Green Energy and Technology

Helen Treichel
Gislaine Fongaro
Thamarys Scapini
Aline Frumi Camargo
Fábio Spitza Stefanski
Bruno Venturin

Utilising Biomass in Biotechnology

A Circular Approach discussing the Pretreatment of Biomass, its Applications and Economic Considerations

Springer

Green Energy and Technology

Climate change, environmental impact and the limited natural resources urge scientific research and novel technical solutions. The monograph series Green Energy and Technology serves as a publishing platform for scientific and technological approaches to “green”—i.e. environmentally friendly and sustainable—technologies. While a focus lies on energy and power supply, it also covers “green” solutions in industrial engineering and engineering design. Green Energy and Technology addresses researchers, advanced students, technical consultants as well as decision makers in industries and politics. Hence, the level of presentation spans from instructional to highly technical.

Indexed in Scopus.

More information about this series at http://www.springer.com/series/8059

Helen Treichel · Gislaine Fongaro ·
Thamarys Scapini · Aline Frumi Camargo ·
Fábio Spitza Stefanski · Bruno Venturin

Utilising Biomass in Biotechnology

A Circular Approach discussing the Pretreatment of Biomass, its Applications and Economic Considerations

Springer

Helen Treichel
Laboratory of Microbiology and Bioprocess
Department of Environmental Science
and Technology
Federal University of Fronteira Sul
Erechim, Rio Grande do Sul, Brazil

Thamarys Scapini
Laboratory of Microbiology and Bioprocess
Department of Environmental Science
and Technology
Federal University of Fronteira Sul
Erechim, Rio Grande do Sul, Brazil

Fábio Spitza Stefanski
Laboratory of Microbiology and Bioprocess
Department of Environmental Science
and Technology
Federal University of Fronteira Sul
Erechim, Rio Grande do Sul, Brazil

Gislaine Fongaro
Laboratory of Virology
Universidade Federal de Santa Catarina
Florianópolis, Santa Catarina, Brazil

Aline Frumi Camargo
Laboratory of Microbiology and Bioprocess
Department of Environmental Science
and Technology
Federal University of Fronteira Sul
Erechim, Rio Grande do Sul, Brazil

Bruno Venturin
Department of Agricultural Science
Agricultural Engineering Post-Graduate
Program (UNIOESTE)
Universidade Estadual do Oeste do Paraná
Cascavel, Paraná, Brazil

ISSN 1865-3529 ISSN 1865-3537 (electronic)
Green Energy and Technology
ISBN 978-3-030-22852-1 ISBN 978-3-030-22853-8 (eBook)
https://doi.org/10.1007/978-3-030-22853-8

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Chapter 1
Circular Economy Based on Residue Valorization

To meet the population's demand for products and services, it is inevitable that industry will interact with the environment during the search for inputs and outputs in the production processes. With the globalization of the market, it is no longer efficient or sustainable for companies to employ linear economic models in which the fundamental idea is to extract raw material, transform it, and discard it at the end of its life cycle [1].

For industries to adopt new models, fundamental concepts of the laws of nature must be pursued; the natural cycle of materials is one of the foundations of the circular economy. The expression *circular economy* has been used since the 1970s. It is based on the concept of an economy being restorative and regenerative with continuous development based on natural preservation and reduction [2]. Circular economics seeks to link business in the form of a network when, a material is no longer, it can be reallocated within a company or may be shared with another organization or segment that can take advantage of it and thereby generate revenue streams [3].

The development of the circular economy requires the adoption of systems that tend to move away from the traditional modules of food production, energy, and water use, seeking integrative adoption of practices that increase the conversion and valuation of residues into products that add value. Furthermore, it seeks to reduce the use of natural resources and wastes generated in agrobusiness, livestock farming, and other industries [4].

The benefits of a circular production system are categorized as short term and long term. In most cases, the advantages of modifying a production process are more intense at the end; in the short term, these changes require social and cultural transformations within organizations. Because this is a system based on the natural cycle, enterprises that adopt it must internalize the fact that the regeneration of resources such as water and energy requires time and space [5]. The values added to the circular economy, in addition to sustainability, are based on environmental quality, economic prosperity, and social equity, based on amenity values, economic resources, sinks for residual flows and support for increasing life cycles; all these bases are intertwined (Fig. 1.1) [6].

H. Treichel et al., *Utilising Biomass in Biotechnology*,
Green Energy and Technology, https://doi.org/10.1007/978-3-030-22853-8_1

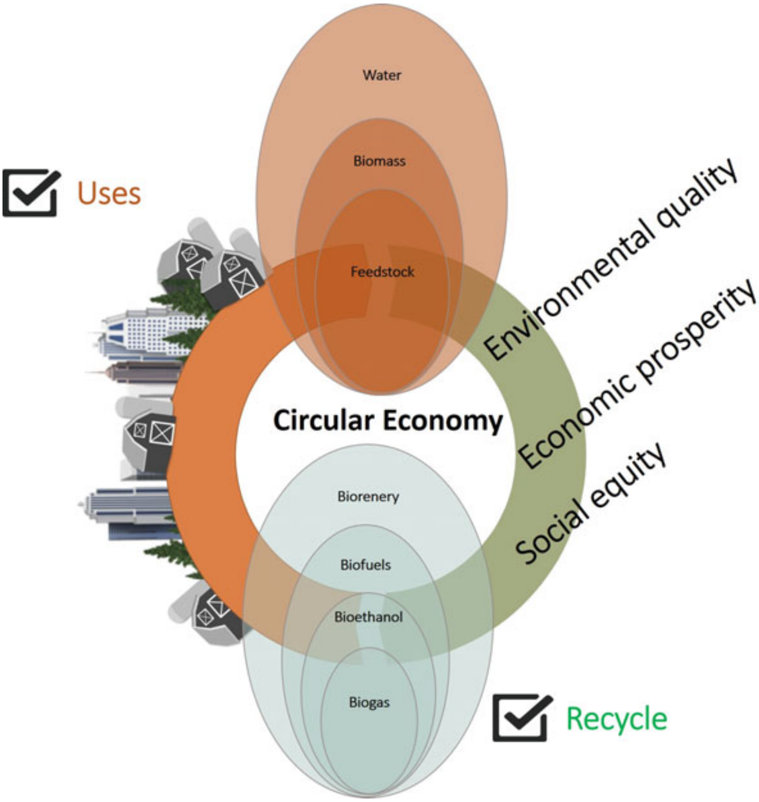

Fig. 1.1 Summary of the context of circular economy with an emphasis on biomass valorization. *Source* Authors

Many efforts have been made globally. The AgroCycle European program has sought to fully evaluate the capacity for recycling and valuation of agricultural waste to create new sustainable chains, accounting for the economic outlook [7, 8]. Worldwide, main products are identified using the circular chain approach and are used to valorize agro-industrial waste, mainly agriculture and livestock, for the production of biofuels, biofertilizers, water recycling, and reusable steam [9–11]. Residues such as lignocellulose derivatives and animal effluents are used for recovery of struvite, phosphorus [12], and noble metals [13] as well as serving as substrates for highly valued products [14, 15], including noble enzymes.

Throughout the last decade, the circular economy has become a doctrine for the agricultural, livestock, and agro-industrial sectors, using minimum inputs into the system to reduce costs and to close cycles of nutrients, water resources, and energy, reducing negative impacts to the biome, such as the emission of greenhouse gases and release of wastes. The circular economy will be more profitable with long-term implementation. Reuse and water recycling stand out among economic, social, and environmental strategies. The use of steam is an example because it is a by-product of several productive processes, permitting heat transfer and water recycling [16].

Reuse in agriculture and livestock is carried out in culture, animal supply, agro-industrial supply, and rural populations and is adopted to reduce the demand for drinking water. Water reuse is tied to its nutritional characteristics for fertilization and ferti-irrigation purposes. It is necessary to consider that, in these forms of water reuse, there is re-utilization of nutrients that augment growth and yield of crops because the wastewater contains large quantities of organic matter. Another source of recycling is bioenergy, using biogas and bioethanol, an emerging technology for valorization and optimization of biomass and water resources. Nevertheless, it is necessary to consider the need for control of bacterial, parasitic, and viral pathogens and toxic substances, such as heavy metals [16, 17].

Worldwide, biogas is generated mainly from food debris, animal waste, and human waste used for anaerobic digestion. Residues of livestock (poultry, cattle, and swine) have high energy potential for biogas, as do wastes from the sugar and alcohol industries, biodiesel production processes, dairy industry, citriculture, brewing, paper industries and pulp, urban solid waste, sewage treatment, and urban cleaning [18, 19].

In the production of first-generation ethanol (1G), sugarcane, corn, beets, and other cultivars are used. This practice has been widely debated, with a focus on environmental and socioeconomic problems associated with production processes and the need for exclusive cultivation of raw material affecting food demand, water security, and biodiversity. Second-generation and third-generation (2G and 3G) bioethanol focuses on the reuse of agricultural residues, such as, waste from the paper industry, glycerol, and by-products of the food industry [20]. It should be noted that, within the circular economy, ethanol production demands large amounts of water, requiring a reduction of the water footprint. There is a demand for water recirculation in the ethanol production chain and a constant emphasis on the reduction of freshwater use and its replacement by seawater, by wastewater and use algal biomass from treatment systems [21, 22].

It should be noted that the economic balance is essential for there to be interest and investment in new technologies based on the circular economy. The global energy appeal and cost–benefit pressures for such productions require efficient biotechnological methods with business models that are plausible for companies and industries in a company, business, or industry, focusing on reducing material flows and interconnected management [23].

The economic feasibility of reclaiming biomass lies precisely in the capacity to reuse goods and services and the use of biodiversity, microorganism technology, and recycled water resources. These processes are necessary to maintain environmental health and safety. The circular economy becomes a model largely based on ecosystems that produce energy, minimizing the impacts of waste [24].

In this sense, it is necessary to understand the biomass to be used with respect to its nutritional, structural, and molecular composition. These characteristics are essential to delineate the recycling of the biomass and the technology to be used, as well as its energy value. Advanced analytical methods are employed for structural and non-structural characterization of biomasses to be evaluated, using chromatography, mass spectrometry, electron microscopy, and X-ray diffraction (Chap. 2). Based on the characteristics of the biomass, it is necessary to choose pretreatments essential for the biomass to be bioavailable in conditions suitable for particular bioprocesses, such as biofuel production based on microbial activities. Within the concept of circularity and its economic aspects, it is emphasized that pretreatments (physical, chemical, biological, or combined) require investment in time and energy; this is the crucial step for the success of bioprocesses (Chap. 3). In addition, many inhibitory by-products can be generated that are inherent to pretreatments and detoxification processes (mainly employing enzymes) many of which are necessary to reduce inhibitory impacts (Chap. 4).

From this perspective, this book presents worldwide tendencies of productive sectors, focusing on agriculture, agroindustries, and livestock, in the ambit of economic circularity, because food producers and nutritional sources demand a great deal of natural resources, generating large amounts of waste that can be valorized by material engineering and biotechnological studies.

References

1. Murray A, Skene K, Haynes K (2017) The circular economy: an interdisciplinary exploration of the concept and application in a global context. J Bus Ethics 140:369–380. https://doi.org/10.1007/s10551-015-2693-2
2. Twigger HA (2016) Shifting perceptions: the reknit revolution. In: Centre for circular design circular transitions. University of the Arts of London, pp 57–67
3. Bonciu F (2014) The European Economy; From a linear to a circular economy. Rom J Eur Aff 14:78–91
4. Winkler MS, Krieger GR, Divall MJ, Singer BH, Utzinger J (2012) Health impact assessment of industrial development projects: a spatio-temporal visualization. Geospat Health 6:299–301. https://doi.org/10.4081/gh.2012.148
5. Park H-S, Behera SK (2014) Methodological aspects of applying eco-efficiency indicators to industrial symbiosis networks. J Clean Prod 64:478–485. https://doi.org/10.1016/j.jclepro.2013.08.032
6. Molina-Moreno V, Leyva-Díaz CJ, Llorens-Montes JF, Cortés-García JF (2017) Design of indicators of circular economy as instruments for the evaluation of sustainability and efficiency in wastewater from pig farming industry. Water 9
7. Toop TA, Ward S, Oldfield T, Hull M, Kirby ME, Theodorou MK (2017) AgroCycle—developing a circular economy in agriculture. Energy Procedia 123:76–80. https://doi.org/10.1016/j.egypro.2017.07.269
8. AgroCycle (2019) AgroCycle protocol rules 2019. http://www.agrocycle.eu/#project
9. Wang X, Yang G, Feng Y, Ren G, Han X (2012) Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresour Technol 120:78–83. https://doi.org/10.1016/j.biortech.2012.06.058
10. Guendouz J, Buffière P, Cacho J, Carrère M, Delgenes J-P (2010) Dry anaerobic digestion in batch mode: design and operation of a laboratory-scale, completely mixed reactor. Waste Manag 30:1768–1771. https://doi.org/10.1016/j.wasman.2009.12.024
11. Pawelczyk A (2005) EU policy and legislation on recycling of organic wastes to agriculture. International Society for Animal Hygiene

12. Liu Y, Kwag J-H, Kim J-H, Ra C (2011) Recovery of nitrogen and phosphorus by struvite crystallization from swine wastewater. Desalination 277:364–369. https://doi.org/10.1016/j.desal.2011.04.056
13. Fleming, C.A., Ferron CJ, Dreisinger DB, O'Kane PT (2000) A process for the simultaneous leaching and recovery of gold, platinum group metals and base metals from ores and concentrates. In: Taylor P (ed) EPD Proc TMS, Warrendale, 419–443
14. Venturin B, Frumi Camargo A, Scapini T, Mulinari J, Bonatto C, Bazoti S, Pereira Siqueira D, Maria Colla L, Alves SL, Paulo Bender J, Luís Radis Steinmetz R, Kunz A, Fongaro G, Treichel H (2018) Effect of pretreatments on corn stalk chemical properties for biogas production purposes. Bioresour Technol 266. https://doi.org/10.1016/j.biortech.2018.06.069
15. Sanchez S, Demain AL (2017) Chapter 1: Useful microbial enzymes—an introduction. In: Brahmachari G (ed) B of ME. Academic Press, pp 1–11
16. Shakir E, Zahraw Z, Al-Obaidy AHMJ (2017) Environmental and health risks associated with reuse of wastewater for irrigation. Egypt J Pet 26:95–102. https://doi.org/10.1016/j.ejpe.2016.01.003
17. Fongaro G, Viancelli A, Magri ME, Elmahdy EM, Biesus LL, Kich JD, Kunz A, Barardi CRM (2014) Utility of specific biomarkers to assess safety of swine manure for biofertilizing purposes. Sci Total Environ 479–480:277–283. https://doi.org/10.1016/j.scitotenv.2014.02.004
18. Guerini Filho M, Lumi M, Hasan C, Marder M, Leite LCS, Konrad O (2018) Energy recovery from wine sector wastes: a study about the biogas generation potential in a vineyard from Rio Grande do Sul, Brazil. Sustain Energy Technol Assess 29:44–49. https://doi.org/10.1016/j.seta.2018.06.006
19. Krzywika A, Szwaja S (2017) Putrid potatoes as biomass charge to an agricultural biomass-to-biogas power plant. Energy Procedia 118:40–45. https://doi.org/10.1016/j.egypro.2017.07.008
20. Neves PV, Pitarelo AP, Ramos LP (2016) Production of cellulosic ethanol from sugarcane bagasse by steam explosion: effect of extractives content, acid catalysis and different fermentation technologies. Bioresour Technol 208:184–194. https://doi.org/10.1016/j.biortech.2016.02.085
21. Milano J, Ong HC, Masjuki HH, Chong WT, Lam MK, Loh PK, Vellayan V (2016) Microalgae biofuels as an alternative to fossil fuel for power generation. Renew Sustain Energy Rev 58:180–197. https://doi.org/10.1016/j.rser.2015.12.150
22. Nizami AS, Rehan M, Waqas M, Naqvi M, Ouda OKM, Shahzad K, Miandad R, Khan MZ, Syamsiro M, Ismail IMI, Pant D (2017) Waste biorefineries: enabling circular economies in developing countries. Bioresour Technol 241:1101–1117. https://doi.org/10.1016/j.biortech.2017.05.097
23. Clift R, Allwood J (2011) Rethinking the economy. The Chemical Engineer
24. Pauli GA (2010) The blue economy 10 years, 100 innovations, 100 million jobs. Paradigm Publications, Taos, New Mexico

Chapter 2
Structure of Residual Biomass Characterization

2.1 Primary Constituents of Biomass

Agro-industrial waste is essentially made up of oxygen-containing organic polymers. Unlike fossil fuels, organic waste biomass is highly oxygenated owing to the presence of carbohydrates. In decreasing order of predominance, carbon (C) is the main constituent (ranging from 30 to 60%) on a dry basis (db). Next is oxygen (O) (30–40%) by weight (db), hydrogen (H) (5–6%) (db), and finally nitrogen (N), sulfur (S), and chlorine (Cl) that are generally present in lower quantities (1%) (db) [1]. All these elements are organized in the form of sugars and other compounds (here I would like to add a few more) providing an attractive source for exploiting valuable resources in the energy and biorefinery sector.

These chemical elements in waste biomass are usually organized in an orderly fashion, forming complex structures such as lignocellulosic material. Lignocellulosic structures represent recalcitrant complexes that are biopolymeric and difficult to hydrolyze because they are associated with hemicellulose and are surrounded by lignin, which has a limited covalent bond with hemicellulose. There remains in its structure the potential for the formation of six hydrogen bonds (four intramolecular and two intermolecular), giving it a highly ordered and compact structure [2]. This structure is highly crystalline and tangled, requiring pretreatment steps to convert the raw material into products of interest [3].

Generally, lignocellulosic biomass consists of 35–50% cellulose, 20–35% hemicellulose, and 10–25% lignin. Cellulose is the main component of lignocellulosic biomass, representing a structure formed by subunits of sugars that form chains of elementary fibrils linked by hydrogen bonds and van der Waals forces [4, 5]. It is estimated that about half of the organic carbon in the biosphere is present in the form of cellulose and the exploitation by this resource is of fundamental importance for obtaining biofuels and valuable chemicals [6–8].

Hemicellulose represents the second most abundant polymer in lignocellulosic material; its molecular weight is lower than that of cellulose [4]. The main difference

H. Treichel et al., *Utilising Biomass in Biotechnology*,
Green Energy and Technology, https://doi.org/10.1007/978-3-030-22853-8_2

between the two is that hemicellulose branches consist of short side chains with different sugars, whereas cellulase possesses oligomers that hydrolyze easily [5].

Lignan is an aromatic polymer synthesized from phenylpropanoid precursors [5]. It acts as a resistant and rigid glue on the cell wall, preventing the passage of pathogens and insects as well as contributing to the strengthening of plant tissue [9].

At the other end of the biomass spectrum, the microalgae are primarily the focus of the food and cosmetic sector, not the energy sector. Nevertheless, microalgae biomass has shown potential as an alternative raw material in biotechnological processes, including biogas, bioethanol, biodiesel, bioherbicide, among others [10–12].

Algae are organisms that inhabit aquatic environments and settle with ease, owing to their metabolic diversity. Within this large group of organisms are microalgae, particularly photosynthetic prokaryotes and unicellular eukaryotes [13].

The possibilities of application of microalgal biomass are directly related to their characteristics, because this biomass is mainly composed of lipids (2–90% dry matter), proteins (10–60% dry matter), and carbohydrates (5–50% of dry matter). These values are dependent variables of the microalgae species [11]. This biomass diffuses rapidly in a biased way because of several advantages: environmental conditions of rapid cultivation, even in nutrient-rich wastewater, high growth rates, being even more efficient than terrestrial biomass [14, 15], with efficiency in fixing CO_2 and lack of competition with arable land for food crops [16], in addition to being a low-cost biomass [17].

Biomass from food waste appears abundantly in the global scenario, representing the increase in population and problems associated with disposal of these wastes [18, 19]. Most of the time, this biomass is composed of fruit remnants, vegetables, and leftovers from food. The non-utilization of these residues can be considered a waste of energy, because the composition of this biomass presents great value as an energetic resource [20].

Biomass composed of fruit residues adds substantial value to biotechnological processes because of the presence of fermentable sugars that serve as sources for the production of energy and environmentally sustainable fuels. The residual biomass of fruit consists of pectin (3–12%), cellulose (20–50%), hemicellulose (1–50%), and low lignin content (1–20%) [21, 22].

In the structure of these components, there a number of other elements, mainly sugars. Pectin can be subdivided into glucose, fructose, galacturonic acid, arabinose and galactose [23], glucose and cellobiose [24], whereas hemicellulose is composed of xylose, arabinose (pentoses), mannose, glucose, and galactose (hexoses) [25].

The composition of fruit biomass makes it clear why it is such a valuable biomass: The sugars by which it is formed are precursors to biotechnological processes, including second-generation ethanol. This biomass also presents low lignin content and, therefore, lower energy costs of pretreatment methods [26].

2.2 Lignocellulosic Biomass Characterization Methods

One of the most important steps during lignocellulosic biomass pretreatment processes is its characterization and evaluation of the structural changes that occur in this material after various pretreatment techniques. Among the forms of characterization, we highlight the analysis of the structural composition of biomass, using imaging techniques (SEM, TEM, and AFM) and crystallinity indices (FTIR and XRD). These techniques help determine whether pretreatment was effective.

2.2.1 Analysis of the Structural and Non-structural Composition of Biomass

The basic composition of lignocellulosic biomass can be divided into extractives, fixed solids (ashes), structural carbohydrates (cellulose and hemicellulose), and lignin. The mass percentage of cellulose is quantified mainly by the sum of monomers, glucose, cellobiose, and 5-hydroxymethylfurfural. Hemicellulose is quantified as the sum of the monomers xylose, arabinose, furfural, and acetic acid. Total lignin is calculated by the sum of its two fractions, soluble in acid and not soluble.

It should be noted that, at the end of the structural characterization process, the mass percentage will barely reach 100.0%, because several compounds can be formed during characterization steps, some of which are not possible to quantify by the methods described below.

2.2.1.1 Quantification of Extractives and Fixed Solids (Ashes)

Extractives' determination has two purposes: to quantify the extractives for the composite analysis and to remove the non-structural material before the quantification of lignin and carbohydrates such that there is no interference in these analytical steps [27].

First, one must determine the moisture content of the material [28]. Subsequently, approximately 4.0 g of the sample is added, discounting the moisture content, to the extraction cartridge having its mass duly noted. After extractor assembly, the biomass-filled cartridge should be properly inserted into the Soxhlet extraction tube.

Extractables should be extracted for 6 h in the Soxhlet with distilled water and then for an additional 6.0 h with 95.0% ethanol. A total of 200.0 mL of each solution is added to each extraction flask together with glass beads. The temperature should be set to a cycle of 10 siphonings per hour. The process is considered finished when the liquid around the extraction cartridge becomes colorless, indicating the removal of biomass extractives. At the end of extraction, the solids should be washed with

100.0 mL of ethanol and dried at 105.0 °C to constant mass using an analytical balance.

The content of fixed solids or ashes may be determined after burning the muffle samples at 800.0 °C for 20.0 min and repeated for a further 15.0 min until a constant mass is found [29].

2.2.1.2 Quantification of Structural Carbohydrates and Lignin

To determine the cellulose, hemicellulose, and total lignin content in the lignocellulosic biomass, aggressive hydrolysis of the material with 72% H_2SO_4 is required, requiring extra care in the analysis.

Work with particles smaller than 20 mesh should be sought; particles larger than this can lead to misleading results if hydrolysis is not complete. If the lignocellulosic material has a particle size larger than 20 mesh, it must be ground again for its characterization [30].

About 0.3 g dry sample (discounting moisture content) should be treated with 3.0 mL 72% H_2SO_4 ($v\ v^{-1}$) for one hour at 30 °C in a thermostatic bath. The mixture should be stirred every 5 min with a glass rod without removing it from the test tube, always keeping the biomass in contact with the acidic solution and in the bath. Then, the contents of the test tube are quantitatively transferred to a 250-mL flask with using 84 mL water for dilute acid hydrolysis. The mixture should be autoclaved at 121 °C for one hour. It is essential that the Erlenmeyer flasks are sealed to prevent loss of reaction liquid. After the period of dilute acid hydrolysis, the Erlenmeyer flasks should be cooled before opening to avoid evaporation of the reaction liquid inside [30]. The reaction liquid should be filtered through pre-calcined and weighed glass Gooch crucibles through 45-μm fiberglass membranes using a vacuum pump. Material retained on the membrane should be washed with approximately 1.5 L of distilled water and dried to constant mass in an oven at 105 °C. This residue corresponds to the acid-insoluble lignin after weighing. This residue can be recalculated to determine the fixed solid content present in this fraction. The filtrate is then analyzed for carbohydrate content in a chromatographic system [30].

Chromatographic analysis for the determination of glucose, xylose, cellobiose, arabinose, and acetic acid may be performed using liquid chromatography with a mass spectrometer (CL-MS) equipped with column-operated refractive index detector (DIR-10 A). Aminex® Biorad HPX87H using as mobile phase H_2SO_4 0.005 mol L^{-1}, isocratic mode, temperature 45.0 °C, injection volume 20.0 μL, and flow 0.6 mL min^{-1}. Samples should be properly diluted in the eluent and filtered through a 45-μm cellulose acetate filter. The eluent should be vacuum filtered with a 0.45-μm polytetrafluoroethylene (PTFE) membrane and then degassed in an ultrasonic bath for 15 min. The concentration of compounds should be determined using calibration curves with specific LC-MS standards [31, 32].

The quantification of furfural and 5-hydroxymethylfurfural (HMF) can be performed in CL-MS equipped using a diode array detector (DAD) operated with a C18 column using 1:8 acetonitrile/water mobile phase with the addition of 1% acetic acid.

Table 2.1 Absorptivity constants for acid-soluble lignin measurement for select biomass types

Biomass type	Recommended wavelength (nm)	Absorptivity at recommended wavelength (L g^{-1} cm^{-1})
Pinus radiata-NIST SRM 8493	240	12
Bagasse-NIST SRM 8491	240	25
Corn stover-NREL supplied feedstock	320	30
Populus deltoides-NIST SRM 8492	240	25

Oven temperature should be 30.0 °C, isocratic mode, with an injection volume of 20 μL and a flow rate of 0.8 mL min^{-1}. Samples should be properly diluted in the eluent and filtered through 45-μm cellulose acetate syringe filters. The eluent should be filtered with 0.45-μm polytetrafluoroethylene (PTFE) membranes and then degassed. The concentration of compounds is again determined using calibration curves with LC-MS specific standards [31, 32].

Acid-soluble lignin content can be determined using UV spectrometry, adopting an approximately 100-fold dilution of the reaction liquid to maintain absorbance values less than 1. The absorbance standard is expressed as L g^{-1} cm^{-1} and can be found in Table 2.1 [30].

The absorptive value most appropriate for the type of biomass being analyzed should be determined. Lignin content is expressed as the sum of the insoluble and acid-soluble fractions.

The procedure presented by Sluiter et al. [30] is optimal for the analysis of typical lignocellulosic matrices. However, it has not been optimized to characterize biomass such as urban solid waste, manure, or algae. Nevertheless, the indiscriminate use of analysis of a non-recommended number of substrates has been reported in the literature; in some cases, adverse reactions occurred, affecting the results of the analysis and causing distrust of the responses obtained [33].

2.2.2 *Image Analysis*

After an analysis of biomass composition, it is sometimes necessary to investigate the effects of pretreatment of the lignocellulosic matrix using other methods, because it is not sufficient to know how much lignin is in a given sample; it is also important to know where lignin is located, as well as how it interacts with other components, celluloses, and hemicelluloses.

Currently, SEM, TEM, and AFM are widely used to investigate the structures of nanoscale lignocellulosic materials. Although they are qualitative methods, they can provide insight into biomass structures that cannot be obtained using further analysis, e.g., images of microcracks and cell walls [33].

2.2.2.1 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is one of the most widely used imaging techniques for investigating the surface of the lignocellulosic matrix [34]. It provides information regarding the superficial and morphological characterization of the microstructure.

SEM equipment can be adjusted to perform energy-dispersive spectroscopy (EDS). This analysis can determine elemental composition with some precision. It is possible to observe surface erosions, deconstruction, and relocation of cell wall components to approximate the accessibility of this biomass [35].

Samples can be subjected to SEM which must possess conductivity. To make the samples conductive, they can be coated with a vaporized metal (gold) or carbon. The electron beam can damage samples, and this is the primary limitation of the technique [36].

Avoid drying of samples in ovens and kilns, as this may result in significant deformation and surface collapse. The key recommendation is lyophilization of the samples to avoid surface tensions that distort the analysis. Staining can also be used to illuminate various fractions of biomass using $KMnO_4$ and to localize lignin within the cell wall [37].

2.2.2.2 Transmission Electron Microscopy (TEM)

TEM is used to detect deconstruction of the cell wall. The staining of samples for traditional TEM is done using $KMnO_4$, thereby providing information regarding changes that occur in the lignocellulosic matrix after pretreatments [38]. Electronic tomography, an extension of TEM, is used to capture the three-dimensional structures of lignocellulosic biomass [39]. Donohoe and colleagues [40] formulated the most suitable methods for sample preparation to obtain better imaging results using TEM. It should be noted that great care is needed in preparing biomass with specific resin and appropriate thickness; otherwise, the results obtained will be inconclusive [33].

2.2.2.3 Atomic Force Microscopy (AFM)

AFM, also known as scanning force microscopy, is used to investigate the topographic, physical, and chemical properties of lignocellulosic biomass. In typical AFM, the interaction forces a 1–10 nm probe tip, allowing the sample to be measured. Using topographic imaging, it is possible to follow the structural changes of biomass fibers (e.g., fiber surface characteristics and fiber cross-sectional areas) throughout the hydrolysis or pretreatment process [33].

Cell wall boundaries, middle lamellae, and microfiber arrangement can be observed as closely as possible to the native state [36]. AFM can be used to investigate surface properties, e.g., by measuring the roughness of biomass. In addition, AFM can be used to directly evaluate the interaction forces between various components (cellulose, lignin, and hemicellulose). Hydrophilic and hydrophobic regions of lignocellulose can also be mapped using AFM.

SEM and TEM can provide two-dimensional images, while high-resolution three-dimensional images can be obtained using AFM without sample preparation, staining, dehydration, or metal coating. AFM can be combined with a variety of optical microscopy techniques, including fluorescent microscopy for greater applicability. Some of the limitations of AFM are very slow scanning speeds and image analysis that is more difficult than SEM and TEM images [33].

2.2.3 Analysis of the Crystallinity Index

The crystallinity index is a parameter used to indicate the relative amount of crystalline (ordered) and amorphous (less ordered) regions of a cellulosic structure. Such values strongly depend on the method of information collection and subsequent methodology of data analysis.

2.2.3.1 Analysis of Fourier-Transform Infrared Spectroscopy (FTIR)

To determine chemical changes in lignocellulosic biomass, FTIR spectra can be obtained using an infrared spectrometer. Dried samples should be compressed into tablets containing KBr. The mass ratio of KBr for biomass samples should be approximately 100:1. Sample spectra should be obtained by scanning from 4000 to 400 cm^{-1} with a spectral resolution of 2.0 cm^{-1}, with an average of 40 scans. Table 2.2 presents a compilation of the meaning of the main absorbance peaks presented in a typical lignocellulosic biomass analysis [41–44].

Typical appearance of an infrared spectrum of a corn stalk biomass before and after different chemical pretreatments can be seen in Fig. 2.1.

2.2.3.2 X-Ray Diffraction (XRD)

XRD provides information related to crystalline and amorphous fractions of cellulose. The crystalline cellulosic fraction returns strong signals, while the non-crystalline cellulose fraction shows broader and weaker signals in the diffraction pattern [45]. Obtaining XRD data is easier than other methods; however, data analysis is quite challenging [33].

In 1959, Segal and colleagues [46] developed a method for analyzing XRD data based on the use of focusing and transmission techniques. In this method, known as peak height, the crystallinity index is calculated simply by dividing the peak height (200) (the maximum interference; I200) by the minimum height between the peaks (200) and (110) (intensity in 28:18°; I_{AM}):

Table 2.2 Wavelengths that correspond to a given functional group and respond to infrared spectroscopy

Wavelength (cm^{-1})	Assignment
3348	O-H stretch (hydrogen cellulose connection bond)
2900	C-H stretch (methyl/methylene cellulose group)
1734	Carbonyl bonds (associated with removal of the lignin side chain)
1716	Carboxylic acids/ester groups
1633	Aromatic ring stretch (associated with lignin removal)
1604	Aromatic ring stretch (changes in lignin structure)
1516	Generic lignin
1516/897	Lignin/cellulose ratio
1373	Phenolic O-H stretch (changes in lignin structure)
1319	Syringyl ring stretch (changes in the lignin monomer)
1251	C-O absorption (result of acetyl-lignin groups' cleavage)
1110	Crystalline cellulose
1059	C-O-C stretch (cellulose and hemicellulose)
897	Amorphous cellulose
1110/897	Crystalline/amorphous cellulose ratio
833	C-H flexion of syringyl
771	Crystalline cellulose (Iα)
719	Crystalline cellulose (Iβ)
771/719	Ratio of crystalline cellulose polymorphs (Iα/Iβ)

Source Reference [31]

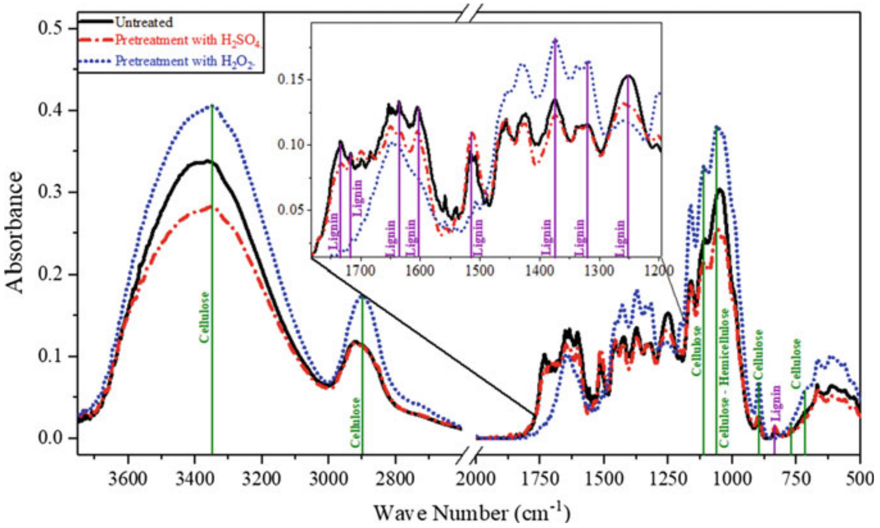

Fig. 2.1 FTIR spectra of untreated and pretreatment biomass. *Source* Reference [31]

$$\text{Crystallinity Index(CrI)} = (I_{200} - I_{AM})/I_{200} * 100$$

This method is most widely used for calculating CrI. Nevertheless, the exact amount of crystalline water may be higher at the peak area than at its height [47].

Another method used to measure the crystallinity index is based on the deconvolution (curve fitting) of the peak for the crystalline and amorphous fractions. The calculation is based on peak intensity for Iβ cellulose and a single broad peak for amorphous fractions. In this method, it is assumed that the widening of the peak is due to the increase in the amorphous region. The difficulty in selecting the appropriate peaks is the main challenge of this technique variation [47].

It should be emphasized that XRD data obtained from the same sample and analyzed using various methods may lead to conflicting results, and this requires some caution in the use of such technique [33, 48].

In addition to the aforementioned characterization techniques, there are many variations of the ones presented above, each with its own peculiarities and purposes.

References

1. Virmond E, De Sena RF, Albrecht W, Althoff CA, Moreira RFPM, José HJ (2012) Characterisation of agroindustrial solid residues as biofuels and potential application in thermochemical processes. Waste Manag 32:1952–1961. https://doi.org/10.1016/j.wasman.2012.05.014
2. Demain AL, Báez-Vásquez MA (2013) Chapter 14: Biofuels of the present and the future. In: Suib SL (ed) N and FD in C. Elsevier, Amsterdam, pp 325–370
3. Kim D (2018) Physico-chemical conversion of lignocellulose: inhibitor effects and detoxification strategies: a mini review. Molecules 23
4. Isikgora FH, Becer CR (2015) Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polym Chem 6:4497–4559. https://doi.org/10.1039/c5py00263j
5. Sánchez C (2009) Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnol Adv 27:185–194. https://doi.org/10.1016/j.biotechadv.2008.11.001
6. Zhou C-H, Xia X, Lin C-X, Tong D-S, Beltramini J (2011) Catalytic conversion of lignocellulosic biomass to fine chemicals and fuels. Chem Soc Rev 40:5588–5617. https://doi.org/10.1039/C1CS15124J
7. Somerville C, Youngs H, Taylor C, Davis SC, Long SP (2010) Feedstocks for lignocellulosic biofuels. Science (80-) 329:790–792. https://doi.org/10.1126/science.1189268
8. Taarning E, Osmundsen CM, Yang X, Voss B, Andersen SI, Christensen CH (2011) Zeolite-catalyzed biomass conversion to fuels and chemicals. Energy Environ Sci 4:793–804. https://doi.org/10.1039/C004518G
9. Rubin EM (2008) Genomics of cellulosic biofuels. Nature 454:841
10. Varol A, Ugurlu A (2016) Biogas production from microalgae (Spirulina platensis) in a two stage anaerobic system. Waste Biomass Valoriz 7:193–200. https://doi.org/10.1007/s12649-015-9442-8
11. Colla LM, Margarites ACF, Decesaro A, Magro FG, Kreling N, Rempel A, Machado TS (2019) Waste biomass and blended bioresources in biogas production. In: Treichel H, Fongaro G (eds) Improving biogas production: technological challenges, alternative sources, future developments. Springer International Publishing, Cham, pp 1–23
12. Costa JAV, Freitas BCB, Cruz CG, Silveira J, Morais MG (2019) Potential of microalgae as biopesticides to contribute to sustainable agriculture and environmental development. J Environ Sci Heal Part B 54:366–375. https://doi.org/10.1080/03601234.2019.1571366

13. Miranda MT, Sepúlveda FJ, Arranz JI, Montero I, Rojas CV (2018) Physical-energy characterization of microalgae Scenedesmus and experimental pellets. Fuel 226:121–126. https://doi.org/10.1016/j.fuel.2018.03.097
14. Kröger M, Müller-Langer F (2012) Review on possible algal-biofuel production processes. Biofuels 3:333–349. https://doi.org/10.4155/bfs.12.14
15. González-González LM, Correa DF, Ryan S, Jensen PD, Pratt S, Schenk PM (2018) Integrated biodiesel and biogas production from microalgae: towards a sustainable closed loop through nutrient recycling. Renew Sustain Energy Rev 82:1137–1148. https://doi.org/10.1016/j.rser.2017.09.091
16. Suali E, Sarbatly R (2012) Conversion of microalgae to biofuel. Renew Sustain Energy Rev 16:4316–4342. https://doi.org/10.1016/j.rser.2012.03.047
17. Raheem A, Prinsen P, Vuppaladadiyam AK, Zhao M, Luque R (2018) A review on sustainable microalgae based biofuel and bioenergy production: recent developments. J Clean Prod 181:42–59. https://doi.org/10.1016/j.jclepro.2018.01.125
18. Mahmood A, Iguchi R, Kataoka R (2019) Multifunctional food waste fertilizer having the capability of Fusarium-growth inhibition and phosphate solubility: a new horizon of food waste recycle using microorganisms. Waste Manag 94:77–84. https://doi.org/10.1016/j.wasman.2019.05.046
19. Neugebauer M (2018) The use of biological waste as a source of low-temperature heat for hotbeds in spring in north-eastern Poland. J Environ Manage 225:133–138. https://doi.org/10.1016/j.jenvman.2018.07.076
20. Zhao C, Yan H, Liu Y, Huang Y, Zhang R, Chen C, Liu G (2016) Bio-energy conversion performance, biodegradability, and kinetic analysis of different fruit residues during discontinuous anaerobic digestion. Waste Manag 52:295–301. https://doi.org/10.1016/j.wasman.2016.03.028
21. Choi IS, Lee YG, Khanal SK, Park BJ, Bae H-J (2015) A low-energy, cost-effective approach to fruit and citrus peel waste processing for bioethanol production. Appl Energy 140:65–74. https://doi.org/10.1016/j.apenergy.2014.11.070
22. Mahato N, Sharma K, Sinha M, Cho MH (2018) Citrus waste derived nutra-/pharmaceuticals for health benefits: Current trends and future perspectives. J Funct Foods 40:307–316. https://doi.org/10.1016/j.jff.2017.11.015
23. Cárdenas-Pérez S, Chanona-Pérez JJ, Güemes-Vera N, Cybulska J, Szymanska-Chargot M, Chylinska M, Kozioł A, Gawkowska D, Pieczywek PM, Zdunek A (2018) Structural, mechanical and enzymatic study of pectin and cellulose during mango ripening. Carbohydr Polym 196:313–321. https://doi.org/10.1016/j.carbpol.2018.05.044
24. Laluce C, Roldan IU, Pecoraro E, Igbojionu LI, Ribeiro CA (2019) Effects of pretreatment applied to sugarcane bagasse on composition and morphology of cellulosic fractions. Biomass Bioenerg 126:231–238. https://doi.org/10.1016/j.biombioe.2019.03.002
25. Ferreira AD, Mussatto SI, Cadete RM, Rosa CA, Silva SS (2011) Ethanol production by a new pentose-fermenting yeast strain, *Scheffersomyces stipitis* UFMG-IMH 43.2, isolated from the Brazilian forest. Yeast 28:547–554. https://doi.org/10.1002/yea.1858
26. Cypriano DZ, da Silva LL, Tasic L (2018) High value-added products from the orange juice industry waste. Waste Manag 79:71–78. https://doi.org/10.1016/j.wasman.2018.07.028
27. Sluiter A, Ruiz R, Scarlata C, Sluiter J, Templeton D (2008) Determination of extractives in biomass. Laboratory Analytical Procedure (LAP)
28. Sluiter A, Hames B, Hyman D, Payne C, Ruiz R, Scarlata C, Sluiter J, Templeton D, Nrel JW (2008) Determination of total solids in biomass and total dissolved solids in liquid process samples. Natl Renew Energy Lab 9. doi:NREL/TP-510–42621
29. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D (2008) Determination of ash in biomass. Laboratory Analytical Procedure (LAP). Nrel/Tp-510-42622 18. doi: NREL/TP-510-42619
30. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D (2012) Determination of structural carbohydrates and lignin in biomass

31. Venturin B, Frumi Camargo A, Scapini T, Mulinari J, Bonatto C, Bazoti S, Pereira Siqueira D, Maria Colla L, Alves SL, Paulo Bender J, Luís Radis Steinmetz R, Kunz A, Fongaro G, Treichel H (2018) Effect of pretreatments on corn stalk chemical properties for biogas production purposes. Bioresour Technol 266. https://doi.org/10.1016/j.biortech.2018.06.069
32. Bazoti SF, Golunski S, Pereira Siqueira D, Scapini T, Barrilli ÉT, Alex Mayer D, Barros KO, Rosa CA, Stambuk BU, Alves SL, Valério A, de Oliveira D, Treichel H (2017) Second-generation ethanol from non-detoxified sugarcane hydrolysate by a rotting wood isolated yeast strain. Bioresour Technol 244:582–587. https://doi.org/10.1016/j.biortech.2017.08.007
33. Karimi K, Taherzadeh MJ (2016) A critical review of analytical methods in pretreatment of lignocelluloses: composition, imaging, and crystallinity. Bioresour Technol 200:1008–1018. https://doi.org/10.1016/j.biortech.2015.11.022
34. Amiri H, Karimi K (2015) Improvement of acetone, butanol, and ethanol production from woody biomass using organosolv pretreatment. Bioprocess Biosyst Eng 38:1959–1972. https://doi.org/10.1007/s00449-015-1437-0
35. Donohoe BS, Vinzant TB, Elander RT, Pallapolu VR, Lee YY, Garlock RJ, Balan V, Dale BE, Kim Y, Mosier NS, Ladisch MR, Falls M, Holtzapple MT, Sierra-Ramirez R, Shi J, Ebrik MA, Redmond T, Yang B, Wyman CE, Hames B, Thomas S, Warner RE (2011) Surface and ultrastructural characterization of raw and pretreated switchgrass. Bioresour Technol 102:11097–11104. https://doi.org/10.1016/j.biortech.2011.03.092
36. Yarbrough JM, Himmel ME, Ding S-Y (2009) Plant cell wall characterization using scanning probe microscopy techniques. Biotechnol Biofuels 2:17. https://doi.org/10.1186/1754-6834-2-17
37. Karp EM, Resch MG, Donohoe BS, Ciesielski PN, O'Brien MH, Nill JE, Mittal A, Biddy MJ, Beckham GT (2015) Alkaline Pretreatment of Switchgrass. ACS Sustain Chem Eng 3:1479–1491. https://doi.org/10.1021/acssuschemeng.5b00201
38. Donohoe BS, Selig MJ, Viamajala S, Vinzant TB, Adney WS, Himmel ME (2009) Detecting cellulase penetration into corn stover cell walls by immuno-electron microscopy. Biotechnol Bioeng 103:480–489. https://doi.org/10.1002/bit.22281
39. Ciesielski PN, Matthews JF, Tucker MP, Beckham GT, Crowley MF, Himmel ME, Donohoe BS (2013) 3D electron tomography of pretreated biomass informs atomic modeling of cellulose microfibrils. ACS Nano 7:8011–8019. https://doi.org/10.1021/nn4031542
40. Donohoe BS, Ciesielski PN, Vinzant TB (2012) Preservation and preparation of lignocellulosic biomass samples for multi-scale microscopy analysis. In: Himmel ME (ed) Biomass conversion: methods and protocols. Humana Press, Totowa, NJ, pp 31–47
41. Zhao C, Shao Q, Ma Z, Li B, Zhao X (2016) Physical and chemical characterizations of corn stalk resulting from hydrogen peroxide presoaking prior to ammonia fiber expansion pretreatment. Ind Crops Prod 83:86–93. https://doi.org/10.1016/j.indcrop.2015.12.018
42. Li P, Cai D, Luo Z, Qin P, Chen C, Wang Y, Zhang C, Wang Z, Tan T (2016) Effect of acid pretreatment on different parts of corn stalk for second generation ethanol production. Bioresour Technol 206:86–92. https://doi.org/10.1016/j.biortech.2016.01.077
43. Kumar R, Mago G, Balan V, Wyman CE (2009) Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. Bioresour Technol 100:3948–3962. https://doi.org/10.1016/j.biortech.2009.01.075
44. Yao Y, Bergeron AD, Davaritouchaee M (2018) Methane recovery from anaerobic digestion of urea-pretreated wheat straw. Renew Energy 115:139–148. https://doi.org/10.1016/j.renene.2017.08.038
45. Terinte N, Ibbett R, Schuster KC (2011) Overview on native cellulose and microcrystalline cellulose i structure studied by X-ray diffraction (WAXD): comparison between measurement techniques. Lenzing Beritchte 89:118–131. https://doi.org/10.1163/156856198X00740
46. Segal L, Creely JJ, Martin AE, Conrad CM (1959) An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. Text Res J 29:786–794. https://doi.org/10.1177/004051755902901003

47. Ju X, Bowden M, Brown EE, Zhang X (2015) An improved X-ray diffraction method for cellulose crystallinity measurement. Carbohydr Polym 123:476–481. https://doi.org/10.1016/j.carbpol.2014.12.071
48. Vieira FS, Pasquini C (2014) Determination of cellulose crystallinity by terahertz-time domain spectroscopy. Anal Chem 86:3780–3786. https://doi.org/10.1021/ac4035746

Chapter 3
Waste Biomass Pretreatment Methods

Pretreatment of residual biomass is one of the key elements in integrated conversion processes such as biorefineries. Biotechnology projects rely heavily on the efficient, technical, economic, and environmental feasibility of pretreatments. Pretreatment is a unitary operation that precedes a certain process, and it is not itself the ultimate goal of the activity. Nevertheless, the inclusion of this step increases the efficiency of subsequent processes by increasing accessibility to the primordial biomass structure, facilitating access to enzymes and reagents used in the development of high added-value products.

The need for cost-effective pretreatment technologies is the highlight of newly developed biotechnological processes, always seeking treatments that are efficient, with a high degree of activation of key biomass components, the formation of low by-product concentrations and low reagent consumption [1]. Among the pretreatment methods reported in recent literature are physical pretreatments (mechanical, ultrasound, microwave, thermal), chemical methods (alkaline, acidic, oxidative), biological methods (microorganisms and enzymes), and combined processes that include two or more pretreatment methods. The mechanisms of action of each method will be detailed and discussed throughout this section.

3.1 Physical

Physical pretreatment comprises unitary operations designed to modify physical properties of waste biomass without the addition of chemical reagents or microorganisms. Physical pretreatment techniques include mechanics (grinding, screening, extruding), ultrasound, microwave, and heat.

H. Treichel et al., *Utilising Biomass in Biotechnology*,
Green Energy and Technology, https://doi.org/10.1007/978-3-030-22853-8_3

3.1.1 Mechanics

Mechanical pretreatments are widely used to reduce particle size and to increase porosity and biomass surface area. Grinding and screening are highlighted in the literature for waste biomass for simplicity and ease of use. The absence of chemical reagents, as well as the non-generation of inhibitors and toxic products, makes the mechanical processes attractive, especially when subsequent biological processes such as bioenergy production or enzyme production are applied. Mechanical methods are among the most suitable for preliminary treatment of biomass in industrial expansion, resulting in reduced structure stiffness and crystallinity and increasing the concentration of available nutrients in biomass cells facilitating the subsequent processes [2].

Grinding is often used as a preliminary mechanical treatment in combination with other processes such as heat, ultrasound, chemical treatments, acting on biomass through physical force, increasing the diffusion of biomass compounds by increasing surface area and sample uniformity, thereby enhancing system performance [3, 4]. For high lignin structures such as corn husk, when fragmented by particle grinding of 40–60 mm, the cell wall is destroyed, causing disruption in cell structure such that cellulose layers become more available, resulting in particles with high reactivity due to the increase in accessible surface area and due to structural disorder [5].

All processes that occur with biomass are heterogeneous and the reaction rate and yield to obtain the final product strongly depend on the surface area available for the reaction. This is the case of lignocellulosic biomass which when applied in substance extraction processes limits the process of direct diffusion through the complex lignocellulosic matrix. As such, large-scale processing usually includes first-stage milling [1].

The most widespread equipment in the milling process is knives and hammers that differ depending on the size of the milling bodies; however, there remain others such as disk mills, ball mills, bead mills, and vibration mills.

In the hammer mill, the grinding process is performed in two steps. The first occurs by the impact of biomass with the grinding bodies, and in the second, the rotor presses the particles against the screen mesh of the equipment, where the material is crushed. This equipment is widely used and has high productivity and relatively low power consumption. The main limiting factor is that reducing the size of the screen mesh reduces the performance of the equipment, creating the difficulty of developing a mesh capable of supporting the mechanical load during grinding. Nevertheless, this is the most commonly used equipment for preliminary biomass treatment, reducing particle sizes from tens of centimeters to tens of millimeters [1].

Destruction models and force theories for biomass grinding processes are myriad. In the application of these devices, the mechanism that explains particle reduction is based on the type of mechanical action, and the same equipment involves several mechanisms. The main types of action in grinding processes are cleaving, shearing, crushing, cracking, cutting, sawing, abrasion, limited impact, and free impact [1, 6]. For the process of cleavage, shearing, cracking, and sawing, it is necessary that the biomass particles have sizes comparable to the equipment working body. With units above millimeters, these are generally used for primary biomass preparation. The crushing action is suitable for fragile biomass. Cutting and sawing reduce particle size by several centimeters and are usually applied in robust biomass grinding equipment. Finally, actions such as cutting, abrasion, and impact are used to grind rigid biomass such as plants [1].

Pretreatment using a knife mill has been described by Lee and Mani [7] as fiber shear action for nanofibril cellulose production, with relatively lower specific energy expenditure than other equipment used for this same process. Pirich et al. [8] compared various mechanical pretreatment processes, including the colloid milling process, characterized by grinding action, and shown to be essential in the isolation of cellulose nanofibrils. In this context, it is important to highlight that the economic costs involved in the grinding processes do not make the process unfeasible and must be evaluated for each reality and biomass varying for each production; therefore, the optimization of processes and characteristics of biomass determine much of the consumption of this stage.

Another mechanical process is extrusion, used for fractionation of residual biomass. This is a simple process, with mild temperature conditions, not necessarily needing the addition of chemicals, and operating continuously. This process is effective and versatile, effectively mixing pretreated biomass. The yield is continuous, and there is the possibility of adaptation to various process configurations [9, 10].

In the extrusion process, the biomass passes through an extrusion barrel, where it is subjected to high shear. As a result, regions of high pressures and temperatures develop that cause the defibrillation and shortening of the fibers that compose the biomass [10–12]. During the process, the moisture of the biomass comes out of steam as a result of the sudden drop in pressure, causing pore expansion and opening [12, 13]. The high mechanical shear developed inside the extruder barrel breaks down the biomass structure, and this process facilitates contact between residual biomass structures and subsequent processing agents [11, 12].

Extrusion stands out among the mechanical methods because it is a viable technology, without generation of by-products, acting under mild conditions, reducing the degradation of compounds, and capable of fractionation with high solids loads. It works with considerably larger biomass sizes than other methods, leading to greater economic profitability than processes aimed at reducing biomass size [10, 12].

Compared to residual wheat and soybean meal biomasses, mechanical milling processes using hammer mills and extrusion have shown to be promising. The extrusion process showed higher yields compared to residual wheat bran biomass. Higher temperatures for shorter times and lower temperatures for longer times gave rise to extrusion process with higher yields [11].

3.1.2 Ultrasound

Ultrasonic energy accomplishes pretreatment process by cavitation, the result of the propagation of strong waves of frequencies higher than 20 kHz that propagate longitudinally in liquid with alternating pressure periodically in a continuous cycle of rarefaction and compression; this generates negative and positive pressures, respectively [14–16]. As acoustic energy propagates in the liquid and generates negative pressures in the medium, gaseous microbubbles form and fill with vapor; dissolved gases distribute throughout the liquid [15, 17]. The negative pressure that results in microbubble formation is called the cavitation threshold [15]. Microbubbles expand and retract, reaching a maximum diameter of 4–300 mm, varying according to the frequency of the ultrasonic wave pulse. This phenomenon is called stable cavitation when microbubbles are generated under conditions where the maximum sound pressure. In the rarefaction cycle, it is not strong enough to force the bubble to expand its collapse radius [15, 16, 18]. The compression and rarefaction (radial oscillation) cycles due to the pressure oscillation in the medium caused by the ultrasonic waves cause the microbubbles to continue to grow over a few cycles until they reach a critical diameter, high temperature (5000 K), and high pressure (100 mPa), enter an unstable stage, and collapse violently, generating a microjet with dominant shear stress, turbulence, increased pressure and temperature at the site. This process is referred to as "transient cavitation" and is referred to when acoustic pressure exerted on the microbubble causes it to expand at its resonant radius amid several acoustic cycles, resulting in collapse. This force is sufficiently strong to destabilize structures, disrupt cells, and increase mass transfer [15, 16, 19–21]. Collapse occurs within approximately 400 μs [16, 22].

Cavitation occurs in a liquid system, and when applied to solid biomass emerging from the liquid, the cavitation bubbles collapse and generate high-velocity microjets toward the biomass surface, causing flaking, erosion, breakdown of cell walls, so as to increase the diffusion of biomass matrix compounds, inducing intense macroturbulence, micromixing, and consequent collision [15, 23]. These phenomena increase particle reactivity. Mass transfer is also increased because of increased surface area [23].

Ultrasound describes the result of the transformation of electrical energy into thermal and vibrational energy that is then converted into cavitation and is also lost by sound reflection. It is through the combination of pressure, temperature, and turbulence that we obtain a variety of effects in ultrasonic systems in the most diverse biomass and end products.

The efficiency of ultrasound in residual biomass pretreatment techniques depends on several factors. Solvent viscosity is a parameter that should be considered when using ultrasound in pretreatment processes, because cavitation requires negative pressure that must overcome the natural cohesion forces of the liquid. Cavitation is diminished in viscous liquids, where the natural cohesive forces are stronger than the negative pressure of cavitation formation. This factor may reduce the process yield. To increase the cavitation threshold, simply increase the viscosity of the liquid

[14, 24–26]. Increasing polysaccharides in the solvent may reduce the cavitation process by increasing the viscosity of the medium [25]. The increase of the solid/liquid ratio in a system pretreated with ultrasound can be achieved to a point where the increased solids in the medium correlate with decreased system yield because of the increase of viscosity, resulting in difficulty of cavitation formation [24].

Another factor that affects the process and should be noted with caution is the temperature of the solvent, which changes viscosity and surface tension, inducing an increase in vapor pressure. As a result, the rate of chemical reactions changes, affecting the threshold and intensity of cavitation and resulting in a greater number of bubbles that will collapse less violently and reduce the effects of cavitation processes [14, 23]. High temperatures near the boiling point of the solvent used in the process may negatively affect ultrasound pretreatment [23]. Optimization of the process temperature is important, considering that, for residual biomass, ultrasonic pretreatment can be conducted at relatively high temperatures giving higher yields. Yield decreases would occur only in regions near the boiling point of the solvent.

Two devices apply conventional high-power ultrasound pretreatment techniques: ultrasonic baths and probes. These systems use a transducer as a power source. The ultrasonic bath is widely used for its simplicity, low cost, and ability to treat several samples simultaneously. This equipment usually operates at 40 kHz and has temperature control. The amount of energy dissipated in the ultrasonic bath is not easily quantifiable and depends on the size of the equipment, the vessel in which the reaction occurs, and the position of the sample within the tank. These factors influence the reproducibility and potency of ultrasonic waves in pretreatment samples [23].

The ultrasonic probe is more powerful than the bath because of the intensity provided by the system that is delivered only by the probe tip that releases energy directly into the solvent where the biomass is emerging, resulting in less energy loss from the cavitation waves. The selection of the ultrasonic probe model should be made by studying the application, biomass, and volume desired [23].

3.1.3 Microwave

Microwave irradiation is a widely used process for biomass pretreatment. Electromagnetic irradiation of microwave equipment operates in the range 0.3–300 GHz, within the electromagnetic spectrum between infrared and radio frequency [27, 28].

Microwaves work via two mechanisms: thermal and non-thermal. Thermal effects are governed by temperature differences, and non-thermal effects (acceleration, change in reactivity and selectivity of biomass) refer to the circumstances of a synthesis that are microwave conditions [28]. There are arguments against the non-thermal effects, particularly that microwave photon energy (0.0016 eV) is not sufficient to break chemical bonds [29].

The electrical component of the electromagnetic field is responsible for heating (thermal effect) during the microwave process through three primary mechanisms:

dipolar polarization, ion conduction, and interfacial polarization. Generally, these mechanisms occur simultaneously [28–30].

The dipolar polarization mechanism is the primary principle of microwave heating that involves the process of dielectric loss heating [30]. When biomass is exposed to microwave frequency, a dipole sensitive to external electric fields tries to align with the applied electric field. When this applied field oscillates, an attempt is made to realign the dipole field to the alternating electric field, though in the presence of a field. High-frequency electrical power does not have enough time to respond to the oscillating field, causing a phase delay that results in fields colliding with one another. In this process, the energy is dissipated as heat by molecular friction and dielectric loss [29, 30]. The amount of heat generated during this process is directly related to the ability to align between the matrix and frequency of the applied field. That is, there is a quick orientation with the applied field, and if the dipole has sufficient time to realign itself, warming will occur [29].

The interfacial polarization mechanism, also called Maxwell–Wagner Polarization, should be considered when applying the microwave process to non-homogeneous biomasses such as biological materials in suspensions or colloids. This process consists of components with varying conductivities and dielectric constants. In the interface area of these components, polarization induces the formation of a charge accumulation region that causes field distortions and dielectric loss, resulting in heating [28].

The conduction mechanism is the heating principle that involves the creation of an induced electric current resulting from the movement of mobile charge carriers (electrons, ions, etc.) under the influence of a microwave electric field. These induced currents are responsible for heating as a result of electrical resistance caused by collisions between charged molecules [30]. The effect of conductivity has a greater influence on system warming than does the dipolar rotation mechanism [28].

Microwave heating differs from conventional heating in that, by means of ion conduction and dipolar polarization mechanisms, the heat wave is generated from within the biomass structure evenly and rapidly [31]. The use of microwaves as a pretreatment technique is based on the heating of biomass by “microwave dielectric heating,” which depends on the ability of materials to absorb microwave energy and convert it into heat. The heating characteristics of the biomass subjected to microwave pretreatment depend on the dielectric properties of the material [27–29]. The dielectric properties of a material are a function of the dielectric constant and the dielectric loss factor. The first relates to the ability of the material to be polarized by an electric field and represents the amount of energy that is stored in the material; the second suggests the efficiency with which electromagnetic energy is converted to heat [28].

The use of microwaves as lignocellulosic pretreatment has been performed over the last 30 years. In some cases, transitions from laboratory to pilot scale have already been seen [31]. Compared to conventional heating, this technology has low sugar degradation and by-product formation [32].

3.1.4 Hydrothermal

Thermal pretreatment processes are techniques based on the use of thermal energy to cause agitation and molecular changes in the structure of biomass that may cause defibrillation, chemical bond cleavage, increased surface area, and reduced recalcitrance.

Of all biomass pretreatment technologies, steam explosion has been considered as a potential process for numerous products, primarily because of the low or nonexistent chemical consumption, low monosaccharide degradation under mild process, and neutral conditions. The pH that prevents equipment corrosion as well as the energy consumption is relatively low compared to those of other physical pretreatment processes. Nevertheless, steam explosion has disadvantages such as inhibitor generation and weight loss of the initial dry mass of biomass [33].

Steam explosion operates at temperatures of 140–240 °C and high-pressure conditions at incubation times of seconds to 20 min. The mechanism of action is the result of the mechanical effect of an adiabatic expansion of water absorbed by biomass and the chemical effect of the action of organic acids released during the process, occurring at the first moment of the solubilization of hemicellulose at high temperatures and pressures [34, 35]. The mechanical effect results from the rapid decompression of the equipment where the process occurs, resulting in the internal explosion of biomass fibrils disrupting the structure of the lignocellulosic complex and causing structural changes, particle size reduction, and biomass pore enlargement [33, 35–37]. The pressure and temperature conditions cleave the hemicellulose and lignin bonds, and the secondary explosion caused by the sudden reduction in temperature and pressure disrupts the biomass structure, increasing the solid surface area and causing a disturbance in the crystalline region of the structure [35]. At the end of the steam explosion process, a solid fraction containing partially modified cellulose and lignin is obtained, with a low pH liquid fraction containing oligomers, monomers, organic acids, and phenolic compounds [34].

Another promising technique for thermal pretreatment is liquid hot water (LHW), based on the application of high pressures to maintain liquid water and temperatures from 160 to 240 °C. This is a process with high removal capacity of the hemicellulose fraction as well as changes in lignin structure of the lignocellulosic complex without the addition of chemical reagents, making cellulose more accessible for hydrolysis processes [34, 38]. It has been suggested that, when temperature and pressure increase in the water, there is penetration in pores of the biomass, causing destructuring that leads to acidification of the environment. The latter is caused by the release of organic acids present in the structure, especially acetic acid that acts on hydrolysis of the structure during the process, releasing mainly oligosaccharides in the liquid fraction [34, 39, 40]. The main difference between the LHW and the steam blast technique is that the former does not use rapid decompression for structure defibrillation.

Steam heat pretreatment is a technique with similar mechanisms to the previous ones; the primary difference is the arrangement of the biomass in the reactor, because the material remains at the top, having no direct contact with the water in the liquid state. This crucial difference causes steam pretreatment to allow higher solids charges than steam blast and LHW techniques. The hemicellulosic fraction is the main target of this type of pretreatment and is removed by contact with steam at high pressure and temperature [34, 41].

Process parameters are crucial for the results obtained in the thermal pretreatment technique, with temperature, pressure, and incubation time being the main factors to be studied. These techniques are considered green and cost-effective because of the process yield and not the chemical application.

3.2 Chemicals

Chemical pretreatment is operations designed to modify physical and chemical properties of residual biomass in the presence of a chemical catalyst aiming for conformational changes in the biomass structure such as cleavage of bonds and generation of specific products. The techniques of chemical pretreatment include alkalis, acids, organosolv, ionic liquids, and oxidative processes.

3.2.1 Alkaline

Alkaline media are popularly used for pretreatment of biomass because they are relatively less expensive than other reagents and have less compound degradation. Among the most widely used bases are sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), potassium hydroxide (KOH), and ammonium hydroxide (NH_4OH).

The efficiency of alkaline pretreatments is associated with the ability of the base to perform biomass delignification and reduction of cellulose crystallinity. It is generally associated with the pretreatment of lignocellulosic biomass for bioenergy production. In addition to acting efficiently in delignification, alkaline pretreatment has advantages such as lower sugar degradation, especially compared to other chemical treatments such as acids or oxidizing agents, and can be conducted at low temperatures and pressures. Nevertheless, depending on the complexity of biomass, longer periods may be required for higher yields [42, 43].

Table 3.1 displays the bases applied in pretreatment processes, aiming to demonstrate recent applications in biomass for various biotechnological purposes. All alkaline pretreatment techniques in lignocellulosic biomass have the common effect of increasing biomass digestibility by altering the lignocellulosic complex.

The action of alkaline pretreatments remains much discussed in recent works. It is believed that the mechanism of alkaline pretreatments in lignocellulosic biomass is based on two principles: the swelling of the biomass structural complex and the reduction of crystallinity, resulting in partial cleavage of lignin structure and solubilization of hemicellulose acetyl groups [50, 51]. Removal of hemicellulose is relevant

Table 3.1 Pretreatment processes applied in biomasses for biotechnological purposes

Reaction	Reaction conditions	Biomass	Main effect	Reference
Sodium hydroxide	2–8% (m m^{-1}) NaOH 35, 55, and 121 °C 24–24 h; 1 h	*Pennisetum hybrid*	– Reduction of hemicellulose and lignin content and increase of cellulose content – Increased surface porosity – Breakage of intra- and intermolecular hydrogen bonds and methyl/methylene of cellulose	[44]
Sodium hydroxide	0.5, 1.0 and 2.0% p v^{-1} NaOH 121, 50 and 21 °C 0.25–1, 1–48 e 1–96 h	Switchgrass	– Reduction in lignin was closely related to temperature: 85.8%—121 °C, 77.8%—50 °C, 62.9%—21 °C, at higher NaOH concentrations and longer times	[45]
Calcium hydroxide	0.02–0.12 g g^{-1} $Ca(OH)_2$ 25–55 °C 7–28 days	*Spartina alterniflora*	– The pH has been neutralized in some samples by acids formed in the process due to the breakdown of ester bonds and neutralizing structural carboxylic acids formed by deacetylation of hemicellulose – Selective removal of hemicellulose and lignin, with cellulose recovery yield between 91 and 98.7%	[46]
Ammonium hydroxide	0.5–50% mass NH_3 30 °C 4–12 weeks	Corn straw	– Preservation of carbohydrates – 55% delignification with the highest NH_3 load – Increased digestibility of biomass in the enzymatic process	[47]
Potassium hydroxide	0.5–12.5 g KOH 20 °C 24 h	Wheat straw	– Lignin and hemicellulose content decreased with increasing KOH load – Sugar concentration after enzymatic hydrolysis of pretreated biomass	[48]

(continued)

Table 3.1 (continued)

Reaction	Reaction conditions	Biomass	Main effect	Reference
Sodium hydroxide	0.07 g NaOH/g of biomass 140 °C 30 min	Corn straw	– High efficiency in lignin removal from biomass – Low structural alteration of lignin in lower-molecular-weight compounds – High concentration of lignin in the pretreatment liquid fraction	[49]

to the effect of pretreatment on cellulose structure. Hemicellulose losses occur for degradation products that may have an inhibitory effect on the subsequent process [50].

Cellulose structure is disturbed by the action of alkaline pretreatment because of the action of solvation process forces, which, in the presence of swelling of the lignocellulosic structure and increase of the internal surface area, causes intermolecular forces of the cellulose to be smaller than forces due to solvation, disrupting the hydrogen bonds of adjacent chains of the cellulosic structure [43]. This process may favor the penetration and propagation of reagents and enzymes in subsequent processes. It is common for the cellulose crystallinity index to increase [42, 44] as a result of the removal of amorphous cellulose that is more susceptible to alkaline hydrolysis.

The intermolecular saponification process is a possible action of alkaline pretreatment, and this is the process that results in the removal of lignin. The increase in the internal biomass surface allows the alkaline agent to access the xylan-linked ester bonds present in hemicellulose with other components. These breakdowns produce charged carboxyl groups that cleave bonds with lignin and other lignocellulosic complex compounds. It is these structural cleavages that allow the breakdown of cellulose adjacent hydrogen bonds reported in the solvation process [42, 43, 50].

In particular, calcium hydroxide promotes lignin reduction through the formation of a calcium–lignin complex, where calcium ions (two positive charges) tend to attract negative lignin charges under alkaline conditions because of ionization of functional groups, and this mechanism avoids intensive lignin solubilization [45, 52]. However, increased calcium loading may reduce lignin dissolution and may attenuate carbohydrate loss, possibly due to the binding of calcium ions with these compounds under alkaline conditions. [45].

Sodium hydroxide is the most often used catalyst, with high delignification efficiency, increased biomass digestibility, relatively fast reaction rate, less formation of biological process inhibitors, and increased surface area of biomass [53]. Nevertheless, between sodium hydroxide and calcium hydroxide, the latter is preferable; because of its safety profile, relatively lower cost, ease of recovery, compatibility with oxidizing agents, and selectivity for the structure of lignocellulosic biomass [46, 51, 54], the residence time of calcium hydroxide pretreatment needs to be longer, as noted in the studies reported in the table below. This factor has to do with the low solubility of the reagent that requires longer residence time to achieve the same yield as the other alkali reagents discussed in this section.

Ammonia-based pretreatments have been highlighted for easy reagent recovery, non-corrosive and non-toxic nature, economically viability, and wide use in fertilizers. In addition, their use and industrial recovery are well-established procedures, with viable processing options for recovery. The effects of ammonia on biomass include delignification, increased surface area, and structural modification of cellulose and hemicellulose [55, 56].

Alkaline pretreatments are interesting for high lignin and hemicellulose residual biomass, as well as in processes where the preservation of carbohydrate structure is desired. These methods are easy to apply, with low generation of inhibitors, and can be applied to various biotechnological processes.

Recent studies have evaluated the use of alkaline pretreatment (NaOH) in maize straw for chemical production. The authors used SEM and found that cell walls of the structure were swollen and that the rupture caused by the pretreatment of biomass exposed the internal areas of the structure, triggering an increase in the porosity of the material. The authors were able to remove between 63.90 and 81.91% of lignin at temperatures at 60 °C, with 1-h pretreatment, and low NaOH concentrations (0.25–0.75 mol dm^{-3}, respectively) [57].

Hashemi et al. [58] studied the effects of ethanolic ammonia pretreatment on sugarcane bagasse for biogas production. The authors observed that lignin–carbohydrate and lignin bonds were hydrolyzed and lignin was removed from the biomass. They also found that glucan and xylan recovery were higher with pretreatment supplemented with ethanol than with ammonia alone. The effect of ethanol on this system is believed to cause soluble xylan to precipitate into solid structures that facilitates the recovery of this sugar for anaerobic digestion processes [58, 59]. Another similar work defined pretreatment for ethanol and aqueous ammonia immersion, now focusing on corn straw biomass, and found that the effect of ethanol contributed to the preservation of cellulose in solid form. The addition of ethanol at 20% (by weight) resulted in an optimal concentration for glucan and xylan digestibility [59].

Alkaline pretreatment (NaOH) in wheat straw reduced the lignin fraction by 36% and significantly reached hemicellulose (35% reduction) after pretreatment [60]. The predicted effects for the reduction of lignin and hemicellulose were associated with saponification and cross-linking cleavage processes between xylan and lignin that also caused increases in internal surface area, as previously reported [61, 62].

3.2.2 Acids

Pretreatment techniques that use acids as catalysts are commonly used for various biomasses at high or diluted concentrations, with inorganic acids such as sulfuric acid (H_2SO_4), hydrochloric acid (HCl), and phosphoric acid (H_2PO_4) being most common.

Pretreatment with an inorganic acid in lignocellulosic biomass is widespread on the industrial scale, because it is a chemical reaction facilitated in complex structures and because the process is efficient in fractionating the hemicellulose of the biomass structure by increasing the surface area and accessibility of the biomass structure in downstream processes [32, 63]. Furthermore, this pretreatment may be useful in dissolving lignin in lignocellulosic biomass, even though it generally acts less efficiently with respect to removal of this compound compared to hemicellulose cleavage [64]. Nevertheless, biological process inhibitors such as alcoholic fermentation for ethanol production form during hemicellulose and lignin cleavage, solubilization, and degradation, including furans because of dehydration of monomers and phenolic compounds [65].

When the technique is performed by applying concentrated acid, the temperature is generally low and results in high yield of monosaccharides in lignocellulosic structures derived from the cellulose structure. However, the hydrolysis rate of amorphous cellulose is slow compared to that of hemicellulose that has an amorphous structure; therefore, the concentrated acid technique generates high concentrations of furan inhibitors due to dehydration of monomers of the chemical structure of hemicellulose. Another disadvantage is equipment corrosion, high chemical consumption, high toxicity for the environment, and high energy demand for acid recovery [43].

Another application of the chemical pretreatment is the hydrolysis using dilute acid, where high-temperature and high-pressure applications are required to increase the yield from crystalline cellulose, consuming fewer chemicals than the prior technique. However, this usually results in the degradation of sugars because of more severe temperature and pressure conditions, causing solubilization of hemicellulose chains and dehydration of pentoses and hexoses in inhibitors [43, 66–68].

With the search of continuous advances in biomass pretreatment processes, organic acids have become alternatives to inorganic acids for some biotechnological processes, including dicarboxylic (malic, oxalic, fumaric) and monocarboxylic acids (acetic acid). Compared to inorganic acids, organics have advantages such as lower equipment corrosive capacity and less environmental damage, lower energy demand for compost recovery, longer long-chain cellulose insulation capacity, lower inhibitor generation, and higher pH in relation to inorganic acids [43, 69, 70]. Organic acids are an efficient alternative to the pretreatment of high cellulose and hemicellulose biomasses such as aquatic plants and rice straw [71, 72].

The interaction mechanisms between biomass and acid in pretreatment processes strongly depend on the factors involved in the process, especially the temperature and composition of the material subjected to pretreatment. The main purpose of acid application in biomass pretreatment reactions is to weaken or disrupt the chemical structure, being generally based on hemicellulose hydrolysis and reduction of crystallinity of other compounds [73, 74]. It is suggested that this action occurs through the cleavage of aryl ether bonds, a set of abundant bonds in lignin macromolecules that have low binding energy that facilitates direct cleavage in this branch. Through covalent bonds, lignin remains in the lignocellulosic complex associated with polysaccharides, especially hemicellulose. Lignin cleavage into macromolecules causes depolymerization of biomass and exposes the structural chain of hemicellulose and cellulose, facilitating access to hydrolysis of these polysaccharides [75–78]. Under optimized process conditions, it is possible to remove lignin without causing severe disruption of the monosaccharides of interest from cellulose and hemicellulose. In processes with dilute acid applied at high temperatures, the system is influenced by the thermal process that results in pore opening in the biomass structure; cleavage of the structure is performed by acid as the chemical catalyst of the process [50].

Application of acids in the pretreatment process is a widely explored technique, with established industrial processes; there nevertheless remains a need for improvements aimed at cost reductions, lower generation of process inhibitors, higher yields, less degradation of essential compounds in the production system, lower reagent consumption, and reduction of environmental impacts.

3.2.3 Oxidizers

Oxidative pretreatment techniques are performed using agents such as hydrogen peroxide (H_2O_2), ozone, and oxygen, involving processes with high yield in biomass delignification. These techniques are associated with electrophilic substitution processes, chemical bond displacement, alkyl-aryl ether bond cleavage, and/or aromatic ring cleavage [4, 50, 79].

Oxidative processes occur because of the high reactivity of these oxidizing agents to aromatic rings. They have a direct effect on lignin structure, and they consequently access the hemicellulose structure of the lignocellulosic complex. Because there is no selectivity, for the chemical structure of lignin, these oxidizing compounds can aggressively attack hemicellulose and cellulose, causing loss of valuable compounds to the downstream process; therefore, strict control in use of this technique is essential [4, 50].

Hydrogen peroxide is a widely applied oxidizing agent for this technique and has great potential for pretreatment of biomass, mainly because it leaves no residue in the material as it degrades in oxygen and water [79, 80]. Even so, its lack of selectivity can lead to high concentrations of inhibitors from lignin and hemicellulose [50].

The H_2O_2 delignification potential is related to the release of hydroxyl ions and superoxides released by reagent degradation at pH 11.5–11.6, a range determined by Gould [81], who demonstrated that at pH less than 10 and greater than 12.5, there is no efficiency in the pretreatment process of lignocellulosic biomass by H_2O_2.

The mechanism of action of H_2O_2 in lignocellulosic biomass is the result of the presence of hydroxyl ions and superoxides released in the degradation of H_2O_2 that have high reactivity, resulting in the immediate cleavage of bonds with low energy activation in the lignocellulosic complex, resulting in mass loss of the structure [4, 35, 79]. The H_2O_2 concentration should be greater than 1%, and the reagent-to-biomass ratio should be 0.25 for good yields [81]. In addition to the loss of lignin by cleavage resulting from the release of reagent ions, cleavage of the hemicellulosic structure was reported for pretreatment of lignocellulosic biomass by H_2O_2 and solid with high cellulose percentage [80].

Wet oxidation is an oxidative pretreatment technique where oxygen is injected into the liquid phase under high temperatures (120–325 °C) and high pressures (0.5–20 MPa) to increase oxygen solubility and velocity of reaction, leading to contact biomass oxidation, resulting in the formation of organic salts, simple biodegradable compounds, or complete oxidation forming carbon dioxide and water [4, 82–86]. The reaction mechanism of the wet oxidation process in relation to biomass is generally explained by free radical chain reactions in the system and/or by reaction pathways based on self-catalysis by the release of intermediate products of structure degradation of biomass such as acetic acid and formic acid [84, 87, 88].

Ozonolysis is a promising oxidative pretreatment technique based on the use of ozone to reduce biomass recalcitrance. It is widely studied for bleaching processes in the pulp industry and for wastewater treatment, and it is currently gaining prominence as an alternative for pretreatment of lignocellulosic biomass [89]. The ozonolysis technique is considered green because ozone is a powerful oxidant with high oxidation potential in contact with biomass and when decomposed forms oxygen and can be safely released into the environment; the system is run at environmental temperatures and pressures [90, 91].

Ozone is highly reactive to lignin; however, like other oxidative reagents, it has low selectivity and can react with carbohydrates and other compounds, causing degradation of products of interest to the downstream reaction system [89]. The reaction between ozone and lignocellulosic biomass is thought to be mediated by mechanisms involving C–C bonds and cleavage of aromatic centers and glycosidic bonds [89, 92]. High ozone reactivity is a result of electron deficiency in a terminal molecule during resonance, directly affecting compounds with high electron density (C–C bonds and aromatic rings), resulting in attack on lignin and carbohydrate structures [89, 91, 93]. Travaini et al. [94] studied sugarcane biomass pretreated with ozone and reported partial attack of insoluble lignin, transforming it into soluble lignin, with loss of total lignin, and demonstrating the efficiency of delignification in ozonolysis process.

The application of this technique has advantages such as low generation of inhibitory compounds and low degradation of hemicellulose and cellulose under mild and mild operating conditions. Usually, ozone is produced and used on site, reducing the need for reagent transport. Furthermore, there is no need for the addition of chemical catalysts; this reduces pollution by depleting residual ozone as oxygen. By contrast, ozone has exothermic characteristics that may require a cooling system; it is highly reactive, flammable, and corrosive, requiring resistant materials for reactor construction [89].

3.2.4 Ionic Liquids

Ionic liquids (ILs) are pure salts with low melting points (<100 °C), composed of organic cations and organic or inorganic anions. ILs with melting points below room temperature are known as ionic liquids. Most ILs are non-flammable and have low or negligible vapor pressure, chemical, and thermal stability, and their physical properties can be adjusted for a specific task by varying the cation and anion amounts [95].

There are several types of ILs, including acidic ionic liquids (AILs) that turn can be subdivided into Lewis acidic ionic liquids (LAILs) and Bronsted acidic ionic liquids (BAILs) [96]; protic ionic liquids [97], and dicationic ionic liquids [95].

The mechanism of action of ILs in biomass usually involves lignin attack, dissolving it by the deconstruction of the lignocellulosic matrix by breaking the bonds between the basic units. Lignin consists of seven different types of linkage bonds, including β-O-4, α-O-4, β-5, 5-5, 4-O-5, β-1, and β-β. However, the β-O-4 bond accounts for between 50 and 60% of total bonds. During cleavage of the β-O-4 bond, an intermediate β-1 interlock is formed prior to further degradation, while the β-5 bond is converted to stilbene. Stilbene is a comparatively non-reactive and colorless compound initially present in lignin that is insoluble in water and has mainly two isomers [95].

According to molecular dynamics simulations, the interaction between ILs and biomass depends to a large extent on IL solubility [98]. Lignin degradation performance through ILs depends on solvation parameters, as predicted by the Kamlet–Taft solubility model. The model is widely used to predict three empirical parameters of IL polarity in biomass pretreatment: hydrogen-bond acidity, hydrogen-bond basicity, and dipolarity/polarity [99].

Because of their low vapor pressure, ILs are thought to be green alternatives to volatile organic solvents. ILs that contain anions with high hydrogen-bond basicity such as chloride, phosphates, phosphonates, and carboxylates are excellent solvents for cellulose dissolution [100]. They also have high potential for energy-efficient biomass pretreatment, recyclability, and various properties that can be adjusted to the product that needs to be obtained. Some liquids can be prepared from renewable biomass raw materials [95]. One of their disadvantages is that liquids can become more viscous in pretreatment processing, making them difficult to use and opening up their operational processes [101]. Anther difficultly with pretreatment with ionic liquids is that the largest of these is toxic to cellulase and should be removed prior to subjecting the biomass to enzymatic hydrolysis [102].

Pretreatment of lignocellulosic biomass generally involves heating the dry and milling biomass to moderate temperatures, containing the excess ionic liquid in question, such that the LI can be recycled later. These pretreatment processes must be further refined, modified, optimized, and expanded. This process has been studied for many years [100].

Studies have reported successes in biomass pretreatment experiments using IL. Da Costa Lopes et al. [101] reported that lignin content and cellulose crystallinity decreased significantly when wheat straw was treated with 1-ethyl-3-methylimidazole acetate. Monosaccharide yield increased to 81–97%, and the liquid has been reused more than 20 times. The fractionation of completely dissolved wheat straw materials led to cellulose-rich and hemicellulose-rich components. Pretreatment also produced high-purity lignin [103].

Semerci and Güler [97] investigated IL 1-butylimidazolium hydrogen sulfate ($HBIMHSO_4$) as pretreatment of cotton stalks. They used 20% ($m\ m^{-1}$) of water and 15% ($m\ m^{-1}$) of biomass at 120 °C for four hours. Pretreatment resulted in significant structural changes in biomass. The lignin content of cotton stalks was reduced by 35%, and the cellulose content increased from 36 to 55%. Enzymatic hydrolysis of biomass increased almost fivefold despite an increase in biomass crystallinity. Morphological changes in cotton stalks observed using SEM analysis revealed differences in composition and enzymatic accessibility of biomass samples subjected to pretreatment.

3.2.5 Organosolv

The pretreatment technique using organosolv has applications for many compounds including methanol, ethanol, acetic acid, peracetic acid, and acetone, being mixed in water and exposed to biomass. This technique is notable for high yields in the removal of lignin and hemicellulose from lignocellulosic complexes, generating substances of high purity as well as keeping the cellulose solid and relatively intact [104].

The action of organosolv in lignocellulosic biomass pretreatment processes occurs through the separation of the lignocellulosic complex in the cleavage of internal lignin and hemicellulose bonds that are isolated in low-molecular-weight fragments and generally dissolved in the supernatant. Cellulose maintains solid structure susceptible to hydrolysis processes [77, 105]. The process of removing lignin and hemicellulose structure increases pore volume in biomass and surface area and reduces recalcitrance [105].

Biomass pretreatment with organosolv is a promising technique for the generation of high-purity, low-molecular-weight lignin and xylose polymers. This structural fractionation is directly related to the effects of solvent property on delignification and precipitation. The use of organosolv in process integration concepts is compelling because it generates purity by-products that can be used for various purposes [104, 105]. Organosolv has high economic value; therefore, the use of the pretreatment technique depends on the reagent recovery processes, usually performed by distillation and recycling to the system, considerably increasing energy consumption and requiring high throughput at this stage to make the technique viable [104]. Another obstacle to the application of the technique is the need for extreme rigor to avoid solvent volatilization owing to the high flammability potential of these substances [77, 104].

To reduce the energy consumption of the operation of this technique, some investigators addressed the addition of a catalyst, usually an inorganic acid, to improve the delignification process and to increase the efficiency of the technique [77, 104, 106]. The technique was applied at high temperatures (>185 °C), where the release of organic acids from the structures is believed to act as a catalyst in the process of rupture and stabilization of lignin macromolecules [107]. In this context, it is understood that the pretreatment process with organosolv can be evaluated in three fractions, the lignin fraction, the solubilized hemicellulose fraction, and the solid medium cellulose fraction.

The most commonly used organosolv in pretreatment techniques are alcohols, mainly methanol and ethanol, because of their low boiling point, facilitating the recovery process and generating low acquisition costs. High-boiling alcohols were also studied, although they require high energy consumption for recovery. Other compounds such as organosolv (e.g., formic acid and acetic acid), organic peracids (e.g., peracetic acid and pericetic acid), cetone (e.g., acetone), and others such as dioxane, phenol, and ethylenediamine have also been applied in the technique [104, 106, 107].

The mechanism of pretreatment with organosolv is the result of three chemical reactions: (1) degradation of lignin and hemicellulose by cleavage of internal bonds of ether, ester, and 4-O-methylglucuronic acid ester bonds; (2) disruption of the glycosidic bonds of hemicellulose and the amorphous regions of cellulose, this process being dependent on the solvent and the conditions applied to the pretreatment; (3) under severe conditions, mono- and oligosaccharides are dehydrated in HMF and furfural, or in acids such as levulinic and formic, followed by condensation between lignin and reactive aldehydes [104, 108–110]. In pretreatments using organosolv, the swelling of the crystalline cellulose region has been reported, increasing the surface area of this structure and facilitating hydrolysis [106, 111].

Pretreatment with organosolv is a process of removal of lignin and hemicellulose by solvation and solubilization of compounds. Selective cleavages of the structures of the lignocellulosic complex are the most efficient mechanisms within this technique, as well as the generation of compounds with high purity. However, because the biomass to which pretreatment is applied varies in terms of source and composition, and because solvents that can be used for pretreatment are also diverse, further investigation is required as to the behavior of the mechanism of action, the technique, and the delignification process [104].

Organosolv pretreatment is still considered a technique with high economic costs. Nevertheless, within current concepts of the circular economy and biorefineries, this technique can provide high added-value by-products and may be considered for integration and optimization of processes, making the use of organosolv a promising technique for pretreatment of various biomasses.

3.2.6 Thermochemicals

Thermochemical pretreatment is a technique used to modify biomass properties in the presence of catalysts. Thermochemical pretreatment techniques include supercritical CO_2 and ammonia fiber explosion (AFEX).

Among pretreatment processes using supercritical fluids, carbon dioxide (CO_2) stands out for its high performance in chemical structure compound extraction processes, because it is non-flammable and toxic, recoverable, low cost, and inert, in addition to presenting safer critical pressure and temperature conditions than other solvents [112, 113].

The supercritical point is reached when the temperature and pressure applied to the system are higher than the critical values, such that the fluid can be considered an expanded liquid or a compressed gas [114]. CO_2 has a critical point in temperature of 304.2 K and a critical pressure of 7.38 MPa, and at this point, it has near liquid density and near gas viscosity [112]. The biomass moisture has a positive influence on the supercritical CO_2 process, being responsible for good yields in hemicellulose partial hydrolysis reactions. This is because wet biomass in contact with supercritical CO_2 dissociates hydrogen bonds of microfibrils of hemicellulose and cellulose, resulting in increased accessibility and contact surface. Water present in biomass under supercritical conditions causes biomass swelling, facilitating deeper access of CO_2 molecules to the biomass structure and increasing reaction yield. Subsequently, an explosive release of pressure occurs and supercritical CO_2 breaks the biomass fibers, reducing structure recalcitrance [113, 115–117].

AFEX is a technology that utilizes high pressure (e.g., 250–300 psi) and moderate temperature (e.g., 90–100 °C) associated with chemical catalyst (ammonia) to increase accessibility to the biomass structure [118]. The action mechanism of this technique is based on the cleavage of lignocellulosic complex bonds through structural modifications in the biomass cell wall, gradually increasing the internal pores of the biomass and solubilizing the structures of the lignocellulosic complex. Two reactions occur simultaneously, ammonolysis and hydrolysis, converting acetyl and ester bonds into amines and organic acids [119, 120]. A major advantage of AFEX is that nitrogenous residues serve as an important source of nitrogen in biological processes that may be of interest to the system [121]. AFEX reduces lignin recalcitrance and promotes partial depolymerization of hemicellulose and decrystallization of cellulose [118].

3.3 Biological

Biological pretreatments offer a potential alternative to ensure the unlocking of complex lignin structures and thereby enabling access to sugars and products of interest [122]. Access may be possible through lignolytic enzymes that are capable of disrupting complex lignin structures and making energy-bearing organic carbons accessible

[122]. The by-products produced during the biological pretreatment stage generally do not affect subsequent hydrolytic processes, because the pretreatment conditions are generally mild. Furthermore, in this process, the use of chemicals is not involved, and there is no need for recycling of chemicals in the final phase of the process [123]. However, some of the greatest challenges to be overcome in this type of model are the high cost (application of special enzyme) and the time. Other disadvantages such as substantial loss of holocellulose (cellulose and hemicellulose) and scale-up (reactor design and decontamination) also need to be addressed to make an industrial application viable [124].

Chen et al. [122] found that white-rot fungi were the most effective microorganism model of lignin degradation. For biofuel production, these microorganisms are preferred for fungal pretreatment because they ensure highly delignified cellulose-rich biomass. The mechanism of degradation is mainly associated with the action of enzymes through oxidative processes such as laccases (benzenediol oxygen oxidoreductase, EC 1.10.3.2) and various types of peroxidases (lignin peroxidases (LiPs; EC 1.11.1.14), manganese peroxidases (MnPs; EC 1.11.1.13), versatile peroxidases (VPs; E.C.1.11.1.16), and manganese-independent peroxidases (MiPs; EC 1.11.1.7)) [122, 125, 126]. In addition to these enzymes, some low-molecular-weight metabolites may also be associated with lignin biodegradation, including chemical oxidizing agents and natural mediators of ligninolytic enzymes [127, 128].

Lignin peroxidases are known for the oxidative capacity of high potential redox aromatic rings, including compounds such as veratryl (3,4-dimethoxybenzyl) alcohol, methoxybenzenes, and non-phenolic lignin model dimers. For this enzyme, non-phenolic aromatic substrates are preferred [126]. It has also been seen that oxidation of phenolic compounds may be possible with the presence of veratryl alcohol that provides a cation radical to act as a redox mediator [129, 130].

Manganese peroxidases act on both phenolic and non-phenolic lignin units through lipid peroxidation reactions [131]. The mechanism of action is associated with the oxidation of Mn^{2+} to Mn^{3+} that oxidizes phenol rings to phenoxy radicals, thereby leading to decomposition of compounds [123].

Lignin peroxidase and manganese peroxidase are the two main supporting enzymes acting on the lignolytic system. Both are heme-containing glycoproteins that require hydrogen peroxide as an oxidizing agent. Some of the fungi that produce them are *Phanerochaete chrysosporium, Pycnoporus cinnabarinus, Ceriporiala cerata, Ceriporiopsis subvermispora, Cyathus stercoreus,* and *Pleurotus ostreatus* [123, 132].

Laccases are another class of enzymes that have effects on lignin. Catalysis is associated with the oxidation of phenolic units of lignin and phenolic compounds and aromatic amines to radical. The action of laccases on this material, together with lignin peroxidase and manganese peroxidase, may lead to complete degradation of lignin. However, they also require a redox mediator for potential effects on lignocellulosic materials such as 3-hydroxyanthranilic acid, 2,2-β-azino-bis (3-ethylthiazoline-6-sulfonate) [123].

Studies reported promising effects with an 82% increase in hydrolysis rate with the use of white-rot fungus *Irpex lacteus* after 28 days of pretreatment on corn stalks.

The effect of this significant increase was associated with the variety of extracellular, hydrolytic, and oxidative enzymes produced by the fungus during pretreatment. Such enzymes and other metabolites left in pretreated corn stalks could also continue to play important roles during material hydrolysis at later stages [133].

In general, the use of fungi during biological pretreatment is a suitable option for the delignification processes. Nevertheless, their slow growth, lasting for several weeks or months, is the principle disadvantages of fungal pretreatment: the issue of time and loss of holocellulose previously reported. The use of enzymes for direct treatment of biomass is an encouraging alternative to overcome such challenges. The action of enzymes during the process is closely associated with the mechanism for each of them as described above; however, in this case, they are not produced by microorganisms in the same system.

Factors such as temperature, pH and enzyme concentration are important during enzyme pretreatment. Ramos et al. [134] tested the effect of crude enzymatic extract of *P. chrysosporium* on sugarcane bagasse for mechanical pulp production. They found that crude enzyme extracts (containing lignin peroxidase, manganese peroxidase, and laccase) were more advantageous than pretreatments using fungi. In this system, 36 h of enzymatic pretreatment with H_2O_2 addition resulted in a higher pulp yield than fungal pretreatment for two weeks.

In some cases, it has been shown that pretreatment with crude extracts resulted in a synergistic action of various enzymes on the substrate [135, 136]. For example, enzymatic pretreatment of wheat straw with lignolytics (laccase and peroxidase) and cellulolytics (carboxymethylcellulase (CMCase) and avicelase) contributed to an in vitro degradation of wheat straw cell walls [136]. Nevertheless, further studies are needed to examine the influence and characteristics of various types of enzymes, as well as their interactions with the substrate, when mixed in the biological pretreatment of biomasses.

3.4 Combined Pretreatments

In general, pretreatment methods aim to intensify the results of subsequent steps, including hydrolysis of biomass to produce fermentable sugars, among others.

Combined pretreatments, such as grinding, screening, use of dilute aqueous ammonia and ultrasound have been employed on lignocellulosic biomass, including corn cob, sorghum stalk, and corn husk. The biomass was ground and sieved in 20–40 mesh, ammonia was added at 1.0–4.0% and the samples were inserted in a 90 W and 59 kHz ultrasonic processor where the temperature was maintained at 40–70 °C for 1–4 h. The crystallinity of the samples was monitored in their crude form and pretreated using an X-ray diffractometer, and surface analyses were observed using scanning electron microscopy (see Session 2.2). In corn husks, alkaline pretreatment was positive and the concentration of 2.0%, 60 °C and intermediate time of 2 h obtained the highest sugar yields; however, the removal of lignin and hemicellulose was very low. For the corn cob and sorghum stalk, the trend was the same as those of pretreatments, because lignin and hemicellulose contents decreased when the combination of ultrasound and alkaline treatment was used, improving the sugar yield, that is, coupled with increased contact surface between biomass and hydrolyzing enzymes, an effect linked to ultrasound. Electron micrographs demonstrated the effect of pretreatment, because the raw biomass appears compactly, and after treatment, it has a rough and looser surface, suggesting that parts of the hemicellulose and lignin were removed during the process [137].

In term of industrial-scale processes, the application of wood chips for power generation presents a promising logic, especially when it comes to constant charge flows. The benefits and limitations of this technology were evaluated using ultrasonic pretreatment, and its effectiveness was tested as the physical characteristics of biomass (SEM, see Sect. 2.2.4.1) and bio-oil yield. Waste from a pulp and paper mill was milled on a 5-mm grid and subjected to an ultrasonic bath (250 W L^{-1}) with a capacity of 34 L, and the wood chip biomass treatment was carried out under the following conditions: 0.5 h and 170 kHz followed by 1.5 h to 40 kHz with power of 1000 W and maximum temperature of 76 °C [138]. From the analysis of biomass morphology by scanning electron microscopy, it was observed that the treated wood presented openings in its surface and no chemical or inhibitor formation was observed during the process. More specifically, ultrasound treatment improved the accessibility and caused small erosions on the surface of the particles, improving heat and mass transfer rates, favoring the subsequent processes of use of this biomass, and enhancing the yield. Nevertheless, in this type and treatment, attention must be paid to factors such as exposure time and energy invested, as they can degrade the material to the point of making it non-usable for the process.

The use of combined pretreatment technologies presents the essence of the possibility of positively developing the expected result. Generally, there is a synergistic effect aiming to pretreat substrates with high efficiency; for example, the combination of ultrasound and alkaline and acidic treatment techniques is compelling for the disintegration of organic particles and improving their solubilization [139].

Studies aimed at improving the efficiency of anaerobic digestion for biogas production have focused on the combination of chemical and microwave pretreatments. The addition of a chemical compound (sodium citrate) initially disintegrates the structure of biomass and the input of energy provided by the microwaves facilitates the solubilization of organic matter; consequently, the synergy between the pretreatments helps permeabilize the biomass. The yield was considerably higher when combining treatment techniques, proving the beneficial effect of these processes acting together [140].

In similar fashion, studies have combined NaOH with ultrasonic treatment to accelerate biomass biodegradability [141]. To study effective waste management in the cosmetic industry, investigators combined thermo-alkaline, thermo-sonication, and thermo-alkaline-sonication treatments. Combined treatment with thermo-alkaline-sonication showed higher degradation value (66.1%) of biomass and favored methane production by 50%, increasing energy consumption by only 1.1% [142].

The choice of the appropriate pretreatment methodology considers several factors such as biomass characteristics and their potential application in biotechnological processes, especially with respect to cost reduction and development of environmentally viable processes, as well as the generation of products with added value, according to the principles of circular economy.

References

1. Lomovsky O, Bychkov A, Lomovsky I (2016) Chapter 2: Mechanical pretreatment. In: Mussatto SI (ed) BFT for a LFBB. Elsevier, Amsterdam, pp 23–55
2. Dahunsi SO (2019) Mechanical pretreatment of lignocelluloses for enhanced biogas production: methane yield prediction from biomass structural components. Bioresour Technol 280:18–26. https://doi.org/10.1016/j.biortech.2019.02.006
3. Elliott A, Mahmood T (2012) Comparison of mechanical pretreatment methods for the enhancement of anaerobic digestion of pulp and paper waste activated sludge. Water Environ Res 84:497–505. https://doi.org/10.2175/106143012X13347678384602
4. Venturin B, Bonatto C, Damaceno FM, Mulinari J, Fongaro G, Treichel H (2019) Physical, chemical, and biological substrate pretreatments to enhance biogas yield. In: Fongaro G, Treichel H (eds) Improving biogas production: technological challenges, alternative sources, future developments. Springer International Publishing, Cham, pp 25–44
5. Bychkov AL, Ryabchikova EI, Korolev KG, Lomovsky OI (2012) Ultrastructural changes of cell walls under intense mechanical treatment of selective plant raw material. Biomass Bioenerg 47:260–267. https://doi.org/10.1016/j.biombioe.2012.09.035
6. Karinkanta P, Ämmälä A, Illikainen M, Niinimäki J (2018) Fine grinding of wood—overview from wood breakage to applications. Biomass Bioenerg 113:31–44. https://doi.org/10.1016/j.biombioe.2018.03.007
7. Lee H, Mani S (2017) Mechanical pretreatment of cellulose pulp to produce cellulose nanofibrils using a dry grinding method. Ind Crops Prod 104:179–187. https://doi.org/10.1016/j.indcrop.2017.04.044
8. Pirich CL, Picheth GF, Machado JPE, Sakakibara CN, Martin AA, de Freitas RA, Sierakowski MR (2019) Influence of mechanical pretreatment to isolate cellulose nanocrystals by sulfuric acid hydrolysis. Int J Biol Macromol 130:622–626. https://doi.org/10.1016/j.ijbiomac.2019.02.166
9. Duque A, Manzanares P, Ballesteros I, Negro MJ, Oliva JM, Saez F, Ballesteros M (2014) Study of process configuration and catalyst concentration in integrated alkaline extrusion of barley straw for bioethanol production. Fuel 134:448–454. https://doi.org/10.1016/j.fuel.2014.05.084
10. Duque A, Manzanares P, Ballesteros M (2017) Extrusion as a pretreatment for lignocellulosic biomass: fundamentals and applications. Renew Energy 114:1427–1441. https://doi.org/10.1016/j.renene.2017.06.050
11. Lamsal B, Yoo J, Brijwani K, Alavi S (2010) Extrusion as a thermo-mechanical pretreatment for lignocellulosic ethanol. Biomass Bioenerg 34:1703–1710. https://doi.org/10.1016/j.biombioe.2010.06.009
12. Karunanithy C, Muthukumarappan K (2013) Thermo-mechanical pretreatment of feedstocks. In: Green biomass pretreatment for biofuels production. In: Gu T (ed) Springer Netherlands, Dordrecht, pp 31–65
13. de Vrije T, de Haas GG, Tan GB, Keijsers ERP, Claassen PAM (2002) Pretreatment of Miscanthus for hydrogen production by *Thermotoga elfii*. Int J Hydrogen Energy 27:1381–1390. https://doi.org/10.1016/S0360-3199(02)00124-6

14. Pilli S, Bhunia P, Yan S, LeBlanc RJ, Tyagi RD, Surampalli RY (2011) Ultrasonic pretreatment of sludge: a review. Ultrason Sonochem 18:1–18. https://doi.org/10.1016/j.ultsonch.2010.02.014
15. Wen C, Zhang J, Zhang H, Dzah CS, Zandile M, Duan Y, Ma H, Luo X (2018) Advances in ultrasound assisted extraction of bioactive compounds from cash crops—a review. Ultrason Sonochem 48:538–549. https://doi.org/10.1016/j.ultsonch.2018.07.018
16. Tan SX, Lim S, Ong HC, Pang YL (2019) State of the art review on development of ultrasound-assisted catalytic transesterification process for biodiesel production. Fuel 235:886–907. https://doi.org/10.1016/j.fuel.2018.08.021
17. Soria AC, Villamiel M (2010) Effect of ultrasound on the technological properties and bioactivity of food: a review. Trends Food Sci Technol 21:323–331. https://doi.org/10.1016/j.tifs.2010.04.003
18. Bundhoo ZMA, Mohee R (2018) Ultrasound-assisted biological conversion of biomass and waste materials to biofuels: a review. Ultrason Sonochem 40:298–313. https://doi.org/10.1016/j.ultsonch.2017.07.025
19. Ohl C-D, Wolfrum B (2003) Detachment and sonoporation of adherent HeLa-cells by shock wave-induced cavitation. Biochim Biophys Acta Gen Subj 1624:131–138. https://doi.org/10.1016/j.bbagen.2003.10.005
20. Show K-Y, Mao T, Lee D-J (2007) Optimisation of sludge disruption by sonication. Water Res 41:4741–4747. https://doi.org/10.1016/j.watres.2007.07.017
21. Qiu W-Y, Cai W-D, Wang M, Yan J-K (2019) Effect of ultrasonic intensity on the conformational changes in citrus pectin under ultrasonic processing. Food Chem 297:125021. https://doi.org/10.1016/j.foodchem.2019.125021
22. Luo J, Fang Z, Smith RL (2014) Ultrasound-enhanced conversion of biomass to biofuels. Prog Energy Combust Sci 41:56–93. https://doi.org/10.1016/j.pecs.2013.11.001
23. Chemat F, Rombaut N, Sicaire A-G, Meullemiestre A, Fabiano-Tixier A-S, Abert-Vian M (2017) Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason Sonochem 34:540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035
24. Xu Y, Zhang L, Bailina Y, Ge Z, Ding T, Ye X, Liu D (2014) Effects of ultrasound and/or heating on the extraction of pectin from grapefruit peel. J Food Eng 126:72–81. https://doi.org/10.1016/j.jfoodeng.2013.11.004
25. Chen C, You L-J, Abbasi AM, Fu X, Liu RH (2015) Optimization for ultrasound extraction of polysaccharides from mulberry fruits with antioxidant and hyperglycemic activity in vitro. Carbohydr Polym 130:122–132. https://doi.org/10.1016/j.carbpol.2015.05.003
26. Zheng Z, Huang Q, Luo X, Xiao Y, Cai W, Ma H (2019) Effects and mechanisms of ultrasound- and alkali-assisted enzymolysis on production of water-soluble yeast β-glucan. Bioresour Technol 273:394–403. https://doi.org/10.1016/j.biortech.2018.11.035
27. Kappe CO, Pieber B, Dallinger D (2013) Microwave effects in organic synthesis: myth or reality? Angew Chemie Int Ed 52:1088–1094. https://doi.org/10.1002/anie.201204103
28. Asomaning J, Haupt S, Chae M, Bressler DC (2018) Recent developments in microwave-assisted thermal conversion of biomass for fuels and chemicals. Renew Sustain Energy Rev 92:642–657. https://doi.org/10.1016/j.rser.2018.04.084
29. Kappe CO (2004) Controlled microwave heating in modern organic synthesis. Angew Chemie Int Ed 43:6250–6284. https://doi.org/10.1002/anie.200400655
30. Sun J, Wang W, Yue Q (2016) Review on microwave-matter interaction fundamentals and efficient microwave-associated heating strategies. Materials 9
31. Li H, Qu Y, Yang Y, Chang S, Xu J (2016) Microwave irradiation—a green and efficient way to pretreat biomass. Bioresour Technol 199:34–41
32. Hassan SS, Williams GA, Jaiswal AK (2018) Emerging technologies for the pretreatment of lignocellulosic biomass. Bioresour Technol 262:310–318. https://doi.org/10.1016/j.biortech.2018.04.099
33. Wang K, Chen J, Sun S-N, Sun R-C (2015) Chapter 6: Steam explosion. In: Pandey A, Negi S, Binod P, Larroche C (eds) P of B. Elsevier, Amsterdam, pp 75–104

34. Silva AS da, Teixeira RSS, Moutta R de O, Ferreira-Leitão VS, Barros R da RO de B, Ferrara MA, Bon EP da S (2013) Sugarcane and woody biomass pretreatments for ethanol production. In: Chandel AK, Silva SS da (eds) Sustainable degradation of lignocellulosic biomass—techniques, applications and commercialization. IntechOpen, Rijeka, Ch. 3
35. Paudel SR, Banjara SP, Choi OK, Park KY, Kim YM, Lee JW (2017) Pretreatment of agricultural biomass for anaerobic digestion: current state and challenges. Bioresour Technol 245:1194–1205. https://doi.org/10.1016/j.biortech.2017.08.182
36. Wang K, Jiang J-X, Xu F, Sun R-C (2009) Influence of steaming explosion time on the physic-chemical properties of cellulose from Lespedeza stalks (*Lespedeza crytobotrya*). Bioresour Technol 100:5288–5294. https://doi.org/10.1016/j.biortech.2009.05.019
37. Yu Q, Liu R, Li K, Ma R (2019) A review of crop straw pretreatment methods for biogas production by anaerobic digestion in China. Renew Sustain Energy Rev 107:51–58. https://doi.org/10.1016/j.rser.2019.02.020
38. Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol 101:4851–4861. https://doi.org/10.1016/j.biortech.2009.11.093
39. Pérez JA, Ballesteros I, Ballesteros M, Sáez F, Negro MJ, Manzanares P (2008) Optimizing liquid hot water pretreatment conditions to enhance sugar recovery from wheat straw for fuel-ethanol production. Fuel 87:3640–3647. https://doi.org/10.1016/j.fuel.2008.06.009
40. Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–686. https://doi.org/10.1016/j.biortech.2004.06.025
41. Laser M, Schulman D, Allen SG, Lichwa J, Antal MJ, Lynd LR (2002) A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. Bioresour Technol 81:33–44. https://doi.org/10.1016/S0960-8524(01)00103-1
42. Bali G, Meng X, Deneff JI, Sun Q, Ragauskas AJ (2015) The effect of alkaline pretreatment methods on cellulose structure and accessibility. Chemsuschem 8:275–279. https://doi.org/10.1002/cssc.201402752
43. Rabemanolontsoa H, Saka S (2016) Various pretreatments of lignocellulosics. Bioresour Technol 199:83–91. https://doi.org/10.1016/j.biortech.2015.08.029
44. Kang X, Sun Y, Li L, Kong X, Yuan Z (2018) Improving methane production from anaerobic digestion of Pennisetum hybrid by alkaline pretreatment. Bioresour Technol 255:205–212. https://doi.org/10.1016/j.biortech.2017.12.001
45. Xu J, Cheng JJ, Sharma-Shivappa RR, Burns JC (2010) Lime pretreatment of switchgrass at mild temperatures for ethanol production. Bioresour Technol 101:2900–2903. https://doi.org/10.1016/j.biortech.2009.12.015
46. Liang Y, Zheng Z, Luo X, Si Y, Cao D, Nie E, Cheng B (2013) Lime pretreatment to improve methane production of smooth cordgrass (*Spartina alterniflora*). Chem Eng J 217:337–344. https://doi.org/10.1016/j.cej.2012.11.135
47. Li X, Kim TH (2011) Low-liquid pretreatment of corn stover with aqueous ammonia. Bioresour Technol 102:4779–4786. https://doi.org/10.1016/j.biortech.2011.01.008
48. Liu X, Zicari SM, Liu G, Li Y, Zhang R (2015) Pretreatment of wheat straw with potassium hydroxide for increasing enzymatic and microbial degradability. Bioresour Technol 185:150–157. https://doi.org/10.1016/j.biortech.2015.02.047
49. Li M, Eskridge K, Liu E, Wilkins M (2019) Enhancement of polyhydroxybutyrate (PHB) production by 10-fold from alkaline pretreatment liquor with an oxidative enzyme-mediator-surfactant system under Plackett-Burman and central composite designs. Bioresour Technol 281:99–106. https://doi.org/10.1016/j.biortech.2019.02.045
50. Hendriks ATWM, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100:10–18. https://doi.org/10.1016/j.biortech.2008.05.027
51. Sierra R, Holtzapple MT, Granda CB (2011) Long-term lime pretreatment of poplar wood. AIChE J 57:1320–1328. https://doi.org/10.1002/aic.12350
52. Soares Rodrigues CI, Jackson JJ, Montross MD (2016) A molar basis comparison of calcium hydroxide, sodium hydroxide, and potassium hydroxide on the pretreatment of switchgrass

and miscanthus under high solids conditions. Ind Crops Prod 92:165–173. https://doi.org/10.1016/j.indcrop.2016.08.010

53. Taherzadeh JM, Karimi K (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int J Mol, Sci, p 9
54. Singh J, Suhag M, Dhaka A (2015) Augmented digestion of lignocellulose by steam explosion, acid and alkaline pretreatment methods: a review. Carbohydr Polym 117:624–631. https://doi.org/10.1016/j.carbpol.2014.10.012
55. Kim TH, Gupta R, Lee YY (2009) Pretreatment of biomass by aqueous ammonia for bioethanol production. In: Mielenz JR (ed) Biofuels: methods and protocols. Humana Press, Totowa, NJ, pp 79–91
56. Kim JS, Lee YY, Kim TH (2016) A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresour Technol 199:42–48. https://doi.org/10.1016/j.biortech.2015.08.085
57. Hong E, Kim D, Kim J, Kim J, Yoon S, Rhie S, Ha S, Ryu Y (2015) Optimization of alkaline pretreatment on corn stover for enhanced production of 1.3-propanediol and 2,3-butanediol by *Klebsiella pneumoniae* AJ4. Biomass Bioenerg 77:177–185. https://doi.org/10.1016/j.biombioe.2015.03.016
58. Sajad Hashemi S, Karimi K, Majid Karimi A (2019) Ethanolic ammonia pretreatment for efficient biogas production from sugarcane bagasse. Fuel 248:196–204. https://doi.org/10.1016/j.fuel.2019.03.080
59. Kim TH, Nghiem NP, Hicks KB (2009) Pretreatment and fractionation of corn stover by soaking in ethanol and aqueous ammonia. Appl Biochem Biotechnol 153:171–179. https://doi.org/10.1007/s12010-009-8524-0
60. Mancini G, Papirio S, Lens PNL, Esposito G (2018) Increased biogas production from wheat straw by chemical pretreatments. Renew Energy 119:608–614. https://doi.org/10.1016/j.renene.2017.12.045
61. Mirahmadi K, Mohseni Kabir M, Jeihanipour A, Karimi K, Taherzadeh M (2010) Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production. Bioresources 5(2)
62. Zheng Y, Zhao J, Xu F, Li Y (2014) Pretreatment of lignocellulosic biomass for enhanced biogas production. Prog Energy Combust Sci 42:35–53. https://doi.org/10.1016/j.pecs.2014.01.001
63. Mathew AK, Parameshwaran B, Sukumaran RK, Pandey A (2016) An evaluation of dilute acid and ammonia fiber explosion pretreatment for cellulosic ethanol production. Bioresour Technol 199:13–20. https://doi.org/10.1016/j.biortech.2015.08.121
64. Yu H, Xiao W, Han L, Huang G (2019) Characterization of mechanical pulverization/phosphoric acid pretreatment of corn stover for enzymatic hydrolysis. Bioresour Technol 282:69–74. https://doi.org/10.1016/j.biortech.2019.02.104
65. Jönsson LJ, Martín C (2016) Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. Bioresour Technol 199:103–112. https://doi.org/10.1016/j.biortech.2015.10.009
66. Ravindran R, Jaiswal AK (2016) A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: challenges and opportunities. Bioresour Technol 199:92–102. https://doi.org/10.1016/j.biortech.2015.07.106
67. Sun S, Sun S, Cao X, Sun R (2016) The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. Bioresour Technol 199:49–58. https://doi.org/10.1016/j.biortech.2015.08.061
68. Amiri H, Karimi K (2018) Pretreatment and hydrolysis of lignocellulosic wastes for butanol production: challenges and perspectives. Bioresour Technol 270:702–721. https://doi.org/10.1016/j.biortech.2018.08.117
69. Lee J-W, Jeffries TW (2011) Efficiencies of acid catalysts in the hydrolysis of lignocellulosic biomass over a range of combined severity factors. Bioresour Technol 102:5884–5890. https://doi.org/10.1016/j.biortech.2011.02.048

70. Liu X, Wei W, Wu S (2019) Synergism of organic acid and deep eutectic solvents pretreatment for the co-production of oligosaccharides and enhancing enzymatic saccharification. Bioresour Technol 290:121775. https://doi.org/10.1016/j.biortech.2019.121775
71. Amnuaycheewa P, Hengaroonprasan R, Rattanaporn K, Kirdponpattara S, Cheenkachorn K, Sriariyanun M (2016) Enhancing enzymatic hydrolysis and biogas production from rice straw by pretreatment with organic acids. Ind Crops Prod 87:247–254. https://doi.org/10.1016/j.indcrop.2016.04.069
72. Rabemanolontsoa H, SAKA S (2012) Characterization of Lake Biwa macrophytes in their chemical composition. J Jpn Inst Energy 91:621–628. https://doi.org/10.3775/jie.91.621
73. Zhang Y, Wang B, Zhang W, Xu W, Hu Z (2019) Effects and mechanism of dilute acid soaking with ultrasound pretreatment on rice bran protein extraction. J Cereal Sci 87:318–324. https://doi.org/10.1016/j.jcs.2019.04.018
74. Fang H, Deng J, Zhang T (2011) Dilute acid pretreatment of black spruce using continuous steam explosion system. Appl Biochem Biotechnol 163:547–557. https://doi.org/10.1007/s12010-010-9061-6
75. Buranov AU, Mazza G (2010) Extraction and characterization of hemicelluloses from flax shives by different methods. Carbohydr Polym 79(1):17–25. https://doi.org/10.1016/j.carbpol.2009.06.014
76. Pan X, Gilkes N, Kadla J, Pye K, Saka S, Gregg D, Ehara K, Xie D, Lam D, Saddler J (2006) Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: optimization of process yields. Biotechnol Bioeng 94:851–861. https://doi.org/10.1002/bit.20905
77. Zhang K, Pei Z, Wang D (2016) Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: a review. Bioresour Technol 199:21–33. https://doi.org/10.1016/j.biortech.2015.08.102
78. Ouyang X, Zhu G, Huang X, Qiu X (2015) Microwave assisted liquefaction of wheat straw alkali lignin for the production of monophenolic compounds. J Energy Chem 24:72–76. https://doi.org/10.1016/S2095-4956(15)60286-8
79. Rabelo SC, Filho RM, Costa AC (2008) A comparison between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol production. Appl Biochem Biotechnol 148:45–58. https://doi.org/10.1007/s12010-008-8200-9
80. Rabelo SC, Carrere H, Maciel Filho R, Costa AC (2011) Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. Bioresour Technol 102:7887–7895. https://doi.org/10.1016/j.biortech.2011.05.081
81. Gould JM (1984) Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. Biotechnol Bioeng 26:46–52. https://doi.org/10.1002/bit.260260110
82. Schmidt AS, Thomsen AB (1998) Optimization of wet oxidation pretreatment of wheat straw. Bioresour Technol 64:139–151. https://doi.org/10.1016/S0960-8524(97)00164-8
83. Varga E, Klinke HB, Réczey K, Thomsen AB (2004) High solid simultaneous saccharification and fermentation of wet oxidized corn stover to ethanol. Biotechnol Bioeng 88:567–574. https://doi.org/10.1002/bit.20222
84. Fu D, Chen J, Liang X (2005) Wet air oxidation of nitrobenzene enhanced by phenol. Chemosphere 59:905–908. https://doi.org/10.1016/j.chemosphere.2004.11.004
85. Martín Medina CO, Marcet M, Thomsen AB (2008) Comparison of wet oxidation and steam explosion as pretreatment methods for bioethanol production from sugarcane bagasse. BioResources 3:670–683. https://doi.org/10.15376/BIORES.3.3.670-683
86. Kaparaju P, Felby C (2010) Characterization of lignin during oxidative and hydrothermal pre-treatment processes of wheat straw and corn stover. Bioresour Technol 101:3175–3181. https://doi.org/10.1016/j.biortech.2009.12.008
87. Jing G, Luan M, Chen T (2016) Progress of catalytic wet air oxidation technology. Arab J Chem 9:S1208–S1213. https://doi.org/10.1016/j.arabjc.2012.01.001
88. Delgado JJ, Pérez-Omil JA, Rodríguez-Izquierdo JM, Cauqui MA (2006) The role of the carbonaceous deposits in the Catalytic Wet Oxidation (CWO) of phenol. Catal Commun 7:639–643. https://doi.org/10.1016/j.catcom.2006.02.003

89. Travaini R, Barrado E, Bolado-Rodríguez S (2016) Effect of ozonolysis pretreatment parameters on the sugar release, ozone consumption and ethanol production from sugarcane bagasse. Bioresour Technol 214:150–158. https://doi.org/10.1016/j.biortech.2016.04.102
90. Wan Omar WNN, Amin NAS (2016) Multi response optimization of oil palm frond pretreatment by ozonolysis. Ind Crops Prod 85:389–402. https://doi.org/10.1016/j.indcrop.2016.01.027
91. Panneerselvam A, Sharma-Shivappa RR, Kolar P, Ranney T, Peretti S (2013) Potential of ozonolysis as a pretreatment for energy grasses. Bioresour Technol 148:242–248. https://doi.org/10.1016/j.biortech.2013.08.129
92. Bule MV, Gao AH, Hiscox B, Chen S (2013) Structural modification of lignin and characterization of pretreated wheat straw by ozonation. J Agric Food Chem 61:3916–3925. https://doi.org/10.1021/jf4001988
93. Perrone OM, Rossi JS, Moretti MM de S, Nunes C da CC, Bordignon SE, Gomes E, Da-Silva R, Boscolo M (2017) Influence of ozonolysis time during sugarcane pretreatment: effects on the fiber and enzymatic saccharification. Bioresour Technol 224:733–737. https://doi.org/10.1016/j.biortech.2016.11.043
94. Travaini R, Otero MDM, Coca M, Da-Silva R, Bolado S (2013) Sugarcane bagasse ozonolysis pretreatment: effect on enzymatic digestibility and inhibitory compound formation. Bioresour Technol 133:332–339. https://doi.org/10.1016/j.biortech.2013.01.133
95. Halder P, Kundu S, Patel S, Setiawan A, Atkin R, Parthasarthy R, Paz-Ferreiro J, Surapaneni A, Shah K (2019) Progress on the pre-treatment of lignocellulosic biomass employing ionic liquids. Renew Sustain Energy Rev 105:268–292. https://doi.org/10.1016/j.rser.2019.01.052
96. Asim AM, Uroos M, Naz S, Sultan M, Griffin G, Muhammad N, Khan AS (2019) Acidic ionic liquids: promising and cost-effective solvents for processing of lignocellulosic biomass. J Mol Liq 287:110943. https://doi.org/10.1016/j.molliq.2019.110943
97. Semerci I, Güler F (2018) Protic ionic liquids as effective agents for pretreatment of cotton stalks at high biomass loading. Ind Crops Prod 125:588–595. https://doi.org/10.1016/j.indcrop.2018.09.046
98. Li Y, Liu X, Zhang S, Yao Y, Yao X, Xu J, Lu X (2015) Dissolving process of a cellulose bunch in ionic liquids: a molecular dynamics study. Phys Chem Chem Phys 17:17894–17905. https://doi.org/10.1039/C5CP02009C
99. El Seoud OA, da Silva VC, Possidonio S, Casarano R, Arêas EPG, Gimenes P (2011) Microwave-assisted derivatization of cellulose, 2–The surprising effect of the structure of ionic liquids on the dissolution and acylation of the biopolymer. Macromol Chem Phys 212:2541–2550. https://doi.org/10.1002/macp.201100348
100. Bhutto AW, Qureshi K, Harijan K, Abro R, Abbas T, Aqeel Ahmed B, Sadia K, Yu G (2017) Insight into progress in pre-treatment of lignocellulosic biomass. Energy. https://doi.org/10.1016/j.energy.2017.01.005
101. da Costa Lopes AM, João KG, Rubik DF, Bogel-Łukasik E, Duarte LC, Andreaus J, Bogel-Łukasik R (2013) Pre-treatment of lignocellulosic biomass using ionic liquids: wheat straw fractionation. Bioresour Technol 142:198–208. https://doi.org/10.1016/j.biortech.2013.05.032
102. Sathitsuksanoh N, George A, Zhang Y-HP (2013) New lignocellulose pretreatments using cellulose solvents: a review. J Chem Technol Biotechnol 88:169–180. https://doi.org/10.1002/jctb.3959
103. Tian S-Q, Zhao R-Y, Chen Z-C (2018) Review of the pretreatment and bioconversion of lignocellulosic biomass from wheat straw materials. Renew Sustain Energy Rev 91:483–489. https://doi.org/10.1016/j.rser.2018.03.113
104. Zhao X, Cheng K, Liu D (2009) Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Appl Microbiol Biotechnol 82:815. https://doi.org/10.1007/s00253-009-1883-1
105. Koo B-W, Kim H-Y, Park N, Lee S-M, Yeo H, Choi I-G (2011) Organosolv pretreatment of *Liriodendron tulipifera* and simultaneous saccharification and fermentation for bioethanol production. Biomass Bioenerg 35:1833–1840. https://doi.org/10.1016/j.biombioe.2011.01.014

106. Smit A, Huijgen W (2017) Effective fractionation of lignocellulose in herbaceous biomass and hardwood using a mild acetone organosolv process. Green Chem 19:5505–5514. https://doi.org/10.1039/C7GC02379K
107. Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83:1–11. https://doi.org/10.1016/S0960-8524(01)00212-7
108. Yáñez-S M, Matsuhiro B, Nuñez C, Pan S, Hubbell CA, Sannigrahi P, Ragauskas AJ (2014) Physicochemical characterization of ethanol organosolv lignin (EOL) from *Eucalyptus globulus*: effect of extraction conditions on the molecular structure. Polym Degrad Stab 110:184–194. https://doi.org/10.1016/j.polymdegradstab.2014.08.026
109. Jang S-K, Kim H-Y, Jeong H-S, Kim J-Y, Yeo H, Choi I-G (2016) Effect of ethanol organosolv pretreatment factors on enzymatic digestibility and ethanol organosolv lignin structure from *Liriodendron tulipifera* in specific combined severity factors. Renew Energy 87:599–606. https://doi.org/10.1016/j.renene.2015.10.045
110. Tan X, Zhang Q, Wang W, Zhuang X, Deng Y, Yuan Z (2019) Comparison study of organosolv pretreatment on hybrid Pennisetum for enzymatic saccharification and lignin isolation. Fuel 249:334–340. https://doi.org/10.1016/j.fuel.2019.03.117
111. Guo Y, Zhou J, Wen J, Sun G, Sun Y (2015) Structural transformations of triploid of *Populus tomentosa* Carr. lignin during auto-catalyzed ethanol organosolv pretreatment. Ind Crops Prod 76:522–529. https://doi.org/10.1016/j.indcrop.2015.06.020
112. Brunner G (2005) Supercritical fluids: technology and application to food processing. J Food Eng 67:21–33. https://doi.org/10.1016/j.jfoodeng.2004.05.060
113. Narayanaswamy N, Faik A, Goetz DJ, Gu T (2011) Supercritical carbon dioxide pretreatment of corn stover and switchgrass for lignocellulosic ethanol production. Bioresour Technol 102:6995–7000. https://doi.org/10.1016/j.biortech.2011.04.052
114. Reverchon E, De Marco I (2006) Supercritical fluid extraction and fractionation of natural matter. J Supercrit Fluids 38:146–166. https://doi.org/10.1016/j.supflu.2006.03.020
115. Peter van Walsum G, Shi H (2004) Carbonic acid enhancement of hydrolysis in aqueous pretreatment of corn stover. Bioresour Technol 93:217–226. https://doi.org/10.1016/j.biortech.2003.11.009
116. Kim TH, Lee YY (2007) Pretreatment of corn stover by soaking in aqueous ammonia at moderate temperatures. In: Mielenz JR, Klasson KT, Adney WS, McMillan JD (eds) Applied biochemistry and biotechnology: the twenty-eighth symposium proceedings of the twenty-eight symposium on biotechnology for fuels and chemicals held Apr 30–May. Humana Press, Totowa, NJ, pp 81–92
117. Zhao M, Xu Q, Li G, Zhang Q, Zhou D, Yin J, Zhan H (2019) Pretreatment of agricultural residues by supercritical CO^2 at 50–80 °C to enhance enzymatic hydrolysis. J Energy Chem 31:39–45. https://doi.org/10.1016/j.jechem.2018.05.003
118. Balan V, Bals B, Chundawat SPS, Marshall D, Dale BE (2009) Lignocellulosic biomass pretreatment using AFEX. In: Mielenz JR (ed) Biofuels: methods and protocols. Humana Press, Totowa, NJ, pp 61–77
119. Weimer PJ, Chou YCT, Weston WM, Chase DB, Scott CD (eds) (1986) Effect of supercritical ammonia on the physical and chemical structure of ground wood. United States
120. Chundawat SPS, Vismeh R, Sharma LN, Humpula JF, da Costa Sousa L, Chambliss CK, Jones AD, Balan V, Dale BE (2010) Multifaceted characterization of cell wall decomposition products formed during ammonia fiber expansion (AFEX) and dilute acid based pretreatments. Bioresour Technol 101:8429–8438. https://doi.org/10.1016/j.biortech.2010.06.027
121. Lau MW, Dale BE (2009) Cellulosic ethanol production from AFEX-treated corn stover using *Saccharomyces cerevisiae* 424A(LNH-ST). Proc Natl Acad Sci 106:1368–1373. https://doi.org/10.1073/pnas.0812364106
122. Chen S, Zhang X, Singh D, Yu H, Yang X (2010) Biological pretreatment of lignocellulosics: potential, progress and challenges. Biofuels 1:177–199. https://doi.org/10.4155/bfs.09.13
123. Sindhu R, Binod P, Pandey A (2016) Biological pretreatment of lignocellulosic biomass—an overview. Bioresour Technol 199:76–82. https://doi.org/10.1016/j.biortech.2015.08.030

124. Wan C, Li Y (2012) Fungal pretreatment of lignocellulosic biomass. Biotechnol Adv 30:1447–1457. https://doi.org/10.1016/j.biotechadv.2012.03.003
125. Moreira MT, Feijoo G, Mester T, Mayorga P, Sierra-Alvarez R, Field JA (1998) Role of organic acids in the manganese-independent biobleaching system of Bjerkandera sp. Strain BOS55. Appl Environ Microbiol 64:2409–2417
126. Martínez AT (2002) Molecular biology and structure-function of lignin-degrading heme peroxidases. Enzyme Microb Technol 30:425–444. https://doi.org/10.1016/S0141-0229(01)00521-X
127. Calcaterra A, Galli C, Gentili P (2008) Phenolic compounds as likely natural mediators of laccase: a mechanistic assessment. J Mol Catal B Enzym 51:118–120. https://doi.org/10.1016/j.molcatb.2007.11.023
128. Evans CS, Dutton MV, Guillén F, Veness RG (1994) Enzymes and small molecular mass agents involved with lignocellulose degradation. FEMS Microbiol Rev 13:235–239. https://doi.org/10.1111/j.1574-6976.1994.tb00044.x
129. Harvey PJ, Palmer JM (1990) Oxidation of phenolic compounds by ligninase. J Biotechnol 13:169–179. https://doi.org/10.1016/0168-1656(90)90102-H
130. Chung N, Aust SD (1995) Veratryl alcohol-mediated indirect oxidation of phenol by lignin peroxidase. Arch Biochem Biophys 316:733–737. https://doi.org/10.1006/abbi.1995.1097
131. Binod P, Janu KU, Sindhu R, Pandey A (2011) Chapter 10: Hydrolysis of lignocellulosic biomass for bioethanol production. In: Pandey A, Larroche C, Ricke SC, Dussap C-G, Gnansounou E(eds) Biofuels. Academic Press, Amsterdam, pp 229–250
132. Kumar R, Wyman CE (2009) Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. Biotechnol Prog 25:302–314. https://doi.org/10.1002/btpr.102
133. Du W, Yu H, Song L, Zhang J, Weng C, Ma F, Zhang X (2011) The promoting effect of byproducts from *Irpex lacteus* on subsequent enzymatic hydrolysis of bio-pretreated cornstalks. Biotechnol Biofuels 4:37. https://doi.org/10.1186/1754-6834-4-37
134. Ramos J, Rojas T, Navarro F, Dávalos F, Sanjuán R, Rutiaga J, Young RA (2004) Enzymatic and fungal treatments on sugarcane bagasse for the production of mechanical pulps. J Agric Food Chem 52:5057–5062. https://doi.org/10.1021/jf030728+
135. Tabka MG, Herpoël-Gimbert I, Monod F, Asther M, Sigoillot JC (2006) Enzymatic saccharification of wheat straw for bioethanol production by a combined cellulase xylanase and feruloyl esterase treatment. Enzyme Microb Technol 39:897–902. https://doi.org/10.1016/j.enzmictec.2006.01.021
136. Rodrigues MAM, Pinto P, Bezerra RMF, Dias AA, Guedes CVM, Cardoso VMG, Cone JW, Ferreira LMM, Colaço J, Sequeira CA (2008) Effect of enzyme extracts isolated from white-rot fungi on chemical composition and in vitro digestibility of wheat straw. Anim Feed Sci Technol 141:326–338. https://doi.org/10.1016/j.anifeedsci.2007.06.015
137. Xu Q-Q, Zhao M-J, Yu Z-Z, Yin J-Z, Li G-M, Zhen M-Y, Zhang Q-Z (2017) Enhancing enzymatic hydrolysis of corn cob, corn stover and sorghum stalk by dilute aqueous ammonia combined with ultrasonic pretreatment. Ind Crops Prod 109:220–226. https://doi.org/10.1016/j.indcrop.2017.08.038
138. Cherpozat L, Loranger E, Daneault C (2019) Ultrasonic pretreatment of soft wood biomass prior to conventional pyrolysis: scale-up effects and limitations. Biomass Bioenerg 124:54–63. https://doi.org/10.1016/j.biombioe.2019.03.009
139. Wenjing L, Chao P, Lama A, Xindi F, Rong Y, Dhar BR (2019) Effect of pre-treatments on biological methane potential of dewatered sewage sludge under dry anaerobic digestion. Ultrason Sonochem 52:224–231. https://doi.org/10.1016/j.ultsonch.2018.11.022
140. Peng L, Appels L, Su H (2018) Combining microwave irradiation with sodium citrate addition improves the pre-treatment on anaerobic digestion of excess sewage sludge. J Environ Manage 213:271–278. https://doi.org/10.1016/j.jenvman.2018.02.053

141. Tian X, Ng WJ, Trzcinski AP (2018) Optimizing the synergistic effect of sodium hydroxide/ultrasound pre-treatment of sludge. Ultrason Sonochem 48:432–440. https://doi.org/10.1016/j.ultsonch.2018.07.005
142. Demichelis F, Fiore S, Onofrio M (2018) Pre-treatments aimed at increasing the biodegradability of cosmetic industrial waste. Process Saf Environ Prot 118:245–253. https://doi.org/10.1016/j.psep.2018.07.001

Chapter 4
Subproducts and Inhibitors

In order for the conversion of lignocellulosic biomass to biofuels to be efficient, the use of pretreatments of the raw materials is fundamental to transform stable lignocellulosic structures, overcoming the recalcitrance of lignocelluloses, chemical composition, and digestibility in soluble sugars, thereby improving the subsequent process of fermentation [1–3]. As seen in the previous sections, there are several types of pretreatments (biological, physical, chemical) as well as the possibility of combining these. Nevertheless, some of the approaches used may suffer from significant disadvantages in terms of economic production, industrial scales, and ecological impacts [4].

Lignocellulosic resources avoid competition with food agriculture and are very abundant, reducing the costs of raw materials, because they can be obtained from a wide range of agricultural, forestry, and energy crop residues [5]. The action of pretreatments in this type of biomass can trigger a series of undesirable products and by-products that need to be evaluated and monitored when the objective is the production of biofuels and enzymatic production (Fig. 4.1). Often, the choice of the appropriate type of pretreatment should consider not only the transformation efficiency of the complex structures, but also the generation of by-products and inhibitors that interfere with the metabolism of microorganisms in the stages of hydrolysis and fermentation by which the bioconversion happens.

Bellido et al. [6] found that the concentration and composition of inhibitors formed are influenced by the types of raw materials used and the intensity of the pretreatment employed. Jönsson and Martín [7] reported that, in addition to chemical differences in raw materials, inhibition problems are often more significant because by-products accumulate via water recirculation and the high solid loads that are used to obtain the concentrated sugar.

In this section, we will present the generation processes for inhibitors associated with pretreatments to be applied to the residual biomasses. We will also discuss the existing models for the removal of these undesirable compounds and the interest of their application for other purposes, allowing added value of the by-product. We will

H. Treichel et al., *Utilising Biomass in Biotechnology*,
Green Energy and Technology, https://doi.org/10.1007/978-3-030-22853-8_4

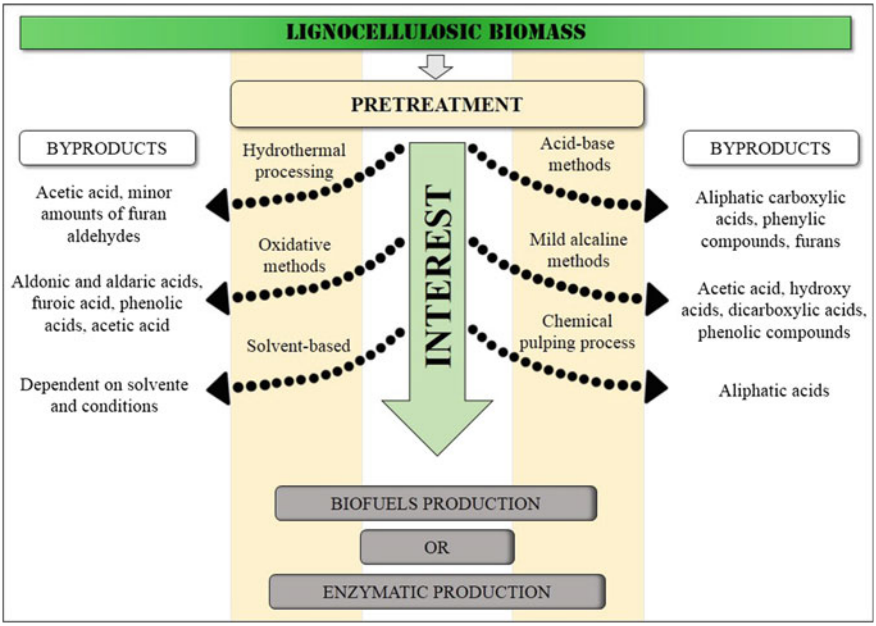

Fig. 4.1 Schematization of the main by-products and inhibitors formed from the use of pretreatments of residual lignocellulosic biomass. *Source* Authors

also discuss the major challenges and the major advances in this important stage that precede the hydrolytic and fermentative processes, as well as the enzymatic production.

4.1 By-products and Inhibitors from Physical and Chemical Pretreatments

Pretreatments such as acid hydrolysis, steam blasting, and ammonia expansion on lignocellulosic materials produce a number of inhibitors because of the severity with which they are applied to promote fiber rupture. Chandra et al. [8] analyzed the various characteristics of the lignocellulosic substrate at the level of fibers, fibrils, and microfibrils that were modified during pretreatment. They found that the initial characteristics of the biomass and the pretreatment applied played significant roles in the properties of the substrate that in turn governed the effectiveness of the enzymatic hydrolysis. The use of ammonia fiber expansion, for example, facilitated cellulose hydrolysis by cellulase, but generated amide inhibitors such as feruloyl amide derived by ferulic acid that attack biofuel models, while presenting new potential biological properties [9–11].

Most of the unwanted by-products present after pretreatment comprise three major groups: dehydrated sugar monomers (furans), degraded lignin polymers (phenols), and small organic acids [12].

4.1.1 Furans

By-products such as 5-(hydroxymethyl)-2-furaldehyde (5-HMF) and furan-2-carbaldehyde (furfural) are the major degradation compounds of xylose and glucose, respectively [13, 14]. In addition to these, various other products such as aldehydes and aliphatic and aromatic acids can also be formed by producing an inhibitory action on enzymes and yeasts (Fig. 4.1) [14].

Pretreatments with high temperatures and high acid concentrations are commonly used to promote the removal of lignin in the structure and convert the hemicellulose into simple sugars such as xylose, arabinose, lactose, and mannose [15]. These characteristics are the main factors related to the formation of undesirable compounds such as furans [16–20]. Together with the characteristics of this type of pretreatment, the amount of the carbon atoms in the monomeric sugar structures has influence on the type of compound formed, because 5-HMF may be the result of the dehydration of hexoses (six carbon molecules) and furfural, resulting from the dehydration of pentoses (five carbon molecules) [12]. At least, four routes for the formation of HMF from glucose and three routes for the formation of furfural from xylose are possible [14]. However, under neutral and basic conditions, the degradation of these sugars occurs through alternative routes [19].

The high concentration of furans mixed with other compounds in the same system as acetic acid, furfural, and lignin derivatives can be harmful to microbial growth and cause losses in the fermentation response [21]. It was found that the synergistic combination of acetic acid, furfural, and lignin derivatives resulted in greater decreases in ethanol yield and productivity than caused by the combined inhibition of single compounds [22]. In fermentations involving ethanol production, furfural may be more toxic than HMF, promoting the inhibition of enzymes acting on carbon catalysis, including acetaldehyde dehydrogenase, alcohol dehydrogenase, aldehyde dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase, and pyruvate dehydrogenase [23]. It was also found that 2.0 g L^{-1} furfural, 2.0 g L^{-1} HMF, and 3.0 g L^{-1} acetic acid formed after acid treatment were sufficient to compromise the efficiency of fermentation [13]. A study showed that the production of furfural was lower with the use of dilute organic acids such as fumaric acid and maleic acid than with sulfuric acid (most commonly used) [24].

The choice of alkaline pretreatment methods such as wet oxidation and AFEX that are carried out at high pH may result in the formation of relatively low furan compounds [12]. In general, AFEX degradation products increase metabolic yield by reducing formation of fermentation by-products [10].

Furans are probably the most undesirable group of inhibitors, because the fermentability of hydrolysates obtained by acidification is inversely related to the concentration of these compounds [25]. Most of the time, the concentrated acid must be recovered from the system in order to make the process economically viable. In some cases, enzymatic hydrolysis can be avoided because the acid itself hydrolyzes the biomass into fermentable sugars [26].

Technological innovations such as the use of molecular dynamics simulations and quantum dynamics modeling along with experimental evidence of various mechanisms of degradation of monosaccharides in furans suggest that the formation of products can be controlled by adjusting process parameters [14]. This idea generated strategies to form efficient industrial processes that are more attractive with minimization of inhibitory by-products such as furans.

The choice of hydrothermal and alkaline pretreatment methods such as wet oxidation and AFEX that are performed at high pH may result in the relatively low formation of furanic compounds (Fig. 4.1) [27, 28]. In general, AFEX degradation products increase metabolic yield by reducing formation of fermentation by-products [10].

4.1.2 Phenolic Compounds

Formation of phenolic compounds is associated with the molecular weight, polarity, and side-chain characteristics of the lignin structure as well as the influence of some types of pretreatments applied in the lignocellulosic biomass, among which are acidic, basic, mild alkaline, and oxidative methods (Fig. 4.1) [7, 21]. The great problem with the presence of these compounds after the pretreatment of the biomass is the precipitation and strong irreversible inhibition that they cause in the enzymes [29]. Studies have shown that high concentrations of phenolic compounds present after acid treatment cannot be exclusively attributed to lignin breakage [30]. Phenols produced under alkaline conditions exhibit very high reactivity due to the ionization of hydroxyl groups [31]. Michelin et al. [32] evaluated the effect of phenolic compounds of pretreated sugarcane bagasse in liquid hot water (180–200 °C) on cellulolytic and hemicellulolytic activities. The effect of this pretreatment technique solubilized hemicellulose, acetic acid, and sugar oligomers from lignocellulose, while simultaneously solubilizing the phenolic compounds. Phenolic compounds inhibit cellulolytic enzymes and increase the pretreatment severity with liquid hot water, causing the solubilization of phenolic compounds to increase [29, 32]. In addition to the degradation of lignin, these compounds may be derived from phenolic ester groups associated with hemicellulose [33]. In addition, phenolic compounds are capable of promoting loss of integrity in biological membranes, reducing cell growth, affecting the ability to serve as barriers and enzymatic matrices, decreasing the assimilation of sugars, and interfering with protein synthesis by breaking DNA and inhibiting RNA [34, 35]. DNA damage may be associated with the formation of reactive oxygen species because it has a high positive charge on one side of the aldehyde group, especially if this group is bound to the next carbon with a double bond [36].

Studies have shown that phenols produced during pretreatment of sugarcane bagasse through wet oxidation are intermediate compounds during the process, because they are reactive and can condense to a greater extent during pretreatment to form carboxylic acids [30, 37]. The concentration of total phenolic compounds was higher under acid pretreatment conditions than under alkaline conditions. The most abundant phenols are derived from G units (vanillin, vanillic acid, acetovanillone, vanillyl alcohol, guaiacol, and homovanillic acid), S units (syringaldehyde, syringic acid, acetosyringone, and syringol), and H units (p-hydroxybenzaldehyde, p-hydroxybenzoic acid, p-hydroxyacetophenone, and p-hydroxybenzyl alcohol) [30]. Some of these, including 4-hydroxybenzaldehyde, vanillin, syringaldehyde, acetosyringone, vanillic acid, syringic acid, and (4-hydroxy-3,5-dimethoxyacetophenone), were found in wheat straw pretreated with alkaline wet oxidation in the range 0.04–0.12 g 100 g_{straw}^{-1} [37].

In addition to phenolic compounds, some non-phenolic but aromatic compounds can be found in pretreated lignocellulosic hydrolysates [7]. Among the most frequent are benzoic acid [38, 39], para- and ortho-toluic acid [19], benzyl alcohol [39], cinnamic acid [40], cinnamaldehyde [41], and 3,4-dimethoxycinnamic acid [39]. The reason that some of the respective acids are clustered in the phenolic groups, and not in aliphatic carboxylic acid groups, is explained by the fact that they have phenylpropanoid structures, suggesting that they originate from lignin or from the hydrolysis of esterified phenols. They are generally found at low concentrations in the lignocellulosic hydrolysates, and the inhibitory effect becomes more potent than the aliphatic carboxylic acid species [7].

Rasmussen et al. [42] discovered and quantified 28 oligophenolic inhibitors of cellulase hydrothermal pretreatment of wheat straw biomass. The authors suggested that pentoses are directly involved in the formation of compounds and proposed that the hydrothermal pretreatment contributes to the formation via reactions with auto-condensation of pentoses involving aldol condensations, 1,4 additions to α, β unsaturated carbonyl compounds, decarboxylation, and oxidation of 3-ketoacid. Xylose is the precursor in the reaction pathways for the formation of these inhibitors, and many may still trigger the formation of pseudo-lignin. Therefore, protecting the reactive anomeric center of xylose could reduce the formation of inhibitors. The process of protection of the reactive anomeric center was carried out by the authors using ethylene glycol ether together with hydrothermal pretreatment, effectively reducing the level of oligo-phenols by 73%. That study provided a new way to develop reactions that hinder the formation of inhibitors of the lignocellulosic source [42]. Nevertheless, technological improvement is still required for recovery of xylose and separation of ethylene glycol monobutyl ether from the aqueous reaction mixture [42].

The occurrence of p-hydroxyphenyl derivatives after pretreatment techniques can be attributed to the lignin solubilization of the secondary wall of lignocellulosic biomasses that is rich in p-hydroxyphenyl units [30, 43]. The existence of p-coumaric acid in the cell wall as a non-constitutional part of the lignins may undergo oxidative pretreatment and may be one of the reasons for the formation of p-hydroxyphenyl derivative compounds [30].

Other types of pretreatments have been associated with the formation of these compounds. Steam blasting can trigger the generation of syringaldehyde, 4-hydroxybenzaldehyde, and vanillin [44]. Dry pretreatments (AFEX and pretreatment with ethylenediamine) may even reduce water consumption, while increasing the concentrations of soluble phenolics for enzymatic hydrolysis [45, 46]. Hydrothermal processes based on hot water pretreatment release phenolic compounds that affect β-glycosidase or β-xylosidase components of the enzyme cellulase and hemicellulose [32].

4.1.3 Organic Acids

Acetic acid is a by-product of the cleavage of acetylated groups present in hemicellulose during thermal and chemical pretreatments [19], including acid pretreatment [13]. For example, acetyl and uronic acid groups may be present at the 2′ and 3′ position of sugar in wheat straw residues [47]. In other cases, with the use of hydrothermal pretreatment of the biomass, the formation of acetic acid can occur by means of acetylated galactoglucomannans that result from hydrolytic cleavage of the acetyl substitutions during pretreatment of acetylic bonds in xylan [14]. Acetic acid, although considered a small organic acid, is the most abundant in most cases. In addition to acetic acid, uronic acids, formic acid, and levulinic acid can also be formed from the degradation of sugars such as xylose and glucose [7, 13] and degradation of HMF and furfural [48]. In wet oxidation, monomeric sugar molecules can be oxidized to formic acid and acetic acid [37]. Pretreatments with organic acids can fractionate the lignocellulosic biomass in cellulosic solid (greater than 85% cellulose and lignin contents below 10%), hemicellulosic syrup, and high-purity lignin. However, the delignification process triggered an acylated (acetylated or formylated) cellulose. The presence of the acetyl group and its degree of acylation may increase the cellulose chain diameter, limit the enzymatic hydrolysis of cellulose, and also impair the recognition of the cellulase enzyme for this substrate [49–51].

Levulinic acid was produced under strongly acid pretreatment conditions, while negligible concentrations were produced at higher pH. This trend was similar to the previously reported 5-HMF and furfural productions. The most likely explanation for this phenomenon is that levulinic acid is formed after further degradation of 5-HMF under strongly acidic conditions [19, 48, 52]. Formic, acetic, and levulinic acids were also detected after pretreatment by steam explosion on cellulosic material; it was observed that, after the rinsing of the treated raw material, formic acid and levulinic acid affected the activity of cellulase enzymes [44]. Other studies also reported that severe pretreatment conditions with steam explosion generate inhibitors such as aromatic compounds and dehydration by-products such as weak acids and their weak derivatives that influence subsequent hydrolysis processes [53–56]. Recent studies that verified the potential of corn straw in methane production suggested that the inhibition caused by the rigorous pretreatment conditions of steam explosion may be due to the formation of pseudo-lignin; high lignin contents that are present in the pretreated material affect methane yield [57]. One of the reasons found for the formation of pseudo-lignin with severe explosion conditions may involve condensation and re-polymerization reactions that lead to increased acid-insoluble lignin fractions

[57, 58]. One of the ways to minimize the generation of inhibitory compounds and improve the efficiency of vapor explosion pretreatment is the addition of catalysts such as H_2SO_4, H_3PO_4, SO_2, or CO_2 [59–62]. However, this effect combined with the high severity also affects the formation of inhibitors such as carboxylic acids (acetic acid, formic, among others), as well as aromatic aldehydes (5-HMF, furfural, phenols) (Fig. 4.1) [62].

In ethanol fermentations, the use of combined severity (CS), consisting of a single parameter involving residence time, temperature, and sulfuric acid concentration as pretreatment conditions, showed that the additional increase of CS and formation of formic and levulinic acid simultaneously increased along with increased yields of mannose and glucose under these conditions [63].

As seen so far, acid pretreatment of biomasses is thought to be one of the most critical in the formation of inhibitory by-products. In addition to furan aldehydes and phenyl aldehydes, it is likely that small aliphatic aldehydes are also ubiquitous in biomass after this type of pretreatment [64]. Even though they are considered volatile compounds and are easy to vaporize, more research should be needed to understand the meaning of the aliphatic aldehydes present in this system [7].

A recent study applied data modeling to systematically evaluate the prediction of the inhibitors present in the pretreated lignocellulosic biomass in the fermentation of bioethanol using the yeast *Saccharomyces cerevisiae*. With the objective of reducing experimental costs, the authors used mathematical models of nineteen representative inhibitors in the fermentation of bioethanol individually and in combination. According to the authors, this was an innovative methodology to evaluate inhibition and to eliminate the stronger inhibitors that exist in pretreated lignocellulosic hydrolysates [3].

4.2 Oxidative Pretreatments: From Challenges to Trends

Oxidative pretreatments have been shown to be effective in making the solid cellulose fraction more accessible for enzymatic hydrolysis and fermentation. These methods include wet oxidation and oxidative lime with liquid hot water [19, 65, 66]. The combination of wet oxidation with alkaline compounds minimizes the formation of furanic and phenolic aldehydes [7].

Among the alternatives to existing pretreatments, ozonolysis is one of the most promising methods of oxidative pretreatments of lignocellulosic biomass for the degradation of lignin, generating reduced effects on hemicellulose and cellulose. Its mode of action is preferentially on the structure of lignin because the deficiency of electrons in the terminal oxygen causes its action to be in an electron-rich substrate such as lignin that presents many more electrons than do other carbohydrates [67]. For applications that require the use of delignified biomass such as biofuels (biogas, bioethanol, butanol, biohydrogen) and even the production of enzymes, the use of this method is compelling [68].

The challenges that are encountered in prior processes such as high generation of inhibitors during pretreatment processes are minimized with this technology. Furfural and HMF, commonly found as sugar-degrading compounds, are not detected. However, ozonolysis during the degradation of sugar generates mainly oxalic acid, formic acid, acetic acid, and levulinic acid [67, 69]. For all processes of treatment of lignocellulosic biomass using O_3, acetic acid is a by-product. This does not have to be considered a serious problem, because acetic acid can be converted to biogas that may be used for the production of heat and electricity [70].

Ozonolysis for the pretreatment of sugarcane bagasse generated acetic acid, formic acid, lactic acid, and xylitol, however, at lower concentrations than other published pretreatments of sugarcane [68].

The inhibitory effect of carboxylic acids is directly related to pH. For these compounds to penetrate the cell membrane of microorganisms, they must be present in a no dissociable form. Because intracellular pH is commonly higher, there is dissociation of carboxylic acids, leading to decreased internal pH, consequently affecting cellular functions. For subsequent hydrolysis and fermentation processes not to be affected, it is proposed that this form of pretreatment could be improved with increasing pH [67].

One of the great challenges that this method must overcome is scaling-up. Nevertheless, technological advances are reducing costs for ozone production by increasing prospects for application in the near future [70].

4.3 From Biological Methods

Phenolic compounds, furan derivatives, and weak acids are toxic inhibitors formed after the application of chemical, physical, and physicochemical pretreatments that must be removed in an additional stage of detoxification [71]. The formation of these inhibitors, in addition to causing disadvantages to hydrolysis, contributes to the corrosion of equipment that results in a compromise of the process. The use of biological pretreatments is an alternative because it reduces the additional steps of residual flows for detoxification and is considered an environmentally more promising model. Nevertheless, some disadvantages have been reported, including the need for longer durations of lignin degradation; furthermore, some of the cellulose and hemicellulose can be degraded [72, 73].

According to Carrillo-Nieves et al. [73], the goals of consolidated bioprocesses are as follows: to find a microorganism that has the capacity to promote rupture by means of enzymatic forms of lignin, avoiding the use of aggressive chemicals that subsequently need treatment; to tolerate the high inhibitory concentrations produced during the process; and to produce enzymes necessary to transform cellulose and hemicellulose into sugars.

Wang et al. [74] investigated a biological pretreatment of corn straw using ligninolytic enzymes and reported that the formation of microbial inhibitors was reduced with this technique.

4.4 Methods for Detoxification

Several methods of detoxification are used to remove toxic compounds from plant biomass hydrolysates, including enzymatic biological treatments (laccase), physical treatments, evaporation and extraction, chemical treatments with alkaline agents (NaOH, CaO, $Ca(OH)_2$), and physical–chemical treatments involving adsorption with activated carbon and ion-exchange resins [25].

According to Travaini et al. [67], in several publications, the use of washing water resulted in an effective phase of removal of inhibitory compounds, increasing glucose release after enzymatic hydrolysis when pretreatment with ozonolysis was used. In ozonated wheat straw washing water, Schultz-Jensen et al. [75] found various lignin degradation products, including phenolic carboxylic acids, phenolic, vanillic, acetic, citric, and other products.

Several methods to detoxify the culture medium can be used, including physical (detoxification-mediated), chemical (addition of $Ca(OH)_2$), adsorption (using activated carbon ion-exchange resins), and biological (microbial and enzymatic) to combat the inhibitors of xylitol production [76].

Cetoxification may also reduce the amount of sugars and consequently decrease the efficiency of fermentation [77]. Piotrowski et al. [11] showed that the search for new strains that decrease the inhibitory effects of degraded lignocellulosic biomass is an economically more favorable strategy than applying expensive detoxification methods that reduce the sugar content of biomass.

4.5 By-products and Inhibitors: A Form of Valorization

Given the great impasse that the by-products and inhibitors of the degradation of lignocellulosic materials cause in enzymatic production processes and biofuels, there is a need to search for solutions to add value to the by-product. Often, the purpose of a pretreatment is not to form monomeric sugars and raw materials for biofuel synthesis or enzymatic production, but rather to pay attention to the valorization advantages of other chemical compounds formed during pretreatment.

Furfural is a chemical solvent that has been used to separate saturated and unsaturated compounds in oil, gas, oil and diesel refining industries [78]. The aldehyde group present in the structure and the unsaturated bonds make this material a highly versatile compound for the production of a wide range of industrial chemicals, including plastics, pharmaceuticals, and agrochemicals [79]. The production of this compound involves the acid-catalyzed hydrolysis of the hemicellulosic biomass fractions of pentose (such as cornstalks and corncobs, oat and peanut husks, as well as other agricultural surpluses) and consecutive cyclodehydration of pentose monomers (xylose being the most predominant pentose in most feedstocks) [80].

HMF can be transformed into a number of compounds, including 5-ethoxymethyl furfural [81], 5-arylaminomethyl-2-furanmethanol, 5-hydroxymethylfuroic acid, furfuryl alcohol, levulinic acid, and levulinate esters [82]. 5-HMF and furfural derivatives also have the potential to replace petroleum-based chemicals and building blocks, offering more sustainable routes for the production of polymers, fuels, and fine chemicals [83].

According to Luo et al. [84], furfural production with high selectivity and productivity is a challenging but crucial process to alleviate dependence on petroleum-derived chemicals. The production of furfural with high selectivity and yield requires catalysts; organic solvents are the most common methods for the depolymerization of dissolved hemicellulose. In particular, the adjustment of biphasic systems of organic solvents and NaCl is a compelling choice to produce furfural because it avoids secondary reactions and the formation of undesirable compounds, directing the balance of the system exclusively toward the formation of furfural [84].

There are no synthetic routes available for the production of furfuraldehyde; it is exclusively produced from renewable biomass resources through acid-catalyzed dehydration of pentoses [85].

Value-added products can also be obtained from the conversion of lignin with the probability of offsetting the additional costs caused by pretreatment processes. The technologies of pretreatment provide refining of biomass, and further facilitating recovery of lignin, together with processes in engineering, will allow the use of biopolymers such as low-cost carbon fibers, plastics and thermoplastic elastomers, polymer foams, and other fungible fuels [86].

Lignin pretreatment processes that involve oxidative methods can trigger the formation of aromatic compounds and aldehydes that do not have much market value, because they contain aromatic rings. However, oxidation also results in open-ring organic acid productions that are more highly valued. Natural oxidative pretreatments, based on the depolymerization of lignin via oxidative enzymatic process with the use of laccases and peroxidases secreted by white-rot fungi, have been a new strategy for obtaining aromatic compounds of low molecular weight [86–89].

Carbon and energy storage compounds such as hydroxyalkanoate polyester, in this case polyhydroxybutyrate (PHB), present in alkaline pretreatment liquors, can be synthesized through aromatic rings by means of bacteria and may be comparable with the mechanical properties of petroleum-based plastics or in the pharmaceutical industry because of their biocompatibility and degree of polymerization. Studies in this area reinforce the notion that sustainable models in the context of biorefineries with enzyme-mediated supplementation strategies in synthesis processes can provide a more attractive economic outlook.

For some types of waste pretreatment methods, adjustments are made to allow a more affordable fraction of products with high added value. Phenolic compounds have wide use in food additives, the pharmaceutical and cosmetics industries, energies, diverse chemicals, and phenolic resins [90]. The residue of grape marc is a raw material with great potential that is a rich source of phenolic acids, anthocyanins, colored flavonoids, and tannins. The pretreatments of this material are aimed at extraction of phenolic compounds, lactic acid, xylitol, bioadsorbents, and oligosaccharides [91]. The extraction of phenolic compounds from this type of residue is a focus of study because of its compelling activities including antioxidants [91, 92].

To date, more than 90% of phenol production is derived from the synthesis of benzene, obtained from fossil fuels and triggering concern for the limitation of this resource and the aggravating factors of environmental pollution [93]. By contrast, production of phenol via the degradation of lignin is a method of manufacturing phenol from renewable raw material [94].

Studies of the role of phenolic compounds in the secondary metabolism of plants, including several biological functions, have associated the antioxidant properties of these substances with existing benefits such as healthy diets and prevention of chronic diseases through the consumption of fruits and vegetables rich in phenolic compounds [95, 96].

Phenolic compounds can also positively or negatively affect the sensory characteristics of foods, including color, taste, and astringency. This impact needs to be evaluated and becomes important so that health-promoting products are also palatable and are widely consumed [95]. In addition to antioxidant functions, phenolic compounds prevent diseases through other mechanisms, including cell signaling, gene expression, and modulation of enzymatic activity [97].

Although phenolic compounds can be transformed into high-value food additives, antioxidant recovery has not yet been achieved on an industrial scale [98]. One of the most valuable phenolic compounds resulting from lignin is vanillin, presenting good prospects in the polymer industries [99].

Levulinic acid, another compound that has received substantial attention in the preparation of pharmaceuticals, dyes, plastics, pesticides, additives, resins, and lubricating additives, is considered by the US Department of Energy to be among the 12 most valuable platform compounds [100]. Among other possible applications, upgrades of liquid fuels such as levulinic acid and methanol can produce methyl and ethyl esters under the action of acid catalysts [101]. In food, it can serve as a raw material for jasmine flavors. In addition, when mixed with 2-methyltetrahydrofuran, these compounds can also be added to gasoline (up to 70%) without the need for engine modification [101, 102].

Other modes of levulinic acid valorization, described by Li et al. [101], include the synthesis of pyridinyl levulinate, 2-methyl-3-indole acetic acid, pesticidal intermediates, 2-mercapto-4-methyl-5-thiazole acetic acid, and indomethacin pharmaceutical intermediates. In addition, calcium decanoate obtained by levulinic acid and calcium carbonate can be useful in the medical field for intravenous injections to maintain the excitability of muscles and nerves and to assist in bone formation. They can also be used in skin care products and cosmetics for the treatment of acne, sebum, and even as anti-inflammatory and antibacterial treatments [101].

The recovery of the liquid fraction after pretreatment by steam explosion and enzymatic hydrolysis of the lignocellulosic biomass was shown to be beneficial for biogas production, because the energy recovery can be associated with the digestibility of lignin inhibitors and polymers in the transformation of methane [5].

References

1. Demain AL, Báez-Vásquez MA (2013) Chapter 14: Biofuels of the present and the future. In: Suib SLBT-N and FD in C. Elsevier, Amsterdam, pp 325–370
2. Sambusiti C, Monlau F, Antoniou N, Zabaniotou A, Barakat A (2016) Simultaneous detoxification and bioethanol fermentation of furans-rich synthetic hydrolysate by digestate-based pyrochar. J Environ Manage 183:1026–1031. https://doi.org/10.1016/j.jenvman.2016.09.062
3. Hou J, Tang J, Chen J, Deng J, Wang J, Zhang Q (2019) Evaluation of inhibition of lignocellulose-derived by-products on bioethanol production by using the QSAR method and mechanism study. Biochem Eng J 147:153–162. https://doi.org/10.1016/j.bej.2019.04.013
4. Hassan SS, Williams GA, Jaiswal AK (2018) Emerging technologies for the pretreatment of lignocellulosic biomass. Bioresour Technol 262:310–318. https://doi.org/10.1016/j.biortech.2018.04.099
5. Cotana F, Cavalaglio G, Petrozzi A, Coccia V (2015) Lignocellulosic biomass feeding in biogas pathway: state of the art and plant layouts. Energy Procedia 81:1231–1237. https://doi.org/10.1016/j.egypro.2015.12.334
6. Bellido C, Bolado S, Coca M, Lucas S, González-Benito G, García-Cubero MT (2011) Effect of inhibitors formed during wheat straw pretreatment on ethanol fermentation by *Pichia stipitis*. Bioresour Technol 102:10868–10874. https://doi.org/10.1016/j.biortech.2011.08.128
7. Jönsson LJ, Martín C (2016) Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. Bioresour Technol 199:103–112. https://doi.org/10.1016/j.biortech.2015.10.009
8. Chandra RP, Bura R, Mabee WE, Berlin A, Pan X, Saddler JN (2007) Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics? In: Olsson L (ed) Biofuels. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 67–93
9. Chundawat SPS, Vismeh R, Sharma LN, Humpula JF, da Costa Sousa L, Chambliss CK, Jones AD, Balan V, Dale BE (2010) Multifaceted characterization of cell wall decomposition products formed during ammonia fiber expansion (AFEX) and dilute acid based pretreatments. Bioresour Technol 101:8429–8438. https://doi.org/10.1016/j.biortech.2010.06.027
10. Lau MW, Dale BE (2009) Cellulosic ethanol production from AFEX-treated corn stover using *Saccharomyces cerevisiae* 424A(LNH-ST). Proc Natl Acad Sci 106:1368–1373. https://doi.org/10.1073/pnas.0812364106
11. Piotrowski JS, Zhang Y, Bates DM, Keating DH, Sato TK, Ong IM, Landick R (2014) Death by a thousand cuts: the challenges and diverse landscape of lignocellulosic hydrolysate inhibitors. Front Microbiol 5:90. https://doi.org/10.3389/fmicb.2014.00090
12. van der Pol EC, Bakker RR, Baets P, Eggink G (2014) By-products resulting from lignocellulose pretreatment and their inhibitory effect on fermentations for (bio)chemicals and fuels. Appl Microbiol Biotechnol 98:9579–9593. https://doi.org/10.1007/s00253-014-6158-9
13. Damião Xavier F, Santos Bezerra G, Florentino Melo Santos S, Sousa Conrado Oliveira L, Luiz Honorato Silva F, Joice Oliveira Silva A, Maria Conceição M (2018) Evaluation of the simultaneous production of xylitol and ethanol from sisal fiber. Biomolecules 8
14. Rasmussen H, Sørensen HR, Meyer AS (2014) Formation of degradation compounds from lignocellulosic biomass in the biorefinery: sugar reaction mechanisms. Carbohydr Res 385:45–57. https://doi.org/10.1016/j.carres.2013.08.029
15. Avci A, Saha BC, Kennedy GJ, Cotta MA (2013) High temperature dilute phosphoric acid pretreatment of corn stover for furfural and ethanol production. Ind Crops Prod 50:478–484. https://doi.org/10.1016/j.indcrop.2013.07.055
16. Venkateswar Rao L, Goli JK, Gentela J, Koti S (2016) Bioconversion of lignocellulosic biomass to xylitol: an overview. Bioresour Technol 213:299–310. https://doi.org/10.1016/j.biortech.2016.04.092
17. Lacerda TM, Zambon MD, Frollini E (2013) Effect of acid concentration and pulp properties on hydrolysis reactions of mercerized sisal. Carbohydr Polym 93:347–356. https://doi.org/10.1016/j.carbpol.2012.10.039

18. Haghighi Mood S, Hossein Golfeshan A, Tabatabaei M, Salehi Jouzani G, Najafi GH, Gholami M, Ardjmand M (2013) Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renew Sustain Energy Rev 27:77–93. https://doi.org/10.1016/j.rser.2013.06.033
19. Du B, Sharma LN, Becker C, Chen S-F, Mowery RA, van Walsum GP, Chambliss CK (2010) Effect of varying feedstock–pretreatment chemistry combinations on the formation and accumulation of potentially inhibitory degradation products in biomass hydrolysates. Biotechnol Bioeng 107:430–440. https://doi.org/10.1002/bit.22829
20. Kabel MA, Bos G, Zeevalking J, Voragen AGJ, Schols HA (2007) Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw. Bioresour Technol 98:2034–2042. https://doi.org/10.1016/j.biortech.2006.08.006
21. Kim D (2018) Physico-chemical conversion of lignocellulose: inhibitor effects and detoxification strategies: a mini review. Molecules 23
22. Nigam JN (2001) Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. J Biotechnol 87:17–27. https://doi.org/10.1016/S0168-1656(00)00385-0
23. Guo G-L, Chen W-H, Chen W-H, Men L-C, Hwang W-S (2008) Characterization of dilute acid pretreatment of silvergrass for ethanol production. Bioresour Technol 99:6046–6053. https://doi.org/10.1016/j.biortech.2007.12.047
24. Kootstra AMJ, Beeftink HH, Scott EL, Sanders JPM (2009) Comparison of dilute mineral and organic acid pretreatment for enzymatic hydrolysis of wheat straw. Biochem Eng J 46:126–131. https://doi.org/10.1016/j.bej.2009.04.020
25. de Albuquerque TL, da Silva IJ, de Macedo GR, Rocha MVP (2014) Biotechnological production of xylitol from lignocellulosic wastes: a review. Process Biochem 49:1779–1789. https://doi.org/10.1016/j.procbio.2014.07.010
26. Kumar AK, Sharma S (2017) Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresour Bioprocess 4:7. https://doi.org/10.1186/s40643-017-0137-9
27. Karimi K, Taherzadeh MJ (2016) A critical review of analytical methods in pretreatment of lignocelluloses: composition, imaging, and crystallinity. Bioresour Technol 200:1008–1018. https://doi.org/10.1016/j.biortech.2015.11.022
28. Donohoe BS, Selig MJ, Viamajala S, Vinzant TB, Adney WS, Himmel ME (2009) Detecting cellulase penetration into corn stover cell walls by immuno-electron microscopy. Biotechnol Bioeng 103:480–489. https://doi.org/10.1002/bit.22281
29. Ximenes E, Kim Y, Mosier N, Dien B, Ladisch M (2011) Deactivation of cellulases by phenols. Enzyme Microb Technol 48:54–60. https://doi.org/10.1016/j.enzmictec.2010.09.006
30. Martín C, B KH, Marcelo M, Luis G, Ena H, Belinda TA (2007) Study of the phenolic compounds formed during pretreatment of sugarcane bagasse by wet oxidation and steam explosion. Holzforschung 61:483. https://doi.org/10.1515/hf.2007.106
31. Packer J, Vaughan JA (1958) A modern approach to organic chemistry. Clarendon Press, Oxford
32. Michelin M, Ximenes E, de Lourdes Teixeira de Moraes Polizeli M, Ladisch MR (2016) Effect of phenolic compounds from pretreated sugarcane bagasse on cellulolytic and hemicellulolytic activities. Bioresour Technol 199:275–278. https://doi.org/10.1016/j.biortech.2015.08.120
33. Chen S-F, Mowery RA, Scarlata CJ, Chambliss CK (2007) Compositional analysis of water-soluble materials in corn stover. J Agric Food Chem 55:5912–5918. https://doi.org/10.1021/jf0700327
34. Klinke HB, Thomsen AB, Ahring BK (2004) Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. Appl Microbiol Biotechnol 66:10–26. https://doi.org/10.1007/s00253-004-1642-2
35. Kang L, Lee YY, Yoon SH, Smith AJ, Krishnagopalan GA (2012) Ethanol production from the mixture of hemicellulose prehydrolysate and paper sludge. BioResources 7:3607–3626
36. Feron VJ, Til HP, de Vrijer F, Woutersen RA, Cassee FR, van Bladeren PJ (1991) Aldehydes: occurrence, carcinogenic potential, mechanism of action and risk assessment. Mutat Res Toxicol 259:363–385. https://doi.org/10.1016/0165-1218(91)90128-9

37. Klinke HB, Ahring BK, Schmidt AS, Thomsen AB (2002) Characterization of degradation products from alkaline wet oxidation of wheat straw. Bioresour Technol 82:15–26. https://doi.org/10.1016/S0960-8524(01)00152-3
38. Martín C, Galbe M, Nilvebrant N-O, Jönsson LJ (2002) Comparison of the fermentability of enzymatic hydrolyzates of sugarcane bagasse pretreated by steam explosion using different impregnating agents. In: Finkelstein M, McMillan JD, Davison BH (eds) Biotechnology for fuels and chemicals: the twenty-third symposium. Humana Press, Totowa, NJ, pp 699–716
39. Mitchell VD, Taylor CM, Bauer S (2014) Comprehensive analysis of monomeric phenolics in dilute acid plant hydrolysates. BioEnergy Res 7:654–669. https://doi.org/10.1007/s12155-013-9392-6
40. Kim Y, Kreke T, Hendrickson R, Parenti J, Ladisch MR (2013) Fractionation of cellulase and fermentation inhibitors from steam pretreated mixed hardwood. Bioresour Technol 135:30–38. https://doi.org/10.1016/j.biortech.2012.10.130
41. Persson P, Andersson J, Gorton L, Larsson S, Nilvebrant N-O, Jönsson LJ (2002) Effect of different forms of alkali treatment on specific fermentation inhibitors and on the fermentability of lignocellulose hydrolysates for production of fuel ethanol. J Agric Food Chem 50:5318–5325. https://doi.org/10.1021/jf025565o
42. Rasmussen H, Tanner D, Sørensen HR, Meyer AS (2017) New degradation compounds from lignocellulosic biomass pretreatment: routes for formation of potent oligophenolic enzyme inhibitors. Green Chem 19:464–473. https://doi.org/10.1039/C6GC01809B
43. Sun J-X, Sun X-F, Sun R-C, Fowler P, Baird MS (2003) Inhomogeneities in the chemical structure of sugarcane bagasse lignin. J Agric Food Chem 51:6719–6725. https://doi.org/10.1021/jf034633j
44. Cantarella M, Cantarella L, Gallifuoco A, Spera A, Alfani F (2004) Effect of inhibitors released during steam-explosion treatment of poplar wood on subsequent enzymatic hydrolysis and SSF. Biotechnol Prog 20:200–206. https://doi.org/10.1021/bp0257978
45. Qin L, Li W-C, Zhu J-Q, Liang J-N, Li B-Z, Yuan Y-J (2015) Ethylenediamine pretreatment changes cellulose allomorph and lignin structure of lignocellulose at ambient pressure. Biotechnol Biofuels 8:174. https://doi.org/10.1186/s13068-015-0359-z
46. Li B-Z, Balan V, Yuan Y-J, Dale BE (2010) Process optimization to convert forage and sweet sorghum bagasse to ethanol based on ammonia fiber expansion (AFEX) pretreatment. Bioresour Technol 101:1285–1292. https://doi.org/10.1016/j.biortech.2009.09.044
47. Sun JX, Sun XF, Sun RC, Su YQ (2004) Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses. Carbohydr Polym 56:195–204. https://doi.org/10.1016/j.carbpol.2004.02.002
48. Ulbricht RJ, Northup SJ, Thomas JA (1984) A review of 5-hydroxymethylfurfural (HMF) in parenteral solutions. Fundam Appl Toxicol 4:843–853. https://doi.org/10.1016/0272-0590(84)90106-4
49. Zhao X, Li S, Wu R, Liu D (2017) Organosolv fractionating pre-treatment of lignocellulosic biomass for efficient enzymatic saccharification: chemistry, kinetics, and substrate structures. Biofuels, Bioprod Biorefining 11:567–590. https://doi.org/10.1002/bbb.1768
50. Zhao X, Zhang L, Liu D (2012) Biomass recalcitrance. Part I: The chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. Biofuels Bioprod Biorefining 6:465–482. https://doi.org/10.1002/bbb.1331
51. Zhao X, Liu D (2013) Fractionating pretreatment of lignocellulosic biomass
52. Palmqvist E, Hahn-Hägerdal B (2000) Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresour Technol 74:25–33. https://doi.org/10.1016/S0960-8524(99)00161-3
53. Vivekanand V, Olsen EF, Eijsink VGH, Horn SJ (2013) Effect of different steam explosion conditions on methane potential and enzymatic saccharification of birch. Bioresour Technol 127:343–349. https://doi.org/10.1016/j.biortech.2012.09.118
54. Sun C, Liu R, Cao W, Yin R, Mei Y, Zhang L (2015) Impacts of alkaline hydrogen peroxide pretreatment on chemical composition and biochemical methane potential of agricultural crop stalks. Energy Fuels 29:4966–4975. https://doi.org/10.1021/acs.energyfuels.5b00838

55. Verardi A, Blasi A, Marino T, Molino A, Calabrò V (2018) Effect of steam-pretreatment combined with hydrogen peroxide on lignocellulosic agricultural wastes for bioethanol production: analysis of derived sugars and other by-products. J Energy Chem 27:535–543. https://doi.org/10.1016/j.jechem.2017.11.007
56. Baruah J, Nath BK, Sharma R, Kumar S, Deka RC, Baruah DC, Kalita E (2018) Recent trends in the pretreatment of lignocellulosic biomass for value-added products. Front Energy Res 6:141. https://doi.org/10.3389/fenrg.2018.00141
57. Lizasoain J, Trulea A, Gittinger J, Kral I, Piringer G, Schedl A, Nilsen PJ, Potthast A, Gronauer A, Bauer A (2017) Corn stover for biogas production: effect of steam explosion pretreatment on the gas yields and on the biodegradation kinetics of the primary structural compounds. Bioresour Technol 244:949–956. https://doi.org/10.1016/j.biortech.2017.08.042
58. Li J, Henriksson G, Gellerstedt G (2007) Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. Bioresour Technol 98:3061–3068. https://doi.org/10.1016/j.biortech.2006.10.018
59. Neves PV, Pitarelo AP, Ramos LP (2016) Production of cellulosic ethanol from sugarcane bagasse by steam explosion: effect of extractives content, acid catalysis and different fermentation technologies. Bioresour Technol 208:184–194. https://doi.org/10.1016/j.biortech.2016.02.085
60. Negro MJ, Alvarez C, Ballesteros I, Romero I, Ballesteros M, Castro E, Manzanares P, Moya M, Oliva JM (2014) Ethanol production from glucose and xylose obtained from steam exploded water-extracted olive tree pruning using phosphoric acid as catalyst. Bioresour Technol 153:101–107. https://doi.org/10.1016/j.biortech.2013.11.079
61. Guerrero AB, Ballesteros I, Ballesteros M (2017) Optimal conditions of acid-catalysed steam explosion pretreatment of banana lignocellulosic biomass for fermentable sugar production. J Chem Technol Biotechnol 92:2351–2359. https://doi.org/10.1002/jctb.5239
62. Wang Z, Wu G, Jönsson LJ (2018) Effects of impregnation of softwood with sulfuric acid and sulfur dioxide on chemical and physical characteristics, enzymatic digestibility, and fermentability. Bioresour Technol 247:200–208. https://doi.org/10.1016/j.biortech.2017.09.081
63. Larsson S, Palmqvist E, Hahn-Hägerdal B, Tengborg C, Stenberg K, Zacchi G, Nilvebrant N-O (1999) The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. Enzyme Microb Technol 24:151–159. https://doi.org/10.1016/S0141-0229(98)00101-X
64. Cavka A, Stagge S, Jönsson LJ (2015) Identification of small aliphatic aldehydes in pretreated lignocellulosic feedstocks and evaluation of their inhibitory effects on yeast. J Agric Food Chem 63:9747–9754. https://doi.org/10.1021/acs.jafc.5b04803
65. Kim S, Holtzapple MT (2005) Lime pretreatment and enzymatic hydrolysis of corn stover. Bioresour Technol 96:1994–2006. https://doi.org/10.1016/j.biortech.2005.01.014
66. Klinke HB, Olsson L, Thomsen AB, Ahring BK (2003) Potential inhibitors from wet oxidation of wheat straw and their effect on ethanol production of *Saccharomyces cerevisiae*: wet oxidation and fermentation by yeast. Biotechnol Bioeng 81:738–747. https://doi.org/10.1002/bit.10523
67. Travaini R, Martín-Juárez J, Lorenzo-Hernando A, Bolado-Rodríguez S (2016) Ozonolysis: an advantageous pretreatment for lignocellulosic biomass revisited. Bioresour Technol 199:2–12. https://doi.org/10.1016/j.biortech.2015.08.143
68. Travaini R, Marangon-Jardim C, Colodette JL, Morales-Otero M, Bolado-Rodríguez S (2015) Chapter 7: Ozonolysis. In: Pandey A, Negi S, Binod P, Larroche C (eds) P of B. Elsevier, Amsterdam, pp 105–135
69. Travaini R, Otero MDM, Coca M, Da-Silva R, Bolado S (2013) Sugarcane bagasse ozonolysis pretreatment: effect on enzymatic digestibility and inhibitory compound formation. Bioresour Technol 133:332–339. https://doi.org/10.1016/j.biortech.2013.01.133
70. Schultz-Jensen N, Leipold F, Bindslev H, Thomsen AB (2011) Plasma-assisted pretreatment of wheat straw. Appl Biochem Biotechnol 163:558–572. https://doi.org/10.1007/s12010-010-9062-5
71. Ibrahim MF, Ramli N, Kamal Bahrin E, Abd-Aziz S (2017) Cellulosic biobutanol by Clostridia: challenges and improvements. Renew Sustain Energy Rev 79:1241–1254. https://doi.org/10.1016/j.rser.2017.05.184

72. Isroi I, Millati R, Syamsiah S, Niklasson C, Nur Cahyanto M, Lundquist K, Taherzadeh MJ (2011) Biological pretreatment of lignocelluloses with white-rot fungi and its applications: a review. BioResources 6:5224–5259
73. Carrillo-Nieves D, Rostro Alanís MJ, de la Cruz Quiroz R, Ruiz HA, Iqbal HMN, Parra-Saldívar R (2019) Current status and future trends of bioethanol production from agro-industrial wastes in Mexico. Renew Sustain Energy Rev 102:63–74. https://doi.org/10.1016/j.rser.2018.11.031
74. Wang F-Q, Xie H, Chen W, Wang E-T, Du F-G, Song A-D (2013) Biological pretreatment of corn stover with ligninolytic enzyme for high efficient enzymatic hydrolysis. Bioresour Technol 144:572–578. https://doi.org/10.1016/j.biortech.2013.07.012
75. Schultz-Jensen N, Kádár Z, Thomsen AB, Bindslev H, Leipold F (2011) Plasma-assisted pretreatment of wheat straw for ethanol production. Appl Biochem Biotechnol 165:1010–1023. https://doi.org/10.1007/s12010-011-9316-x
76. Chandel AK, da Silva SS, Singh OV (2013) Detoxification of lignocellulose hydrolysates: biochemical and metabolic engineering toward white biotechnology. BioEnergy Res 6:388–401. https://doi.org/10.1007/s12155-012-9241-z
77. Ping Y, Ling H-Z, Song G, Ge J-P (2013) Xylitol production from non-detoxified corncob hemicellulose acid hydrolysate by *Candida tropicalis*. Biochem Eng J 75:86–91. https://doi.org/10.1016/j.bej.2013.03.022
78. Mamman AS, Lee J-M, Kim Y-C, Hwang IT, Park N-J, Hwang YK, Chang J-S, Hwang J-S (2008) Furfural: hemicellulose/xylosederived biochemical. Biofuels Bioprod Biorefining 2:438–454. https://doi.org/10.1002/bbb.95
79. Dias AS, Lima S, Pillinger M, Valente AA (2006) Acidic cesium salts of 12-tungstophosphoric acid as catalysts for the dehydration of xylose into furfural. Carbohydr Res 341:2946–2953. https://doi.org/10.1016/j.carres.2006.10.013
80. Zeitsch KJBT-SS (2000) 10. Furfural processes. In: The chemistry and technology of furfural and its many by-products. Elsevier, Amsterdam, pp 36–74
81. Kumari PK, Rao BS, Padmakar D, Pasha N, Lingaiah N (2018) Lewis acidity induced heteropoly tungustate catalysts for the synthesis of 5-ethoxymethyl furfural from fructose and 5-hydroxymethylfurfural. Mol Catal 448:108–115. https://doi.org/10.1016/j.mcat.2018.01.034
82. Hu X, Jiang S, Wu L, Wang S, Li C-Z (2017) One-pot conversion of biomass-derived xylose and furfural into levulinate esters via acid catalysis. Chem Commun 53:2938–2941. https://doi.org/10.1039/C7CC01078H
83. Rout PK, Nannaware AD, Prakash O, Kalra A, Rajasekharan R (2016) Synthesis of hydroxymethylfurfural from cellulose using green processes: a promising biochemical and biofuel feedstock. Chem Eng Sci 142:318–346. https://doi.org/10.1016/j.ces.2015.12.002
84. Luo Y, Li Z, Li X, Liu X, Fan J, Clark JH, Hu C (2019) The production of furfural directly from hemicellulose in lignocellulosic biomass: a review. Catal Today 319:14–24. https://doi.org/10.1016/j.cattod.2018.06.042
85. Rong C, Ding X, Zhu Y, Li Y, Wang L, Qu Y, Ma X, Wang Z (2012) Production of furfural from xylose at atmospheric pressure by dilute sulfuric acid and inorganic salts. Carbohydr Res 350:77–80. https://doi.org/10.1016/j.carres.2011.11.023
86. Ragauskas AJ, Beckham GT, Biddy MJ, Chandra R, Chen F, Davis MF, Davison BH, Dixon RA, Gilna P, Keller M, Langan P, Naskar AK, Saddler JN, Tschaplinski TJ, Tuskan GA, Wyman CE (2014) Lignin Valorization: improving lignin processing in the biorefinery. Science (80-) 344:1246843. https://doi.org/10.1126/science.1246843
87. Salvachúa D, Prieto A, López-Abelairas M, Lu-Chau T, Martínez ÁT, Martínez MJ (2011) Fungal pretreatment: an alternative in second-generation ethanol from wheat straw. Bioresour Technol 102:7500–7506. https://doi.org/10.1016/j.biortech.2011.05.027
88. Bugg TDH, Ahmad M, Hardiman EM, Singh R (2011) The emerging role for bacteria in lignin degradation and bio-product formation. Curr Opin Biotechnol 22:394–400. https://doi.org/10.1016/j.copbio.2010.10.009
89. Martínez ÁT, Speranza Mariela, Ruiz-Dueñas Francisco J, Ferreira Patricia, Camarero Susana, Guillén Francisco, Martínez María J, Gutiérrez Ana, del Río José C (2005) Biodegradation

of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin Lignocellulosic materials. Int Microbiol 8:195–204

90. Kim J-S (2015) Production, separation and applications of phenolic-rich bio-oil—a review. Bioresour Technol 178:90–98. https://doi.org/10.1016/j.biortech.2014.08.121
91. Romaní A, Michelin M, Domingues L, Teixeira JA (2018) Chapter 16: Valorization of wastes from agrofood and pulp and paper industries within the biorefinery concept: Southwestern Europe scenario. In: Bhaskar T, Pandey A, Mohan SV, Lee D-J, Khanal SK (eds) WB. Elsevier, Amsterdam, pp 487–504
92. Max B, Torrado AM, Moldes AB, Converti A, Domínguez JM (2009) Ferulic acid and p-coumaric acid solubilization by alkaline hydrolysis of the solid residue obtained after acid pre-hydrolysis of vine shoot prunings: effect of the hydroxide and pH. Biochem Eng J 43:129–134. https://doi.org/10.1016/j.bej.2008.09.015
93. Schmidt RJ (2005) Industrial catalytic processes—phenol production. Appl Catal A Gen 280:89–103. https://doi.org/10.1016/j.apcata.2004.08.030
94. Omoriyekomwan JE, Tahmasebi A, Yu J (2016) Production of phenol-rich bio-oil during catalytic fixed-bed and microwave pyrolysis of palm kernel shell. Bioresour Technol 207:188–196. https://doi.org/10.1016/j.biortech.2016.02.002
95. Oliveira L de L de, Carvalho MV de, Melo L (2014) Health promoting and sensory properties of phenolic compounds in food. Rev Ceres 61:764–779
96. Tsao R, Yang R (2003) Optimization of a new mobile phase to know the complex and real polyphenolic composition: towards a total phenolic index using high-performance liquid chromatography. J Chromatogr A 1018:29–40. https://doi.org/10.1016/j.chroma.2003.08.034
97. Milenkovic D, Deval C, Dubray C, Mazur A, Morand C (2011) Hesperidin displays relevant role in the nutrigenomic effect of orange juice on blood leukocytes in human volunteers: a randomized controlled cross-over study. PLoS ONE 6:e26669
98. Ramful D, Tarnus E, Aruoma OI, Bourdon E, Bahorun T (2011) Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. Food Res Int 44:2088–2099. https://doi.org/10.1016/j.foodres.2011.03.056
99. Putro JN, Soetaredjo FE, Lin S-Y, Ju Y-H, Ismadji S (2016) Pretreatment and conversion of lignocellulose biomass into valuable chemicals. RSC Adv 6:46834–46852. https://doi.org/10.1039/C6RA09851G
100. Terinte N, Ibbett R, Schuster KC (2011) Overview on native cellulose and microcrystalline cellulose i structure studied by X-ray diffraction (Waxd): comparison between measurement techniques. Lenzing Beritchte 89:118–131. https://doi.org/10.1163/156856198X00740
101. Li M, Eskridge K, Liu E, Wilkins M (2019) Enhancement of polyhydroxybutyrate (PHB) production by 10-fold from alkaline pretreatment liquor with an oxidative enzyme-mediator-surfactant system under Plackett-Burman and central composite designs. Bioresour Technol 281:99–106. https://doi.org/10.1016/j.biortech.2019.02.045
102. Obregón I, Gandarias I, Al-Shaal MG, Mevissen C, Arias PL, Palkovits R (2016) The role of the hydrogen source on the selective production of γ-valerolactone and 2-methyltetrahydrofuran from levulinic acid. Chemsuschem 9:2488–2495. https://doi.org/10.1002/cssc.201600751

Chapter 5
Biotechnology Application of Pretreated Biomass

Among the proposals for the application of pretreated biomasses, those that make possible the realization of the circular economy appear as alternatives in several areas of biotechnology [1]. Given this circular economy proposal, processes for this purpose can be adapted. When referring to pretreatment methods, some indicators should be considered, including energy cost, formation of inhibitors, sugar content (in some cases as production of ethanol), yield and its effects on the environment, and if necessary, existing methods can be adapted [2, 3].

In the global scenario, the loss of food and consequently the generation of waste is a major challenge. Some 222 million tons of waste are produced by developed countries, because approximately 40% of these losses derive from post-harvest and processing of food [4]. Some residues may be returned to supplement the food chain; however, others will be transformed into a source of biomass for processes with a biotechnological bias. In this development of technology, it is essential that these residues are raw materials with potential for industrial application that may reduce the impacts of inadequate disposal of waste in the environment, and that instead add value [5, 6].

The spectrum of application of these biomass possibilities is broad; focusing on biotechnological processes can subdivide the fate of these raw materials into three major categories: (a) enzyme production, (b) bioenergy, and (c) waste management. Figure 5.1 illustrates some of the application paths for these pretreated biomasses.

5.1 Production of Enzymes from Pretreated Biomasses

Industry is attracted to enzymes as biomolecules with specific actions and biocatalysis potentials in various reactions. The search for increasingly optimized and efficient processes drives the demand for rapid, low cost, and dynamic catalysis reactions; in this regard, enzymes perform admirably [7].

The commercially produced enzyme sector is consolidated worldwide and commands the market with enzymes of industrial interest that act in specific ways in

H. Treichel et al., *Utilising Biomass in Biotechnology*,
Green Energy and Technology, https://doi.org/10.1007/978-3-030-22853-8_5

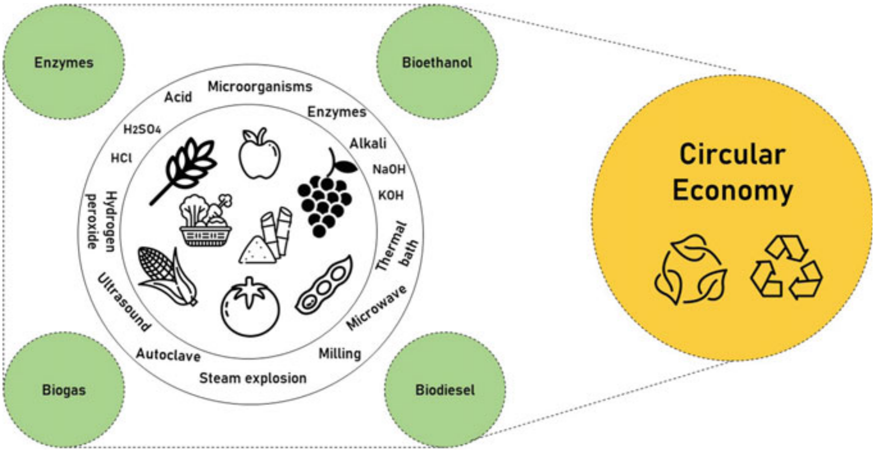

Fig. 5.1 Possibilities of application of the biomasses available in the world matrix, as well as viable pretreatments used for biotechnological processes within the principles of the circular economy. *Source* Authors

various processes. Biomasses are compelling alternatives, performing essential functions. In biotechnological processes, they create the potential to generate lower-cost enzymes. They must possess specificity and capacity to adapt to adverse situations, in addition to stimulating the principles of the circular economy, obtaining new products through residues [8–11].

In the scenario of enzymatic production through residues, microbial strains appear as an essential part of the process, because the production of enzymes by fermentation occurs through the action of microorganisms (mainly fungi and bacteria) in these substrates. As products, there is a range of enzymatic production. Various (pretreated or untreated) residues are sources of enzyme production with high added value that are essential for industrial and technological processes. These include amylase, cellulases, xylanase, and laccase, among others, all of which are produced by microorganisms [5].

Cellulases have the potential to hydrolyze biomasses composed of cellulose. These in turn are popularly known as lignocellulosic biomasses and because of their complex nature. This is because of the simultaneous action of three other enzymes (endoglucanase, cellobiohydrolase, and β-glycosidase) that act on specific parts of the cellulose, breaking long chains in short chains, until obtaining glucose [12].

Xylanases aid in the breakdown of xylan formed by xylose (pentose) and present in biomasses containing hemicellulose. The literature suggests that the microorganisms such as *Trichoderma reesei*, *Humicola isolens,* and *Bacillus* are excellent producers of this enzyme; cellulases and xylanases also form an enzymatic complex, composed of endo-1,4-β-xylanase, β-xylosidase, α-arabinofuranosidases, and esterases. Xylan is considered heterogeneous; therefore, the synergistic action of an enzymatic pool is necessary [12].

Peroxidases are reductive oxidizing enzymes that consist of the enzyme lignin peroxidase and manganese peroxidase. The former acts on biomasses containing phenolic and non-phenolic aromatic compounds and produces cations and the latter acts by oxidizing phenolic substrates [12, 13].

Following the oxidative enzymes, the laccases act by oxidizing biomass that contains copper, in plants, insects, and microorganisms. Laccases are widely used in the degradation of lignin present in the biomass used in bioprocesses. In this sense, white-rot fungus acts through laccase [12].

Keratinases are in the class of proteases that have potential for performance in complex substrates, especially keratinous ones, with heterogeneous structures that are difficult to degrade [14–16].

Lipases act on water-insoluble substrates, catalyzing reactions such as esterification, hydrolysis. They structurally modify oils and fats by specifically catalyzing these substrates. These enzymes are responsible for the breakdown of long lipid chains into smaller chains of fatty acids [17].

Amylases catalyze the breakdown of long starch molecules into smaller chains such as maltose and glucose. These enzymes are of great industrial interest, mainly in the productive sectors of paper, detergents, syrups, and sweeteners. Because of their wide use, they represent a significant portion of the world market for enzymes, about 25% [18–21]. Pectinases are of great importance mainly to the food industry, because they can be used at several stages of fruit processing to facilitate stages of development of the final product, essentially those related to the clarification of juices, wines, coffees, and teas [22, 23].

It is possible to obtain an enzymatic cocktail by means a series of biomass; the formation of enzymes is only possible with the presence of microbial strains capable of using these biomasses as source for their development and enzymatic production. In some cases, the microorganisms are not able to access them, so the pretreatments act as facilitators of these processes [5, 12].

Studies of enzymatic production by means of residual biomass aim not only to obtain new products, but also to evaluate wastes that would be discarded, thereby stimulating the development of the circular economy. In this sense, the possibilities of biomass are amplified. Some of them are summarized below, highlighting enzymes that can be obtained from these sources. The need or lack of need for pretreatment of biomass is displayed in Table 5.1.

5.2 Production of Bioenergies from Pretreated Biomass

The world's energy demand has experienced exponential growth, on account of which high prices of fossil fuels and reduction of natural resources becomes inevitable. Therefore, the development of environmentally sustainable technologies using alternative and low cost of biomass presents greater potential than conventional fossil sources [7].

Table 5.1 Compilation of enzymatic production from the evaluation of residual biomasses in the absence or presence of pretreatment methods

Enzyme	Source of biomass	Pretreatment	Reference
Cellulase	Soybean hulls and waste paper	Autoclave	[24, 25]
	Corn cobs, carrot, orange, potato, pineapple peelings, wheat bran, wheat, straw, sawdust, and rice husk	Alkali	[24, 26]
	Rice husk, soybean hulls, sugarcane bagasse, powder toothpick, and yerba mate	Autoclave	[1]
	Tobacco solid waste	Milling 1% H_2SO_4 NaOH in ratio 1:5 (w v^{-1}).	[27]
Xylanase	White and red grape, vine shoots trimming and grape stalks from the winery industry. Organic crude olive pomace and exhausted olive pomace, and brewer's spent grain	*	[28]
	Grape pomace	Autoclave	[24, 29]
Peroxidase	By-product of olive oil extraction	*	[30]
	Rice bran	Milling Sodium phosphate buffer (pH 5.0) at a ratio of 1:10 (w v^{-1}) under mechanical agitation	[31, 32]
Laccase	Fruit juice waste	*	[33]
	Soybean pod and coffee husk	NaClO 0.5% Dry, crush and sieve	[34]
	Olive leaves	Milling and sieve	[35]
Keratinase	Human hair, pig hair, chicken feather meal, raw chicken feathers, and bovine horn	*	[16, 36–38]
Lipase	Waste cooking oil	Ultrasound	[39]
	Mango residues from juice preparation (peel and seed)	Milling	[40]
Amylase	By-products of wheat	Milling	[20]
	Kitchen waste and peels of vegetables	Alkali (NaOH) and autoclave	[24, 41]
	Banana peel	Wash (H_2O), milling and autoclave	[24, 42]

(continued)

Table 5.1 (continued)

Enzyme	Source of biomass	Pretreatment	Reference
Pectinase	Citrus pulp of floater	*	[43]
	Melon peel	Chemical (citric acid)	[44]
	Potato pulp	Acid (sulfuric, hydrochloric and citric)	[45]

* Not provided

To exploit these biotechnologies in realistic scales, it is necessary to study the stages of the process, especially with regard to the raw material being used. Lignocellulosic biomasses are notable for their potential to produce bioenergies; however, they often require high energy and process costs introduced by pretreatment [7, 46, 47]. Therefore, the search is underway for biomasses that are cheaper, more efficient and that allow promotion of the circular economy. Biomass from the food sector has been presented as an opportunity for innovation, primarily in the sense of having low costs and acting as a substrate for the synthesis of biotechnological products with high added value [7, 48, 49].

Considering the imminent shortage of fossil fuel reserves and the high price of automotive fuels, as well as the large amount of food lost during processing, there is considerable interest in renewable biomass with high energy. Studies regarding the transformation of this biomass as substrate for the application in biorefineries have already been conducted [7, 50]. The concept of biorefineries refers to all biomass (including biofuels) that can be used for the energy sector. The principle of this process is the premise of obtaining technological, economically viable, and environmentally sustainable processes [7, 51].

Second-generation biofuels are those that use residual biomass from other processes, mainly lignocellulosic ones; for this reason, they require additional stages, i.e., pretreatments. The efficiency of pretreatment is related to the potential of simplifying biomass through low-cost techniques, low energy demand, high yields, and the lowest possible impact on the environment [3]. Lignocellulosic biomass is also compelling in the sense of reducing greenhouse gas emissions and non-competition with agricultural crops, consequently directly affecting the food sector, because this occurs when treating biomass as first-generation biofuels [12, 52].

Bensah, Kádár and Mensah [3] used lignocellulosic biomass in your study, rubberwood (*Hevea brasiliensis*), bamboo wood (*Bambusa vulgaris*), Siam weed (*Chromolaena odorata*), and elephant grass (*Pennisetum purpureum*). The biomass underwent a combination of pretreatments: milling in the knife mill (2-mm), alkaline treatment (KOH), and glycerol, after which the samples were autoclaved, followed by enzymatic hydrolysis (cellulase and xylanase). Ethanol fermentation was carried out using *Saccharomyces cerevisiae*. KOH pretreatment was the most promising, producing the highest amount of ethanol (9.8 g 100 $\mathrm{g}_{\mathrm{biomass}}^{-1}$).

Some studies have also evaluated the potential of eucalyptus biomass for the production of second-generation ethanol. The biomass was pretreated with a combination of the solvent and acid method (50 mM H_2SO_4). As a result, the rate of enzymatic hydrolysis was increased by 94.3% for one kilo of pretreated eucalyptus biomass, suggesting that the method was promising for efficient conversion of cellulose to glucose for subsequent application in the production of bioethanol, as well as for generation of high-value-added products, including xylitol and phenolic lignin resins that are widely used industrially [53].

Agricultural biomass residues are usually harvested from crops and are transported to a central depot to continue to biorefineries, their final destination. Recent studies have sought to simplify the logistics processing of this type of biomass, decentralizing the pretreatment stage, such that the costs of transportation would be significantly reduced [54–59]. The pretreatment, in this case, must be dry, because the biomass needs to be dry for transportation; furthermore, if water is used in the process, it will need to undergo treatment, in the same, way pretreatments that require high energy consumption (such as steam explosion) not are viable [54, 60]. The modified diluted sulfuric acid (dry acid) pretreatment was applied to crop biomass residuals involving corn, wheat, and rice straw; this increased the initial biomass solids content by 70% by reducing residual water. As a final result, 260 kg of ethanol can be produced from one metric ton of pretreated biomass, a relevant yield when compared to the yield of liquid ammonia pretreatment, in which a maximum yield of 205 kg of ethanol from one metric ton of biomass [57, 61, 62].

In spite of the environmental appeal surrounding the use of residual biomasses in high value-added biotechnological processes, care must be taken with respect to the pretreatment used, because it may end up making the whole process unfeasible. Therefore, a comparative approach is needed and aimed at investigating environmental performance of various pretreatments and estimating the consequences of these techniques. What is required is a scenario in which lignocellulosic biomass undergoes various pretreatments, and identifying the technique that presents the lowest environmental impact and greater biomass conversion efficiency for ethanol. Such studies would quantify the entrances and exits of the process, including indicators such as energy consumption and products and emissions generated. In one such study, the pretreatment techniques used were ammonia, sodium hydroxide, sulfuric acid, and methanol; with regard to the emission of greenhouse gases, the pretreatment with sodium hydroxide was the one that expressed the greatest amount of these gases, about 12.03 kg CO_2, followed by sulfuric acid (7.77 kg CO_2) and methanol (0.0019 kg CO_2). A relevant fact in this study is that methanol was also the most effective treatment for the conversion of biomass to ethanol, presenting a smaller carbon footprint, demonstrating the potential to become a product for pretreatment [63].

It is worth highlighting that the environmental impact assessment is specific to each process, biomass and type of pretreatment used. In this sense, the important thing is to verify the possibility of reuse, reduction, and recycling of products or by-products during the pretreatment process with the purpose of minimizing the generation of effluents and the use of drinking water, stimulating environmentally safe processes.

Microalgae are alternative biomasses for the production of biofuels, primarily on account of their formation containing carbohydrates that can be transformed into starch, glucose, polysaccharides, and absence of lignin, facilitating their hydrolysis; however, in the same way as with lignocellulosic biomass, microalgae need to go through pretreatment, which in this case aims to break their cell wall and release compounds of interest for ethanolic fermentation as the starch that is trapped within the cell [64–66]. There are a number of pretreatment methods that can be employed for this purpose, some of which have already been mentioned in Sect. 3 of this volume: acid treatment, ultrasonic, hydrothermal, biological, and enzymatic, among others. Recent studies have used the combined treatment of acid and water at high temperature and pressure (hydrothermal) of the biomass of *Chlorella* spp. to evaluate the production of ethanol. Using experimental and statistical analysis, the authors obtained a maximum yield of 5.1 $g L^{-1}$ of ethanol concentration, with intermediate temperature (130 °C), maximum time (40 min), and maximum concentration of sulfuric acid (1.5 $v v^{-1}$) among the trials in the studied range. The concentration of the acid was the only variable that had a significant effect on ethanol production from the pretreated microalgae biomass; this can be explained by the tests in which no acid was used and also no ethanol production, possibly due to the low concentration of glucose in the treated biomass; therefore, it is understood that with the presence of the acid, rupture of the cell wall occurred, facilitating the release of sugars [66].

In addition to the production of bioethanol, the lignocellulosic biomass also has potential for generation of bioenergies by means anaerobic biodigestion of these residues, that is, for the production of biogas. Other biomasses including agricultural, landfill, food, and aquatic biomass residues (e.g., microalgae) can be used as substrates for energy production [67].

Raposo et al. [68] compiled information regarding the type of biomass, pretreatment, and biogas yield. The most promising biomass were corn straw and empty fruit bunches, both pretreated in alkaline form, as well as straw from beans, pretreated in an autoclave. They reported yields of 372.4, 404, and 440 mL CH_4 gVS_{added}^{-1}, respectively.

Venturin et al. [69] studied only one biomass, corn stalks, that were subjected to various pretreatments: varying concentrations of sulfuric acid and hydrogen peroxide in an orbital shaker. The authors obtained interesting results. With the alkaline pretreatment (peroxide) there was an increase in biogas production of 22%, in addition to removing 71.6% of the lignin and 19.3% hemicellulose providing a cellulose content of 73.4%.

Using biological pretreatments, it is possible to use the natural microbial functions in favor of the treatment process, which in the case of lignocellulosic biomass is to degrade the lignin or to the microalgae, to break down the components of the cell wall [70]. Most fungal pretreatments refer directly to studies for lignocellulosic biomass, aiming to degrade lignin, for which the fungi of white rot, brown rot (Basidiomycetes family), and rotting fungi (Ascomycetes group) are considered ideal for these processes. A disadvantage of this pretreatment is the possibility of removal of components important for the later stages, e.g., significant losses of cellulose and hemicellulose caused by use of non-selective fungal strains. Some studies have identified potential fungal strains pretreatments considering the high efficiency of lignin removal and the low losses of cellulose and hemicellulose during treatment. The fungus *Ceriporiopsis subvermispora* stands out in this scenario because of its potential to remove lignin with very low loss of cellulose. In these studies, the biomasses pretreated by the fungus were corn straw, rubber, grasses, and hardwoods [71–73], obtaining high overall process efficiencies. This was due to the selectivity of the fungi that secrete lignolytic enzymes and assist in the hydrolysis of the biomass.

In a study aimed at biogas production, a pool of lignocellulosic biomass, corn straw, wheat straw, flax, hemp, miscanthus, and willow were used. All underwent enzymatic treatment with laccase and peroxidase enzymes, both produced by microorganisms (*Trametes versicolor* and *Bjerkandera adusta*, respectively). Biogas production through the addition of a bovine manure effluent in these pretreated biomasses has enhanced the yield and quality of biogas and treated effluent [74].

Observing the yield of the fermentation process after the pretreatment step gives us the information on how effective the chosen method was. The literature suggests higher yields in microalgae biomass, because their structure to be obtained by the pretreatment is more flexible; by contrast, the lignocellulosic biomasses present lower yields and this is due to their structure being more rigid and more difficult to access by enzymes (cellulases, xylanases, among others) or hydrolytic microorganisms. This can be seen in studies in which raw biomass yields were quantified untreated in their crude form. The differences between the two types of raw materials are presented in Table 5.2.

It is difficult to reach objective conclusions about these processes. It is necessary to have a broad view of the whole structure and composition of biomass, conditions of pretreatment processes, yields, as well as technological and economic viabilities, among others. One can see the vast possibility of applying pretreated biomass to stimulate a world energy sector that is facing a serious crisis. These approaches allow the insertion of frequently unused biomasses into biotechnological processes with high benefit.

5.3 Valuation of Residues from Pretreated Biomass

Usually, waste management is carried out in the most well-known ways, composting, incineration, or disposed in landfills. Nevertheless, this does not mean that these are the most appropriate practices, despite worldwide acceptance [7, 78].

The valuation of residues appears as a management possibility, mediated by sustainable conversion of these residues. This approach offers a number of advantages, especially in the economic and environmental sector, as it reduces waste disposal in landfills and consequently decreases the possibility of contamination, stimulating a circular economy culture [7].

Table 5.2 Studies showing the yield differences between lignocellulosic and microalgae biomass

	Biomass	Pretreatment	Yield and application	Reference
Lignocellulosic	Rice straw	Fungal (*Pleurotus ostreatus* and *Trichoderma reesei*)	Yield 120% for methane yield	[75]
	Corn straw	Fungal (*Phanerochaete chrysosporium*)	Yield 263% for ethanol	[73]
	Switchgrass	Fungal (*Pycnoporus* sp.)	Yield 50% for sugar	[71]
Microalgae	*Scenedesmus obliquus*	Enzyme (cellulase, esterase, protease, and endogalactouronase)	Yield 485% for methane	[76]
	Scenedesmus obliquus	Enzyme (esterase and protease)	Yield 273% for methane	[76]
	Nannochloropsis gaditana	Bacteria (*Raoultella ornithinolytica*)	Yield 114–159% for methane	[77]

Management of the vast accumulation of waste requires efficient strategies. In this sense, it is desirable to focus on the conversion of waste biomass for use in processes of biotechnological interest such as the bioenergy sector [7]. By 2050, it is estimated that the increase in population will lead to an increase in global food production, a determining factor for the implementation of the circular economy. For these reasons, transforming residues into substrates that do not need to be burned or grounded and which have priority to close the cycle are extremely relevant [28].

Among the possibilities of waste valuation, the production of enzymes from residual biomasses appear as compelling low-cost alternatives, but with high added value, because these enzymes often have potential application in biotechnological processes. In this sense, Leite et al. [28] evaluated the production of the enzymes β-glycosidase, cellulase, and xylanase by combining the biomasses of olive mill, winery, and brewery wastes using solid-state fermentation with filamentous fungi (*Aspergillus ibericus*, *Rhizopus oryzae,* and *Aspergillus niger*). They found high activities of cellulase, xylanase, and β-glycosidase, respectively, in the fermentation products. In addition, the enzymes produced oxidative phenolic compounds. Biological pretreatment and production of an enzymatic pool permitted generation of antioxidant by-products in a low cost and environmentally viable biotechnological process.

Another example of biomass valuation is the use of chicken feathers from the poultry industry. A study carried out in Malaysia in 2016 found that about 43 thousand tons of waste feathers were generated during the processing of cut poultry. This waste has a most common destination landfills and incinerators, contributing to greenhouse gases and environmental contamination [79]. Studies have focused on alternative solutions for the management of residue, highlighting the valuation of this residue as a form of biomass for use in biotechnological processes. However, feathers have rigid structures, and keratin must be pretreated so that it can be used in other processes, as described by Cheong et al. [79]. The authors combined pretreatments (alkaline NaOH, microwave, autoclave, and enzymatic with commercial protease) for feather solubilization and protein recovery. They found that degradation of the feathers was low, possibly because of disulfide bonds between the keratin chains that hampered the access of enzymes [80]; by contrast, the tests with pretreated NaOH and microwave presented more efficient results of protein recovery; these techniques may be promising for the utilization of chicken feathers from the protein processing industries.

Animal protein processing demonstrates that keratin-rich residues constitute biomass of great interest for the development of new processes and product valorizations, particularly chicken feathers and swine hairs. Recycling these wastes expands the range of biotechnological applications (production of enzymes, biomolecules, fertilizers, biogas, among others), considering the principles of the circular economy, as well as the environmental and economic sectors involved in these processes [16, 81–84].

India invests heavily in waste management, as a populous country with relatively small territory. Studies of urban waste valuation in these regions are relevant and are technology drivers. Recently, food waste, fruit peel, vegetable peels, and garden pruning have been used as raw materials for biogas production. The total substrate composition was 50% cooked food waste, 25% vegetable peel and fruit peel, and 25% garden waste. A combination of milling and hydrothermal pretreatments was applied at temperatures of 80, 100, 120, 140, and 160 °C with durations of 0, 15, 30, 60, and 120 min. After pretreatment, authors observed better dissolution of carbohydrates, proteins, and lipids. At lower temperatures (80 and 100 °C), the biomass pH also decreased, suggesting thermal hydrolysis; nevertheless, these temperatures were not sufficient to degrade cellulose or hemicellulose. This was made possible under the most severe pretreatment conditions, in which methane production was also increased, reaching a yield of 200 mL g_{VS}^{-1} [85]. This suggests that hydrothermal pretreatment allowed the solubilization of complex organic matter, making the degradation of microorganisms and enzymes present in the biodigestion process more accessible, thereby reducing retention time, and making the process more efficient.

Residual biomass is placed in the waste valuation scenario as an important source in the most diverse biotechnological processes, with the potential to produce high value-added by-products. Although the waste valuation process offers an opportunity and is compelling from an environmental and economic point of view, it presents limitations regarding an efficient conversion. This is basically due to the heterogeneous constitution of these biomasses. In this scenario, studies appear as alternatives to find suitable strategies in which it would be possible to carry out the waste management while extracting value from them [24].

References

1. Astolfi V, Astolfi AL, Mazutti MA, Rigo E, Di Luccio M, Camargo AF, Dalastra C, Kubeneck S, Fongaro G, Treichel H (2019) Cellulolytic enzyme production from agricultural residues for biofuel purpose on circular economy approach. Bioprocess Biosyst Eng 42:677–685. https://doi.org/10.1007/s00449-019-02072-2
2. Bensah EC, Mensah M (2013) Chemical pretreatment methods for the production of cellulosic ethanol: technologies and innovations. Int J Chem Eng 2013:21. https://doi.org/10.1155/2013/719607
3. Bensah EC, Kádár Z, Mensah MY (2019) Alkali and glycerol pretreatment of West African biomass for production of sugars and ethanol. Bioresour Technol Reports 6:123–130. https://doi.org/10.1016/j.biteb.2019.02.013
4. FAO (2011) Food loss &waste
5. Bharathiraja S, Suriya J, Krishnan M, Manivasagan P, Kim S-K (2017) Chapter Six: Production of enzymes from agricultural wastes and their potential industrial applications. In: Kim S-K, Toldrá F (eds) A in F and NR. Marine enzymes biotechnology: production and industrial applications, Part III—Application of marine enzymes. Academic Press, pp 125–148
6. Wang B, Dong F, Chen M, Zhu J, Tan J, Fu X, Wang Y, Chen S (2016) Advances in recycling and utilization of agricultural wastes in China: based on environmental risk, crucial pathways, influencing factors, policy mechanism. Procedia Environ Sci 31:12–17. https://doi.org/10.1016/j.proenv.2016.02.002
7. Bilal M, Iqbal HMN (2019) Sustainable bioconversion of food waste into high-value products by immobilized enzymes to meet bio-economy challenges and opportunities—a review. Food Res Int 123:226–240. https://doi.org/10.1016/j.foodres.2019.04.066
8. Kim D (2018) Physico-chemical conversion of lignocellulose: inhibitor effects and detoxification strategies: a mini review. Molecules 23
9. Zhou C-H, Xia X, Lin C-X, Tong D-S, Beltramini J (2011) Catalytic conversion of lignocellulosic biomass to fine chemicals and fuels. Chem Soc Rev 40:5588–5617. https://doi.org/10.1039/C1CS15124J
10. Somerville C, Youngs H, Taylor C, Davis SC, Long SP (2010) Feedstocks for lignocellulosic biofuels. Science (80-) 329:790–792. https://doi.org/10.1126/science.1189268
11. Taarning E, Osmundsen CM, Yang X, Voss B, Andersen SI, Christensen CH (2011) Zeolite-catalyzed biomass conversion to fuels and chemicals. Energy Environ Sci 4:793–804. https://doi.org/10.1039/C004518G
12. Binod P, Gnansounou E, Sindhu R, Pandey A (2019) Enzymes for second generation biofuels: recent developments and future perspectives. Bioresour Technol Reports 5:317–325. https://doi.org/10.1016/j.biteb.2018.06.005
13. Schoemaker HE, Piontek K (1996) On the interaction of lignin peroxidase with lignin. Pure Appl Chem 68:2089–2096. https://doi.org/10.1351/pac199668112089
14. Brandelli A, Daroit DJ, Riffel A (2010) Biochemical features of microbial keratinases and their production and applications. Appl Microbiol Biotechnol 85:1735–1750. https://doi.org/10.1007/s00253-009-2398-5
15. Gegeckas A, Šimkutė A, Gudiukaitė R, Čitavičius DJ (2018) Characterization and application of keratinolytic paptidases from Bacillus spp. Int J Biol Macromol 113:1206–1213. https://doi.org/10.1016/j.ijbiomac.2018.03.046
16. Brandelli A, Sala L, Kalil SJ (2015) Microbial enzymes for bioconversion of poultry waste into added-value products. Food Res Int 73:3–12. https://doi.org/10.1016/j.foodres.2015.01.015
17. Preczeski KP, Kamanski AB, Scapini T, Camargo AF, Modkoski TA, Rossetto V, Venturin B, Mulinari J, Golunski SM, Mossi AJ, Treichel H (2018) Efficient and low-cost alternative of lipase concentration aiming at the application in the treatment of waste cooking oils. Bioprocess Biosyst Eng 41. https://doi.org/10.1007/s00449-018-1919-y
18. Simair AA, Bhutto MA, Lu C, Qureshi AS, Khushk I, Lashari S, Mangrio GS, Ali CH (2017) Production and partial characterization of α-amylase enzyme from Bacillus sp. BCC 01-50 and potential applications. Biomed Res Int 9. https://doi.org/10.1155/2017/9173040

19. Naili B, Sahnoun M, Bejar S, Kammoun R (2016) Optimization of submerged *Aspergillus oryzae* S2 α-amylase production. Food Sci Biotechnol 25:185–192. https://doi.org/10.1007/s10068-016-0028-4
20. Frantz SC, Paludo LC, Stutz H, Spier MR (2019) Production of amylases from *Coprinus comatus* under submerged culture using wheat-milling by-products: optimization, kinetic parameters, partial purification and characterization. Biocatal Agric Biotechnol 17:82–92. https://doi.org/10.1016/j.bcab.2018.11.004
21. Sindhu R, Binod P, Madhavan A, Beevi US, Mathew AK, Abraham A, Pandey A, Kumar V (2017) Molecular improvements in microbial α-amylases for enhanced stability and catalytic efficiency. Bioresour Technol 245:1740–1748. https://doi.org/10.1016/j.biortech.2017.04.098
22. Kapoor M, Panwar D, Kaira GS (2016) Chapter 3: Bioprocesses for enzyme production using agro-industrial wastes: technical challenges and commercialization potential. In: Dhillon GS, Kaur S (eds) A-IW as F for EP. Academic Press, San Diego, pp 61–93
23. Martins ES, Silva D, Da Silva R, Gomes E (2002) Solid state production of thermostable pectinases from thermophilic Thermoascus aurantiacus. Process Biochem 37:949–954. https://doi.org/10.1016/S0032-9592(01)00300-4
24. Sindhu R, Gnansounou E, Rebello S, Binod P, Varjani S, Thakur IS, Nair RB, Pandey A (2019) Conversion of food and kitchen waste to value-added products. J Environ Manage 241:619–630. https://doi.org/10.1016/j.jenvman.2019.02.053
25. Julia BM, Belén AM, Georgina B, Beatriz F (2016) Potential use of soybean hulls and waste paper as supports in SSF for cellulase production by *Aspergillus niger*. Biocatal Agric Biotechnol 6:1–8. https://doi.org/10.1016/j.bcab.2016.02.003
26. Bansal N, Tewari R, Soni R, Soni SK (2012) Production of cellulases from *Aspergillus niger* NS-2 in solid state fermentation on agricultural and kitchen waste residues. Waste Manag 32:1341–1346. https://doi.org/10.1016/j.wasman.2012.03.006
27. Buntić AV, Milić MD, Stajković-Srbinović OS, Rasulić NV, Delić DI, Mihajlovski KR (2019) Cellulase production by *Sinorhizobium meliloti* strain 224 using waste tobacco as substrate. Int J Environ Sci Technol. https://doi.org/10.1007/s13762-019-02230-9
28. Leite P, Silva C, Salgado JM, Belo I (2019) Simultaneous production of lignocellulolytic enzymes and extraction of antioxidant compounds by solid-state fermentation of agro-industrial wastes. Ind Crops Prod 137:315–322. https://doi.org/10.1016/j.indcrop.2019.04.044
29. Botella C, Diaz A, de Ory I, Webb C, Blandino A (2007) Xylanase and pectinase production by *Aspergillus awamori* on grape pomace in solid state fermentation. Process Biochem 42:98–101. https://doi.org/10.1016/j.procbio.2006.06.025
30. Reina R, Liers C, Ocampo JA, García-Romera I, Aranda E (2013) Solid state fermentation of olive mill residues by wood- and dung-dwelling Agaricomycetes: effects on peroxidase production, biomass development and phenol phytotoxicity. Chemosphere 93:1406–1412. https://doi.org/10.1016/j.chemosphere.2013.07.006
31. Gautério GV, Garda-Buffon J, Kalil SJ (2018) Single-step purification of peroxidases from rice bran: evaluation of the main expanded-bed chromatography parameters. Ind Crops Prod 126:440–448. https://doi.org/10.1016/j.indcrop.2018.10.039
32. Feltrin ACP, Garcia SDO, Caldas SS, Primel EG, Badiale-Furlong E, Garda-Buffon J (2017) Characterization and application of the enzyme peroxidase to the degradation of the mycotoxin DON. J Environ Sci Heal Part B 52:777–783. https://doi.org/10.1080/03601234.2017.1356672
33. Sondhi S, Saini K (2019) Response surface based optimization of laccase production from *Bacillus* sp. MSK-01 using fruit juice waste as an effective substrate. Heliyon 5:e01718. https://doi.org/10.1016/j.heliyon.2019.e01718
34. Gonzalez JC, Medina SC, Rodriguez A, Osma JF, Alméciga-Díaz CJ, Sánchez OF (2013) Production of *Trametes pubescens* Laccase under submerged and semi-solid culture conditions on agro-industrial wastes. PLoS ONE 8:e73721
35. Aydınoğlu T, Sargın S (2013) Production of laccase from *Trametes versicolor* by solid-state fermentation using olive leaves as a phenolic substrate. Bioprocess Biosyst Eng 36:215–222. https://doi.org/10.1007/s00449-012-0777-2

36. Fakhfakh-Zouari N, Haddar A, Hmidet N, Frikha F, Nasri M (2010) Application of statistical experimental design for optimization of keratinases production by *Bacillus pumilus* A1 grown on chicken feather and some biochemical properties. Process Biochem 45:617–626. https://doi.org/10.1016/j.procbio.2009.12.007
37. Daroit DJ, Corrêa APF, Brandelli A (2011) Production of keratinolytic proteases through bioconversion of feather meal by the Amazonian bacterium *Bacillus* sp. P45. Int Biodeterior Biodegradation 65:45–51. https://doi.org/10.1016/j.ibiod.2010.04.014
38. e Silva LAD, Macedo AJ, Termignoni C (2014) Production of keratinase by Bacillus subtilis S14. Ann Microbiol 64:1725–1733. https://doi.org/10.1007/s13213-014-0816-0
39. Mulinari J, Venturin B, Sbardelotto M, Dall Agnol A, Scapini T, Camargo AF, Baldissarelli DP, Modkovski TA, Rossetto V, Dalla Rosa C, Reichert FW, Golunski SM, Vieitez I, Vargas GDLP, Dalla Rosa C, Mossi AJ, Treichel H (2017) Ultrasound-assisted hydrolysis of waste cooking oil catalyzed by homemade lipases. Ultrason Sonochem 35. https://doi.org/10.1016/j.ultsonch.2016.10.007
40. da Pereira AS, Fontes-Sant'Ana GC, Amaral PFF (2019) Mango agro-industrial wastes for lipase production from *Yarrowia lipolytica* and the potential of the fermented solid as a biocatalyst. Food Bioprod Process 115:68–77. https://doi.org/10.1016/j.fbp.2019.02.002
41. Hasan MM, Marzan LW, Hosna A, Hakim A, Azad AK (2017) Optimization of some fermentation conditions for the production of extracellular amylases by using Chryseobacterium and Bacillus isolates from organic kitchen wastes. J Genet Eng Biotechnol 15:59–68. https://doi.org/10.1016/j.jgeb.2017.02.009
42. Radha Krishna P, Srivastava AK, Ramaswamy NK, Suprasanna P, D'Souza SF (2012) Banana peel as substrate for α-amylase production using *Aspergillus niger* NCIM 616 and process optimization. Indian J Biotechnol 11:314–319
43. Cypriano DZ, da Silva LL, Tasic L (2018) High value-added products from the orange juice industry waste. Waste Manag 79:71–78. https://doi.org/10.1016/j.wasman.2018.07.028
44. Raji Z, Khodaiyan F, Rezaei K, Kiani H, Hosseini SS (2017) Extraction optimization and physicochemical properties of pectin from melon peel. Int J Biol Macromol 98:709–716. https://doi.org/10.1016/j.ijbiomac.2017.01.146
45. Yang J-S, Mu T-H, Ma M-M (2018) Extraction, structure, and emulsifying properties of pectin from potato pulp. Food Chem 244:197–205. https://doi.org/10.1016/j.foodchem.2017.10.059
46. Bilal M, Iqbal HMN, Hu H, Wang W, Zhang X (2018) Metabolic engineering pathways for rare sugars biosynthesis, physiological functionalities, and applications—a review. Crit Rev Food Sci Nutr 58:2768–2778. https://doi.org/10.1080/10408398.2017.1341385
47. Arevalo-Gallegos A, Ahmad Z, Asgher M, Parra-Saldivar R, Iqbal HMN (2017) Lignocellulose: a sustainable material to produce value-added products with a zero waste approach—a review. Int J Biol Macromol 99:308–318. https://doi.org/10.1016/j.ijbiomac.2017.02.097
48. Chen SS, Maneerung T, Tsang DCW, Ok YS, Wang C-H (2017) Valorization of biomass to hydroxymethylfurfural, levulinic acid, and fatty acid methyl ester by heterogeneous catalysts. Chem Eng J 328:246–273. https://doi.org/10.1016/j.cej.2017.07.020
49. Yu IKM, Ong KL, Tsang DCW, Haque MA, Kwan TH, Chen SS, Uisan K, Kulkarni S, Lin CSK (2018) Chemical transformation of food and beverage waste-derived fructose to hydroxymethylfurfural as a value-added product. Catal Today 314:70–77. https://doi.org/10.1016/j.cattod.2018.01.011
50. Tuck CO, Pérez E, Horváth IT, Sheldon RA, Poliakoff M (2012) Valorization of biomass: deriving more value from waste. Science (80-) 337:695–699. https://doi.org/10.1126/science.1218930
51. Cherubini F (2010) The biorefinery concept: using biomass instead of oil for producing energy and chemicals. Energy Convers Manag 51:1412–1421. https://doi.org/10.1016/j.enconman.2010.01.015
52. Searchinger T, Heimlich R, Houghton RA, Dong F, Elobeid A, Fabiosa J, Tokgoz S, Hayes D, Yu T-H (2008) Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. Science (80-) 319:1238–1240. https://doi.org/10.1126/science.1151861

53. Li Y-J, Li H-Y, Sun S-N, Sun R-C (2019) Evaluating the efficiency of γ-valerolactone/water/acid system on Eucalyptus pretreatment by confocal Raman microscopy and enzymatic hydrolysis for bioethanol production. Renew Energy 134:228–234. https://doi.org/10.1016/j.renene.2018.11.038
54. Eranki PL, Bals BD, Dale BE (2011) Advanced regional biomass processing depots: a key to the logistical challenges of the cellulosic biofuel industry. Biofuels Bioprod Biorefining 5:621–630. https://doi.org/10.1002/bbb.318
55. Hoover AN, Tumuluru JS, Teymouri F, Moore J, Gresham G (2014) Effect of pelleting process variables on physical properties and sugar yields of ammonia fiber expansion pretreated corn stover. Bioresour Technol 164:128–135. https://doi.org/10.1016/j.biortech.2014.02.005
56. Kim S, Dale BE (2015) Comparing alternative cellulosic biomass biorefining systems: centralized versus distributed processing systems. Biomass Bioenerg 74:135–147. https://doi.org/10.1016/j.biombioe.2015.01.018
57. Liu G, Bao J (2019) Constructing super large scale cellulosic ethanol plant by decentralizing dry acid pretreatment technology into biomass collection depots. Bioresour Technol 275:338–344. https://doi.org/10.1016/j.biortech.2018.12.061
58. Martelli R, Bentini M (2015) REMOVED: harvest storage and handling of round and square bales of giant reed and switchgrass, an economic and technical evaluation. Biomass Bioenerg 73:67–76. https://doi.org/10.1016/j.biombioe.2014.12.008
59. Sokhansanj S, Mani S, Tagore S, Turhollow AF (2010) Techno-economic analysis of using corn stover to supply heat and power to a corn ethanol plant—Part 1: Cost of feedstock supply logistics. Biomass Bioenerg 34:75–81. https://doi.org/10.1016/j.biombioe.2009.10.001
60. Zhang J, Shao S, Bao J (2016) Long term storage of dilute acid pretreated corn stover feedstock and ethanol fermentability evaluation. Bioresour Technol 201:355–359. https://doi.org/10.1016/j.biortech.2015.11.024
61. Liu G, Bao J (2017) Evaluation of electricity generation from lignin residue and biogas in cellulosic ethanol production. Bioresour Technol 243:1232–1236. https://doi.org/10.1016/j.biortech.2017.07.022
62. Uppugundla N, da Costa Sousa L, Chundawat SPS, Yu X, Simmons B, Singh S, Gao X, Kumar R, Wyman CE, Dale BE, Balan V (2014) A comparative study of ethanol production using dilute acid, ionic liquid and AFEX™ pretreated corn stover. Biotechnol Biofuels 7:72. https://doi.org/10.1186/1754-6834-7-72
63. Smullen E, Finnan J, Dowling D, Mulcahy P (2019) The environmental performance of pretreatment technologies for the bioconversion of lignocellulosic biomass to ethanol. Renew Energy 142:527–534. https://doi.org/10.1016/j.renene.2019.04.082
64. Hernández D, Riaño B, Coca M, García-González MC (2015) Saccharification of carbohydrates in microalgal biomass by physical, chemical and enzymatic pre-treatments as a previous step for bioethanol production. Chem Eng J 262:939–945. https://doi.org/10.1016/j.cej.2014.10.049
65. Kim KH, Choi IS, Kim HM, Wi SG, Bae H-J (2014) Bioethanol production from the nutrient stress-induced microalga *Chlorella vulgaris* by enzymatic hydrolysis and immobilized yeast fermentation. Bioresour Technol 153:47–54. https://doi.org/10.1016/j.biortech.2013.11.059
66. Ngamsirisomsakul M, Reungsang A, Liao Q, Kongkeitkajorn MB (2019) Enhanced bio-ethanol production from *Chlorella* sp. biomass by hydrothermal pretreatment and enzymatic hydrolysis. Renew Energy 141:482–492. https://doi.org/10.1016/j.renene.2019.04.008
67. Colla LM, Margarites ACF, Decesaro A, Magro FG, Kreling N, Rempel A, Machado TS (2019) Waste biomass and blended bioresources in biogas production. In: Fongaro G, Treichel H (eds) Improving biogas production: technological challenges, alternative sources, future developments. Springer International Publishing, Cham, pp 1–23
68. Raposo F, De la Rubia MA, Fernández-Cegrí V, Borja R (2012) Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. Renew Sustain Energy Rev 16:861–877. https://doi.org/10.1016/j.rser.2011.09.008
69. Venturin B, Frumi Camargo A, Scapini T, Mulinari J, Bonatto C, Bazoti S, Pereira Siqueira D, Maria Colla L, Alves SL, Paulo Bender J, Luís Radis Steinmetz R, Kunz A, Fongaro G, Treichel H (2018) Effect of pretreatments on corn stalk chemical properties for biogas production purposes. Bioresour Technol 266. https://doi.org/10.1016/j.biortech.2018.06.069

70. Zabed HM, Akter S, Yun J, Zhang G, Awad FN, Qi X, Sahu JN (2019) Recent advances in biological pretreatment of microalgae and lignocellulosic biomass for biofuel production. Renew Sustain Energy Rev 105:105–128. https://doi.org/10.1016/j.rser.2019.01.048
71. Liu J, Wang ML, Tonnis B, Habteselassie M, Liao X, Huang Q (2013) Fungal pretreatment of switchgrass for improved saccharification and simultaneous enzyme production. Bioresour Technol 135:39–45. https://doi.org/10.1016/j.biortech.2012.10.095
72. Nazarpour F, Abdullah KD, Abdullah N, Zamiri R (2013) Evaluation of biological pretreatment of rubberwood with white rot fungi for enzymatic hydrolysis. Materials 6
73. Wan C, Li Y (2010) Microbial delignification of corn stover by *Ceriporiopsis subvermispora* for improving cellulose digestibility. Enzyme Microb Technol 47:31–36. https://doi.org/10.1016/j.enzmictec.2010.04.001
74. Schroyen M, Vervaeren H, Vandepitte H, Van Hulle SWH, Raes K (2015) Effect of enzymatic pretreatment of various lignocellulosic substrates on production of phenolic compounds and biomethane potential. Bioresour Technol 192:696–702. https://doi.org/10.1016/j.biortech.2015.06.051
75. Mustafa AM, Poulsen TG, Sheng K (2016) Fungal pretreatment of rice straw with *Pleurotus ostreatus* and *Trichoderma reesei* to enhance methane production under solid-state anaerobic digestion. Appl Energy 180:661–671. https://doi.org/10.1016/j.apenergy.2016.07.135
76. Ometto F, Quiroga G, Pšenička P, Whitton R, Jefferson B, Villa R (2014) Impacts of microalgae pre-treatments for improved anaerobic digestion: thermal treatment, thermal hydrolysis, ultrasound and enzymatic hydrolysis. Water Res 65:350–361. https://doi.org/10.1016/j.watres.2014.07.040
77. Muñoz C, Hidalgo C, Zapata M, Jeison D, Riquelme C, Rivas M (2014) Use of cellulolytic marine bacteria for enzymatic pretreatment in microalgal biogas production. Appl Environ Microbiol 80:4199–4206. https://doi.org/10.1128/aem.00827-14
78. Lin CSK, Pfaltzgraff LA, Herrero-Davila L, Mubofu EB, Abderrahim S, Clark JH, Koutinas AA, Kopsahelis N, Stamatelatou K, Dickson F, Thankappan S, Mohamed Z, Brocklesby R, Luque R (2013) Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. Energy Environ Sci 6:426–464. https://doi.org/10.1039/C2EE23440H
79. Cheong CW, Lee YS, Ahmad SA, Ooi PT, Phang LY (2018) Chicken feather valorization by thermal alkaline pretreatment followed by enzymatic hydrolysis for protein-rich hydrolysate production. Waste Manag 79:658–666. https://doi.org/10.1016/j.wasman.2018.08.029
80. Mokrejs P, Svoboda P, Hrncirik J, Janacova D, Vasek V (2010) Processing poultry feathers into keratin hydrolysate through alkaline-enzymatic hydrolysis. Waste Manag Res 29:260–267. https://doi.org/10.1177/0734242X10370378
81. Callegaro K, Brandelli A, Daroit DJ (2019) Beyond plucking: feathers bioprocessing into valuable protein hydrolysates. Waste Manag 95:399–415. https://doi.org/10.1016/j.wasman.2019.06.040
82. Orak T, Caglar O, Ortucu S, Ozkan H, Taskin M (2018) Chicken feather peptone: a new alternative nitrogen source for pigment production by *Monascus purpureus*. J Biotechnol 271:56–62. https://doi.org/10.1016/j.jbiotec.2018.02.010
83. Sinkiewicz I, Śliwińska A, Staroszczyk H, Kołodziejska I (2017) Alternative methods of preparation of soluble keratin from chicken feathers. Waste Biomass Valoriz 8:1043–1048. https://doi.org/10.1007/s12649-016-9678-y
84. Toldrá F, Mora L, Reig M (2016) New insights into meat by-product utilization. Meat Sci 120:54–59. https://doi.org/10.1016/j.meatsci.2016.04.021
85. Dasgupta A, Chandel MK (2019) Enhancement of biogas production from organic fraction of municipal solid waste using hydrothermal pretreatment. Bioresour Technol Reports 100281. doi: https://doi.org/10.1016/j.biteb.2019.100281

Chapter 6
The Future of Biomaterials Engineering and Biomass Pretreatments

The energy and environmental crises that the world is facing are forcing us to reassess the efficient use of natural resources and to identify alternative uses through clean technologies. In this sense, lignocellulosic biomass has considerable potential to meet the current energy demand of the modern world. Trends drive biotechnology in search of improved products. To overcome current energy problems, lignocellulosic biomass, in addition to the circular economy, is expected to be the main focus of research in the near future.

Some technical challenges of biomass pretreatments that need to be addressed to be commercially viable in biomass processing are described below. We cover general and then specific promising techniques, with recommendations that provide directions for future research.

6.1 General Challenges

Laboratory-scale experiments of pretreatments were thoroughly discussed, including the reaction mechanisms, optimal conditions of the various processes, and the degradation kinetics. Future research should focus on optimizing the techniques reported in previous chapters, as well as on how and where they are being used. For example, in catalytic and enzymatic pretreatments, new compounds and enzymes need to be studied to increase efficacy while decreasing long-term toxicity.

Pretreatment process parameters should be studied with process optimization techniques, including experimental design, to increase production and maximize energy consumption [1]. Various response difficulties can be resolved using alternative techniques, sequentially or concomitantly. The combination of two or three pretreatment methods can be considered for commercial-scale process development. A physical pretreatment method such as extrusion can be integrated with biological and ionic pretreatment methods. An integrated method can overcome many economic, environmental, and technological problems of a single pretreatment method. An example of this may be the integration of ionic pretreatment with microwave

H. Treichel et al., *Utilising Biomass in Biotechnology*,
Green Energy and Technology, https://doi.org/10.1007/978-3-030-22853-8_6

or ultrasound methods instead of conventional heating, dilute acid or alkaline treatments, and steam explosion. In so doing, the advantages of each technique can be realized and the disadvantages can be minimized.

Another challenge is the establishment of scale-up rules and rheological studies. The study of hydrodynamics and kinetics of reactions occurring during pretreatments is extremely important for the development of large-scale processes. Studies involving the design and optimization of pretreatment reactors are rare in the literature. Normally, dimensional analyses are performed to establish scaling rules. Dimensional analysis is a mathematical method involving measurements of mass, length, and time to establish scaling rules. We recommend that the knowledge obtained on the laboratory and bench scales be used to establish scaling rules and to study hydrodynamics and reaction kinetics on a large scale [2].

Rheological investigation of pretreatment reactions to understand mass and heat transfer mechanisms and identify ways to improve process efficiency and solvent recovery, where appropriate, is of paramount importance. Future work should focus on the effects of shear rate, temperature, concentration, and viscosity of the employed fluids. We should leverage our understanding of Newtonian, non-Newtonian, and pseudoplastic behavior of reaction liquids under different operating conditions [2].

6.2 One-Off Challenges

Some pretreatment techniques deserve to be highlighted as promising and considered the main alternatives for the future. However, in addition to opportunities, they nevertheless present challenges that need to be overcome.

6.2.1 Microwave

Microwave can be considered an affordable and environmentally friendly technology, yielding gains over those of conventional heating reactions. Microwave pretreatments lead to high yields and reduce process times. However, there are few studies on the use of microwave reactors for lignocellulosic biomass, requiring pilot- or industrial-scale pretreatments to dedicate efforts in this area. The development of microwave reactors and systems is required to operate at high loads and high pressures to prevent the formation of hot spots that lead to the formation of inhibitor compounds; therefore, homogeneous heat transfer should be performed.

Microwave heating to decrease lignocellulosic biomass recalcitrance is a technology that is just beginning to be developed and can be considered as a technique that is not well established because of the few types of biomass that have been studied. The dielectric properties of lignocellulosic biomass should be studied to select the most suitable microwave materials and to facilitate the establishment of optimal pretreatment conditions [3].

Despite the fact that microwave irradiation has advantages and increases biofuel production, there remain some aspects of technology that require further investigation, including the formation of inhibitors. Biomass pretreatment results in better digestibility and improved biofuel production through anaerobic digestion and fermentation processes; however, pretreatments can also generate inhibitors. Studies of microwave pretreatment in lignocellulosic biomass have reported generation of inhibitors such as 5-HMF, furfural, phenolic compounds, and acetic acids [4–7]. One of the strategies to circumvent this inhibition is the use of activated charcoal for microwave-assisted hydrolysate detoxification; nevertheless, the use of activated charcoal as a detoxifying agent has also resulted in glucose losses [4, 7]. Studies involving inhibitor removal represent an area for future research. It is desirable to document effects of inhibitors managed without influence on carbon production, including the acclimatization of microorganisms to inhibitors before the production of biofuels, using recalcitrant and larger substrates to prevent formation of inhibitors.

Another aspect that remains to be improved regarding microwave irradiation is energy efficiency. Pretreatment was successfully applied to various biomasses improving biofuel production in most studies. However, energy efficiencies were negative in most studies, suggesting that increased biofuel production would not compensate for energy input from microwave systems. Biodiesel production, on the other hand, is an example where it was found that the microwave-assisted was more energy efficient than conventional process [8–12]. The energy efficiency and energy consumption of microwave-assisted pretreatment techniques should be the focus of future research, as this will determine the economic viability and even scalability of this technology.

Technical aspects such as the addition of microwave absorbers should also be studied. Only dielectric compounds are able to absorb microwaves for subsequent heating. Biomass in general has poor dielectric properties; therefore, a microwave absorber becomes indispensable in processes that need to reach high temperatures [13, 14]. Heterogeneous materials can produce non-uniform heating, creating additional problems [15]. Another major challenge of large-scale application is that microwaves cannot penetrate through a large amount of raw material [16, 17]. This imposes a severe restriction on the amount of materials that can be heated. If the amount of raw material that can be processed is very low, this severely affects the viability of pretreatment technology and the process of producing biofuel on a large scale.

Process parameters involving microwave heating for biomass pretreatment will need optimization and should consider the raw material that will be pretreated to minimize inhibitor formation and maximize biofuel production, energy efficiency, and process economics. Mathematical modeling of such processes prior to large-scale implementation will be an important tool in determining the feasibility of technology [18].

6.2.2 Biological

Pretreatments involving microorganisms and enzymatic cocktails have great potential; however, some challenges have yet to be overcome. These challenges include long pretreatment times, non-selective lignin breakdown, and relatively low yields compared to other thermochemical methods. Biological pretreatment results may vary depending on biomass composition and degree of recalcitrance, strains, and variations in the metabolic efficiency of microorganisms, and even the high selectivity of enzymes precludes successful performance for various types of biomass.

On the other hand, some studies reported a higher efficiency of biological pretreatments compared to conventional techniques. Fungal pretreatment is the best approach among biological pretreatments; however, the long incubation time restricts its application on a large scale. By contrast, bacterial growth is faster, resulting in shorter pretreatment times; however, the yields are lower [19–21].

Non-selective lignin removal or loss of cellulose and hemicellulose strongly depends on the fungal strains, the pretreated biomass, and the operating conditions. Although holocellulose loss, especially hemicellulose, was also observed in conventional methods with the formation of several inhibitors, carbohydrate loss appeared to be greater with fungal pretreatments, because microorganisms end up using these fractions as substrates for their growth [22–24].

To overcome such challenges, biological pretreatments must be combined with other techniques to reduce overall pretreatment time and increase efficiency. For example, the combined pretreatment of fungus and milling resulted in a significant improvement in delignification of rice straw from 92% (fungus) to 165% (fungus + milling) [25, 26].

Another interesting strategy is the use of microbial consortia, knowing that such associations are able to reduce pretreatment times. Isolation and use of microbial strains with high selective power have been suggested to minimize carbohydrate loss. Such losses can be further reduced by optimizing the pretreatment conditions and genetic modification of genes encoding ligninolytic enzymes [27, 28].

One of the research trends involving biological pretreatments is the selection of microorganisms residing in the alimentary canal of ruminant animals. These microorganisms hydrolyze various recalcitrant components present in the plant cell wall by producing various extracellular hydrolytic enzymes [29–33].

Additional efforts should be focused on achieving the best efficiency, specificity, and tolerance by applying metabolic engineering, mutagenesis, and genomic mutation. Several techniques are available for gene editing that can also be applied to ligninolytic microorganisms to increase pretreatment efficiency [34–36]. The development and application of genetically modified organisms are intended to overcome the challenges encountered from naturally occurring strains.

In many cases, we are still in the process of developing tools to manipulate particular species; nevertheless, advances are being made on the laboratory and pilot scales [37]. Genetically modified organisms have a unique potential not only to produce more enzymes but also to generate those that can tolerate extreme operating/environmental conditions [37, 38]. Recent research has focused on the direct application of genetic engineering to enzyme production as well as to microorganisms, significantly improving the ability of enzymes to break down lignocellulosic biomass. This technique led to the fusion of enzymes from two species of bacteria to solubilize lignin [39, 40].

Enzyme recycling is another approach to reduce the amount of enzyme consumed during the pretreatment process, consequently minimizing operating costs [41]. Recycling the insoluble biomass fraction after enzymatic pretreatment to the start of the process recovered cellulase activity, and as a result, enzyme consumption was reduced by 30% without significant change in final glucose yield [42]. It is noteworthy that for an industrial plant, the cost of recycling facilities (dewatering, pumping, etc.) will increase the plant's capital cost; therefore, further studies are needed to investigate whether cost savings from enzyme recycling can offset the additional capital investment of extra processing equipment [40, 43].

Again, the implementation of large-scale biological pretreatments remains hampered by various techno-economic issues. Consequently, further studies are needed to develop biological pretreatments. Key issues to be addressed in these scale-up studies should include reactor designs, biomass supply chain, decontamination and cooling of raw materials, inoculum preparation or enzymatic cocktails, microbial growth and metabolism monitoring, temperature control and ventilation, and finally, evaluation of economic factors for cost estimation [26, 28, 40, 44].

Compared to conventional thermochemical pretreatment techniques, biological and enzymatic pretreatments have lower energy consumption, requiring milder operating conditions and less by-product formation. Biological and enzymatic pretreatments are gaining increased attention and will be the main forms of pretreatments in the future.

6.2.3 Ionic Liquids

Some challenges for the use of ionic liquids in pretreatments have yet to be overcome, even with their demonstrated effectiveness.

Generally, ionic liquids tolerate high temperatures; however, there are exceptions, and such solvents have varying tolerance ranges. Biomass pretreatments usually occur at high temperatures (100 °C); therefore, the ionic liquids used should be stable at these temperatures; however, most studies are not concerned with this. The cation of the ionic liquid easily decomposes at elevated temperatures, and such dissociation is favored by the associated anion. Currently, thermogravimetric analysis is the best analysis to determine the stability of the obtained compounds, calculating the weight loss of the sample in relation to the temperature [45, 46].

Another central problem with ionic liquid pretreatments is moisture sensitivity. These substances should not contain water, and even the water content of lignocellulosic biomass should be removed prior to treatment with ionic liquids. The presence of water above 0.15% in ionic liquids causes cellulose precipitation, thereby substantially affecting the dissolution process [47]. The presence of water also affects regeneration and recycling. To avoid such problems, the biomass must be kiln-dried prior to processing, a process that entails an operation prior to the pretreatment itself. New pretreatment techniques that are moisture-tolerant should be sought [48].

Ionic liquid research should recognize the possible dangers that these substances may cause to the environment and to humans. Fortunately, ionic liquids are considered to have low environmental impacts; nevertheless, some of their toxic effects have been observed during ecotoxicological and biodegradation studies, suggesting that these chemicals should be handled with care. Because of their nonvolatile nature, ionic liquids do not enter the environment through the air; however, they are highly miscible in water and can cause damage upon entering receptor bodies [49–54]. The biocompatibility of ionic liquids with enzymes used for fermentation has yet to be improved to allow enzymatic hydrolysis in a combined reactor process [46].

As with all state-of-the-art technologies, ionic liquids are costly because the reagents used for their production are expensive. Therefore, a challenge is precisely in the production of cheap ionic liquids. Alternative methods and resources are being discovered to reduce the cost of synthesis. One alternative is recycling, which can contribute considerably to reducing the effective cost of ionic liquids for biomass pretreatment; this should be done quickly and cheaply [55]. As an example of cost reduction in manufacturing, one study reported synthesis based on various alkyl ammonium cations and hydrogen sulfate anions and their use in pretreatment. In another study, ionic liquid was recovered from the mixture after pretreatment at high percentages. The calculation of the cost of this technique was presented using a technical–economic model [56]. Several authors claimed that ionic liquid is priced relatively low at $1000 per ton [57–59]. Preparation of ionic liquids from natural sources should be sought as this will generate more economical solvents.

Ionic liquids have been studied for some years for the pretreatment of lignocellulosic biomass, because of their high efficiency, reasonable economic viability, eco-friendly, and non-toxic physicochemical characteristics. With high capacity for pretreatment of biomass components, however, it is necessary to develop economical, high-yield, ecologically correct, and viable ionic liquids for process scaling at the industrial level. Doing so will make biomass available and so abundant it can be used properly, applying all the circular economy concepts that should guide future bioprocesses.

References

1. Rodrigues MI, Iemma AF (2014) Experimental design and process optimization, 1st edn. CRC Press, Boca Raton
2. Halder P, Kundu S, Patel S, Setiawan A, Atkin R, Parthasarthy R, Paz-Ferreiro J, Surapaneni A, Shah K (2019) Progress on the pre-treatment of lignocellulosic biomass employing ionic liquids. Renew Sustain Energy Rev 105:268–292. https://doi.org/10.1016/j.rser.2019.01.052

3. Aguilar-Reynosa A, Romaní A, Ma. Rodríguez-Jasso R, Aguilar CN, Garrote G, Ruiz HA (2017) Microwave heating processing as alternative of pretreatment in second-generation biorefinery: an overview. Energy Convers Manag 136:50–65. https://doi.org/10.1016/j.enconman.2017.01.004
4. Luo Y, Li Z, Li X, Liu X, Fan J, Clark JH, Hu C (2019) The production of furfural directly from hemicellulose in lignocellulosic biomass: a review. Catal Today 319:14–24. https://doi.org/10.1016/j.cattod.2018.06.042
5. Rong C, Ding X, Zhu Y, Li Y, Wang L, Qu Y, Ma X, Wang Z (2012) Production of furfural from xylose at atmospheric pressure by dilute sulfuric acid and inorganic salts. Carbohydr Res 350:77–80. https://doi.org/10.1016/j.carres.2011.11.023
6. Ragauskas AJ, Beckham GT, Biddy MJ, Chandra R, Chen F, Davis MF, Davison BH, Dixon RA, Gilna P, Keller M, Langan P, Naskar AK, Saddler JN, Tschaplinski TJ, Tuskan GA, Wyman CE (2014) Lignin valorization: improving lignin processing in the biorefinery. Science (80-) 344:1246843. https://doi.org/10.1126/science.1246843
7. Salvachúa D, Prieto A, López-Abelairas M, Lu-Chau T, Martínez ÁT, Martínez MJ (2011) Fungal pretreatment: an alternative in second-generation ethanol from wheat straw. Bioresour Technol 102:7500–7506. https://doi.org/10.1016/j.biortech.2011.05.027
8. Bugg TDH, Ahmad M, Hardiman EM, Singh R (2011) The emerging role for bacteria in lignin degradation and bio-product formation. Curr Opin Biotechnol 22:394–400. https://doi.org/10.1016/j.copbio.2010.10.009
9. Martínez ÁT, Speranza Mariela, Ruiz-Dueñas Francisco J, Ferreira Patricia, Camarero Susana, Guillén Francisco, Martínez María J, Gutiérrez Ana, del Río José C (2005) Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin Lignocellulosic materials. Int Microbiol 8:195–204
10. Kim J-S (2015) Production, separation and applications of phenolic-rich bio-oil—a review. Bioresour Technol 178:90–98. https://doi.org/10.1016/j.biortech.2014.08.121
11. Romaní A, Michelin M, Domingues L, Teixeira JA (2018) Chapter 16: Valorization of wastes from agrofood and pulp and paper industries within the biorefinery concept: Southwestern Europe scenario. In: Bhaskar T, Pandey A, Mohan SV, Lee D-J, Khanal SK (eds) WB. Elsevier, Amsterdam, pp 487–504
12. Max B, Torrado AM, Moldes AB, Converti A, Domínguez JM (2009) Ferulic acid and p-coumaric acid solubilization by alkaline hydrolysis of the solid residue obtained after acid prehydrolysis of vine shoot prunings: effect of the hydroxide and pH. Biochem Eng J 43:129–134. https://doi.org/10.1016/j.bej.2008.09.015
13. Schmidt RJ (2005) Industrial catalytic processes—phenol production. Appl Catal A Gen 280:89–103. https://doi.org/10.1016/j.apcata.2004.08.030
14. Omoriyekomwan JE, Tahmasebi A, Yu J (2016) Production of phenol-rich bio-oil during catalytic fixed-bed and microwave pyrolysis of palm kernel shell. Bioresour Technol 207:188–196. https://doi.org/10.1016/j.biortech.2016.02.002
15. Oliveira L de L de, Carvalho MV de, Melo L (2014) Health promoting and sensory properties of phenolic compounds in food. Rev Ceres 61:764–779
16. Tsao R, Yang R (2003) Optimization of a new mobile phase to know the complex and real polyphenolic composition: towards a total phenolic index using high-performance liquid chromatography. J Chromatogr A 1018:29–40. https://doi.org/10.1016/j.chroma.2003.08.034
17. Milenkovic D, Deval C, Dubray C, Mazur A, Morand C (2011) Hesperidin displays relevant role in the nutrigenomic effect of orange juice on blood leukocytes in human volunteers: a randomized controlled cross-over study. PLoS ONE 6:e26669
18. Ramful D, Tarnus E, Aruoma OI, Bourdon E, Bahorun T (2011) Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. Food Res Int 44:2088–2099. https://doi.org/10.1016/j.foodres.2011.03.056
19. Putro JN, Soetaredjo FE, Lin S-Y, Ju Y-H, Ismadji S (2016) Pretreatment and conversion of lignocellulose biomass into valuable chemicals. RSC Adv 6:46834–46852. https://doi.org/10.1039/C6RA09851G

20. Li Y-J, Li H-Y, Sun S-N, Sun R-C (2019) Evaluating the efficiency of γ-valerolactone/water/acid system on Eucalyptus pretreatment by confocal Raman microscopy and enzymatic hydrolysis for bioethanol production. Renew Energy 134:228–234. https://doi.org/10.1016/j.renene.2018.11.038
21. Obregón I, Gandarias I, Al-Shaal MG, Mevissen C, Arias PL, Palkovits R (2016) The role of the hydrogen source on the selective production of γ-valerolactone and 2-methyltetrahydrofuran from levulinic acid. Chemsuschem 9:2488–2495. https://doi.org/10.1002/cssc.201600751
22. Zhao X, Peng F, Cheng K, Liu D (2009) Enhancement of the enzymatic digestibility of sugarcane bagasse by alkali–peracetic acid pretreatment. Enzyme Microb Technol 44:17–23. https://doi.org/10.1016/j.enzmictec.2008.07.011
23. Mishra V, Jana AK, Jana MM, Gupta A (2017) Enhancement in multiple lignolytic enzymes production for optimized lignin degradation and selectivity in fungal pretreatment of sweet Sorghum bagasse. Bioresour Technol 236:49–59. https://doi.org/10.1016/j.biortech.2017.03.148
24. Zhang X, Yu H, Huang H, Liu Y (2007) Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culms. Int Biodeterior Biodegrad 60:159–164. https://doi.org/10.1016/j.ibiod.2007.02.003
25. Mustafa AM, Poulsen TG, Xia Y, Sheng K (2017) Combinations of fungal and milling pretreatments for enhancing rice straw biogas production during solid-state anaerobic digestion. Bioresour Technol 224:174–182. https://doi.org/10.1016/j.biortech.2016.11.028
26. Zabed HM, Akter S, Yun J, Zhang G, Awad FN, Qi X, Sahu JN (2019) Recent advances in biological pretreatment of microalgae and lignocellulosic biomass for biofuel production. Renew Sustain Energy Rev 105:105–128. https://doi.org/10.1016/j.rser.2019.01.048
27. Chang AJ, Fan J, Wen X (2012) Screening of fungi capable of highly selective degradation of lignin in rice straw. Int Biodeterior Biodegrad 72:26–30. https://doi.org/10.1016/j.ibiod.2012.04.013
28. Sharma HK, Xu C, Qin W (2019) biological pretreatment of lignocellulosic biomass for biofuels and bioproducts: an overview. Waste Biomass Valoriz 10:235–251. https://doi.org/10.1007/s12649-017-0059-y
29. Aydin S, Yıldırım E, Ince O, Ince B (2017) Rumen anaerobic fungi create new opportunities for enhanced methane production from microalgae biomass. Algal Res 23:150–160. https://doi.org/10.1016/j.algal.2016.12.016
30. Wang Y, McAllister TA (2002) Rumen microbes, enzymes and feed digestion—a review. Asian-Australas J Anim Sci 15:1659–1676. https://doi.org/10.5713/ajas.2002.1659
31. Giménez JB, Aguado D, Bouzas A, Ferrer J, Seco A (2017) Use of rumen microorganisms to boost the anaerobic biodegradability of microalgae. Algal Res 24:309–316. https://doi.org/10.1016/j.algal.2017.04.003
32. Barragán-Trinidad M, Carrillo-Reyes J, Buitrón G (2017) Hydrolysis of microalgal biomass using ruminal microorganisms as a pretreatment to increase methane recovery. Bioresour Technol 244:100–107. https://doi.org/10.1016/j.biortech.2017.07.117
33. Carrillo-Reyes J, Barragán-Trinidad M, Buitrón G (2016) Biological pretreatments of microalgal biomass for gaseous biofuel production and the potential use of rumen microorganisms: a review. Algal Res 18:341–351. https://doi.org/10.1016/j.algal.2016.07.004
34. Fokum E, Zabed HM, Guo Q, Yun J, Yang M, Pang H, An Y, Li W, Qi X (2019) Metabolic engineering of bacterial strains using CRISPR/Cas9 systems for biosynthesis of value-added products. Food Biosci 28:125–132. https://doi.org/10.1016/j.fbio.2019.01.003
35. Magocha TA, Zabed H, Yang M, Yun J, Zhang H, Qi X (2018) Improvement of industrially important microbial strains by genome shuffling: current status and future prospects. Bioresour Technol 257:281–289. https://doi.org/10.1016/j.biortech.2018.02.118
36. Bilal M, Iqbal HMN, Hu H, Wang W, Zhang X (2018) Metabolic engineering and enzyme-mediated processing: a biotechnological venture towards biofuel production—a review. Renew Sustain Energy Rev 82:436–447. https://doi.org/10.1016/j.rser.2017.09.070
37. Lam FH, Ghaderi A, Fink GR, Stephanopoulos G (2014) Biofuels. Engineering alcohol tolerance in yeast. Science 346:71–75. https://doi.org/10.1126/science.1257859

38. Ottenheim C, Werner KA, Zimmermann W, Wu JC (2015) Improved endoxylanase production and colony morphology of *Aspergillus niger* DSM 26641 by γ-ray induced mutagenesis. Biochem Eng J 94:9–14. https://doi.org/10.1016/j.bej.2014.10.020
39. Tumen-Velasquez M, Johnson CW, Ahmed A, Dominick G, Fulk EM, Khanna P, Lee SA, Schmidt AL, Linger JG, Eiteman MA, Beckham GT, Neidle EL (2018) Accelerating pathway evolution by increasing the gene dosage of chromosomal segments. Proc Natl Acad Sci 115:7105–7110. https://doi.org/10.1073/pnas.1803745115
40. Hosseini Koupaie E, Dahadha S, Bazyar Lakeh AA, Azizi A, Elbeshbishy E (2019) Enzymatic pretreatment of lignocellulosic biomass for enhanced biomethane production—a review. J Environ Manage 233:774–784. https://doi.org/10.1016/j.jenvman.2018.09.106
41. Xing W, Xu G, Dong J, Han R, Ni Y (2018) Novel dihydrogen-bonding deep eutectic solvents: pretreatment of rice straw for butanol fermentation featuring enzyme recycling and high solvent yield. Chem Eng J 333:712–720. https://doi.org/10.1016/j.cej.2017.09.176
42. Weiss A (2013) Ancient and modern apocalypse from a genre theory perspective. Brill, Leiden, The Netherlands, pp 1–14
43. Jørgensen H, Pinelo M (2017) Enzyme recycling in lignocellulosic biorefineries. Biofuels Bioprod Biorefining 11:150–167. https://doi.org/10.1002/bbb.1724
44. Tian X, Fang Z, Guo F (2012) Impact and prospective of fungal pre-treatment of lignocellulosic biomass for enzymatic hydrolysis. Biofuels Bioprod Biorefining 6:335–350. https://doi.org/10.1002/bbb.346
45. King AWT, Parviainen A, Karhunen P, Matikainen J, Hauru LKJ, Sixta H, Kilpeläinen I (2012) Relative and inherent reactivities of imidazolium-based ionic liquids: the implications for lignocellulose processing applications. RSC Adv 2:8020–8026. https://doi.org/10.1039/C2RA21287K
46. Asim AM, Uroos M, Naz S, Sultan M, Griffin G, Muhammad N, Khan AS (2019) Acidic ionic liquids: promising and cost-effective solvents for processing of lignocellulosic biomass. J Mol Liq 287:110943. https://doi.org/10.1016/j.molliq.2019.110943
47. Mazza M, Catana D-A, Vaca-Garcia C, Cecutti C (2009) Influence of water on the dissolution of cellulose in selected ionic liquids. Cellulose 16:207–215. https://doi.org/10.1007/s10570-008-9257-x
48. Ogura K, Ninomiya K, Takahashi K, Ogino C, Kondo A (2014) Pretreatment of Japanese cedar by ionic liquid solutions in combination with acid and metal ion and its application to high solid loading. Biotechnol Biofuels 7:120. https://doi.org/10.1186/s13068-014-0120-z
49. Biczak R, Pawłowska B, Bałczewski P, Rychter P (2014) The role of the anion in the toxicity of imidazolium ionic liquids. J Hazard Mater 274:181–190. https://doi.org/10.1016/j.jhazmat.2014.03.021
50. Grzonkowska M, Sosnowska A, Barycki M, Rybinska A, Puzyn T (2016) How the structure of ionic liquid affects its toxicity to *Vibrio fischeri*? Chemosphere 159:199–207. https://doi.org/10.1016/j.chemosphere.2016.06.004
51. Petkovic M, Seddon KR, Rebelo LPN, Silva Pereira C (2011) Ionic liquids: a pathway to environmental acceptability. Chem Soc Rev 40:1383–1403. https://doi.org/10.1039/C004968A
52. Egorova KS, Ananikov VP (2014) Toxicity of ionic liquids: eco(cyto)activity as complicated, but unavoidable parameter for task-specific optimization. Chemsuschem 7:336–360. https://doi.org/10.1002/cssc.201300459
53. Amde M, Liu J-F, Pang L (2015) Environmental application, fate, effects, and concerns of ionic liquids: a review. Environ Sci Technol 49:12611–12627. https://doi.org/10.1021/acs.est.5b03123
54. Reid JESJ, Prydderch H, Spulak M, Shimizu S, Walker AJ, Gathergood N (2018) Green profiling of aprotic versus protic ionic liquids: synthesis and microbial toxicity of analogous structures. Sustain Chem Pharm 7:17–26. https://doi.org/10.1016/j.scp.2017.11.001
55. Tiong YW, Yap CL, Gan S, Yap WSP (2018) Kinetic study on oil palm biomass conversion to levulinic acid via indium trichloride-ionic liquids. IOP Conf Ser Earth Environ Sci 164:12007. https://doi.org/10.1088/1755-1315/164/1/012007

56. Klein-Marcuschamer D, Simmons BA, Blanch HW (2011) Techno-economic analysis of a lignocellulosic ethanol biorefinery with ionic liquid pre-treatment. Biofuels Bioprod Biorefining 5:562–569. https://doi.org/10.1002/bbb.303
57. Prado R, Erdocia X, De Gregorio GF, Labidi J, Welton T (2016) Willow lignin oxidation and depolymerization under low cost ionic liquid. ACS Sustain Chem Eng 4:5277–5288. https://doi.org/10.1021/acssuschemeng.6b00642
58. Khokarale SG, Le-That T, Mikkola J-P (2016) Carbohydrate free lignin: a dissolution-recovery cycle of sodium lignosulfonate in a switchable ionic liquid system. ACS Sustain Chem Eng 4:7032–7040. https://doi.org/10.1021/acssuschemeng.6b01927
59. Achinivu EC, Howard RM, Li G, Gracz H, Henderson WA (2014) Lignin extraction from biomass with protic ionic liquids. Green Chem 16:1114–1119. https://doi.org/10.1039/C3GC42306A

Chapter 7
Final Considerations

Research and development of processes using biomass involve understanding the advantages and disadvantages of each pretreatment and the factors that act on the lignocellulosic matrix recalcitrance breakdown mechanism to improve cellulose, hemicellulose, and lignin separation and/or hydrolysis.

Utilization of the constituents of the various biomasses should be maximized, homogenizing the diversity of physicochemical and constitutional characteristics of biomasses located near production plants, with the purpose of generating products with unique pretreatment requirements. Combined pretreatments tend to provide better yields during enzymatic hydrolysis of lignocellulosic biomass. Nevertheless, rising operating costs may be involved simultaneously. Proper combination pretreatment should not only improve the digestibility of biomass at relatively low operating costs but also maximize the utilization of its components.

In this context, biomaterial engineering applied to waste biomasses uses should consider the lowest possible number of degradation reactions of its components and generation of pollutants, aiming at natural resources optimization, minimization secondary and tertiary residues with technical and economic feasibility, considering aspects of sustainability, and safety of the circular economy approach.

H. Treichel et al., *Utilising Biomass in Biotechnology*,
Green Energy and Technology, https://doi.org/10.1007/978-3-030-22853-8_7