosynthesis.

INTRODUCTION

The research on bioprocess plays an important role in sustainable development. Different microorganisms are known for their ability to produce biomolecules that have practical applications in medicine, agriculture, and food technology. Besides, the advances made in the last decades in terms of understanding the nature and metabolism of different microorganisms have enabled the scientific and technological community to identify, describe and synthesize products of high economic value, such as biosurfactants.

Biosurfactants are amphiphilic compounds with surface-active properties produced intra- or extracellularly by several microorganisms. Low toxicity, high biodegradability, biocompatibility, and stability, are some of the known characteristics of biosurfactants, which allows their application as a substitute for petrochemical synthetic surfactants. Currently, most of the known biosurfactants are produced by bacteria in submerged fermentation, while fungi and yeasts are still poorly explored for this purpose.

The high costs of raw materials and the subsequent downstream processes are the major issues that prevent the large-scale production of biosurfactants. In this context, several studies are focusing on reducing production costs by using low-cost substrates. Oily residues, such as fatty acids, residues from frying oil and vegetable oil refinery, are examples of substrates that can be used for biosurfactant production. These residues constitute an important environmental issue, due to their high pollution capacity and the high volumes disposed of every year. Thus, the increasing concern of the industry about sustainable processes and exploitation of eco-friendly products are the driving force for the recycling of food and agroindustrial wastes. Although food and agroindustrial residues have been usually employed for methane production, the research focused on the utilization of these substrates for biomolecule production has been increased. In this context, this review describes the current state of biosurfactant production using food and agroindustrial wastes as substrate, highlighting producers' microorganisms, metabolic pathways, and challenges and perspectives.

BIOSURFACTANTS

Biosurfactants are surface-active compounds produced intra- or extracellularly by several microorganisms (RAHMAN; GAKPE, 2008). These molecules are usually produced during the stationary growth phase and can be produced from renewable carbon sources such as carbohydrates, hydrocarbons, oils and fats, and agricultural residues (SOUZA; VESSONI-PENNA; SOUZA OLIVEIRA, 2014; MARTINS; KALIL; COSTA, 2008; ROELANTS et al., 2014). Researches on biosurfactant production began in the 1960s and the use of these compounds has increased in recent years (SANTOS et al., 2016) due to their properties, such as biodegradability, low toxicity, and stability under several pH and temperature conditions. These characteristics allow the use of biosurfactants especially in the bioremediation process in areas contaminated with oil and heavy metals (GEYS; SOETAERT; VAN BOGAERT, 2014).

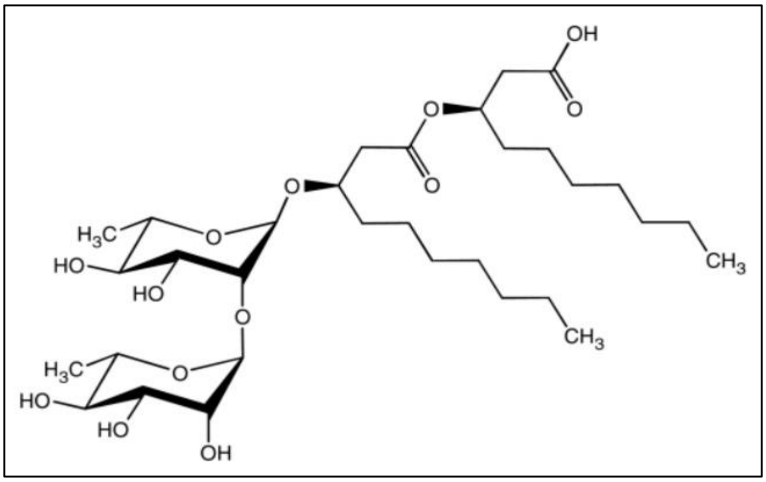
The biosynthesis of biosurfactants depends on the microorganism and the grown medium composition. Besides, it is well known that the use of hydrophobic substrates as a carbon source (e.g. vegetable oil and hydrocarbons) induces biosurfactant production. In these cases, microorganisms produce biosurfactants to improve the bioavailability of the substrate by reducing both surface and interfacial tension between the cell wall and water-insoluble carbon source (SOUZA; VESSONI-PENNA; SOUZA OLIVEIRA, 2014; APARNA; SRINIKETHAN; SMITHA, 2012).

Chemically, the lipophilic portion of biosurfactants is commonly a long-chain fatty acid (from 10 to 18 carbon), a hydroxyl fatty acid, or a α-alkyl-β-hydroxy fatty acid (BANAT; MAKKAR; CAMEOTRA, 2000; SOUZA; VESSONI-PENNA; SOUZA OLIVEIRA, 2014; ARAUJO; FREIRE, 2013; MULLIGAN, 2005). The hydrophilic portion can be a carbohydrate, a cyclic peptide, an amino acid, a carboxylic acid, a phosphate, or an alcohol (BANAT; MAKKAR; CAMEOTRA, 2000; ARAUJO; FREIRE, 2013).

The biosurfactant classification can be made up according to different criteria. It can be classified according to its molar mass into low molecular weight (e.g. glycolipids, lipopeptides, and flavolipids) and high molecular weight (e.g. polysaccharides, proteins, lipopolysaccharides, and lipoproteins) and according to its ionic characteristics, being classified as anionic, cationic, amphoteric, and non-ionic (BANAT; MAKKAR; CAMEOTRA, 2000; ARAUJO; FREIRE, 2013, INÈS; DHOUHA, 2015a). High molecular weight biosurfactants are more effective as stabilizers for water-in-oil emulsions, while low molecular weight biosurfactants are more efficient in reducing the surface and interfacial tension of liquid media (ROSENBERG; RON, 1999). Despite microorganisms that can synthesize a wide variety of high and low molecular weight biosurfactants, just glycolipids and lipopeptides are currently considered economically and industrially relevant (GEYS; SOETAERT; VAN BOGAERT, 2014).

Glycolipids have a fatty acid and a carbohydrate as hydrophobic and hydrophilic portions. Rhamnolipids (Figure 01) are known as the most studied glycolipid and it is usually produced by *Pseudomonas aeruginosa*, having two rhamnose chains bonded to 2-hydroxydecanoic acid (MOYA-RAMÍREZ et al., 2015; SAKTHIPRIYA; DOBLE; SANGWAI, 2015; LEITE et al., 2016; VARJANI; UPASANI, 2016). In addition to their surface-active properties, rhamnolipids have biological activities such as antimicrobial, hemolytic, and antiviral, which make them interesting for medical and therapeutic applications (INÈS; DHOUHA, 2015a).

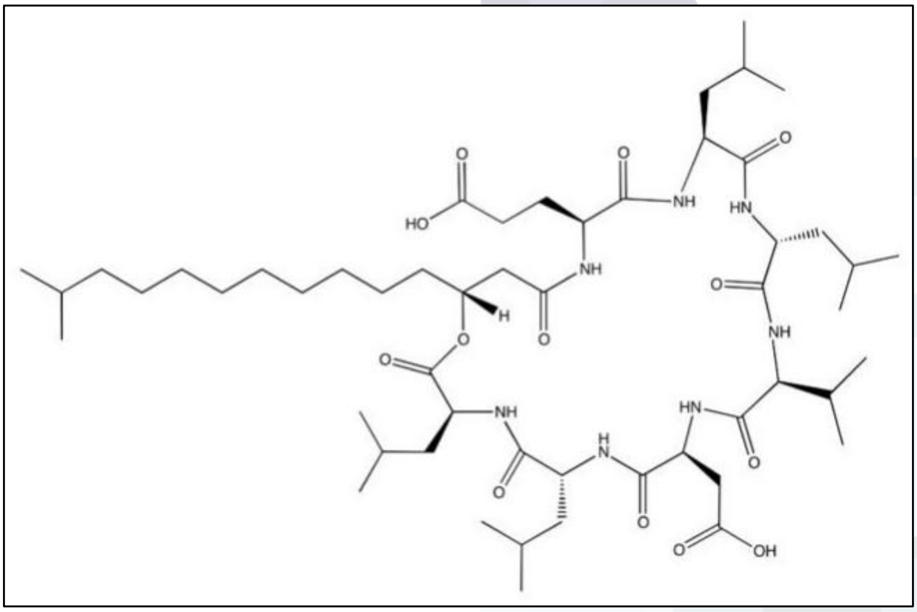
Figure 01: Ramnolipid chemical structure.



Source: GEYS; SOETAERT; VAN BOGAERT (2014).

Lipopeptides are probably the most studied biosurfactants, composed of a fatty acid chain and a peptide chain. Surfactin (Figure 02) is a lipopeptide produced by *Bacillus* sp., and has been extensity reported in the literature (SLIVINSKI et al., 2012; BEN AYED et al., 2015; GURJAR; SENGUPTA, 2015; MAASS et al., 2015). Low critical micellar concentration (CMC) and several surface-active properties involving emulsification, dispersion, and phase solubilization, allow its use as an alternative for petrochemical surfactants (INÈS; DHOUHA, 2015b).

Figure 02: Surfactin chemical structure.



Source: GEYS; SOETAERT; VAN BOGAERT (2014).

MICROORGANISMS

Biosurfactants are produced by several microorganisms (see Chart 01), generally under aerobic conditions in submerged fermentation, using different substrates, such as carbohydrates (EL-SHESHTAWY et al., 2016; MOUAFI; ABOELSOUD; MOHARAM, 2016; FADHILE ALMANSOORY et al., 2017), hydrocarbons (FOOLADI et al., 2016; BEZZA; CHIRWA, 2017), and oils (SANTOS et al., 2016; BAGHERI LOTFABAD et al., 2017; RADZUAN; BANAT; WINTERBURN, 2017). Among the microorganisms, bacteria are widely studied for biosurfactant production. The genus *Bacillus* and *Peseudomonas* are known for their ability to produce rhamnolipids and glycolipids, respectively, using different substrates (KUMARI; SINGH; SINGH, 2012; SAKTHIPRIYA; DOBLE; SANGWAI, 2015; DÍAZ DE RIENZO; KAMALANATHAN; MARTIN, 2016; SLIVINSKI et al., 2012; ZHU et al., 2013).

The production of biosurfactant by yeasts have been studied, highlighting the use of the genus *Candida* (RUFINO et al., 2014; LUNA; RUFINO; SARUBBO, 2016). Santos and collaborators (2016a) evaluated the production of a glycolipid-derivative by Candida lipolytica on large scale, which evidences the technical end economical potential of its use. The use of fungi for biosurfactant production is still incipient and few researchers have attracted their attention to these microorganisms. However, different studies have shown that these microorganisms are capable of synthetize secondary metabolites with excellent surface-active properties (COLLA et al., 2010; SAJNA et al., 2015; VELĠOĞLU, ZULFIYE ; ÖZTÜRK ÜREK, 2015). It is known that different fungi can synthesize glycolipids, however, the identification of the genes responsible for the production of fungal biosurfactants is still unknown (DAS; MUKHERJEE; SEN, 2008).

Chart 01: Microorganisms and oily substrates for biosurfactant production.

Microorganism	Biosurfactant	Substrate	Reference
Aspergillus fumigatus	Undetermined	Soybean oil	Castiglioni; Bertolin; Costa (2009)
Bacillus cereus	Lipopeptide	Peanut oil cake	Nalinia; Parthasarathi; Prabudoss (2016)
Bacillus subtilis.	Surfactin	Two-phase olive mill waste	Maass et al. (2015)
Candida lipolytica	Lipopeptide	Soybean oil refinery residue	Rufino et al. (2014)
Candida sphaerica	Undetermined	Groundnut oil refinery residue	Luna; Rufino; Sarubbo (2016)
Pleurotus ostreatus	Undetermined	Sunflower seed and shell	Velioğlu; Zulfiye; Öztürk Ürek (2015)
Pleurotus sajorcaju	Undetermined	Soybean oil	Alves et al. (2017)
Pseudomonas aeruginosa	Rhamnolipid	Two-phase olive mill waste	Moya-Ramírez et al. (2015)
Pseudomonas aeruginosa	Glycolipid	Soybean oil	Abbasi et al. (2012)
Serratia rubidaea	Rhamnolipids	Mahua oil cake	Nalini; Parthasarathi (2014)
Trametes versicolor	Undetermined	Two-phase olive mill waste	Lourenço et al. (2018)
Ustilago maydis	Glycolipid	Soybean oil, sunflower oil, and olive oil	Cortes-Sanchez; Jaramillo-Flores; Hernandez-Sanchez (2010)

SUBSTRATES

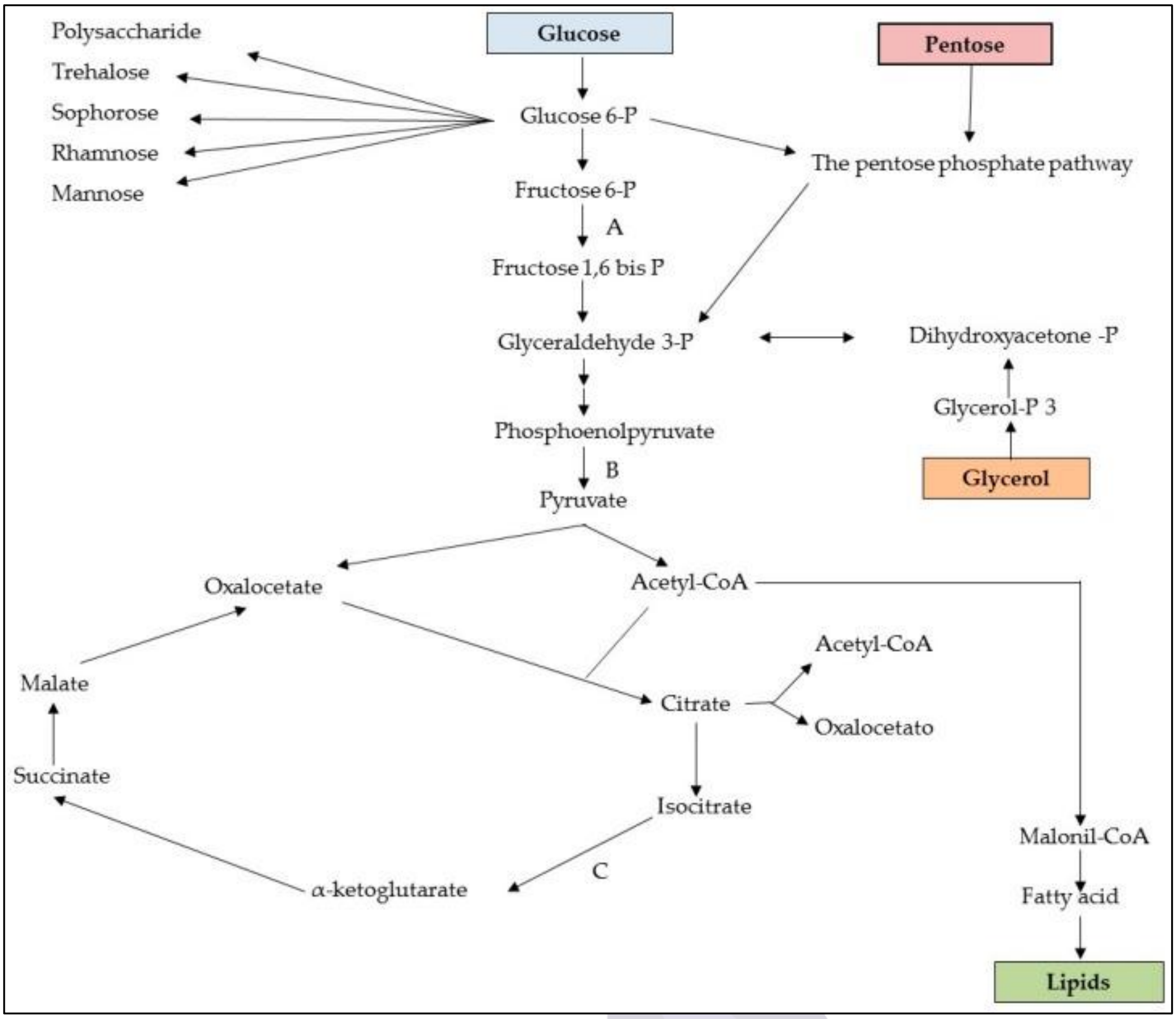
Regarding the culture medium, several authors relate the production of biosurfactants to a survival mechanism on different hydrophobic substrates. However, the production of biosurfactants on water-soluble substrates has been also reported in the literature (EL-

SHESHTAWY et al., 2016; MOUAFI; ABO ELSOUD; MOHARAM, 2016; FADHILE ALMANSOORY et al., 2017). In general, biosurfactants are synthesized by two metabolic pathways, which are involved in the formation of hydrophilic and hydrophobic portions, depending on the nature of the main carbon source (SEN, 2010).

The carbon source influences the synthesis of biosurfactants by induction or repression mechanism. When water-soluble carbohydrates (e.g. glucose) are used as the sole source of energy, the carbon flow is regulated in such a way that both the lipogenic (hydrophobic portion formation) and glycolytic (hydrophilic portion formation) pathways are specially supplied by microbial metabolism (Figure 03). On the other hand, the use of the hydrophobic substrate, such as hydrocarbons, induces microbial metabolism into several mechanisms, including the glycogenesis pathway, as can be seen in Figure 04. Hydrocarbons are oxidized at the endoplasmic reticulum and are subsequently converted to fatty acids. The glycogenesis pathway is then activated, oxidizing the fatty acids by β-oxidation to acetyl-CoA, related to the synthesis of the hydrophilic portions of the biosurfactant. (FONTES; AMARAL; COELHO, 2008; SANTOS et al., 2016).

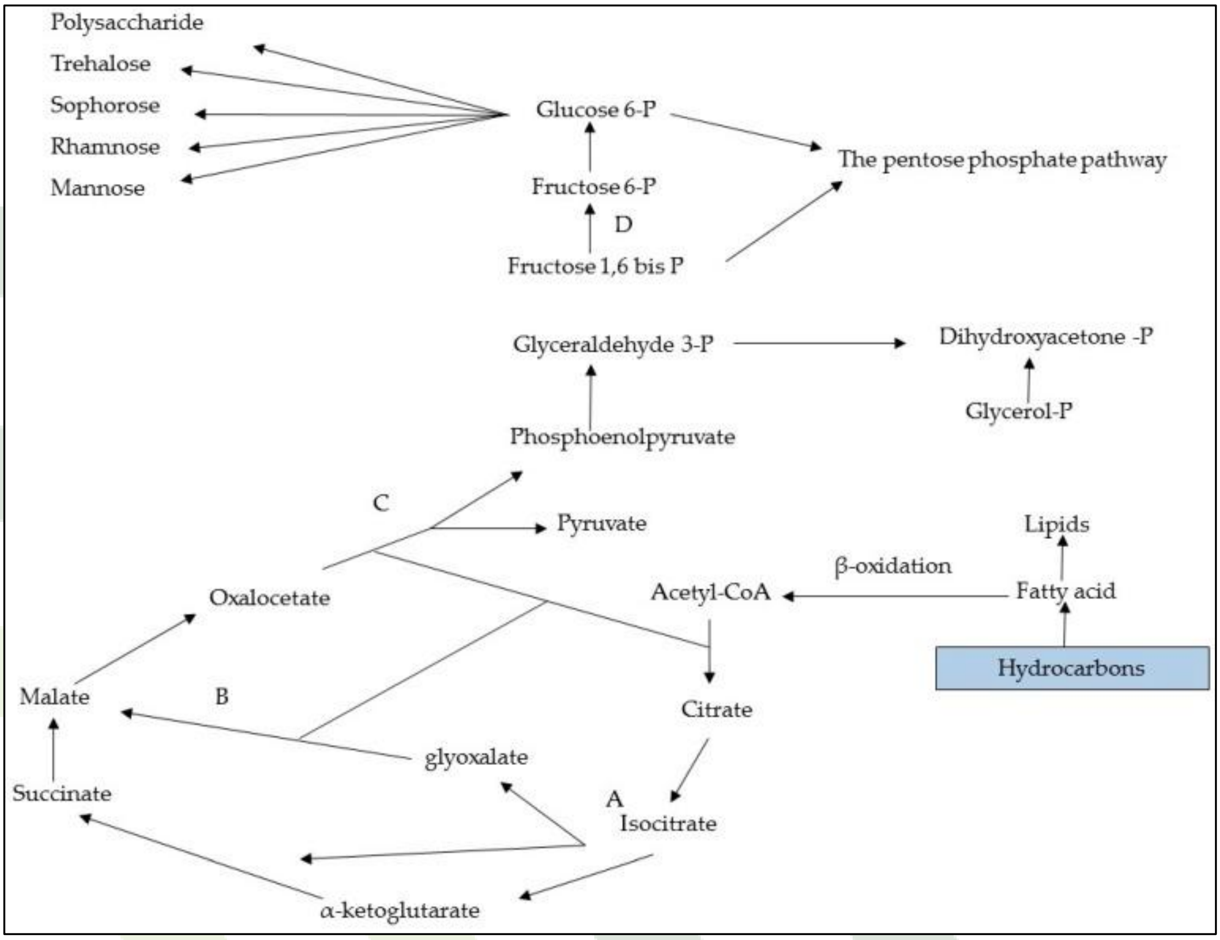
Thus, the selection of the carbon source has an important role in biosurfactant biosynthesis. Besides, the use of low-cost substrate being an interesting alternative, since the cost related to the raw material represents 10 to 30% of the total cost of production (MULLIGAN; SHARMA; MUDHOO, 2014). Several studies are focused on minimizing production costs using substrates from residual sources. The use of waste also confers to the product an eco-friendly brand. The use of oily wastes, residues from frying oil and vegetable oil production, and by-products of oil refining are some of the examples of residual substrates that can be used for biosurfactant production (FONTES; AMARAL; COELHO, 2008; HENKEL et al., 2012).

Figure 03: Biosynthesis mechanism of the production of biosurfactants using carbohydrates as substrate. Enzymes: (A) phosphofructokinase; (B) pyruvate kinase; (C) isocitrate dehydrogenase.



Source: SANTOS et al. (2016)

Figure 04: Biosynthesis mechanism of the production of biosurfactants using hydrophobic substrates. Enzymes: (A) isocitrate lyase; (B) malate synthase; (C) phosphoenolpyruvate; (D) fructose-1.



Source: SANTOS et al. (2016)

Food and Agroindustrial Wastes

Food and agroindustrial are some of the sectors that generate a large amounts of wastes. It is estimated that 1.3 billion tons of food and agricultural are wasted per year (DEHIYA et al., 2018). These residuals are generally disposal of on landfilling or used to obtain energy, which can generate environmental impacts. Therefore, in order to circumvent the drawbacks and contribute to circular economy, the valorization of food and agroindustrial wastes as bioproducts have been reported (GRESES; TOMÁS-PEJÓ; GÓNZALEZ-FERNÁNDEZ, 2020). Thus, oily residues are already beginning to be recognized as potentially recyclable and can be used as a raw material in the manufacture of various products, being also widely investigated as substrates in the most diverse biotechnological processes for the production of biosurfactants (see Chart 01).

These residues are widely used in bioprocess to obtain several compounds (e.g. antibiotics, enzymes, organic acids, biosurfactants) due to their low costs, high availability, and nutrition content, and can be classified according to its physical properties and chemical composition, having many environmental concerns due to their high contaminating capacity. It is estimated that each liter of oil can pollute about one million liters of water (FERREIRA, L.C.; FERNANDES, 2011). Also, these compounds remain on the water surface, compromising the photosynthetic function of plants and the base of the food chain (JAMALY; GIWA; HASAN, 2015). The current Brazilian environmental laws have contributed to minimizing the environmental impacts resulting from the oily wastes discharge, establishing a limit of 50 mg/L for vegetable oils and animal fats wastewater (BRASIL, 2011).

Wastes from food and agroindustrial have contents of oils and residual greases (OGR) in different levels. In the dairy industry, for example, the levels of OGR generally founded in its effluents are between 200 – 4000 mg/L. In the case of the vegetable oil extraction industry, these levels are between 500 and 16000 mg/L (OLIVEIRA et al., 2014). Moreover, every day large amounts of frying residual oils from homes, industries, and commercial establishments are improperly discarded, ending up in sewage systems, causing disturbances in the sanitation network and pollution of water resources.

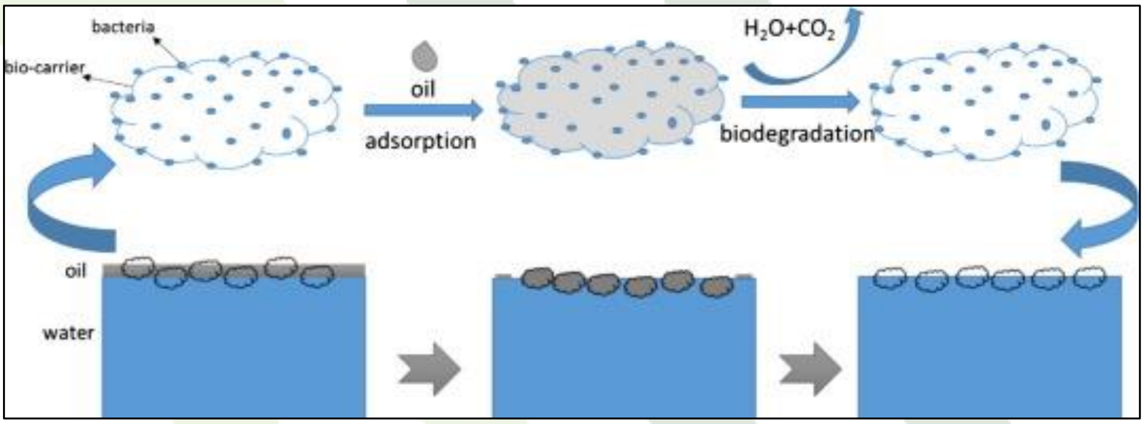
CHALLENGES AND PERSPECTIVES

Despite the wide range of applications and the various advantages mentioned above, the industrial production of biosurfactants is still not a reality. Some commercial products have already included biosurfactants in their formulations, but there are still several issues related to the low yields and production costs. It is estimated that the cost for the production of biosurfactants is approximately three to ten times higher than the cost to produce a chemical surfactant. However, the biosurfactant market is promising, which drives the sector to develop a more efficient process (REIS et al., 2013). According to recent data, the global biosurfactant market was worth USD 43.6 billion in 2017 and is expected to reach USD 66.4 billion by 2025, based on an annual growth rate of 5.4% from 2018 to 2025 (BANAT, et al., 2021).

The main criteria to be considered for the wide production of biosurfactants involve the selection of the substrates and the microorganisms, the proper design of the process and operational parameters, the need to use purification processes, and the yield and properties of the biosurfactant synthetized. The use of crude biosurfactant extracts can be a viable solution, especially if the application is in an environmental context. Biosurfactants, in these cases, do not need to present levels of purity and can be synthesized using a mixture of low-cost carbon sources, which would allow the creation of an economically viable technology for bioremediation processes (MARCHANT; BANAT, 2012).

Moreover, microorganisms that are known as biosurfactant producers can be used in microbial enhanced oil recovery (MEOR) as well as in a synergic oily wastewater biosorption-biodegradation process by the *in situ* biosurfactant production. Adsorption mechanisms in biodegradation systems may justify the sudden reduction in the concentration of hydrophobic pollutants in the initial period of the process. Firstly, the adsorption process is the main mechanism until the contaminant concentration is reduced, following the biodegradation process (Figure 05). As a result, a synergistic action of the two mechanisms leads to a dynamic balance that helps to reduce the overall oil concentration in the system (WANG et al., 2015).

Figure 05: Synergetic biosorption-biodegradation process mechanism.



Source: WANG et al. (2015)

Since the cultivation strategy is a key point in obtaining viable biosurfactants, all accessible variables must be tested, evaluating the kinetics of substrate consumption and product formation, seeking to find an optimized solution of the process conditions (JIRKU et al., 2015). Although improvements in the technology of biosurfactants have allowed the increase in the production of these biomolecules, other significant advances are likely needed to make this technology commercially viable (SANTOS et al., 2016).

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