Chapter 2
Synthesis of Industrial Enzymes from Lignocellulosic Fractions

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Abstract The lignocellulosic material consists of three subunits, hemicellulose, lignin, and cellulose, that are fractionated to extract and produce value-added compounds, such as food additives, organic acids, ethanol, and enzymes. Various biotechnological applications such as effluent bioremediation, hydrolysis and paper bleaching, and construction of biosensors require large quantities of low-cost enzymes. Thus, an appropriate choice for the production of low-cost enzymes is the use of lignocellulosic residues, which contain soluble carbohydrates that can be used as inducers of enzymatic synthesis. However, the depolymerization of lignocellulosic components through various treatments (chemical, physical, physicochemicals, and biological) is necessary for the efficient production of enzymes. Therefore, this book chapter addresses the chemical composition of lignocellulosic residues and their various treatments that allow obtaining value-added products, mainly enzymes. The main types of industrial enzymes, their application in several technological areas, and their market in the world are also addressed.

Keywords Biomass · Lignocellulosic treatment · Industrial enzymes

2.1 Introduction

Different sources of lignocellulosic biomass are produced worldwide due to high agricultural activity, which favors the availability, diversity, and use of agro-industrial waste. The chemical composition of lignocellulosic residues (cellulose, hemicellulose, and lignin) is fractionated and extracted to produce compounds such as organic acids, ethanol, and enzymes (Canilha et al. 2010; Maitan-Alfenas et al. 2015). In addition, the use of these wastes could help to reduce the environmental impact caused by the agricultural and agro-industrial sectors (Ferreira et al. 2011). However, for waste to be used more efficiently, it is necessary to subject them to treatment processes (Ruzene et al. 2008).

Biomass treatments can be grouped into four types: chemical, physical, physicochemical, and biological. From the individual or conjugate use of some of these treatment categories, fractions rich in phenolic compounds, lignin, pentoses, and hexoses, can be used as substrates in bioprocesses using microorganisms mainly as bacteria and fungi. Among the variety of fungi, the white-rot fungi have the ability to degrade lignocellulose components due to their metabolic system that produces lignocellulolytic enzymes such as β-glycosidase, cellulase, xylanase, lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Barakat et al. 2014; Inácio et al. 2015).

In addition to fungi, some groups of bacteria have also been used in an attempt to potentiate the process. Although there are many ligninolytic bacteria, most of them have not been exploited in degradation processes (Davis and Sello 2009). Among those that are already well described regarding the action potential on lignocellulose, phenols, and xenobiotic compounds, the bacteria belonging to the group of

Sphingomonas, *Pseudomonas*, *Rhodococcus*, *Nocardia*, and *Streptomyces* (Bugg and Rahmanpour 2015; Bugg et al. 2016) can be mentioned.

As mentioned previously, several enzymes with function in the degradation of the lignocellulosic residue are released by the metabolic systems of the involved microorganisms. From this, it is possible to highlight the action of the enzymes through the direct application of the microorganisms or to perform extraction and purification steps. In general, these enzymes can be applied in processes of different industrial processes such as food additives, cosmetics, pharmaceuticals, biosensors, and bioremediation.

2.2 Literature Review

2.2.1 Lignocellulosic Residues

Lignocellulose is the main component of cell walls of plants and consequently of the woody plants and dead plant materials and constitutes the most abundant biomass on the planet (Knežević et al. 2013; Barakat et al. 2014), reaching an estimated production of 1.3 billion liters per year worldwide (Silva et al. 2009; Chiu and Lo 2016). The lignocellulosic residue has a complex structure of three main components, lignin cellulose, and hemicellulose, cited by the prevalence of composition (Fig. 2.1) (Song and Ni 2013; Ciolacu 2018). Much of this lignocellulosic waste is produced through forests, agricultural practices, and many agro-industries, causing serious problems of environmental pollution, among them the dispersion of pollutants in the atmosphere caused by the burning of the lignocellulosic material, thus causing the increase of the greenhouse effect and problems which are still evaluated superficially due to the non-identification of many of their potential polluters (Martinelli et al. 2010; Ferreira et al. 2011).

Faced with a decline in nonrenewable resources combined with environmental and economic concerns, the development of new raw materials for the production of

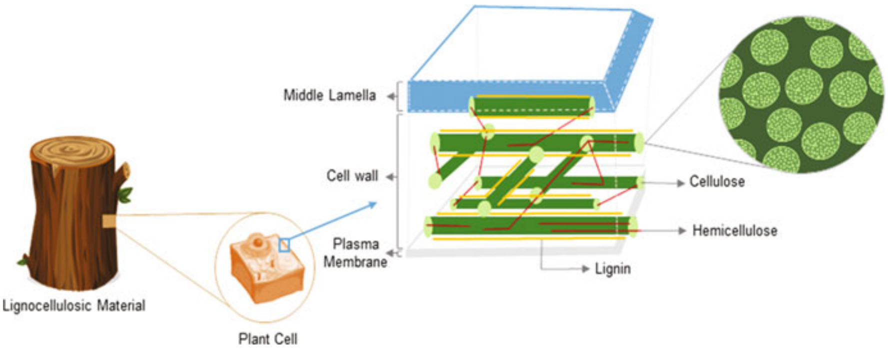


Fig. 2.1 Representation of the source and main structural components of lignocellulosic biomass

Table 2.1 The composition of biomass obtained from different agricultural sources

Lignocellulose	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood	40–55	24–40	18–25
Softwood	45–50	25–35	25–35
Corncobs	40–45	35–45	10–20
Corn Stover	38–42	22–28	7–22
Barley straw	31–45	20–38	8–19
Wheat straw	37–41	27–32	13–15
Rice straw	22–35	10–24	18–22
Bagasse	32–48	19–24	23–32
Soy stalk	40–48	10–20	10–18
Cotton stalk	42–45	5–12	0–15

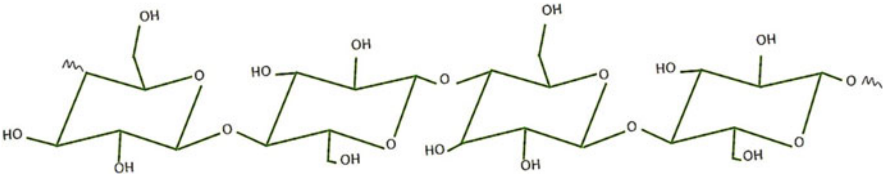


Fig. 2.2 Representative structure of cellulose polymerization from glycosidic bonds

fuels and chemicals from renewable sources as lignocellulose is necessary. In addition, the use of lignocellulose is a potential raw material for obtaining value-added compounds, such as enzymes, ethanol, organic acids, food additives, and others (Canilha et al. 2010; Maitan-Alfenas et al. 2015; Menezes et al. 2017). This fact has attracted the interest of many researchers due to the high potential of lignocellulose residues to obtain new products (Ruqayyah et al. 2013; Zhou et al. 2013).

According to the ECN database Phyllis2 (www.phyllis.nl), which provides reports on biomass and waste composition, it is possible to measure the chemical composition of different lignocellulosic raw materials based on publications between 2016 and 2018 (Table 2.1). Usually, the biomass dry weight contains cellulose (50%), hemicellulose (10–30% in wood or 20–40% in herbaceous plants), and lignin (20–40% in wood or 10–40% in herbaceous plants) (Sharma et al. 2015). However, the composition and proportions of these compounds vary among plants, depending on factors such as age, harvesting season, and growing conditions (McKendry 2002; Prasad et al. 2007).

2.2.1.1 Cellulose

Cellulose, the main component of plant cell walls and algae, is a β-1,4 linker glucose polymer (Fig. 2.2), making it the most abundant carbohydrate in nature, accounting for about 40–45% of the dry weight of lignocellulosic matter. Due to these characteristics, this matter has attracted the interest of researchers in its use for the development of renewable sources (Deswal et al. 2011; Cheroni et al. 2012; Krogell et al. 2013).

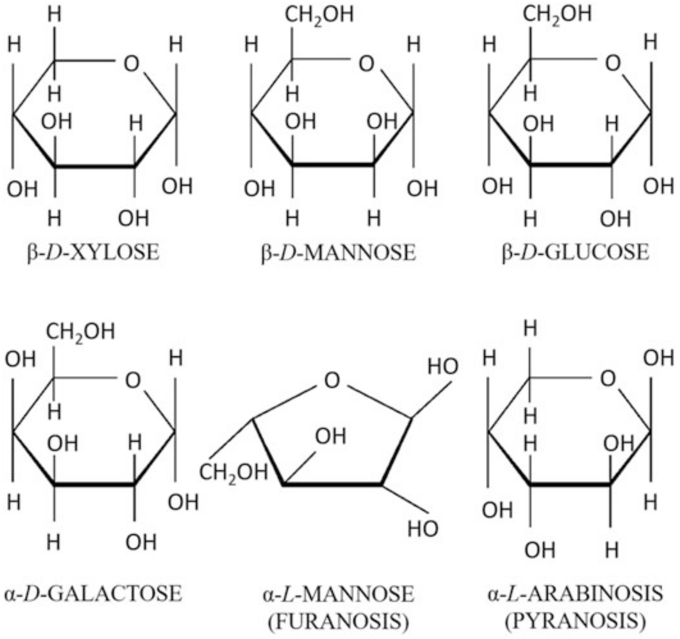
Structurally, cellulose is composed of highly crystallized microfibrils between amorphous matrices, making it difficult to access hydrolysis by enzymes. The use of cellulose as a source of nutrients requires its depolymerization by the cellulase enzymes that promote the cleavage of β-1,4-glycosidic bonds releasing the glucose units (Liu and Cao 2013). The biotechnological potential of cellulolytic enzymes has been demonstrated from basic and applied studies, focusing on several industries, including food, animal feed, beverages, agriculture, biomass refining, pulp and paper, textiles, and laundry. As an example, in recent years, the use of cellulases has become one of the most important measures to improve the performance of food digestion by cattle and poultry and is used as an additive in food (Zhou et al. 2013). In addition, the action of cellulases favors the conversion of cellulose into simple sugars that can be fermented in ethanol (Deswal et al. 2011; Saini et al. 2015).

2.2.1.2 Hemicellulose

Hemicellulose is the second most abundant polysaccharide in plants and may vary depending on the plant species. This polysaccharide presents a flexible character that allows to bind to the surface of the cellulose, forming chains between the microfibrils of cellulose, resulting in a cohesive network of low molar mass, with a degree of polymerization (GP) between 80 and 425 monomeric units (Ayoub et al. 2013). Hemicellulose is associated with other cell wall constituents such as proteins, lignin, and other phenolic compounds formed by covalent bonds and hydrogen interactions. The hemicellulose consists of monosaccharides such as xylose, mannose, arabinose, and galactose (Fig. 2.3), which serve as raw material for the conversion of value-added chemicals such as furfural, 5-hydroxymethylfurfural (HMF), levothyroxine, xylitol, and ethanol (Yi et al. 2013; Zhou et al. 2013; Behera et al. 2014).

The main difference of hemicellulose in relation to cellulose is given by its ramifications composed of chains of several sugars, whereas cellulose has oligomers easily hydrolyzed (Sipos 2010).

Due to the trend towards sustainability in the green industry and a growing interest in the concept of biorefinery, it is possible to use hemicelluloses as a resource to obtain higher added value products such as pectins, ionic polymers, and hydrogels for release (Krogell et al. 2013; Sun et al. 2013; García et al. 2016). There are two determinants for the practical use of hemicellulose isolates: the first one considers chemical composition, and the second suggests that hemicellulose isolation methods influence their structures and, consequently, the possible domains of their applications (Jiang et al. 2014).


β-*D*-XYLOSE β-*D*-MANNOSE β-*D*-GLUCOSE

α-*D*-GALACTOSE α-*L*-MANNOSE (FURANOSIS) α-*L*-ARABINOSIS (PYRANOSIS)

Fig. 2.3 Molecular structure of some monomers commonly found in hemicellulose

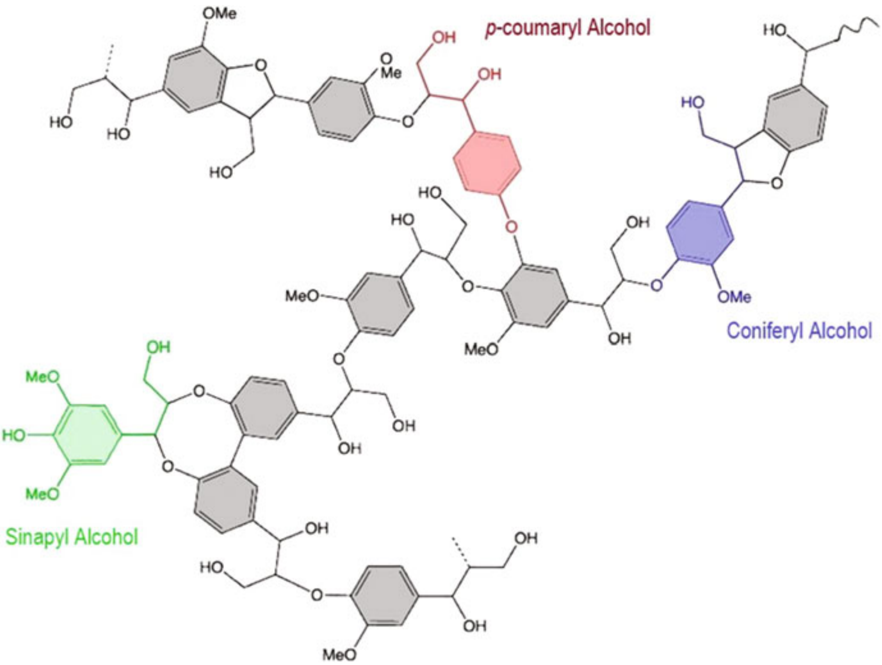


Fig. 2.4 Main connections and functional groups of lignin

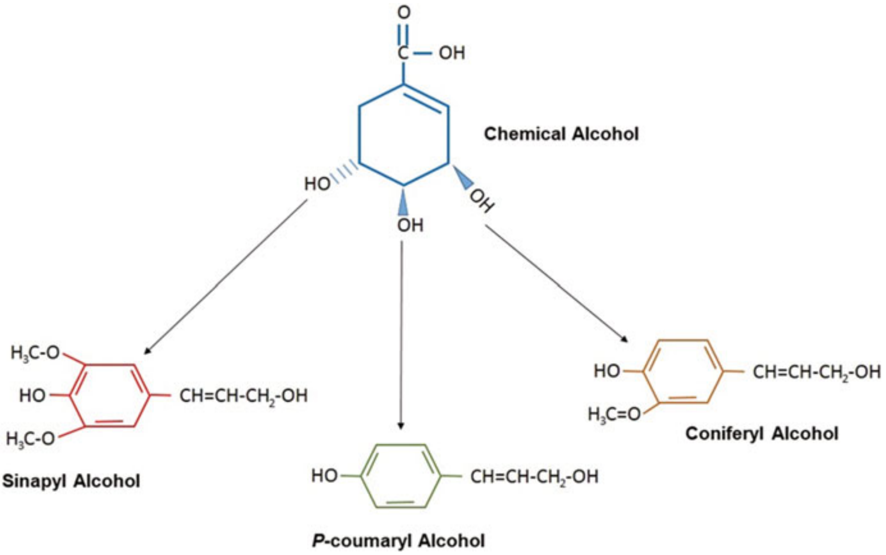


Fig. 2.5 Molecular structure of lignin precursor phenols

2.2.1.3 Lignin

According to Eudes et al. (2014), lignin is a heterogeneous and irregular arrangement of the phenylpropanoid polymer (Fig. 2.4) formed by the polymerization of three monomers: coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, formed by a common precursor (Fig. 2.5), in addition, which represents about 20–30% of the mass total lignocellulosic matter. This composition confers resistance to chemical or enzymatic changes, protecting cellulose from degradation (Krogell et al. 2013). In this way, it is possible to identify that the degradation of lignin is a limiting step for the rate of carbon recycling (Sánchez 2009).

This polymer can be classified as native lignin present in biomass and technical or industrial lignin isolated from biomass by a variety of processes (Bozell et al. 2007).

Due to lignin having a complex polymer structure, the process of mineralization of the structure may take a long time, despite the bacterial and fungi involvement in this process (Bugg et al. 2011; Janusz et al. 2013). These microorganisms have the potential to degrade lignin from agro-industrial and agricultural residues such as corn, converting biomass and synthesizing enzymes with potential use in the paper industry, for example, and in bioremediation of residues (Janusz et al. 2013). In addition to being the fuel for boilers, lignin can be used as polymer additives, surfactants, coloring agents, and as source material for the production of chemicals, fertilizers, adhesives, and dyes. However, lignin is not yet used to its full potential (Sahoo et al. 2011; Ghaffar and Fan 2014).

The fungi present suitable enzymatic mechanisms for the transformation of complex molecules into simple compounds by their ability of selective delignification, which adds value to the bioconversion process. Thus, the microbial conversion of “unused” residues such as corn results in new sources of energy (Elisashvili et al. 2008).

2.2.2 *Pretreatment of Waste*

Considering the high productivity and economic conversion power, several biotechnological processes were developed to use agricultural residues in the production of different products such as alcohol, enzymes, and organic acids. For this to be possible, these residues need to undergo a pretreatment stage, which will allow the fractionation of its components (cellulose, hemicellulose, and lignin), favoring its use more effectively (Phitsuwan et al. 2013; Azelee et al. 2014). In addition, these processes may involve techniques that aim to reduce the recalcitrant character of lignocellulose, making them more reactive and digestible, so that they can be applied in hydrolysis processes (Santos et al. 2012).

In general, the processes of treatment of lignocellulosic materials are classified into four categories: chemical, physical, physicochemical, and biological. Organic solvents (acetone, butanol, toluene, and dichloromethane), mineral acids (HCl, H_2SO_4), and also some bases are generally used within the chemical method. In the physical methods, the parameters temperature, pressure, and mechanical action are applied in a primary way, whereas physicochemical processes involving electrochemistry stand out (Yi et al. 2013; Lee et al. 2014). In addition, there are still biological methods, which make use of microorganisms such as fungi and bacteria, which have metabolisms capable of producing specific enzymes to degrade organic matter. In Fig. 2.6 it is possible to observe a flowchart with the main treatment groups for lignocellulose materials, as well as some of the most used methods in each one of them.

2.2.2.1 Chemical Methods

There are several chemical pretreatment alternatives that can be applied to lignocellulosic materials; however, the main chemical treatment routes used are acids, alkalis, gases, oxidizing agents, and solvents. Although it is possible to obtain high efficiency, in this process, it is necessary to calculate the potential effects against the matter used, since drastic reaction conditions, especially in acidic processes, can modify and/or destroy the components (Zheng et al. 2014; Shirkavand et al. 2016).

Alkaline Extraction

Alkaline pretreatment is often used to increase the digestibility of lignocellulosic materials because of its high capacity to de-structure the lignin bonds and to hydrolyze the hemicelluloses, since this type of treatment promotes the de-

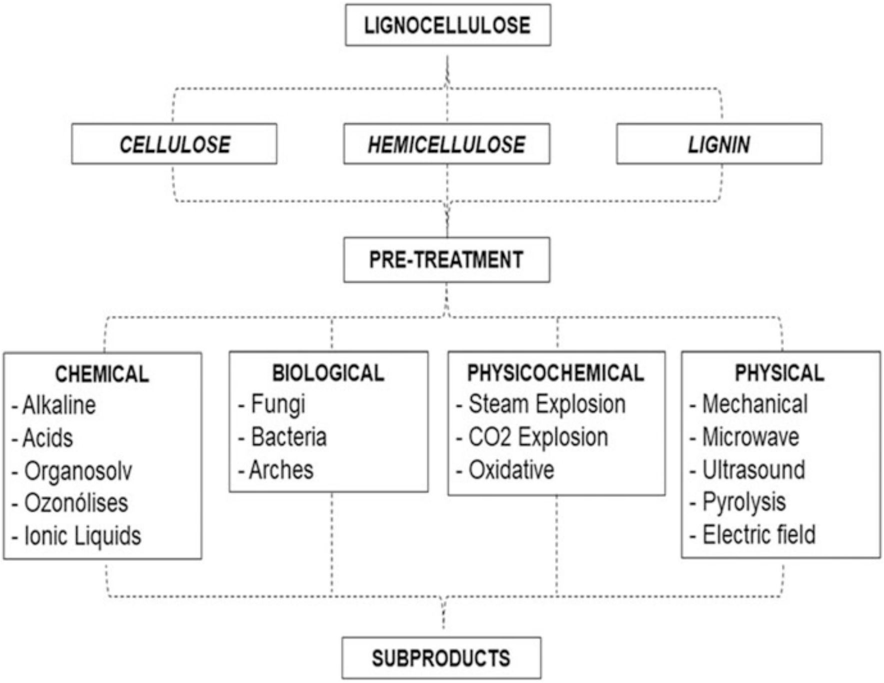


Fig. 2.6 Flowchart for the treatment of lignocellulosic biomass, showing the types of processes that can be applied

structuring of the intermolecular hydrogen bonds between cellulose and hemicellulose and the total or partial cleavage of the ester bonds between ferulic acid and arabinane or galactan in the cell wall, resulting in the dissolution of hemicellulose (Egues et al. 2012; Zhang et al. 2013; Ayoub et al. 2013). In addition, this treatment causes fiber swelling, increasing the internal surface area, and decreasing the crystallinity of the cellulose (Chen et al. 2011). The most bases used in the alkaline treatment are potassium, sodium, calcium, and ammonium hydroxides.

Alkaline processes use lower temperatures and pressures compared to other technologies. They can be performed under environmental conditions, but the reaction time is higher. Compared with acidic processes, this method causes lower sugar degradation, is less corrosive, therefore has a lower reactor load, and allows recovery or regeneration of the salts (Kim et al. 2016). In contrast, the compounds employed are more expensive and are used in higher concentrations. Another problem is related to possible environmental problems, which can increase waste treatment costs and recycling is necessary to make them viable (Prasad et al. 2007). There may also be problems with the biomass itself, which can absorb the bases and hinder fermentation (Hamelinck et al. 2005). The degradation of lignin generates by-products in the form of monomers and phenolic oligomers that negatively affect the fermentation process.

The hydroxide ions present in NaOH provide the release of a portion of the hemicellulosic material in solution. In addition, partial hydrolysis, oxidation or esterification of the hydroxyl groups, and cross-linking can modify the properties of hemicellulose, providing a polymeric raw material for new industrial applications, among them starch products for biodegradable plastics (Ayoub et al. 2013).

Organic Solvents

Pretreatment by organic solvents can be efficient in removing lignin and hemicellulose through the break of the internal bonds. However, disadvantages as solvent recovery, high cost, as well as flammability, and volatility should be considered (Brum et al. 2012). Among the parameters that directly affect the efficiency of this process, temperature, retention time, and solvent concentration can be cited. Organic solvents such as methanol, ethanol, acetone, and ethylene have been used for the pretreatment of different lignocellulosic materials (Kumari and Singh 2018).

In this treatment group, the technique called organosolv, an alternative treatment using a lower concentration of reagents using a mixture of water and organic solvents (Koo et al. 2011; Mood et al. 2013), may be highlighted. This type of delignification process allows the total utilization of the constituents of the vegetal biomass, allowing the recovery of the solvent used (Mesa et al. 2011). Thus, this method reduces the environmental impact of conventional delignification processes, allowing the greater viability for the integral use of the lignocellulosic components, using low investment capital.

Other advantages of organosolv application are the absence of strong odors during the process, ease of recovery of hemicelluloses and lignin that are less degraded, and ease of adjustment of bleaching steps in pulps using non-chlorinated reagents (Mesa et al. 2011). However, the organosolv process does not allow rapid washing of the lignocellulosic residue in water as in conventional processes, due to the precipitation of the lignin on the residue and the volatility of the solvent requiring that the process be well controlled (Sun and Chen 2008).

Acids

Currently, acids have been of interest in the treatment of lignocellulosic materials for the production of bioethanol, since they promote satisfactory results in the hydrolysis of hemicelluloses and the attack on lignin bonds. The most used acids in the pretreatment of biomass are sulfuric acid, nitric acid, hydrochloric acid, and phosphoric acid (Mosier et al. 2005; Tian et al. 2018). The acid pretreatments have advantages over alkaline processes because they are highly reactive, have high efficiency in converting most of the hemicellulose into soluble and fermentable sugars, significantly improve the hydrolysis of cellulose, and are relatively cheaper. The yield in sugars for the fermentation increases significantly. However, the process generates chemicals (such as furfural and phenolic components), which are undesirable in the subsequent processes (Pereira Jr. et al. 2008; Kumar et al. 2009).

For acid treatments of the biomass, usually are used concentrated acids or diluted acids such as H_2SO_4 and HCl. However, despite their efficiency in degrading biomass, these compounds are corrosive, toxic, and require corrosion-resistant reactors in industrial plants, as well as, a recovering process for the concentrated acid, which makes the pretreatment process very expensive (Kumar et al. 2009). In the diluted acid hydrolysis method, further to using a low concentration acid, high temperatures are also used to dissolve hemicelluloses from the cell walls of the biomass, making the cellulose more accessible for subsequent hydrolysis steps. The processing conditions can be adjusted according to the type of raw material, temperature, and reaction time (Alvira et al. 2010).

Ionic Liquids

Ionic liquids (LIs) have shown to be very promising for the treatment of lignocellulosic residues, especially those that have at least one organic ion (cation) and with melting points below or not much above room temperature, which would favor industrial use by reducing energy costs (Seoud et al. 2007; Aung et al. 2018). LIs are referred to as "green" solvents due to exhibit thermal and chemical stability, extremely low vapor pressure, and are non-flammable, allowing the solvent to recycle during the treatment process. These compounds also have the ability to separate organic and inorganic material, as well as variable miscibility in water and inorganic solvents, favoring their use in the treatment of biomass (Seoud et al. 2007; Yoo et al. 2017). The LIs present physical and chemical properties that can be controlled by the combination of cations and anions that compose them, thus favoring the connections between the LI molecules and the residue to be treated. Moreover, it is possible to highlight that the less lipophilic is an ionic liquid, the more efficient its performance in applications, such as the dissolution of cellulose (Wang and Sain 2007; Boujemaoui et al. 2015).

The mechanism of depolymerization of the lignocellulosic material in ionic liquid involves the hydroxyl atoms of the cellulose that forms a donor/receptor electron system, which interacts with the LIs. Within this system, oxygen atoms act as electron donors, while hydrogen acts as a receptor. In this way, the free ions of the LIs associate with the protons of the hydroxyl of the cellulose, whereas the cations complex with the oxygen of the hydroxyl, thus interrupting the hydrogen bonding in the cellulose, promoting its fragmentation (Cao et al. 2009). In addition, it is possible to highlight that imidazolium-based lithium-based lithium may contribute to the dissolution of cellulose, due to its relative acidity and oxygen association capacity of the OH groups of cellulose (Fei et al. 2007).

2.2.2.2 Physical Methods

The physical pretreatment methods can be characterized by the use of techniques that promote the increase of the surface area of the residue as the granulometry decreases, as well as the reduction of the degree of polymerization of part of its components, that can favor the action of treatments. There are several physical treatments that are usually described in the literature, such as mechanical treatment, extrusion, irradiation (ultrasound, microwave), and pyrolysis (Behera et al. 2014).

Mechanical Treatment

Mechanical treatment of biomass is used to reduce particle size and is typically applied primarily to other treatment methods. The reduction of biomass particle size increases the accessible surface area for subsequent hydrolysis processes, reduces the degree of cellulose crystallinity, and decreases the degree of polymerization of cellulose to improve the degradation (Behera et al. 2014). The mechanical action on the biomass can be accomplished using milling machines or grinding machines, including balls, vibro, hammer mills, knife, two rollers, colloids, and friction (Barakat et al. 2014; Bhutto et al. 2017).

The moisture content of the lignocellulose raw material is an important factor in choosing a grinding machine. Biomass with moisture below 15% (wet basis) are usually treated with rolls, hammer, friction, and knife mills, while materials with a moisture content higher than 15% are treated with colloid mills and extruders, already for dry or wet materials are used ball mills and vibro (Miao et al. 2012).

Irradiation

Irradiation, a physical biomass pretreatment, includes several procedures such as microwaves, ultrasound, gamma rays, and electron beam. Among them, microwave pretreatment is widely studied, through which the energy from an electromagnetic field acts directly on the material, providing rapid heating and reduced thermal gradients (Pellera and Gidarakos 2017; Kumari and Singh 2018). The microwave field and material dielectric response are decisive for its heating ability by microwave energy, representing an effective alternative compared to conventional heating. This allows for quick heating besides a reduction in treatment time and energy consumption (Hassan et al. 2018).

Extrusion

Extrusion is a thermo-physical procedure through which the residue is subjected to an association of conditions, such as heating, shearing, pressure, etc. The process can promote the material abrasive wear as a function of the pressure, which promotes the biomass de-structuring, realizing cellulose, hemicellulose, lignin and protein, and sugar and amino acid degradation. This process depends closely on operational parameters including pressure, reaction time, and dry biomass (Chen et al. 2014a; Rodríguez et al. 2017).

As advantages of this process, high shear rate, fast mixing, short residence time, moderate temperature, formation of secondary compounds such as HMF, hydroxymethylfurfural, process adaptability, and easy scaling can be cited. However, the technique has some negative points, among them, the generation of effluents and the increase in costs, due to the additional steps required for the treatment of formed toxic by-products (Gatt et al. 2018).

2.2.2.3 Physicochemical Methods

Steam Explosion

In this process, there is disintegration, and disintegration of the fibers of the material may occur until its rupture. There is also the breakdown of some chemical bonds of the biomass components, which have their activation energies overcome due to the elevated temperature (Duque et al. 2016). What happens in this pretreatment is that the vapor that penetrates the lignocellulosic material condenses and becomes liquid water at high temperatures. If the pressure is withdrawn, the water comes into contact with the vapor and evaporates so rapidly that the explosion takes place (Biswas and Ahring 2016; Lorenzo-Hernando et al. 2018).

Steam blasting acts both chemically and physically in the transformation of the material, involving the treatment with saturated steam at temperatures of 160–240 °C for a reaction time ranging from 2 to 30 min, with or without an acid catalyst. The most commonly used catalysts are SO_2, CO_2, H_2SO_4, and H_3PO_4 (the most commonly used H_2SO_4), and their use leads to more complete removal of hemicelluloses. The process requires low energy consumption, compared to other physical processes, and has lower environmental impact and capital investment (Chen et al. 2014b; Martín et al. 2018).

Explosion of Carbon Dioxide (ECD)

This method applies the supercritical CO_2 (SC-CO_2), which under pressure conditions increases the lignocellulosic biomass digestibility which is subjected at high pressure (1000–4000 psi) and maintained under controlled temperature and time conditions (Carneiro et al. 2016). The CO_2 interacts with the biomass under high pressure, which in contact with water promotes carbonic acid formation, which helps in the hemicellulose hydrolysis. The release of pressurized gas generates the biomass structure disruption, increasing the specific surface area required for the solvent access to the matrix (Dong and Walker 2008). The hydrolytic yield efficiency by

ECD pretreatment requires a certain moisture content in the biomass. As a positive aspect of the process, highlighted can be the low cost of carbon dioxide, the nontoxic by-product generation, and low operating temperatures. However, the high cost of equipment, capable of withstanding high-pressure conditions, restricts its industrial application (Santos et al. 2011; Benazzi et al. 2013).

2.2.2.4 Biological Methods

Based on the complex and heterogeneous structure of lignocellulosic matrices, the biological mechanism of pretreatment is closely associated with the microbial capacity to produce enzymes, whose action can release the biomass components (Behera et al. 2014; Bhutto et al. 2017). Some bacteria, mainly found in soil, present the degradation capacity of the structural components of lignocellulose. Frequently, due to their rapid growth, bacteria are applied in processes for the polymeric molecule degradation, whose products can be recovered to obtain value-added products, including enzymes (Hatakka 2005).

In addition, some bacteria also have the ability to degrade the polymeric structures present in the residue, facilitated mainly by the extracellular xylanases that induce the degradation of the hemicellulose and release simultaneously interconnected lignin components (Vasco et al. 2016). Notwithstanding, bacteria are less applied in processes of lignocellulosic biomass degradation and efficacy when compared to the fungi, due to their inefficiency in producing ligninolytic enzymes, compared to fungi (Akhtar et al. 2015; Xu et al. 2018). Furthermore, some limitations restrict the use of bacteria under environmental stress conditions, such as low nutrient levels, low pH, high contaminant concentrations, and low efficiency in the degradation of water-soluble or soil-bound compounds, in addition to the induction of its enzymatic system to occur only in the presence of the contaminant. Thus, certain contaminant levels could be insufficient to induce the enzyme expression or activity of the enzymes required for the biodegradation process (Moreno et al. 2004).

According to (Kirk et al. 2008), there are approximately 29,914 known species of basidiomycetes, ranging from the popular mushrooms and wooden ears to the coals, rust, gastropods, and jellies. Most species are saprobic, but many obligate or facultative parasites occur as well as mycorrhizal parasites. Basidiomycetes play a crucial role in the nutrient cyclings in nature, especially in the carbon cycle, as efficient lignin degraders, the second most abundant biopolymer on the planet. These fungi also contribute to maintaining the nitrogen, phosphorus, and potassium cycle, incorporated into cell wall insoluble components (Carlile et al. 2002).

Fungi capable of degrading woods and pollutants are divided into three groups: soft, brown, and white fungi. The mild degradation fungi are deuteromycetes and ascomycetes, which have great capacity for degradation of polysaccharides, but they lose performance when they are used for the degradation of lignin. Brown-rot fungi are efficient cellulose and hemicellulose degraders (Sánchez 2009; Sindhu et al. 2016), whereas white-rot fungi degrade lignin. Among fungi, lignolytic enzyme producers, the most studied for the degradation of compounds with lignin is *Phanerochaeta chrysosporium* and the genera *Pleurotus*, *Trametes,* and *Ceriporiopsis*, because they present an enzymatic complex with nonspecific extracellular release (Aguiar and Ferraz 2012).

Studies on lignin degradation by white-rot fungi have revealed three types of extracellular enzymes responsible for its initiating depolymerization, identified as lignin peroxidase (LIP), manganese peroxidase (MnP), and laccase (Lac) (Menezes and Barreto 2015). The enzyme expression is closely related to microorganisms since some secrete LiP and MnP (without Lac), while others can secrete only MnP and Lac. Further, white-rot fungi display efficiency in degrading a range of persistent environmental pollutants such as heterocyclic aromatic hydrocarbons, chlorinated aromatic compounds, dyes, and synthetic polymers (e.g., carbamazepine, diclofenac) (Jayasinghe et al. 2008; Rubilar et al. 2008; Si et al. 2013; Golan-Rozen et al. 2015). This degradation is probably due to a strong oxidative activity and low specificity of ligninolytic enzymes to the substrate. Thereby, white-rot fungi and their enzymes display a wide application in some industrial processes to synthesize bioproducts and in bioremediation procedures (Janusz et al. 2013).

Fermentation

After the initial selection process of the microorganisms with enzymatic potential for degradation, they can be applied in fermentation processes, where they will be inoculated in culture media containing the residue to be treated, associated with controlled conditions of pH, temperature, agitation, and aeration (Farinas 2015). From this, it is possible to obtain the fragmentation of the residue infractions or to promote the removal of toxicity from contaminants. However, this process is generally used as a tool to obtain by-products, such as biofuels and enzymes, which have numerous applications in the most varied industrial sectors (Najafpour 2015).

Fermentation methods include submerged fermentation (SmF) and solid-state fermentation (SSF). SSF can be defined as that developed using solid substrates with low or no water content. Nevertheless, substrates require sufficient moisture to promote microbial growth and metabolic sustainability. This type of fermentation is probably the oldest used by man, with references dating from 1000 BC, when food was already being produced, using agricultural products (wheat, barley, soy) as a substrate (Soccol et al. 2017).

The SSF has advantages such as the use of substrates with low added value, reduced medium volume, reduced investment in bioreactors, direct inoculation, reduced contamination, and facilitated aeration. On the other hand, this type of fermentation presents restrictions regarding its application, which may be associated with the inefficiency of the growth of some microorganisms in systems with low humidity and difficulty in controlling the parameters. In addition, the difficulty of homogenization and diffusion in the reaction medium also present as process problems (Singhania et al. 2009; Martins et al. 2011).

The SmF consists of inoculating a microorganism (inoculum) in a liquid medium (must). This process uses fermenters supplied with several devices to monitor variables such as agitation and aeration, pH, temperature, and dissolved oxygen. Furthermore, in this type of medium the nutrients are dissolved in the liquid medium and are easily accessible for use by microorganisms. Compared with FMS, the submerged processes offer several advantages such as ease of handling and higher volumes of a medium; nutrient absorption and metabolic excretion are performed more efficiently, resulting in shorter fermentation time and, consequently, higher productivity (Fazenda et al. 2008; Hansen et al. 2015).

2.3 Enzymes (Definitions)

Industrial processes involving chemical reactions with polluting potential are present mostly in the manufacture of products or consumer goods by man. Most of these reactions are catalyzed by chemical agents that can be replaced by enzymes. The enzymes are capable of accelerating chemical processes, displaying great advantages over chemical catalysts since enzymes are (i) natural biological and biodegradable products, (ii) feature reaction specificities, (iii) implement nonconsumption during the process, (iv) catalyze chemical reactions by decreasing activation energy, (v) are stereoselective, and (vi) act at mild pH and temperatures. Therefore, enzymes become an environmentally viable solution (Sarrouh et al. 2012). Nowadays, several industries use enzymes as catalysts with emphasis on food, health, and environmental areas (Monteiro and Silva 2009).

Thereby, currently, there is a biotechnological challenge regarding the prospect of new enzyme sources and in improving the performance of those enzymes already known. Concomitantly, the study with enzyme-producing microorganisms these enzymes is constant by the application of molecular biology and genetic engineering tools aimed at microbial prospecting and development of genetically modified organisms. Notwithstanding, several problems impede this development, such as the difficulty of reproducing environmental conditions and the transfer condition from ab-scale to pilot plant-scale or industrial scale, associated with the low research-based industry (Monteiro and Silva 2009; Zhang et al. 2012; Fasim et al. 2021).

According to the International Union of Biochemistry and Molecular Biology, enzymes are classified into six classes based on the type of catalyzed reaction: (i) oxiredutases: catalyze oxidation-reduction or electron transfer reactions; (ii) transferases: transfer functional groups such as amine, phosphate, acyl, carboxyl, between molecules; (iii) hydrolases: catalyze covalent bond hydrolysis reactions; (iv) liases: catalyze addition and elimination reaction; (v) Isomerases: interconversion reactions between optical or geometric isomers; and (vi) ligases: condensation of two molecules, always with the aid of energy, usually ATP (Sarrouh et al. 2012).

2.3.1 World Market

Global enzyme trade is divided into two categories: special enzymes (therapeutic enzymes, diagnostic enzymes, enzymes for chiral chemistry, and enzymes for research) and industrial enzymes (mainly for food and animal feed industries). According to Business Standard, global enzyme demand has grown steadily at an annual rate of 4.5% and is expected to reach a value of US$ 7.2 billion in 2020, referring to the two categories of industrial application enzymes such as amylases, followed by cellulases and lipases. The Latin American market accounts for 3.4% of the enzyme worldwide demand, of which 60% of this consumption corresponds to Brazil. Nevertheless, Brazil represents a small part of the international market, importing 86% of enzymes while exporting only 14% of this input, which reveals technological and strategic backwardness (Valencia and Chambergo 2013; Daiha et al. 2016; Rodrigues et al. 2020).

The global enzyme market was valued at US$ 9.9 billion in 2019 with a compound annual growth rate (CAGR) of 7.1% for the period 2020 to 2027 (Arnau et al. 2020; Sharma and Upadhyay 2020). According to Research and Markets report, “Industrial Enzymes—Global Market Overview,” the growing enzyme global market is by industrial demand from the food and beverage, biofuels, animal feed, and household cleaning sectors (Arnau et al. 2020; Market Analysis Report 2020).

The industrial enzymes applied in the area of food and beverages correspond to the largest segment of the market, absorbing a quarter of the total produced. It was estimated that the market would reach US$ 2.1 billion in 2016, a CAGR of 10.4%. However, it was observed that the projections did not occur as expected and had a 26% share equivalent to US$ 1.4 billion in 2017, evidencing a significant drop, which may be linked to the growth of other segments. In the context of industrial enzymes, it is also possible to highlight the production of biofuels and detergents with 18 and 14%, corresponding to US$ 969.3 and 754.4 million, respectively. However, the enzyme demand for the biofuel sector is growing rapidly compared to other segments with a compound annual growth rate of approximately 7.3% up to 2024.

Nowadays, the demand for enzymes grows rapidly due to the industrial environment transformation promoted by trends in the development of differentiated food and beverages to meet the consumer demand and acceptance attributed to changes in the population’s lifestyles. Furthermore, world governments have encouraged biofuel use as a clean energy-based alternative, which has boosted the industrial enzyme markets. Thereby, biotechnological advances, particularly in the protein engineering area, have driven the biocatalyst sector to meet the enzyme demand for industrial application. Regarding technical enzymes, this category was responsible for the revenue of approximately US$ 1.2 billion in 2011, which corresponds to an annual growth rate of 8.2% (Comyns 2012; Arnau et al. 2020). This explains prospecting studies for enzymes more tolerant to pH and temperature variations to develop industrial processes since these variations directly influence the enzymatic activity

Table 2.2 Enzymes and their applications in several industrial processes

Industry	Enzyme	Application
Cleaning	Amylase	Removal of starch stain
	Cellulase	Cleaning, bleaching, anti-cotton deposition
	Lipases	Fat stain removal
	Protease	Removal of protein stains
	Ligninases	Removal of synthetic dyes
Foods and beverages	Amylase	Production of syrups, reduction of calories from beer
	Cellulase	Lightening of furrows, concentrates of coffee
	Lipases	Flavor to cheeses
	Protease	Milk coagulation, meat sweetening, brightening beer
	Ligninases	Degradation of waste from production
Cosmetics	Amylase	Anti-signs, free radical fighting
	Cellulase	Auxiliary digestion/carbohydrate
	Lipases	Deep cleansing of the skin, treatment of acne and dandruff
	Protease	Peeling, stretch marks, oil control, and seborrhea
Therapies	Amylase	Control of pancreatic juice deficiency
	Cellulase	Support digestion
	Lipases	Restore pancreatic enzymes and control digestion
	Protease	Auxiliary of digestion; debridement of ulcers/protease
	Ligninases	Antimicrobial

Adapted from Ole et al. (2002), Monteiro and Silva (2009)

efficiency and, consequently, its application (Shakeel and Husain 2012). Table 2.2 shows some enzymes of industrial interest and their applications.

The use of enzymatic engineering coupled with recombinant DNA technology and the heterologous expression of enzymes will also represent a new leap forward for enzyme production in the next decade and will be one of the basic points in the development of new enzyme-produced industrial products. Another very promising area for enzyme technology is environmental control to gradually replace chemical processes with enzymatic processes, so-called green technology, playing an important role mainly in industries that apply lignocellulosic raw material to transform it into higher value-added products (Ravindran et al. 2012; Sharma et al. 2018; Leite et al. 2021).

2.3.2 *Enzymes in the Food and Beverage Industry*

As previously mentioned, the food and beverage enzyme market is the largest industrial segment, with an estimated revenue of approximately US$ 2.0 billion up to 2020. Enzymes used in the food industry can be classified into food additives and auxiliary agents during processing which have a significant application in bakery, beverages, and cheeses sectors (Liu and Kokare 2017).

In the beverage industry, it is possible to highlight the use of enzymes in juices, wines, and beer production in order to reduce production costs, energy consumption, besides improving sensorial aspects of the product (Quang et al. 2014). Regarding juice production, a serious problem is the high pectin and starch concentration in ripened fruits, which generates turbidity and higher viscosity in the final product (Danalache et al. 2018). Thus, pectinases and amylases, naturally occurring enzymes, are responsible for the clarification step as well as enhancement of taste and texture, as well as xylanase and cellulase, which are applied to facilitate the release of the juice (Ephrem et al. 2018; Mushtaq 2018; Souza et al. 2020). Enzymes are also applied in wine and brewing processes to improve the raw material component extraction and yields. In addition, enzymes hydrolyze high-molecular-weight substances. This allows solving problems during the filtration process caused by the presence of β-glucans in the malt and controlling the maturation and storage process (Llaubères 2010; Du et al. 2013; Gibson and Newsham 2018).

Concerning the bakery industry, the focus is on the lipolytic enzyme application since studies have shown their use to replace or supplement conventional emulsifiers due to the enzyme's ability to degrade wheat lipids and favor their solubilization (Brites et al. 2018). In addition, there are enzymes such as α-amylases and xylanases that prevent the hardening of loaves, while lipases are related to the degradation of fats, oils, and related compounds, being applied in taste and fragrance control (Bock 2015; Fallahi et al. 2018).

In the dairy sector, enzymes such as proteases, lipases, and lactase are applied. The first stage of the milk coagulation process for cheese production is carried out with rennin, whereas lipases and proteases are added to accelerate cheese maturation (Sobrevilla et al. 2015). Furthermore, lactase hydrolyzes lactose into glucose and galactose, enhancing its solubility and sweetness in several dairy products and reducing the milk allergenic potential (Patel et al. 2017).

2.3.3 Bioremediation

The worldwide population associated with industrial activities has promoted waste accumulation whose polluting capacity has reached critical levels. We are currently interacting more and more with numerous persistent pollutants that are released from various manufacturers, such as industrial solvents, pesticides, heavy metals, petroleum products, dyes, fertilizers, and food additives. The accumulation and recalcitrance of most pollutants are responsible for a severe environmental impact, causing deleterious effects on human and animal health (Das and Dash 2014; Ghosh et al. 2017). Industrial and urban wastes are deposited in landfills or subjected to chemical and/or physical treatments, whose limited efficiency can generate secondary contaminants due to the complex nature of the compounds. Thus, bioremediation is the focus of studies as an ecological and economically sustainable technique to treat contaminated environments (Dzionek et al. 2016; Sharma et al. 2018).

Bioremediation is a strategy that applies the microbial metabolic capacity; therefore, their enzymes play an important role in the maintenance and sustainability of any ecosystem due to microbial ability to degrade or dissimilar toxic compounds transforming them into less toxic molecules to the environment. Thus, bioremediation represents an economical and ecologically friendly tool (Rao et al. 2010, 2014; Pande et al. 2020). In this context, bacteria, algae, fungi, and even plants have been studied to identify species that display xenobiotic-degrading capacity (Sutherland et al. 2004; Liu and Kokare 2017). The research contributes to the development of bioprocess to reduce pollutant accumulation and also obtain added-value substances. The bioremediation effectiveness depends on environmental conditions for the microorganism growth with a metabolic capacity to use the organic pollutant as a carbon source to transform it into CO_2, H_2O, ATP, and simpler molecules, which implies availability or accessibility of the contaminant for its microbial degradation (Khatoon et al. 2017; Sun et al. 2020).

Nonetheless, the use of microorganisms to degrade contaminants with the help is a lengthy process, which, in practice, can affect the bioremediation feasibility. Therefore, isolated enzymes have been used more for bioremediation compared to the direct application of microorganisms (Sharma et al. 2018). Overall, an enzymatic process basically depends on the catalytic activity of the enzyme, while microbial degradation requires an inductive environmental condition. However, microbial adaptability, versatility, and mutability represent an advantage for degrading different compounds (Vallero 2010).

The main classes of enzymes applied directly applied to degrade transform pollutants are oxidoreductases and hydrolases due to their dual function in degrading and protecting (Manubolu et al. 2018). Hydrolases have the ability to reduce complex substrates by transferring electrons from reducers to oxidants, facilitating their microbial metabolism. These enzymes work in cooperation with oxidoreductases which catalyze the breakdown of organic compounds into simpler molecules available for the transformation of the pollutant into CO_2, water, and ATP (Sharma et al. 2018).

2.3.4 Biosensors

A biosensor is an analytical device which may contain biological structures origin, such as antibodies, cells, or enzymes, which are associated with a transducer to convert a biological signal into an electrical signal directly proportional to the target analyte to be monitored (Gianfreda et al. 2016). A biosensor consists of three elements, a bioreactor, a transducer, which can be electrochemical, optical, piezoelectric, and thermometric, as well as a signal processor (Muguruma 2017). Regarding enzymatic biosensors, their initial construction applied immobilization methods by adsorption of enzymes to the support through van der Waals forces, ionic or covalent binding (Mehrotra 2016).

Considering their purpose, biosensors can be highly selective due to the interaction or affinity of the target compound with the sensor substrate. In addition, they offer advantages such as reagent-free tests, quick and preferably reversible responses. To design a biosensor, stability, analysis time, selectivity, and analytical signal are parameters that must be considered (Davis and Higson 2012).

Transducer surfaces are often modified to increase enzyme stability and analytical sensitivity, while this change in electrochemical transducers aims to reduce the sensor's interaction with other analytes (Pacheco et al. 2017). In addition, enzyme immobilization on the surface of the transducer is a factor that ensures long-term performance and stability improvement (Rao et al. 2014). The biosensor manufacture requires the production of its materials, transduction devices, and immobilization methods, representing a multidisciplinary area, involving chemistry, biology, and engineering. The biosensor materials are categorized based on their mechanisms: biocatalytic group, bio-affinity group, and microorganisms. Therefore, biosensors are extensively researched and developed as an application tool in the medical, environmental, food, and pharmaceutical fields (Muguruma 2017).

In the medical field, the biosensor application is growing rapidly, mainly for diabetes mellitus diagnosis and blood glycemic control, which represents 85% of the world market in this segment. Furthermore, biosensors are widely used in the diagnosis of infectious diseases, quantitative determination of cardiac markers, and rapid and accurate detection of cancer markers as well as immunosensor for clinical identification of acute leukemias (Karunakaran et al. 2015).

Concerning the food industry, one of the main requirements is related to quality, safety, maintenance, and processing of the raw material to obtain the final product. This evaluation is performed by chemical and physical traditional techniques, although displaying due to human failures, cost, and long response time. Therefore, the development of biosensors for the food sector emerges as a promising alternative to meet commercial demand since sensors provide simple, selective, and real-time responses (Gao and Lu 2015; Bahadır and Sezgintürk 2017). The use of biosensors allows you to monitor the aging of foods and detect pathogens and pesticides (Pacheco et al. 2017). It is also possible to develop sensors to control parameters such as appearance, taste, smell, texture, and even nutrients during the manufacturing process (Amine et al. 2016).

In the environmental area, the application of biosensors is interesting, since they present important characteristics when environmental monitoring is desired, such as portability, low cost, minimal sample preparation, and stability of the instrument in front of the numerous obstacles in this field. Biosensors in this segment are generally applied to soil and water monitoring and control, identifying the presence of pesticides and heavy metals, proven to be toxic and carcinogenic compounds, which are linked to agribusiness, mining, chemical, cosmetics, and paint industries (Amine et al. 2016).

2.4 Conclusion

The chemical composition of lignocellulosic residues, formed by cellulose, hemicellulose, and lignin, as well as its renewable nature, show the potential of this material to produce value-added compounds as low-cost enzymes for use in various industrial sectors. Hydrolases and oxidoreductases are obtained from fermentation using lignocellulosic biomass as input and, during the previous fermentation process, the choice of the biomass treatment type is extremely important for obtaining these enzymes. These enzymes are required in biotechnological processes applied mainly in the areas of bioremediation, food and beverage industry, paper bleaching, and biosensor construction. Thus, the use of lignocellulosic residues as a source of convertible molecules is promising not only for obtaining enzymes but also for other compounds of industrial interest.

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