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Biosurfactants and Sustainability

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https://doi.org/10.1002/9781119854395.ch4

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4.1 Biorefineries in Contemporary Society

In contemporary society, still dependent on the so-called petroeconomics, the concept of an oil refinery is of fundamental importance. A refinery can be defined as the full use of abundant and low-cost raw material, in this case oil, using physical and/or chemical treatments to produce commercial products with higher added value. It can also be added that a refinery operates through a succession of different stages, starting with fractionation operations, aiming at the separation of different substances. Subsequent to the fractionation, the conversion steps of the substances obtaining products for immediate use or to be made available as raw material for future transformations by the chemical industry will take place. Among the main products of oil refineries are gasoline, diesel oil, liquefied gas, kerosene for aviation, common kerosene, solvents, lubricants, petroleum coke, paraffins, resins, and polymers. Generally these refinery products are of low biodegradability, toxic, and have low compatibility [1].

In recent years, due to the various environmental, economic, and social problems that the oil-dependent society has been facing, the intensification of the call for sustainable development has been highlighted. Several organizations and world powers have sought the concept of bioeconomy and circular economy, based on sustainable processes and products. With this, the advent of the concept of biorefineries was also observed, based on principles of sustainability and green chemistry, whose main raw material in these green refineries is biomass.

Biorefineries can be defined as industrial facilities that integrate processes for the sustainable conversion of biomass (organic materials of starchy, lignocellulosic, oleaginous origin, and others) into value-added products [2–4]. These sustainable industries integrate several conversion routes – biochemical, microbial, chemical, and thermochemical – in the search for the best use of biomass and the energy it contains. The objective of biorefineries is to optimize the use of resources, minimize effluents, and obtain ecofriendly products,

Biosurfactants and Sustainability: From Biorefineries Production to Versatile Applications, First Edition.
Edited by Paulo Ricardo Franco Marcelino, Silvio Silverio da Silva, and Antonio Ortiz Lopez.

maximizing benefits and profit [4]. In the literature, there are several classifications for biorefineries based on: (i) type of raw material (starch, lignocellulosic, oleaginous, algal, and others), (ii) type of technology used in the conversion of biomass (mechanical/physical, biochemical, or thermochemical), (iii) platform technology status (conventional, advanced or first and second generation biorefineries), (iv) main product (bioethanol, biodiesel, etc.) and (v) intermediate production (synthesis gas, sugar, or lignin) [5]. Among the classifications presented, the most common, in the technical literature and in the industrial environment, are related to the type of raw material used and the type of technology used in the conversion of biomass.

4.2 Biomass and Biorefineries: Industrial By-products as Raw Materials for Biorefineries

In recent decades, with the advancement of sustainable research focused on bioprocesses (fermentations and biocatalysis), several industrial sectors, mainly the food, chemical, and pharmaceutical sectors, prioritized the use of materials previously considered industrial by-products, as raw materials for obtaining products with high added value and ecofriendly characteristics, the so-called bio-based products or sustainable products. The adoption of this type of raw material stands out not only for its environmental appeal, but also for its very low cost, enabling a good cost/benefit ratio when compared to those obtained by traditional chemical syntheses, which use expensive raw materials, many of them derivatives of petrochemicals [6].

In addition to adding value to by-products, the following are highlighted as advantages of bioprocesses in relation to traditional chemical syntheses: (i) the use of mild process conditions, as the reactions are carried out by microorganisms and enzymes at a temperature close to room temperature, which entails lower energy expenditure; (ii) pH of the culture or reaction medium close to neutrality; (iii) the low load of waste generated during the process, in addition to having low or zero toxicity, causing no impact on the environment; (iv) regio and stereoselectivity of reactions, mainly in biocatalytic processes, resulting in the production of enantiomerically pure compounds, not requiring the use of expensive resolution (separation) methods, and (v) the possibility of carrying out biological reactions in the solid medium (solid state fermentations or biocatalysis) [7].

The use of by-products in biotechnological processes is not new to society. Although bioprocesses using agro-industrial by-products have only now received greater emphasis, they have already been used, mainly in solid-state fermentation (SSF), since the seventeenth century. It is known that in France, producers of *Agaricus bisporus* (*Champignon*) already cultivated these mushrooms on the “beds” used for storing fruits, made up of different types of straw (rice, wheat, oats) mixed with animal manure (poultry, cattle, horses) [8]. In 1896, Takamine produced a digestive enzyme, takadiastase, in solid-state fermentation by *Aspergillus oryzae* on wheat bran. Between 1900 and 1920, the use of by-products as substrates in the production of microbial enzymes and kojic acid by SSF was observed. In the second half of the twentieth century and the beginning of the twentyfirst century, the production of other microbial metabolites using agricultural and agro-industrial residues as raw material was improved and several other products were obtained, such as: protein-enriched foods for animal and human consumption, antibiotics, alkaloids, organic acids, polyols, biopesticides, and others [9].

In addition to by-products of lignocellulosic and starchy origin, oilseeds, such as residual oils and fats, also stand out as sources of nutrients in fermentation processes. They usually come from the food industry and are widely used in the production of lipases by microorganisms, mainly by yeasts and filamentous fungi [10].

Residual glycerol from biodiesel synthesis has also come to the fore in recent years as a potential raw material for industry. According to Mota *et al.* [11], glycerol from biodiesel production can be used in the production of acetals, ethers, esters, acrolein, acrylic acid, glyceraldehyde, glyceric acid, and glycerin carbonate. On the other hand, application of residual glycerol in biotechnological processes, according to the works of Rivaldi et al. [12] and Mattam et al. [13], can be observed in the production of 1,3 – propanediol, organic acids, ethanol, polyhydroxyalkanoates (PHA), omega 3 polyunsaturated acid, enzymes, antibiotics, microbial biomass, and pigments.

It should also be noted that the by-products rich in nitrogenous compounds are extremely important for bioprocesses. The low-cost nitrogen sources commonly used in fermentation processes are: cornstarch, corn gluten meal, peanut meal, soybean meal, and dairy residues [14]. Soybean and rice bran have been studied and applied in several bioprocesses, such as enzyme production, microbial biomass, bioflavors, second-generation ethanol, and xylitol [15, 16]. The mineral composition of these grains, in addition to supplementing crops with organic nitrogen, makes these extracts promising sources of micronutrients and growth factors. Both in soybean and rice grains, as well as in their extracts, vitamins (niacin, riboflavin, pantothenic acid, and thiamine), and amino acids (arginine, cysteine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine) [17]. Thus, these soybean and rice bran extracts can serve as low-cost alternatives to replace enriched yeast extracts in various bioprocesses. Making a comparison, the price of yeast extract can reach U$ 216.00 per kg, while soy bran costs around U$ 0.47 per kg and rice R$ 0.65 per kg [18, 19].

In addition to the bioproducts mentioned above, biosurfactants are also viable product alternatives to be produced in biorefineries. As previously reported in the introductory chapter of this book, biosurfactants are metabolites of animal, vegetable, or microbial origin, with an amphipathic structure, with outstanding physical-chemical and biological properties, and can be used in various industrial sectors. In addition to the versatility of applications, biosurfactants are considered ecofriendly products and may be possible substitutes for synthetic surfactants [20].

The ecofriendly characteristics of biosurfactants are due to their high biodegradability, low toxicity, biocompatibility and the possibility of production from agro-industrial by-products, making these bioproducts alternatives for biorefineries [20–22].

As can be seen in the topics below, there are studies in the literature that report the production of biosurfactants using oleaginous biomass (rich in lipids), starchy and lignocellulosic (rich in sugars), and residual glycerol from biodiesel as raw materials.

4.3 Biosurfactant Production in the Context of Lignocellulosic Biorefineries

The valorization of residual vegetable biomass in bioprocesses, mainly those of lignocellulosic origin, is due to the fact that they are materials rich in carbohydrates. In the composition of these materials 20–50% of cellulose was found, a linear homopolymer composed of glucose and 15–35% of hemicellulose, a heteropolymer composed mainly of xylose and arabinose. In addition to the glycidic portion, lignin is also found (10–30%), a polyphenolic macromolecule [23–25]. These compounds are commonly found in plant cell walls, arranged so that the cellulose is "cemented" by hemicellulose and lignin (Figure 4.1).

The cellulose, hemicellulose and lignin contents vary according to the parts, ages, and plant species (Table 4.1). The rest of the biomass consists, in a minority, of substances such as proteins, pectin, oils, minerals, terpenes, alkaloids, and various pigments. Due to the rich organic composition, lignocellulosic materials are used in bioprocesses mainly as sources of carbon, nitrogen, sulfur, micronutrients and growth factors of fundamental importance for microbial nutrition [26]. It should be noted that among the lignocellulosic biomasses, the most frequently used in studies to obtain bioproducts has been sugarcane bagasse and straw, mainly with a view to obtaining second-generation ethanol.

The production of biosurfactants using lignocellulosic by-products is already a reality. Various studies in the last decade were carried out using both cellulosic and hemicellulosic fractions, as can be seen in the following sections.

The use of lignocellulosic biomass as a raw material to obtain biosurfactants, or any other bioproducts, requires a pre-treatment step. During the pre-treatment, the recalcitrant fraction, in this case lignin, will be removed, and then the hydrolysis of the polysaccharides into fermentable sugars (glucose and xylose) will be carried out. (Figure 4.2).

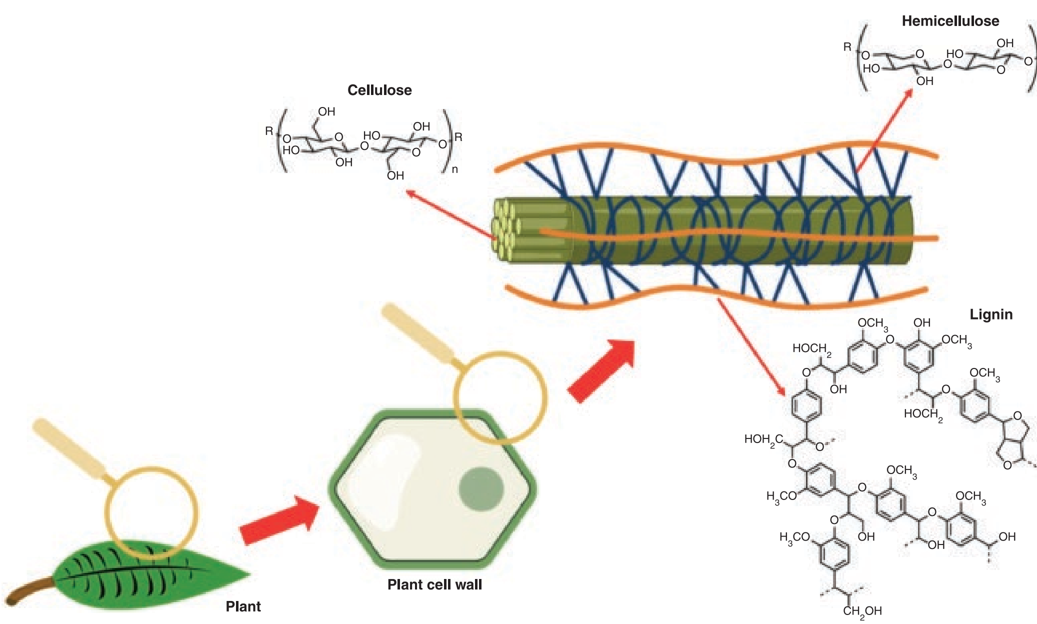

Figure 4.1 Structure and chemical composition of lignocellulosic biomass.

Table 4.1 Chemical composition of some lignocellulosic biomasses.

Lignocellulosic biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Sugarcane straw	40–44	30–32	22–25
Sugarcane bagasse	32–48	19–24	23–32
Hardwood	43–47	25–35	16–24
Softwood	40–44	25–29	25–31
Corn stalk	35	25	35
Corn cob	45	35	15
Cotton	95	2	0,3
Wheat straw	30	50	15
Sisal	73.1	14.2	11
Rice straw	43.3	26.4	16.3
Corn fodder	38–40	28	7–21
Coconut fiber	36–43	0.15–0.25	41–45
Banana fiber	60–65	6–8	5–0
Barley straw	31–45	27–8	14–9

Source: [27].

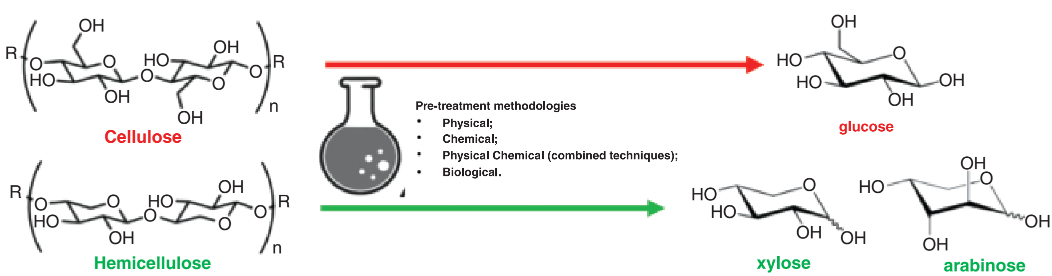

Figure 4.2 Fermentable sugars obtained in the pre-treatment step of lignocellulosic biomass.

In the literature, several pre-treatment methods used to obtain cellulosic or hemicellulosic hydrolysates can be found. However, it should be noted that the adoption of a particular technique will depend on the physical and chemical properties of the lignocellulosic material used as raw materials [28]. In addition, the chosen technique must be capable of producing high yields of pentose sugars, not releasing significant amounts of compounds that act as inhibitors of microbial metabolism (furans and phenols) (Figure 4.3), not producing high levels of residues, being simple and cost-effective [29, 30].

The pre-treatment methods currently used are divided into physical, chemical, physical–chemical and biological processes, as can be seen in Table 4.2.

In studies on the production of biosurfactants using lignocellulosic biomass, one can observe the use of techniques for obtaining the hemicellulosic and cellulosic fraction already used in obtaining other bioproducts, such as second-generation ethanol and xylitol, for example.

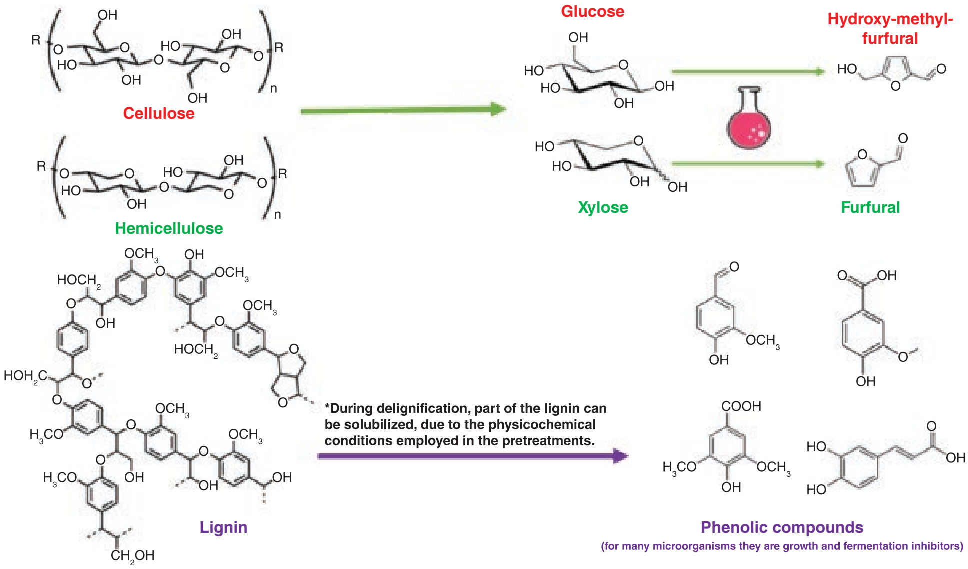

Figure 4.3 Main inhibitor compounds formed during pre-treatment of lignocellulosic biomass.

Table 4.2 Main types of pre-treatments and techniques used in the lignocellulosic biomasses.

Type of pre-treatment	Technique
Physical	Grinding and crushing
	Ultrasonication
	Centrifugal grinding
	Extrusion
Chemical	Pre-treatment with diluted mineral acid
	Pre-treatment with acid-acetone mixture
	Pre-treatment with ionic liquid
	Alkaline pre-treatment with potassium permanganate
	Organosolv
	Pre-treatment with metal chlorides
Physical–chemical	Steam explosion
	Hot water pre-treatment
	Wet oxidation
	Explosion with super critical carbon dioxide
	IHRW pre-treatment
	Plasma
	AFEX
Biological	Microbial consortium
	Pre-treatment with fungal species
	Enzymatic pre-treatment

Source: [30, 31].

Portilla-Rivera et al. [32] and Portilla-Rivera et al. [33] reported the production of biosurfactants by a *Lactobacillus pentosus* strain. using the hemicellulose hydrolysate of grape pomace as the raw material. The characterization of grape pomace showed that this lignocellulosic material has a low sugar content (10.8% cellulose and 11.2% hemicellulose), while the lignin and extractive content are respectively 50.9% and 14.7%, considered high. The pre-treatment used for delignification of the material and the hydrolysis of polysaccharides to obtain fermentable sugars, in this case xylose, was of the acid type. In this study, dilute sulfuric acid concentrations (1–5% $m V^{-1}$) were evaluated at time intervals of 0–180 minutes, as little was known about this biomass. The hydrolytic process was optimized using an acid concentration of 3.3%, at a temperature of 130 °C for 125 minutes. The use of this hemicellulosic hydrolysate as a carbon source in the culture medium showed that after 18 hours of fermentation, 4.8 $mg L^{-1}$ of intracellular surfactin was obtained. This data represents a yield of 0.60 mg of intracellular biosurfactant per g of consumed sugars. It is noteworthy that despite a low yield, the surfactin produced by *L. pentosus* under the studied conditions presented a critical micellar concentration of 2.9 $g L^{-1}$.

One of the probable problems for the low yield in the process reported by Portilla-Rivera et al. [32] is that the lignocellulosic material used had a marked chemical composition of compounds that can generate inhibitors for the fermentation process (lignin and extractives). Furthermore, the conditions adopted for fermentation also contributed to the production of lactic acid, competing with the production of the biosurfactant. Furthermore, the surfactin obtained was intracellular, and the process of extracting an intracellular product may result in lower yields due to several factors intrinsic to the process. It was observed that the severity of the parameters adopted in this pre-treatment also resulted in obtaining significant concentrations of inhibitor compounds (acetic acid, phenolics, and furans).

Studies by Cortés-Camargo [34] showed the production of biosurfactants by a halotolerant *Bacillus tequilensis* ZSB10 strain in cellulosic and hemicellulose hydrolysates from vine-trimming shoots. The pre-treatment of the lignocellulosic material, occurred in three stages, in this case: (i) acid hydrolysis, (ii) an alkaline reaction, and (iii) an enzymatic hydrolysis of cellulignin using cellulases. At the end of the pre-treatment, a cellulosic hydrolysate with 21.57 $g L^{-1}$ of glucose was obtained. The authors reported that after the fermentation process in a 2 L stirred tank reactor, 1.52 $g L^{-1}$ of extracellular biosurfactant was obtained with a critical micellar concentration (CMC) of 177.14 $mg L^{-1}$, lowering the surface tension up to 38.6 $mN m^{-1}$. The emulsifying potential of the biosurfactant obtained in kerosene, measured by the emulsification index (IE), was below 50%. It should be noted that in this work, the authors used a strategy of combining pre-treatments in order to obtain higher concentrations of sugars, since the lignocellulosic material used was not trivial, with pre-treatment conditions previously studied in the literature.

Marcelino and collaborators [35] showed the possibility of the production of glycolipid biosurfactants by yeast in hemicellulosic hydrolysate of sugarcane bagasse. In this study, at first, a screening of several yeasts isolated from Brazilian biomes was carried out. Most of the microorganisms used in the selection were collected from decomposing lignocellulosic materials. Based on this strategy, the strains that produce biosurfactants in detoxified and non-detoxified sugarcane bagasse hemicellulosic hydrolysate can be found. It was observed that the studied yeasts produced biosurfactants in culture medium based on detoxified and non-detoxified hydrolysate. A smaller number of yeasts produced biosurfactant in a medium based on hemicellulosic hydrolysate. This difference can be explained by the ionic strength and the concentration of inhibitor compounds in both situations.

In culture media based on non-detoxified hemicellulosic hydrolysate, the ionic strength is high due to the particles present from the preparation of the hydrolysate. Thus, the osmolarity of the culture medium is altered when compared to synthetic media or media submitted to the detoxification process, in which successive filtrations and centrifugations occur. Furthermore, in culture media based on non-detoxified hemicellulose hydrolysate, there is a significant concentration of inhibitory compounds, such as phenolics, furans, and acetic acid. One of the ways to mitigate the effect of inhibitors in the non-detoxified hydrolysate is the use of some yeasts with the capacity to degrade xenobiotic compounds, which will reduce the concentration of inhibitors in the culture medium. Due to the various disadvantages of working with non-detoxified hydrolysate, the authors continued with studies of biosurfactant production in culture medium based on detoxified hydrolysate, obtaining, after 48 h of fermentation, volumetric productivities of biosurfactants of 0.006–0.167 $g\,L^{-1}\,h^{-1}$ [35].

Chaves et al. [36] reported for the first time the production of biosurfactants in hemicellulosic hydrolysate of sugarcane straw detoxified and non-detoxified by yeasts isolated from the Antarctic continent. The biosurfactant produced by *Naganishia adellienses* L95 showed outstanding emulsifying properties at low temperatures. The results of this study are interesting for biorefineries, as they show the production of biosurfactants in lignocellulosic biomass; the use of extremophile microorganisms; and biosurfactants with different properties that can be applied in industrial processes at low temperatures in the future.

In the study by Barbosa et al. [37], the biosurfactant production by the yeast *Scheffersomyces shehatae* was observed in a culture medium based on hemicellulose hydrolysate of detoxified sugarcane bagasse and residual vegetable oil. The biosurfactant obtained showed outstanding emulsifying characteristics and a volumetric productivity of 0.076 $g\,L^{-1}\,h^{-1}$. It is interesting to emphasize the strategy used in this study, which mixed substrates of different polarities. According to Fontes et al. [38], although the production of biosurfactants occurs in the presence of polar carbon sources, such as sugars, several studies have shown that the highest biosurfactant productions are obtained when non-polar substrates are added. Many works describe the importance of the combination of a substrate insoluble in water and a carbohydrate, as constituents of the culture medium.

In addition to the hemicellulose, cellulosic hydrolysate can also be used as a substrate in biorefineries. The cellulosic hydrolysate can be obtained by enzymatic processes, in which a pool of commercial cellulase enzymes are used, and have a majority composition of glucose. Furthermore, these hydrolysates do not contain inhibitory compounds, since the enzymatic process for obtaining them is specific and uses mild physicochemical conditions. The main disadvantage of cellulosic hydrolysates is that compared to hemicelluloses they can be expensive due to the enzymes used in the hydrolysis process [39].

Faria et al. [40] reported the production of biosurfactants of the type mannosylerythritol lipids (MELs) by a yeast of the genus *Pseudozyma* in semi-synthetic media using purified xylan as a carbon source. This work is important because it shows the possibility of directly converting a hemicellulose into a biosurfactant, without the need for a pre-treatment with drastic physicochemical conditions. Microorganisms that produce cellulases and xylanases can be used in this type of process.

Da Mata et al. [41] reported the production of biosurfactants by *Aureobasidium pullulans* LB83 in SSF. In this work, the authors showed the direct conversion of cellulose from pretreated sugarcane bagasse into biosurfactants, due to the yeast's potential to produce cellulase enzymes.

In the review article by Tan and Li [42] several works were cited that reported the production of rhamnolipids by bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus* and some of the genus *Lactobacillus* using the cellulosic hydrolysate of wheat straw and waste from fruit products.

As can be seen, studies on the production of biosurfactants in the context of lignocellulosic biorefineries have increased, mainly due to the urgency of implementing sustainable processes and products in the scenario of transition from the petroeconomy to the bioeconomy. However, there is still a need for advance in works that focus on the selection of microbial strains that overproduce biosurfactants; in production in bioreactors, from bench to industrial scale, in adjustments of operating parameters such as agitation and aeration; and also solve problems related to foam formation during fermentation.

4.4 Biosurfactant Production in the Context of Oleaginous Biorefineries

In this section, the production of biosurfactants using oleaginous industrial by-products in the context of biorefineries will be emphasized. In the literature, the main oily raw materials used in the production of biosurfactants are animal fat waste, the food processing industry (frying edible oils and fats, olive oil, rape seed oil, sunflower, and vegetable oils) and oil processing mills (coconut cake, canola meal, olive oil mill waste water, palm oil mill, peanut cake, effluent, soybean cake, soapstock, and waste from lubricating oil) [43, 44].

In the production of biosurfactants using oleaginous by-products, mainly waste oils from the food industry, published studies have shown the use of bacteria such as *Pseudomonas*, *Bacillus* and *Serratia*, but focus mainly on yeasts such as *Candida, Starmerella* and *Pseudozyma*. However, in the literature there are also reports of the production of biosurfactants by the yeast genera *Rhodotorula, Pichia, Debaryomyces, Aureobasidium, Kluyveromyces, Issatchenkia, Cryptococcus, Rhizopus, Yarrowia*, and *Trichosporon* [45, 46]. It should be noted that yeasts recently described as positive lipases and oleaginous (accumulators of intracellular lipids) have also stood out as better producers of biosurfactants. In the literature there are also some reports of filamentous fungi producing biosurfactants using oily substrates.

As can be seen yeasts have highlighted in the production of biosurfactants due to the GRAS *status* (generally recognized as safe) of several species used, not presenting risks of pathogenicity and toxicity, allowing the application of biosurfactants without restrictions [38, 47]. In addition, when compared to bacteria, yeasts are more resistant to the biosurfactant secreted and accumulated in the medium during fermentation, suffering fewer morphological changes, mainly in the plasmatic membrane and remaining viable for a longer period of time [48].

Oleaginous raw materials are rich in hydrophobic substrates, which are considered inducers of the production of biosurfactants, and because of this they are known as hydrophobic inducers. Biosurfactant inducers are used in the culture medium in order to promote the induction of the production of these compounds and increase the final yield. They are defined as supplementary sources of carbon, which may be present in low or medium concentrations in the culture medium. Studies show that hydrophobic inducers can cause changes in the chemical structure, and consequently modify the physical-chemical and biological properties of biosurfactants [49–52].

The main substrates considered to be hydrophobic inducers used in the production of biosurfactants are hydrocarbons and vegetable oils. These compounds are considered secondary sources of carbon and can act in microbial growth and also in the synthesis of the non-polar portion of biosurfactants [52–54]. However, one of the main problems in the production of biosurfactants using oleaginous by-products is the low transfer of oxygen, due to the higher viscosity of the oils. The high concentration of these compounds in the fermentation process can lead to low oxygen transfer, in such a way as to affect cell growth and also the production of biosurfactants/bioemulsifiers, so when applying these inducers it is necessary to know the process that is being carried out [52].

Table 4.3 presents a summary of the main microorganisms producing biosurfactants, the oleaginous raw materials used and the type of biosurfactant produced.

The production of biosurfactants using waste oils as raw material is used more routinely than agricultural by-products rich in sugars. Generally, the oils used in these processes must

Table 4.3 Some studies on the production of biosurfactants by yeast using oleaginous industrial by-products.

Microorganism	Raw material/substrate	Biosurfactant produced	References
Candida bombicola	Animal fat and glucose	Glycolipid	[55]
Candida antarctica	Oil refinery residual hydrocarbons	Glycolipid	[56]
Candida bombicola ATCC 22214	Corn oil and honey	Glycolipid	[57]
Candida lipolytica	Waste from vegetable oil industries	Protein-carbohydrate-lipid complex	[58]
Candida bombicola	Waste oil from restaurants	Glycolipid	[59]
Candida lipolytica	Waste from soybean oil industries	Not identified	[60]
Candida sphaerica UCP0995	Residual peanut oil	Not identified	[61]
Scheffersomyces anomala PY1	Soybean oil	Glycolipid	[62]
Candida sp.	Residual peanut oil	Glycolipid	[63]
Candida bombicola	Cane molasses and soybean oil	Glycolipid	[64]
Trichosporon montevideense CLOA 72	Sunflower oil	Glycolipid	[48]
Candida glabrata UCP1002	Waste from vegetable oil industries	Not identified	[65]

also be pre-treated, aiming mainly at the removal of solids that can interfere with fermentation. To remove these solids, unitary operations such as centrifugation or filtration are usually used. In addition, the peroxide/hydroperoxide index of the residual oils must also be analyzed, as high concentrations of these compounds can interfere with the fermentation process [66].

4.5 Biosurfactant Production in the Context of Starchy and Biodiesel Biorefineries

In addition to lignocellulosic and oily by-products, the production of biosurfactants can also be carried out using starchy raw materials. Starch is defined as a renewable, natural, and biodegradable polymer prepared by many plants as a source of stored energy. Two different polysaccharides are present in starch structure: (1) linear (1,4)-linked α-d-glucan amylose, and (2) highly (1,6) branched α-d-glucan amylopectin [67, 68].

Among the starchy agro-industrial by-products that can be used as raw materials in the production of biosurfactants and other bioproducts, we can mention wastes from: soybean, potato, sweet potato, and sweet sorghum. The starch found in these raw materials must be depolymerized, releasing glucose as the main fermentable sugar [43]. Because of this, starchy raw materials are also subjected to pre-treatments aimed at starch hydrolysis [69]. The pre-treatments used for starch hydrolysis can be carried out chemically or enzymatically. Before pre-treatment, the starch gelatinization stage is necessary, in which it will be hydrated at a given temperature in the range 55–80 ºC, depending on the starch source.

In the food industries, acid hydrolysis of starch occurs when a high concentration of starch (30–40 g per 100 g of solids) is treated with diluted inorganic acids at a temperature lower than that of gelatination (30–60 ºC) during one or more hours of reaction [70]. Temperature control in acid hydrolysis is a critical point in the process, since glucose in an acid medium and high temperature can suffer dehydration and form hydroxymethylfurfural.

The enzymatic hydrolysis of starch consists of the action of a pool of enzymes called amylase or amylolytic complex. The starchy raw materials, after the gelatinization process, become a suitable substrate for the action of the amylases that break the existing bonds in the biopolymers, amylose and amylopectin, and releasing glucose molecules and small biopolymers called dextrins into solution, which in turn will also be decomposed. Amylolytic enzymes are mostly produced by fungi or bacteria that modern technologies in bioprocesses have made very efficient and available at affordable costs for industrial use. For an efficient conversion of already gelatinized starch macromolecules to glucose and other low molecular mass derivatives, the coordinated action of the enzymes of the amylolytic complex is necessary: α-amylases, β-amylases, α–D-glucosidase, exo-1, 4-α-D-glucanases, glucosidases, pullulanases, and isoamylases.

The enzymatic hydrolysis of starch is also used in the food industry to produce glucose syrup. In addition, it is also used in the process of producing alcohol from starchy sources (cassava, corn, sweet potato, and others). It should be noted that, when compared to chemical hydrolysis, the enzymatic method still has a high cost and lower yield, requiring studies aimed at optimizing the process to reduce costs [71, 72].

In the literature, works can be found on the use of starchy by-products in the biosurfactant production. Potato processing effluents have been used in the production of surfactin by bacteria of *Bacillus genus* [73–77].

In addition to starchy by-products, another raw material that can be used in the production of biosurfactants is glycerol. In the production of biodiesel, glycerol is the main by-product obtained after the transesterification of vegetable oils and animal fats. Consequently, glycerol production has become increasingly abundant as worldwide biodiesel production increases [78]. Among the microorganisms that produce biosurfactants that can use glycerol as a nutrient, *Bacillus subtilis* and *Pseudomonas aeruginosa* strains have been highlighted [79, 80]. Howerer, There are also studies showing the production of biosurfactants in glycerol-based culture media using *Yarrowia lipolytica*, a type of lipolytic yeast, as a fermentation agent [81].

The main problem with using residual glycerol from biodiesel as a substrate for the production of biosurfactants and other by-products in fermentation processes are impurities such as methanol, fatty acid methyl esters, and salts left over from the transesterification reaction [78]. Thus, a pre-treatment step is required, in which unit operations such as the use of exchange resins acidification and extraction processes can be used [82].

4.6 Conclusion

As can be seen in this chapter, biosurfactants are promising value-added products to be produced in the broader context of biorefineries using different types of raw materials. Through clean processes, these bioproducts can be obtained and contribute to the sustainable development of society, making the transition from the petroeconomy to the bioeconomy smooth. However, there are still several technical and economic obstacles to be studied and overcome, requiring academic and industrial efforts to overcome these barriers and make biosurfactants popular products.

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