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REVIEW

Industrial Lignocellulosic Waste Biomasses: Enormous Substrates for Harnessing Enzymes and Bioethanol Productions

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ABSTRACT: Lignocellulosic waste, consisting mainly of cellulose, hemicellulose and lignin, is the most abundant global biomass. Industries generate millions of tonnes of this waste biomass every year. This includes: sugarcane bagasse, corncob, rice straw and husk, oil palm fibres and empty fruit bunches, wheat straw, brewer's spent grains, among others. These wastes could be valorized to produce high-value industrial lignocellulase enzymes, such as cellulases, hemicellulases and ligninases, by several microorganisms, including bacteria and fungi. Furthermore, the crude or purified lignocellulase enzymes or the microbes producing these enzymes could be used to hydrolyze the lignocellulosic wastes into simple sugars. The sugars are then fermented to bioethanol using simultaneous saccharification and fermentation, or a separate hydrolysis and fermentation technique. However, an initial pretreatment of lignocellulosic biomass greatly facilitates delignification and deconstruction of the lignocellulose complex structure, making the cellulose and/or hemicellulose components more accessible for enzymolysis. This review presents a holistic compilation of the different industrial lignocellulosic waste biomasses. It also highlights the microbial enzymes that can hydrolyze the lignocellulosic biomasses for industrial applications, including the production of fermentable sugars and bioethanol, as well as the different microorganisms producing the enzymes. The processes in the production of these metabolites, and the challenges encountered with the valorization of these wastes, as well as the possible solutions, are also adequately discussed.

KEYWORDS: Lignocellulosic biomass; cellulose; hemicellulose; lignin; cellulase; hemicellulase; ligninase; bioethanol; pretreatment; fermentation

1 Introduction

The amount of lignocellulosic biomass supersedes that of any other biomass on earth. Industries worldwide generate millions of tonnes of lignocellulosic waste annually. These wastes can pose environmental challenges if not properly managed. However, managing them can be challenging due to their inherent recalcitrance. Therefore, using them as substrates to produce essential industrial enzymes and biofuels, like bioethanol, is an interesting concept and a sustainable way of valorizing the wastes [1–4]. To improve yields, lignocellulosic biomass is typically pretreated. Several kinds of pretreatment processes, ranging from physical to chemical methods, have been explored in some studies. In some cases, more than one pretreatment technique is applied to adequately deconstruct the complex and recalcitrant structure of lignocellulosic biomass before proceeding to other processes [5–9].

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This review, therefore, captures the vast number of lignocellulosic wastes from different industries and their constituents, especially those published within the last decade, as opposed to general lignocellulosic waste biomass or only agricultural lignocellulosic waste biomass already captured in the literature. The great potential of using them to produce microbial lignocellulase enzymes—cellulases, hemicellulases and ligninases—is thoroughly discussed using current literature. The conversion of the produced simple sugars into bioethanol using different microbes, either through separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF), is presented holistically. The emphasis of this study is primarily on microbial enzymes (crude or purified), rather than commercial enzymes, for the production of fermentable sugars and bioethanol, thereby making this review unique. Furthermore, the challenges and prospects of using industrial lignocellulosic waste biomass are further elucidated to show the possibilities of second-generation bioethanol through industrial waste valorization.

2 Industrial Lignocellulosic Waste Biomass

Lignocellulosic biomass is the most abundant organic biomass on earth. This biomass accounts for a large share of global daily waste, including industrial waste [1,2]. Lignocellulosic waste biomass consists of three major constituents, viz: cellulose, hemicellulose and lignin. The proportions of these constituents depend on the source of the lignocellulosic waste [1,10–12].

Cellulose is an essential constituent of lignocellulosic waste biomass with multiple industrial applications. It is a complex polymer composed of glucose monomers joined together by β-1,4 glycosidic bonds (Fig. 1). Cellulose chains usually form microfibrils, which can be either unordered or highly ordered, resulting in cellulose with amorphous or crystalline structures, respectively [1,13]. Crystalline cellulose is quite rigid and resistant to both biological and chemical hydrolysis, while the amorphous counterpart is less rigid and can easily be hydrolyzed about 3 to 30 times faster than crystalline cellulose [1,14]. In lignocellulose, the cellulose components are usually shielded by hemicelluloses, which also bind to cellulose microfibrils, making them inaccessible and recalcitrant. Cellulosic biomass is also protected by outer layers of lignin polymers, which must first be removed/degraded to reach it (Fig. 2). And this is usually achieved through several kinds of pretreatment techniques [1,14,15].

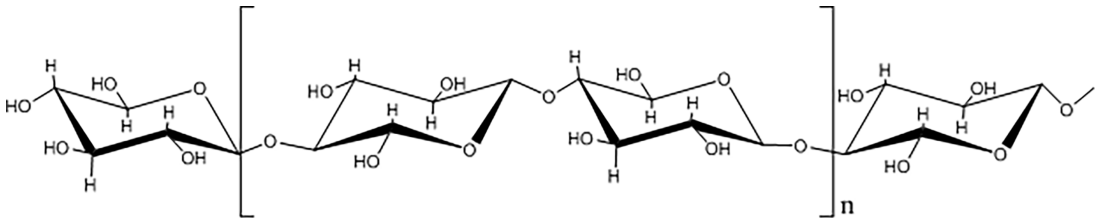

Figure 1: Chemical structure of cellulose.

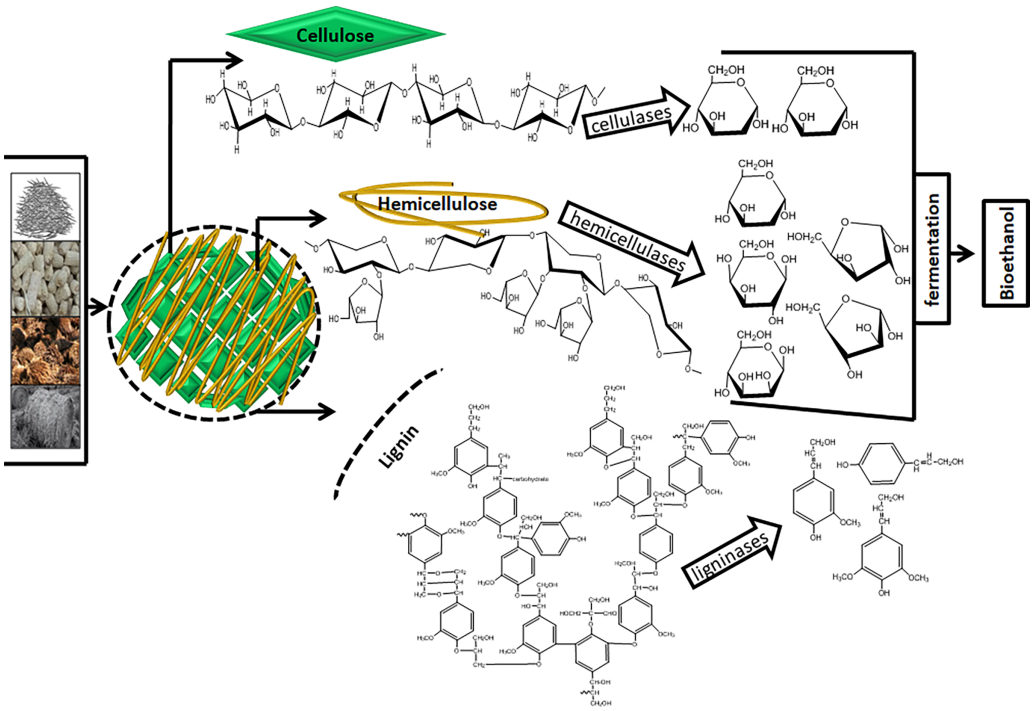

Figure 2: Industrial lignocellulosic waste biomasses used in lignocellulases and bioethanol production.

Hemicelluloses also constitute essential components of lignocellulose. They differ from cellulose and have different monomers. These include hexoses, such as glucose, galactose or mannose; pentoses, such as xylose or arabinose; and other methylated derivatives, such as fucose or rhamnose. Furthermore, hemicelluloses could be branched to form more complex structures. Hemicelluloses are the least resistant lignocellulose constituents due to their branched structures [16,17]. Hemicellulose can be categorized into xylans, mannans, β-glucans and galactans (Figs. 3 and 4). Xylans are mainly linear homopolymers with D-xylose linked by β (1 → 4) or β (1 → 3) bonds. However, xylans could also be heteropolymers with other branched monosaccharides, such as D-glucuronic acid or L-arabinose at positions 2 and 3 to form glucuronoxylans or arabinoxylans, respectively. Mannans are either homopolymers or heteropolymers with D-mannose linked by β (1 → 4) bonds or D-mannose linked to D-glucose (i.e., glucomannans), respectively. Mannans could also be branched at position 6 with D-galactose to form galactomannans. β-glucan hemicellulose is made up of a β-glucoside backbone linked by a β (1 → 4) bond, and D-xylose at the sixth position (i.e., xyloglucan) or a mixed structure with β (1 → 4) and β (1 → 3) linked with a D-glucoside backbone called mixed β-glucan. The galactan hemicelluloses are homopolymers with either β (1 → 3) or β (1 → 4) D-galactose backbones connected to mono- or oligosaccharide side chains of L-arabinose or D-galactose at the sixth position [1,17,18] (Figs. 3 and 4).

The lignin constituent of lignocellulose has no typical structure nor repeatable units like cellulose and hemicellulose. It is usually heterogeneous physically and chemically, with varying monomers. Lignin is synthesized by the combination of different proto-lignins, such as *p*-coumaryl, sinapyl alcohols and coniferyl, through polycondensation and dehydrogenation by enzymes (Figs. 5 and 6). Different plants have different lignin structures, each containing different proportions of the various proto-lignins. The chemical bonds present in lignins include: arylglycerol-*β*-ether linkages (*β*-*O*-4), pinoresinol (*β*-*β*), diarylpropane (*β*-1), phenylcoumaran (*β*-5) and biphenyl (5–5) [1,19].

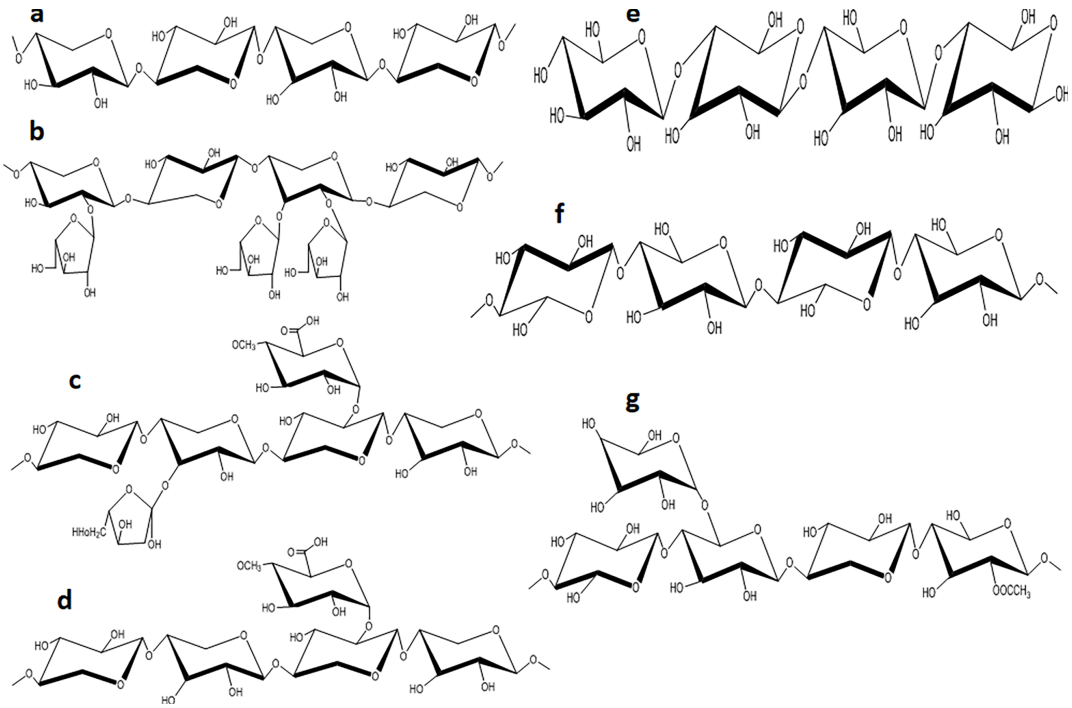

Figure 3: Chemical structures of hemicelluloses: (**a**) homoxylan, (**b**) arabinoxylan, (**c**) (arabino) glucuronoxylan, (**d**) *O*-acetyl-4-O-methylglucuronoxylan, (**e**) mannan, (**f**) glucomannan, and (**g**) *O*-acetylgalactoglucomannan.

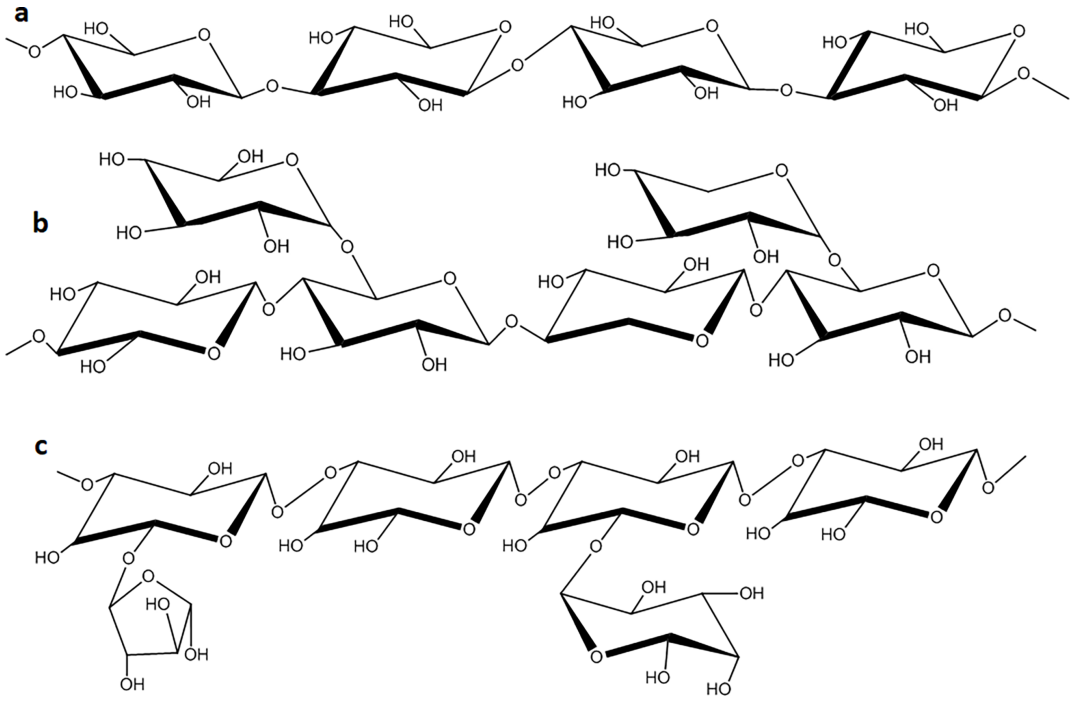

Figure 4: Chemical structures of hemicelluloses: (**a**) β-(1 → 3, 1 → 4)-glucan, (**b**) xyloglucan, and (**c**) arabinogalactan.

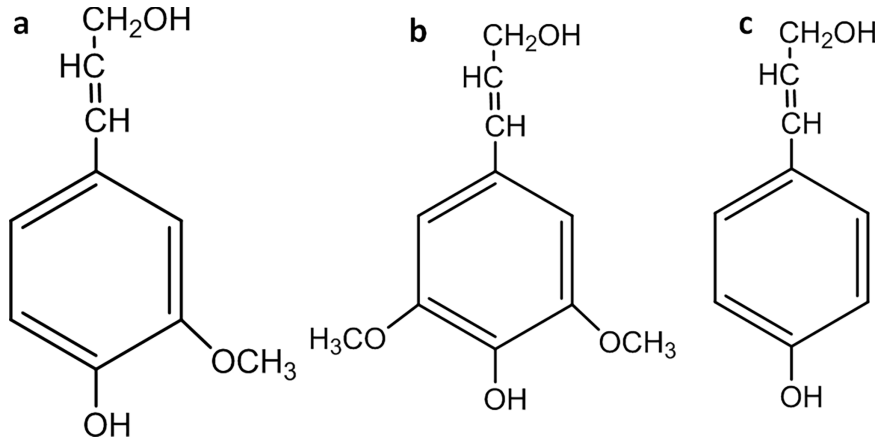

Figure 5: Chemical structures of lignin building blocks: (**a**) coniferyl alcohol, (**b**) sinapyl alcohol and (**c**) *p*-coumaryl alcohol.

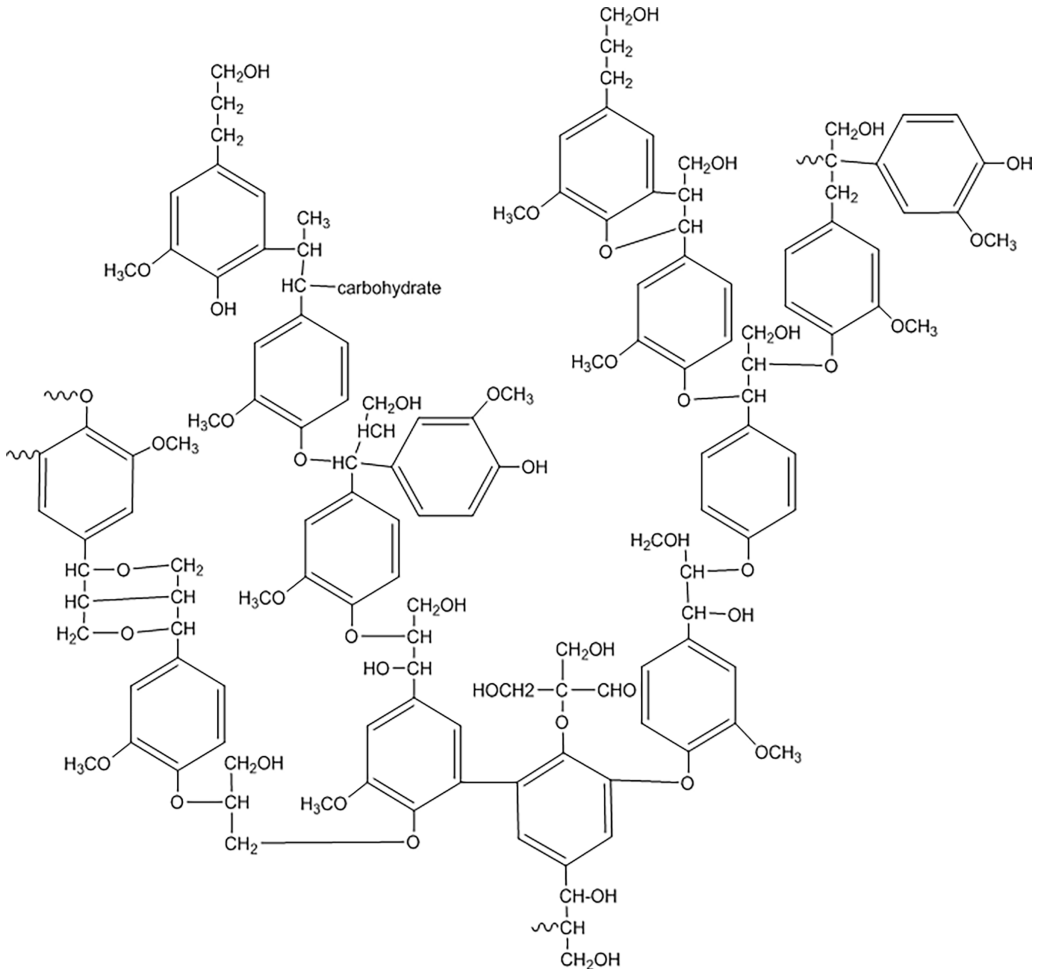

Figure 6: Chemical structure of lignin.

Globally, lignocellulosic wastes comprise a significant portion of industrial and total waste. For example, about 15.7% of lignocellulosic wastes accounts for the total annual waste generated in Europe [1,3]. Industrial lignocellulosic wastes are made of agricultural and forestry (including wood and paper) wastes. Industries involved in the use and/or processing of sugarcane, corn, rice, palm oil, and wheat are among the top producers of lignocellulosic wastes. These industries generate annual waste amounting to millions of tonnes globally. Examples of these wastes include: sugarcane bagasse, corncob, rice straw and husk, oil palm fibres and empty fruit bunches, wheat straw, etc. Closely following the agricultural industry in lignocellulosic waste generation are the wood and paper industries. Sawdust, chippable wastes and wood barks from hard and soft woods are among the top lignocellulosic wastes from the wood industry. The beverage industry, especially the fermentation-based industries, also produces lignocellulosic wastes. For example, beer companies produce a large amount of brewers' spent grains annually. While black liquor, wood fragments in the form of chips and knots, sludge, waste papers and boards, among others, are generated from the paper industry. Other industries that also generate some levels of lignocellulosic wastes are the textile industries, municipal waste management, and others [11,20,21].

If these industrial lignocellulosic wastes are not properly disposed of, they could pose environmental problems, as some are recalcitrant and may take time to biodegrade. On the other hand, these lignocellulosic wastes could significantly increase the cost of municipal waste management, as they require proper treatment and disposal. However, the microbial valorization of these wastes could lead to a win-win scenario. Wastes could become essential raw materials for producing several important industrial products and metabolites, including enzymes, biofuels, pharmaceuticals, nutraceuticals, biofertilizers, etc. And the substrate is also being simultaneously biodegraded during the process, thereby achieving valorization and waste management [4,11,22]. This is indeed an interesting aspect of circular bio-economy and the zero-waste concept, which aligns with several Sustainable Development Goals, and ultimately preserves the ecosystem.

2.1 Microbial Enzymes for Bioethanol Production from Industrial Lignocellulosic Waste Biomass

Biofuels, such as bioethanol, constitute part of the green fuels that can be generated from the valorization of industrial lignocellulosic wastes. This type of bioethanol is known as a second-generation biofuel. Though lignocellulosic wastes are abundant, their use for bioethanol production poses a significant challenge. The core of which is the hydrolysis of the recalcitrant substrate to produce simple sugars that can be easily fermented. Enzymes are usually used for the hydrolysis/saccharification of lignocellulosic biomass after pretreatments [5,6]. However, the cost of commercial enzymes can increase production costs [5].

Therefore, research has focused on obtaining inexpensive microbial enzymes that can be used in crude or partially purified forms [7]. Separate systems of hydrolysis and fermentation could be used, in which the simple sugars produced are used for fermentation in another bioreactor. In some other cases, SSF is preferred. The SSF technique uses either a single organism that saccharifies and ferments (i.e., consolidated bioethanol process), or consortia of organisms that perform both tasks simultaneously [7,8]. Both techniques have their advantages and disadvantages (some are highlighted in Section 3.1).

Some essential enzymes required in the production of bioethanol from industrial lignocellulosic wastes are cellulases, hemicellulases and ligninases. Several research works have demonstrated the prospects of producing these lignocellulase enzymes by different microorganisms, like bacteria and fungi.

Cellulases, hemicellulases and/or ligninases are important lignocellulosic enzymes needed for the biodegradation of lignocellulosic biomass. Fermentable or reducing sugars are part of the essential products that could be generated from the degradation by these enzymes. These sugars are needed as direct substrates for bioethanol production by microorganisms. Several strategies could be taken to generate simple sugars from industrial lignocellulosic waste biomass (Fig. 7). These include pretreatment and detoxification, or only

pretreatment without detoxification, before the use of the lignocellulose. The detoxification process involves removing inhibitory products generated during pretreatment of lignocellulosic substrates. Direct use of lignocellulosic biomass without pretreatment and detoxification is also possible with some microorganisms. However, the actual pathway chosen tends to greatly influence both the yield of fermentable sugars and the eventual production cost.

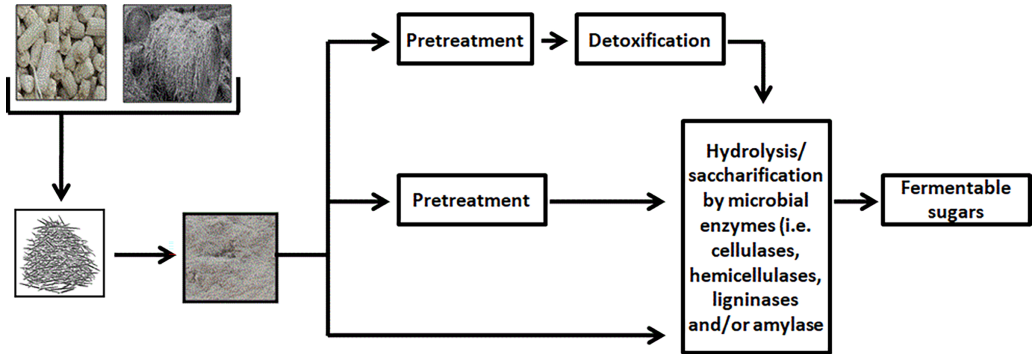

Figure 7: The routes in the production of fermentable sugars from lignocellulosic biomass.

2.1.1 Cellulases

Cellulase is a group of microbial enzymes that can break down cellulose into disaccharides (i.e., cellobiose) and/or monosaccharides, such as simple sugar (i.e., glucose), through a process known as cellulolysis. These enzymes include endoglucanases, exoglucanases and β-glucosidases [1,23–25].

Some microbes, including bacteria and fungi, have been demonstrated to produce different cellulases in the hydrolysis of lignocellulosic waste biomass. Several species of *Bacillus* are among the most frequently reported cellulase producers, e.g., *B. subtilis*, *B. siamensis*, and *B. methylotrophicus* [9,26–28]. Other species of filamentous actinobacteria have also been reported as good cellulase-producing strains, e.g., *Cellulomonas flavigena*, *Streptomyces cavourensis*, *Streptomyces parvus, Streptomyces cavourensis*, among others [29,30] (Table 1).

Multiple fungal strains are among the most popular cellulase-producing microbes, having been shown to produce these enzymes in solid-state fermentation. These include yeasts such as *Filobasidium aff. oriense*, *Candida albicans, Barnettozyma californica, Cyberlindnera jardinii*, and others [28,31,32]. As well as several moulds, mainly from the *Aspergillus* genus (especially *A. niger* and *A. flavus*), which are quite efficient in producing different cellulases [23,33–37]. Other common cellulase-producing moulds are from the *Trichoderma* and *Fusarium* genera [38–44]. The use of the macroscopic fungus, mushroom, in cellulase production has also been reported by several researchers [37,45] (Table 1).

Table 1 gives several industrial lignocellulosic waste biomasses that have been valorized through the production of different microbial cellulases. Most of the reported industrial waste comes from agricultural industries. In some cases, biomass was used as a co-substrate to improve enzyme production [40], while at other times, microbial co-cultures or consortia greatly increased enzyme production [9,26,28,30,31,37].

Some other factors/techniques have helped increase microbial cellulase production. The importance of mass transfer in solid-state fermentation has been shown to affect cellulase production by moulds. Some studies have reported the use of specialized bioreactors to exploit this concept. For example, Grajales et al. [55] demonstrated that cellulase activity from *Myceliophthora thermophila*, using sugarcane bagasse and wheat bran, was increased to 49.12 U/mL using a rotary drum bioreactor compared to 47.78 U/mL obtained from

the static condition. Similarly, endoglucanase, exoglucanase, and β-glucosidase were reported to increase by 1.27-, 1.18-, and 1.06-fold in tray fermentation [33].

Some of the produced cellulases (or the microorganisms producing them directly) were used to hydrolyze/saccharify several lignocellulosic waste biomasses to produce simple sugars and other products with interesting results (Table 1). Very high levels of reducing/simple sugars (over 100 g/L) were reported in some of the studies. For example, 200 g/L from wheat straw [33], 149.25 and 253.00 mg/g from brewer's spent grains [27,32], 338 mg/mL from pea peel [34], 288.06 mg/L from rice straw and soyabean residues [48], among others. In some cases of consolidated bioethanol production or where co-cultures/consortia were used with ethanol-producing ability, some level of ethanol was obtained [28,43,46] (Table 1).

Table 1: Microbial cellulase produced from lignocellulosic waste substrates and the products of enzymolysis.

Cellulase	Microorganism	Lignocellulosic Waste Biomass	Enzyme Activity from Substrate	Hydrolysis Products*	Reference
Cellulase	*Exiguobacterium sibiricum* K1 (bacterium)	Sugarcane bagasse, corn stover, wheat bran, and oil-extracted lemongrass	K1 gave 3.8 U/mL maximum cellulase at pH 5 and 15°C	Several alcohols, ketones, esters, acids, sugars, etc.	[46]
Endoglucanase (CMCase), exoglucanase (FPase) and β-glucosidase	*Trichoderma harzanium* ATCC 20846 (mould)	Surgical cotton and packaging cardboard waste	Enzyme activities: 1.94, 12.47 and 3165.40 IU/mL for FPase, CMCase and β-glucosidase, respectively (in submerged fermentation)		[38]
Endo-β-1,4-glucanase, exo-β-1,4-β-glucanase and β-1,4-glucosidase	*Aspergillus niger* P-19 (mould)	Rice straw	Highest value of 126, 36 and 47 IU/g for CMCase, FPase and β-glucosidase, respectively, after 5 days (in solid state fermentation, SoSF)	70 g/L of reducing sugar from a 100% solid load	[23]
Endoglucanase, exoglucanasese and β-glucosidase	*Aspergillus flavus* ITCC6562 (mould)	Rice straw, rice husk, wheat straw, sugarcane bagasse	Maximum cellulose activity of 12.5, 235 and 190 IU/gds (gram of dry substrate) for FPase, CMCase and β-glucosidase, respectively, at initial pH 5.5 and 30°C at day 5 (in SoSF). Enzyme activities of FPase, CMCase, β-glucosidase, and xylanase were enhanced by approximately 1.27-, 1.18-, 1.06-, and 1.09-fold during tray fermentation.	The highest reducing sugar of about 200 mg/g from wheat straw at 72 h	[33]
Endoglucanase, exoglucanase, cellobiase and β-glucosidase	*Amycolatopsis* sp. GDS. (bacterium)	Wheat straw, sugarcane bagasse, sorghum husk, corn straw, etc.	Wheat straw showed the highest activities for most cellulases: 72.70, 6.72, 8.54 and 1.04 U/mL, for endoglucanase, exoglucanase, cellobiase and FPase, respectively.	The highest reducing sugar of 1890 mg/L was obtained from wheat straw	[24]

(Continued)

Table 1 (continued)

Cellulase	Microorganism	Lignocellulosic Waste Biomass	Enzyme Activity from Substrate	Hydrolysis Products*	Reference
Endoglucanase, exoglucanasese	*Filobasidium aff. oriense* (yeast)	Brewer's spent grains (BSG)	Maximum activities: 3.39 and 2.44 IU/gds (gram of dry substrate) for endoglucanase and FPase, respectively (in SoSF)	Released sugar: 149.25 mg sugars/g dry BSG	[32]
Cellulase	*Lentinus squarrosulus* Mont. (mushroom)	Baby corn husk	Highest cellulose of 6.15 units/mL		[45]
Cellulase	*Aspergillus awamori* MK788209 (mould)	Molokhia stem, pea peel, etc	Highest cellulase activity of 2.03 U/mL (in SoSF)	Reducing sugar: 338 mg reducing sugar/mL from pea peel	[34]
Endoglucanase	*Bacillus subtilis* and *Serratia marcescens* (bacteria) - co-culture	Paddy straw and litchi waste	Activity of 1.6 IU/mL (in SoSF) (Longer enzyme stability-135 min due to nanocatalyst)		[26]
Cellulase	*Streptomyces cavourensis* QT227, *Streptomyces parvus* 5–94 gene and *Streptomyces cavourensis* strain SIF3 (actinomycete)-consortium	Corn cob, sugarcane bagasse and palm leaves	Activity of crude enzyme: 1.34, 1.51 and 0.42 U/mL on corn cob, sugarcane bagasse and palm leaves, respectively	The highest total sugar: 6.9 mg/mL	[30]
Cellulase	*Bacillus siamensis* F2 and *Candida albicans* GP1 (bacterium and yeast)	Fruit waste	Cellulase activity at optimal conditions: 1.1 and 0.0 U/mL for F2 and GP1, respectively (in co-culture consolidated bioprocess)	Maximum ethanol yield of 48.0 g/L and 0.7 g/g of utilized fruit waste	[28]
Endoglucanase, exoglucanase and β-glucosidase	*Aspergillus niger* SCBM1 (mould)	Biomass sorghum and wheat bran	Highest endoglucanase, exoglucanase and β-glucosidase activities: 30.64, 41.47 and 54.90 U/g, respectively (in SoSF)		[35]
Endoglucanase and β-glucosidase	*Lichtheimia ramose* (mould)	Sugarcane bagasse	Cellulase activities: 168.1 and 270.4 U/g of dry substrate for endoglucanase and β-glucosidase, respectively (in SoSF)	Highest glucose yield: 1.45 mg/mL	[47]
Cellulase	*Pseudoxanthonomas taiwanensis*, *Sphingobacterium composti*, *Cyberlindnera jardinii* and *Barnettozyma californica* (bacteria and yeasts) -consortium	Coffee husk	Cellulase activity: 3.1 FPU/g DM for FPase (in SoSF)		[31]

(Continued)

Table 1 (continued)

Cellulase	Microorganism	Lignocellulosic Waste Biomass	Enzyme Activity from Substrate	Hydrolysis Products*	Reference
Endoglucanase and exoglucanase	*Aspergillus niger* (mould)	Urban lignocellulosic wastes: a mixture of dry leaves from trees	Endoglucanase and exoglucanase activities: 413.49 and 230.68 U/L, respectively (in sequential fermentation)	Reducing sugars: 1.19 mg/mL	[36]
Cellulase	*Trichoderma reesei* (mould)	Wheat straw and rice straw	Maximum cellulase activities with wheat and rice straw: 5.4 and 5.3 filter paper units (FPU)/g with standard medium; 4.8 and 5.7 FPU/g with trace element eliminating medium (in SoSF)		[39]
Total cellulase, endoglucanase and β-glucosidase	*Aspergillus* sp. IN5 (mould)	Rice straw, soybean residue	Total cellulase, carboxymethyl cellulase and β-glucosidase activities: 0.26, 3.32 and 196.09 U/g substrate, respectively (in SoSF)	Maximum sugar yield: 288.06 mg/g substrate	[48]
Cellulase	*Bacillus methylotrophicus* (bacterium)	Brewer's spent grain	Maximum cellulose activity: ~0.469 U/mL	Glucose yield: 253 mg/g	[27]
Cellulase	*Virgibacillus salarius* BM02 (bacterium)	Wheat bran	Highest cellulase activity: 44.7 U/mL (using wheat bran)		[49]
Endoglucanase and cellobiase	*Talaromyces verruculosus* IIPC 324 (mould)	Sugarcane bagasse, wheat bran	Endoglucanase and cellobiase activity: 250 and 45 U/g, respectively (in SoSF)	Total reducing sugar and glucose: 9.17 and 7.82 g/L, respectively	[50]
Endoglucanase and exoglucanase	*Zasmidium cellare* CBS 146.36 (moud)	Mustard biomass	Highest endoglucanase and exoglucanase activities: 8.69 IU/mL and 3.48 FPU/mL, respectively	Maximum fermentable sugar: 78.19 mg/mL (glucose: 40.91, xylose: 17.28)	[51]
Total cellulase	*Fusarium solani* and *Fusarium oxysporum* (moulds)	Olive mill waste	High total cellulase activities of 2.88 and 9.36 IU/mL were obtained from *F. oxysporum* and *F. solani* (in consolidated bioprocessing)	Ethanol production and yield: 2.47 g/L and 0.84 g/g, respectively	[43]
Exoglucanase and β-glucosidase	*Trichoderma longiflorum* (mould)	Different waste papers: office paper, corrugated board, tissue paper, magazine paper	The highest FPase and β-glucosidase of 3.21 and 0.80 IU/mL were obtained from waste office paper and mixed carbon (corrugated board + wheat bran), respectively		[40]

(Continued)

Table 1 (continued)

Cellulase	Microorganism	Lignocellulosic Waste Biomass	Enzyme Activity from Substrate	Hydrolysis Products*	Reference
Endoglucanase, exoglucanase and β-glucosidase	*Cladosporium cladosporioides* NS2 (mould)	Sugarcane bagasse	Cellulase activities: 16.9, 150.0 and 200.0 IU/gds for FPase, endoglucanase and β-glucosidase, respectively. Improved production by manganese ion/peptone (nitrogen): 20.0/17.5, 210.0/170.0 and 240.0/225.0 IU/gds, respectively		[25]
Endoglucanase, exoglucanase and β-glucosidase	*Emericella variecolor* NS3 (mould)	Orange peel waste, rice husk	Cellulase activities: 39, 236 and 197 IU/gds for FPase, endoglucanase and β-glucosidase, respectively (in SoSF)	Highest fermentable sugars from alkali-treated rice husk: 47 g/L	[52]
Cellulase	*Cellulomonas flavigena* PR-22 (antinobacterium)	Sugarcane bagasse	Highest cellulase productivity: 101.6 U/L-h	Highest total and reducing sugars: 15.79 and 11.78 g, respectively, with 0.33 g/g fractional conversion	[29]
Cellulase	*Aspergillus terreus* NCFT 4269.10 (mould)	Banana peel, wheat bran, sawdust	The highest activity of 344.88 U/mL was obtained with banana peel. Immobilization of spores increased activity to 809.00 U/mL, still with banana peel (in liquid state surface fermentation)	The highest alcohol of 19.80 g/L was produced from hydrolyzed substrate using *Saccharomyces cerevisiae*	[53]
Endoglucanase, exoglucanase and β-glucosidase	*Chrysoporthe cubensis* (mould)	Eucalyptus wood chips	Activities of 1.3, 1.6 and 2.8 U/mL for FPase, β-glucosidase and endoglucanase, respectively (in semi-solid medium)	Reducing sugar of 160.3 mmol/L (with 17.6 and 7.6 g/L, glucose and xylose, respectively) using 20.0 FPU/g of chip enzyme	[54]
Cellulase	*Myceliophthora thermophila* I-1D3b (mould)	Sugarcane bagasse and wheat bran	Cellulase activity of 49.12 U/mL (obtained from the rotary drum bioreactor, compared to 47.78 U/mL from static conditions)		[55]
Cellulase	*Trichoderma reesei* (MTCC 164) (mould)	Newspaper waste	Maximum cellulase production and activity: 8.64 g/L and 7.82 FPU/mL, respectively (in submerged fermentation)		[41]
Cellulase	*Sphingobacterium* sp. ksn-11 (bacterium)	Corn husk, peanut husk, sugarcane bagasse, rice bran, etc	Cellulase activity: 3.55 U/mL (from corn husk) (in submerged fermentation)		[56]

(Continued)

Table 1 (continued)

Cellulase	Microorganism	Lignocellulosic Waste Biomass	Enzyme Activity from Substrate	Hydrolysis Products*	Reference
Endoglucanase, exoglucanase and β-glucosidase	*Pleurotus ostreatus* PLO6 and *Aspergillus niger* SCBM4 (mushroom and mould-consortium)	Sugarcane bagasse and wheat bran	Endoglucanase: 20.2 U/g Exoglucanase: 13.3 U/g β-glucosidase: 98.5 U/g	–	[37]
Cellulase (CMCase)	*Mucor circinelloides* GL1 and *Fusarium verticillioides* GL5 (moulds)	Coffee husk, areca husk and paddy straw	GL1 gave higher CMCase activities of 11.73, 9.95 and 8.90 IU/mL from paddy straw, areca straw and coffee straw, respectively	–	[44]
Endoglucanase, exoglucanase and β-glucosidase	*Trichoderma harzaianum* (mould)	Sugarcane bagasse	β-1,4 endoglucanase: 53.5 U/mL; β-1,4 exoglucanase: 41.3 U/mL; β-1,4 glucosidase: 46.8 U/mL	More than 50.0 mg/mL glucose	[42]
Endoglucanase, exoglucanase and β-glucosidase	*Aspergillus* sp. (A1) and *Bacillus* sp. (B1) (mould and bacterium)	Sawdust	Highest endoglucanase, exoglucanase and β-glucosidase: 234.79, 17.72 and 61.03 U/g (using co-culture)	Highest reducing sugar: 68.74 mg/g (using co-culture)	[9]

Note: *Products obtained from the enzymolysis of lignocellulosic biomass by microbial lignocellulase enzymes.

2.1.2 Hemicellulases

Hemicellulases are a group of catalytic enzymes produced by microorganisms that can biodegrade hemicelluloses into their monomers, such as xylose, arabinose, mannose, etc. The different kinds of hemicellulases include: xylanases, mannanases, galactanases, xyloglucanases and arabinofuranoidases, which break xylans, mannans, galactans, xyloglucans and arabinofuranosidases, respectively [56,57].

Just like cellulase production, several microorganisms have been reported to produce hemicellulases. These include bacteria, yeasts, moulds and mushrooms. Bacteria such as *Bacillus trypoxylicola, Amycolatopsis* sp., and *Cellulomonas flavigena* have been reported to produce hemicellulases from several industrial lignocellulosic waste substrates, like Agba wood, sugarcane bagasse/sorghum husk and sugarcane bagasse, respectively [24,29,58]. Other lignocellulose substrates that have been explored in xylanase and/or mannanase productions are coffee husks, sawdust, brewer's spent grains, rice straw and husk, waste cotton wool, wheat bran, empty fruit branches, amongst others (Table 2).

Furthermore, moulds such as several species of *Aspergillus, Trichoderma harzanium, Lichtheimia ramose, Trametes lactinea*, etc., have been demonstrated to synthesize hemicellulases in several studies (Table 2). Some mushrooms have also been used alone or in co-culture with several moulds in hemicellulase production [37,59,60]. High xylanase and mannanase activities have been achieved in some studies using microbes, as presented in Table 2. Different kinds of pretreatments—alkaline, acidic and several physical techniques—were used on some of the substrates to delignify them before the enzymatic hydrolysis or enzyme production [9,29,38,47,58].

Several recent works on microbial hemicellulase production or hydrolysis using industrial lignocellulosic waste substrates are presented in Table 2. The different hemicellulases (mostly xylanases) produced and their activities are also recorded. Some studies also reported products of enzymatic hydrolysis/saccharification, such as reducing/simple/total sugars, from the lignocellulosic waste biomasses [9,23,29,58].

Table 2: Microbial hemicellulases produced from lignocellulosic waste biomass and the products of enzymolysis.

Hemicellulase	Microorganism	Lignocellulosic Waste Biomass	Pre-Treatment	Enzyme Activity from Substrate	Hydrolysis Products*	Reference
Hemicellulase	*Bacillus trypoxylicola* (bacterium)	African rose wood and Agba wood	Alkaline	Highest activities: 785.73 U/mg (African rose wood); 493.03 U/mg (Agba wood)	Highest reducing sugar: 2.92 mM (African rose wood); 3.56 mM (Agba wood)	[58]
Xylanase	*Amycolatopsis* sp. GDS (bacterium)	Wheat straw, sugarcane bagasse, sorghum husk, corn straw, etc.	–	Xylanse (U/mL): 530.13 (wheat straw), 280.09 (sugarcane bagasse), 237.54 (sorghum husk)	The highest reducing sugar of 1890 mg/L was obtained from wheat straw	[24]
Xylanase, and mannanase	*Sphingobacterium* sp. ksn-11 (bacterium)	Corn husk, peanut husk, sugarcane bagasse, rice bran, etc.	–	Xylanase: 79.22 U/mL; Mannanase: 12.43 U/mL (in submerged fermentation)		[56]
Xylanase	*Cellulomonas flavigena* PR-22 (antinobacterium)	Sugarcane bagasse	Alkaline	Highest xylanase productivity: 1846.4 U/L-h	Highest total and reducing sugars: 15.79 and 11.78 g, respectively, with 0.33 g/g fractional conversion	[29]
Xylanase	*Pseudoxanthonomas taiwanensis*, *Sphingobacterium composti*, *Cyberlindnera jardinii* and *Barnettozyma californica* (bacteria and yeasts) (consortium)	Coffee husk	–	Xylanase: 48 U/g DM (in SoSF)	–	[31]
Xylanase	*Aspergillus* sp. and *Bacillus* sp. (mould and bacterium)	Sawdust	Oven drying	Highest xylanase: 190.67 U/g (co-culture);	Highest reducing sugar: 68.74 mg/g (in co-culture)	[9]
Xylanase	*Filobasidium aff. oriense* (yeast)	Brewer's spent grains (BSG)	H_2SO_4, H_2O_2 and H_2O	Xylanase: 356.24 IU/gds (in SoSF)	Released sugar: 149.25 mg sugars/g dry BSG	[32]
Xylanase and mannanase	*Aspergillus niger* P-19 (mould)	Raw rice straw	None	Xylanase: 693 IU/g; mannanase: 57 IU/g (in SoSF)	70 g/L of reducing sugar from a 100% solid load	[23]
Xylanases	*Trichoderma harzanium* ATCC 20846 (mould)	Surgical cotton and packaging cardboard waste	Dilute suphuric acid, ammonia	Xylanases: 756.6 IU/mL (in SoSF)	–	[38]
Xylanase	*Aspergillus flavus* ITCC6562 (mould)	Rice straw, rice husk, wheat straw, sugarcane bagasse	Microwave alkaline-best	Xylanase: 180 IU/gds (in SoSF)	The highest reducing sugar of about 200 mg/g from wheat straw at 72 h	[33]
Xylanolytic enzymes	*Aspergillus niger* NRRL3 (mould)	By-products from processing wheat flour; soybean hulls	None	Optimized Xylanolytic activity: 138.3 U/mL (compared to 14.5 U/mL from the unoptimized set-up)	46.7% saccharification of soyabean hulls	[61]

(Continued)

Table 2 (continued)

Hemicellulase	Microorganism	Lignocellulosic Waste Biomass	Pre-Treatment	Enzyme Activity from Substrate	Hydrolysis Products*	Reference
Xylanase	*Aspergillus niger* SCBM1 (mould)	Biomass sorghum and wheat bran	–	Xylanase: 300.07 U/g (in SoSF)	–	[35]
Xylanase	*Lichtheimia ramose* (mould)	Sugarcane bagasse	Microwave and glycerol	Xylanase: 34 U/g (in SoSF)	Highest glucose yield: 1.45 mg/mL	[47]
Xylanase	*Lentinus squarrosulus* Mont. (mushroom)	Baby corn husk	Alkaline	Xylanase: 6.15 U/mL	–	[45]
Xylanase	*Aspergillus fumigatus* RSP-8 (mould)	Sorghum biomass	–	Highest xylanase: 1248 U/mL	–	[62]
Endoxylanase	*Aspergillus niger* ISL-9 (mould)	Wheat bran	Hot-air oven	Endoxylanase: 21.87 U/g [optimum conditions significantly ($p \leq 0.05$) increased production by 1.4-fold] (in SoSF)	–	[63]
Xylanase	*Trametes lactinea* FBW (mushroom)	Empty fruit bunches, rubber wood sawdust	–	Xylanase: 42.26 U/g (SoSF)	–	[60]
Xylanase	*Aspergillus niger* SCBM1, *Aspergillus fumigatus* SCBM6, *Pleurotus ostreatus* PL06 (mould and mushroom) (consortium)	Sugarcane bagasse and wheat bran	–	Highest xylanase: 2582.38 U/gds (in SoSF)	–	[59]
Xylanase	*Pleurotus ostreatus* PLO6 and *Aspergillus niger* SCBM4 (mushroom and mould) (consortium)	Sugarcane bagasse and wheat bran	–	Xylanase: 12.4 U/g (in SoSF)	–	[37]

Note: *Products obtained from the enzymolysis of lignocellulosic biomass by microbial lignocellulase enzymes.

2.1.3 Ligninases

Ligninases or lignin-modifying enzymes are a family of microbial catalytic enzymes that degrade lignin. They include: laccases, lignin peroxidase (LiP), manganese peroxidase (MnP), and others [59,64–67]. Fungi, especially moulds and mushrooms, are the major producers of ligninase, although some studies have shown that bacteria can also produce ligninase [9,44,56,65,68,69]. Examples of these fungi include *Trametes versicolor, Trametes lactinea, Fusarium verticillioides, Aspergillus fumigatus*, among others (Table 3).

Table 3: Microbial ligninases produced from lignocellulosic waste biomass and the products of enzymolysis.

Ligninase	Microorganism	Lignocellulosic Waste Biomass	Enzyme Activity from Substrate	Hydrolysis Products*	Reference
Laccase	*Sphingobacterium* sp. ksn-11 (bacterium)	Corn husk	Laccase: 21.12 U/mL (in submerged fermentation)	–	[56]
Laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP)	*Aspergillus* sp. (A1) and *Bacillus* sp. (B1) (mould and bacteria)	Sawdust	Highest Laccase: 170.83 U/g (co-culture); Highest LiP: 3056.63 U/g (co-culture); and Highest MnP: 47.73 U/g (B1) (in SoSF)	Highest reducing sugar: 68.74 mg/g (co-culture)	[9]

(Continued)

Table 3 (continued)

Ligninase	Microorganism	Lignocellulosic Waste Biomass	Enzyme Activity from Substrate	Hydrolysis Products*	Reference
MnP and LiP	*Pycnoporus sanguineus* MCA16 (mushroom); *Saccharomyces cerevisiae* (yeast) (fermentation)	Sugarcane bagasse, soybean meal and wheat bran	Highest MnP: 14.60 U/g Highest LiP: 10.02 U/g (in SoSF)	3.19 and 2.12 g/L glucose and xylose yields, respectively. 10.0 g ethanol from 8.8 g consumed glucose	[70]
Laccase and LiP	*Fusarium solani* and *Fusarium oxysporum* (mould)	Olive mill waste	Laccase: 0.54 IU/mL (*F.* solani), 0.57 IU/mL (*F. oxysporum*); LiP: 8.43 IU/mL (*F. oxysporum*) (in consolidated bioprocessing)	Ethanol production and yield: 2.47 g/L and 0.84 g/g, respectively	[43]
Laccase, MnP and LiP	*Aspergillus fumigates* G-13 (mould)	Locust, walnut shell and straw	Highest laccase: 972 U/g (locust) and 2332 U/g (walnut shell); Highest MnP: 3054 U/g (locust) and 5611 U/g (walnut shell); Highest LiP: 765 U/g (locust) and 959 U/g (walnut) (SoSF) (in optimized conditions)	–	[68]
Laccase, LiP and MnP	*Mucor circinelloides* GL1 and *Fusarium verticillioides* GL5 (moulds)	Coffee husk, areca husk and paddy straw	GL1 showed higher activity in areca, coffee and paddy husks. Areca husk; Laccase, LiP and MnP: 9.40, 3.50 and 1.45 IU/mL, respectively. Coffee husk; Laccase, LiP and MnP: 12.15, 2.47 and 0.98 IU/mL, respectively. Paddy husk; Laccase, LiP and MnP: 15.83, 4.50 and 0.75 IU/mL, respectively (in SoSF)	–	[44]
Laccase	*Trametes versicolor* (mushroom)	Brewer's spent grain	Highest laccase: 560 U/L (in SoSF)	–	[64]
Laccase, MnP and LiP	*Trametes lactinea* FBW and *Pycnoporus sanguineus* FBR (mushroom)	Empty fruit bunches, rubber wood sawdust	Laccase: 849.40 U/mg; MnP: 42.51 U/mg; LiP: 103.20 U/mg (in SoSF)	–	[60]
Laccase, MnP and LiP	*Schizophyllum commune* IBL-06 (mushroom)	Rice straw	Maximum laccase, MnP and LiP: 316.28, 1846.70 and 1347.20 U/gds, respectively (in SoSF)	–	[42]
Laccase	*Trametes versicolor* (L.) Lloyd (white-rot fungus) (mushroom)	Sawdust, banana peel	Laccase: 573.6 U/L	–	[69]
LiP and MnP	*Phanerochaete chrysosporium*-IBL-03 (white-rot fungus)	Wheat straw	Highest LiP: 1215.15 U/mL; and Highest MnP: 993.90 U/mL (in liquid-solid state fermentation)	–	[71]

(Continued)

Table 3 (continued)

Ligninase	Microorganism	Lignocellulosic Waste Biomass	Enzyme Activity from Substrate	Hydrolysis Products*	Reference
Laccase, LiP and MnP	*Inonotus tropicalis* (M2E) & *Cerrena unicolor* (2a) (mushrooms); *Chaetomium globosum* (GK1) and *Chaetomium brasiliense* (GK2) (moulds)	Sugarcane bagasse and rice straw	Highest laccase from 2a: 946.0 U/L (sugarcane bagasse); Highest LiP from 2a: ~30.0 U/L (rice straw); Highest MnP from M2E: 649.8 U/L (sugarcane bagasse) (in SoSF)	Highest sugar from sugarcane bagasse: 4.39 g/L (using M2E + GK2)	[65]
Laccase and MnP	*Aspergillus niger* SCBM1, *Aspergillus fumigatus* SCBM6 (moulds); *Pleurotus ostreatus* PL06 (mushroom-consortium)	Sugarcane bagasse and wheat bran	Laccase: 25.27 U/gds; MnP: 3.38 U/gds (in SoSF)	–	[59]

Note: *Products obtained from the enzymolysis of lignocellulosic biomass by microbial lignocellulase enzymes.

Several lignocellulosic waste biomasses have been shown to be good substrates for producing different ligninases—laccase, MnP and LiP—using either solid-state or submerged fermentation. These include: olive mill waste, sawdust, walnut shell and straw, coffee husk, rice straw, sugarcane bagasse, wheat straw, etc. (Table 3).

A number of these ligninases, the microorganisms that produce them, and the lignocellulosic waste substrates used have been summarized in Table 3. The hydrolysis products of some of the studies have also been captured in the table.

3 Bioethanol Production from Industrial Lignocellulosic Waste Biomass

The unsustainability of fossil fuels and the current global concern about climate change, driven by greenhouse gases, such as carbon dioxide, have necessitated the search for eco-friendly and sustainable energy sources [72–74]. Biomass energy sources, such as bioethanol, derived from lignocellulosic waste, could be a good substitute, as they generate a net-zero carbon footprint and support adequate waste management [75,76]. Some common industrial sources of lignocellulosic waste that could be channeled into bioethanol production include: the agricultural and forestry industries; the food and beverage industry, including fermentation-based companies like breweries; and the paper and textile industries.

3.1 Bioethanol Production from Agricultural-Based Industrial Lignocellulosic Wastes

Second-generation ethanol involves the production of bioethanol from agricultural-based industrial lignocellulosic waste, and encompasses multiple essential steps, such as pretreatment, saccharification, fermentation, distillation, dehydration and rectification [77,78]. The operations and enzymes required to carry out these steps make it a high-cost technology [7].

Several waste biomasses are generated by agricultural-based industries, including both food- and non-food-based crop fractions. The crops that produce the largest lignocellulosic biomass and are universally cultivated are wheat, rice, sugarcane and maize. These produce over 5300 million tons of dry biomass every year [7]. Most agricultural waste residues, like rice straw, wheat bran, cotton stalk, sugarcane bagasse, and

wheat straw, are lignocellulosic biomass consisting mainly of hemicellulose, cellulose and lignin. However, wheat straw contains a substantial amount of protein and pectin, whereas rice straw contains more silica [79]. Other lignocellulosic wastes that are produced in large quantities by agricultural industries include corncobs, oil palm fibers and empty fruit bunches.

Various agricultural lignocellulosic industrial wastes and residues have been used in the production of bioethanol through enzymatic hydrolysis [6]. These crops are more available for bioethanol production due to their short-term harvest rotation [6,80]. Pseudostem and rachis of banana waste were utilized as a source of biomass for bioethanol production. Enzymatic hydrolysis yielded ~100 g/L glucose concentration in optimized conditions using glucanase of 14.9 and 16.0 FPU/g at solid loading of 15.1% and 17.6%, respectively. The ethanol yields resulted in 74% and 87% of the maximum attainable ethanol yield, respectively [81]. Potato peel has also been investigated in bioethanol production, where enzymatic hydrolysis was steered with a combination of commercial cellulase and alpha-amylase from *Bacillus* sp. This led to a saccharification efficiency of 40.13% and ethanol yields of 0.30 g/g consumed sugars (SSF) and 0.40 g/g consumed sugars (SHF), corresponding to 78% of the maximum theoretical ethanol [82] (Table 4).

Since the application of commercial enzymes has been shown to increase production cost, the use of enzyme-producing microorganisms is a better alternative. *Aspergillus flavus* and *Aspergillus niger*, known as high-cellulase producers, were used to produce raw sugar from lignocellulosic biomass, which was subsequently used for bioethanol. This gave ethanol concentrations of 11.73 mg/mL (*A. flavus*) and 71.39 mg/mL (*A. niger*) from coffee pulp, and concentrations of 68.91 mg/mL (*A. niger*) and 7.81 mg/mL (*A. flavus*) from wheat bran [83] (Table 4). Another study evaluated rice husk for its potential for ethanol production using cellulase produced by *Thermobifida fusca* (Table 4). Optimized conditions resulted in 124.60 g/L of reducing sugar, with 100% consumption when fermented with a consortium of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*, yielding an ethanol concentration of 55.57 g/L [84].

The rate-determining steps that influence the overall process efficacy in the production of bioethanol include: pretreatment, enzymatic hydrolysis and fermentation [85]. High bioethanol yields can be achieved with the appropriate combination of each step in the process, leading to a cost-effective process [86]. The key challenge in bioethanol production is the pretreatment of the lignocellulosic biomass feedstock [86,87]. The lignin and cellulose matrix enclosed by hemicellulose must be fractured during pretreatment to reduce cellulose crystallinity and increase the portion of amorphous cellulose available for enzymatic attack. The pretreatment step comprises the chemical, physical, physicochemical and biological methods [16,17,86].

Pretreatment techniques can improve cellulose access to enzymes during enzymatic hydrolysis by reducing cellulose crystallinity and removing lignin to a large extent, so that the time of hydrolysis and enzyme loading will be minimized [88,89]. Black tea waste was used as a biomass source for bioethanol production. Physical boiling pretreatment method was used to release more fermentable sugars, which were converted to bioethanol using the yeasts, *Brettanomyces claussenii* and *Zygosaccharomyces bailii* [90].

Enzymatic hydrolysis is the most common method of hydrolysis and is applied to the pretreated lignocellulosic biomass. It is a very mild process, requires low maintenance cost and produces high yields [7,86]. It is well-suited to various pretreatment techniques, but toxic inhibitors that are poisonous to enzymes (or the microorganisms producing the enzymes) must be removed by detoxification when chemical or physicochemical pretreatment precedes enzymatic hydrolysis [86,91]. On the other hand, enzymatic hydrolysis could be expensive, especially when using commercial enzymes, and this might affect the cost of hydrolyzing lignocellulosic biomass. Also, due to the very sensitive nature of most enzymes, culture conditions such as temperature, pH, salinity, and others, must be adequately monitored and controlled to prevent enzyme inactivation and denaturation. This could also increase the cost of hydrolysis [7,86,91].

The lignocellulosic biomass pretreatment processes, such as lignin removal, hemicellulose solubility, hydrolysis duration, and enzyme loading, play a significant part in hydrolysis efficiency [6]. Enzymatic hydrolysis is a key step in converting cellulose in pretreated biomass to glucose. Enzymes are the most commonly used for the hydrolysis of cellulose and hemicellulose, although acid and alkali pretreatments can also be applied [92]. The conversion of cellulose, which is stable with a crystalline structure and capable of resisting depolymerization, is accomplished by the enzyme cellulase under slight conditions, like pH (4.5–5.0) and temperature (40°C–50°C) [6,86]. Hemicellulose, in contrast, can be hydrolyzed more effortlessly than cellulose as a result of its amorphous structure, which is more accessible. Two categories of enzymes are required for efficient hydrolysis, namely, depolymerizing core enzymes (1-4-mannosidases, xylan 1,4-xylosidases, endo-1,4-xylanases and endo-1,4-mannanases) capable of cleaving the backbone, and de-branching or auxiliary enzymes (acetyl xylan esterase, glucuronidase, L-arabinofuranosidase, ferulic acid esterase, and p-coumaric acid esterase) capable of eliminating side chains that pose steric hindrances to core enzymes. These help amplify the overall production of fermentable sugars gotten from lignocellulosic biomass [75].

The fermentation process involves the use of microorganisms to metabolize the fermentable sugars liberated from enzyme hydrolysis to generate ethyl alcohol as well as other byproducts [93]. Fermentation can be performed separately or concurrently with enzyme hydrolysis [94,95]. The integration procedure for bioethanol production involves three major approaches. They are SHF, SSF and simultaneous saccharification and co-fermentation (SSCF). The SHF technique entails conducting enzymatic hydrolysis and fermentation steps independently under optimal settings [96,97]. The SSF approach involves carrying out enzyme hydrolysis and fermentation at the same time using the same equipment. This tends to enrich the rate of hydrolysis, yields, and product concentrations. Then, the SSCF technique involves conducting hydrolysis and fermentation at the same time, in the same equipment, with concurrent co-fermentation of hexose and pentose sugars using multiple microorganisms, thereby increasing ethanol yield [98]. Simultaneous production of glucose by enzymes and its conversion to ethanol, as in SSF and SSCF, reduces sugar buildup in the system, and lowers cost as well as the processing time [7,8,98]. They also minimize contamination, increase ethanol productivity and evade cellulase inhibition by sugars, which is seen in the SHF process [7,8]. For example, Song et al. [99] demonstrated that ethanol production from fringe wood using the SSF method yielded a higher ethanol concentration (15.2 g/L) and a better conversion yield (83.1%) than the SHF method, which gave 14.8 g/L ethanol at 80.7% conversion yield.

The effectiveness of the conversion to bioethanol can be accomplished by the optimization of certain parameters, like enzyme loading, solid loading, time of hydrolysis, shaking speed, concentration of inhibitors and the effect of additives [6,75]. Solid loading increases sugar yield (80–100 g/L). However, very high solid loading can lead to viscosity, which affects enzyme efficiency due to poor mixing and impaired heat and mass transfer [92,100]. Similarly, increasing enzyme loading enhances saccharification efficiency, which provides a high glucose yield [92,100,101]. A high solid loading (20%) of hydrogen peroxide and phosphoric acid-pretreated wheat straw substrate gave a maximum glucose concentration of 164.9 g/L at an ethanol conversion rate of 64% with low enzyme input [102].

The shaking speed at an optimal level is required to guarantee optimal mass and heat transfer that translates into elevated glucose yield, but too high a speed causes shearing that can destroy enzymes (and even affect the microbes producing the enzymes), while a lower speed leads to poor mixing and reduced monosugar yields [6,103]. The long time required for complete hydrolysis limits ethanol production on a commercial scale, but the use of engineered microbes or their enzyme cocktails can shorten hydrolysis time [104].

The concentration of inhibitors that were generated during pretreatment can delay or halt enzyme hydrolysis. Therefore, performing detoxification before or during hydrolysis is critical to the process or selecting suitable pretreatment techniques that produce a limited amount of inhibitors [105].

Additives can enhance glucose yield in hydrolysis by obstructing the interactions between enzymes and lignin. This intensifies positive substrate-enzyme communications and recovers operability of cellulose hydrolysis. Examples of such additives are polymer-based polyethylene glycol (PEG), microbial surfactants (red quinoa saponins), non-catalytic protein [bovine serum albumin (BSA)], novel chemical surfactants or non-ionic surfactants (Triton X 100, Tween 80) [92,103,106]. Enzyme hydrolysis of wheat straw and sugarcane bagasse was studied using commercial cellulase, and the addition of Tween 20 to sugarcane bagasse (0.5%) and wheat straw (5.0 g/L) improved hydrolysis yield by decreasing enzyme loading and time of hydrolysis [103].

Table 4: Bioethanol production from agricultural-based industrial lignocellulosic wastes.

Biomass Source	Pretreatment	Fermenting Microbe	Fermentation Conditions	Productivity	References
Rice husk powder	Physical treatment (milling)	Mixed cultures of *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*	Yeast (1.5×10^8 cells/mL) was inoculated into a 32 h-old lignocellulosic waste hydrolysate fortified with 1% (v/v) yeast fermentation broth. The filter-sterilized hydrolysate (200 mL) was inoculated with a yeast cock tail prepared in yeast fermentation broth at 1% (v/v), and incubated for 72 h at 30°C, thereafter centrifuged at 3075× *g* for 15 min	The enzymatic hydrolysate contained 124.60 g/L of reducing sugar, and the yeast combination achieved 100% consumption, giving 55.57 g/L of ethanol	[84]
Wheat bran and coffee pulp	Physical pretreatment (grinding)	Immobilized *S. cerevisiae*	The yeast cells (4×10^7 cells/mL) were inoculated into a sterile fermenting broth (90 mL) containing the following (w/v): 2 g peptone, 2 g yeast extract and the hydrolysate, which were incubated at 30°C for 24 to 72 h	Maximum ethanol concentration of 71.39 mg/mL (*A. niger* hydrolysate) and 11.73 mg/mL (*A. flavus* hydrolysate) from coffee pulp, and a concentration of 68.91 mg/mL (*A. niger* hydrolysate) and 7.81 mg/mL (*A. flavus* hydrolysate) from wheat bran after 72 h of fermentation	[83]
Potato peel	Acid pretreatment	*Wickerhamomyces anomalus* X19	Pre-cultured yeast biomass at 4 g/L was used in sealed vials equipped with 0.22 µm filters, which were then centrifuged at 4500× *g* for 15 min. The pellet was re-suspended in a solution containing KH_2PO_4, $MgCl_2.6H_2O$ and $(NH_4)_2SO_4$, each at concentrations of 1 g/L at a solid loading of 5% w/v, pH 4.8, and temperature of 30°C (SSF) or 50°C for 24 h (SHF). The supernatant was then centrifuged at 6000× *g* for 10 min	With enzymatic treatment, ethanol yield (0.30 g/g consumed sugars) for SSF and (0.40 g/g consumed sugars) for SHF were obtained, which conforms to 78% of the highest theoretical ethanol yield	[82]

(Continued)

Table 4 (continued)

Biomass Source	Pretreatment	Fermenting Microbe	Fermentation Conditions	Productivity	References
Banana waste	Steam explosion pretreatment	*S. cerevisiae*	Glass bottles (250 L) were used, with a total reaction volume of 50 mL and a pH of 4.8 for the hydrolysate. The pre-cultured yeast cell (0.25 g/L) was inoculated, and the bottles were sealed with rubber stoppers. They were incubated in an orbital shaker (150 rpm) at 35°C	An optimized enzymatic hydrolysis condition yielded glucose concentrations of 75 and 100 g/L. Ethanol solutions at 4.8% and 4.0% (v/v) were obtained from banana rachis and pseudostem, respectively, corresponding to 87% and 74% of the maximum achievable ethanol yield	[81]
Sugarcane bagasse and rice husk	Physical treatment (blending) and dilute alkaline treatment.	Mixed cultures of *S. cerevisiae* and *Z. mobilis*	500 mL of glucose syrup solution (hydrolysate) was mixed with the yeast (*S. cerevisiae*) and bacterium (*Z. mobilis*) at pH 5, and the mixture was incubated in a water bath at 60°C for 20 min	The highest glucose concentration of 0.5689 g/L was obtained from sugarcane bagasse with *T. reesei*, and 0.5803 g/L from rice husk with *A. niger*. The highest ethanol yields from rice husk were 9.3000 g/L in 60 h, and 8.1000 g/L from sugarcane bagasse in 48 h	[107]
Mango peel, açaí seeds, peach palm peel	Hydrothermal pretreatment with diluted H_2SO_4	*S. cerevisiae*	Fermentation was carried out using 5 mg fresh yeast/mL in Erlenmeyer flasks containing 20 mL of hydrolysates or liquors in a fermenting medium, incubated in a shaker at 120 rpm and 30°C for 72 h. Fermentation occurred at a pH range of 4.0 to 4.5, and then filtered through sterilizing membranes	Highest glucose level 0.25, 0.26 and 0.34 g/L from peach palm peel, mango peel and acai seeds, respectively. High concentrations of ethanol at 0.119 g/L (mango peel), 0.114 g/L (peach palm peel) and 0.276 g/L (açaí seeds) were obtained	[108]
Rice straw	Alkaline and steam pretreatment	*S. cerevisiae*	Cell suspension of pre-cultured yeast pellets (1×10^8 cells/mL) was inoculated with sugar hydrolysate supplemented with 0.1% each of urea and yeast extract and incubated under static conditions at 30°C for 72 h	The highest sugar production of 70.0 g/L after 120 h was observed with an ethanol yield of 15.6 g/L	[23]

3.2 Bioethanol Production from Fermentation-Based Industrial Lignocellulosic Wastes

The beverage and/or fermentation industries generate some amount of lignocellulosic waste biomass that could be valorized to bioethanol. This plant-based biomass is an important source of raw materials for many fermentation-based industries [1,3,109]. Industrial waste includes both organic and inorganic wastes, and can harm the environment if not properly disposed of; therefore, its management is crucial [110]. The reusability of industrial by-products as raw materials for ethanol production contributes to resource efficiency by reducing energy consumption, lowering greenhouse gas emissions and curbing excessive landfill waste. The valorization of industrial waste supports circular production practices and sustainability [111].

Some of these fermentation-based industries include: breweries, wineries, distilleries, vinegar industries, etc. Lignocellulosic wastes generated from these industries are grape pomace, vine shoots, brewer's spent grains, spent hops, trubs, cereal and grain husks/remains, sugar beets, sugarcane bagasse, grape and berry peels and remains, and others [1,111–113].

Table 5 presents some fermentation-based industrial wastes, their lignocellulosic composition, pretreatment process and ethanol yield using microorganisms. Ethanol yields of over 20% were reported in some of the studies [77,113–115]. Industrial lignocellulosic wastes that were reported include grape and apple pomace, brewer's spent grain, and different distillages, among others.

Table 5: Bioethanol production from fermentation-based industrial lignocellulosic wastes.

Lignocellulosic Fermentation Waste	Industry	Form of Waste	Composition (%)	Pretreatment	Fermentation Conditions	Ethanol Yield (g/L)	References
Brewer's spent grain	Brewery	Solid	Cellulose 38.90, hemicellulose 26.16, lignin 23.90	Enzymatic hydrolysis, acid hydrolysis	pH: 5.5, temperature: 38°C	32.0	[114]
Rye stillage	Distillery	Liquid waste after centrifugation	Cellulose 16.80, hemicellulose 29.60, lignin 15.57	Microwave dilute acid hydrolysis	Temperature: 35°C, pH: 5.5, initial inoculum: 1.2×10^9 CFU/mL	20.0	[113]
Press mud	Distillery	Soft, light weight, spongy solid residue	Cellulose 22.30, hemicellulose 21.67, lignin 12.00, moisture 76.21, ash 18.25, protein 1.65	Acid hydrolysis	Conical flask: 250 mL (100 mL working volume), temperature: 28°C, pH: 4.5, initial inoculum 5% (v/v), shaking: 120 rpm, time: 96 h	11.65	[116]
Sugar beet pulp	Distillery	Solid	Cellulose 21.7, hemicellulose 24.0, lignin 2.4, protein 9.3	Acid hydrolysis	Fermenters: 87.4 m^3, time: 36 h	12.6	[117]
Apple pomace	Vinegar, cider	Solid residue consisting of skin, flesh and seeds	Cellulose 10.47, hemicellulose 26.10, lignin 1.65, protein 6.80, ash 4.82	Hydrothermal, alkaline and acid hydrolysis	Temperature: 50°C, pH 5.0	10.47	[118]
Corn fiber	Liquor, distillery	Solid	Cellulose 15–20, hemicellulose 30–50, starch 10–25.	Alkaline, acid hydrolysis	Temperature: 32°C, shaking: 200 rpm, time ~72 h	28.0	[115]
Pomegranate peel	Wine	Solid	Cellulose 36.36, hemicellulose 28.20, lignin 15.27, ash 11.40, protein 15.60, moisture 7.65.	Alkaline peroxide	Temperature 30°C	14.3	[119]
Rice husk	Distillery	Solid	Cellulose 35.0, hemicellulose 15.0, lignin 16.0, starch 7.0.	Alkaline peroxide	Temperature: 30°C, initial inoculum: 5×10^8 CFU/mL, time: 60 h	23.0	[77]

(Continued)

Table 5 (continued)

Lignocellulosic Fermentation Waste	Industry	Form of Waste	Composition (%)	Pretreatment	Fermentation Conditions	Ethanol Yield (g/L)	References
Corn cobs	Liquor, distillery	Solid	Cellulose 28.0, hemicellulose 42.0, lignin 19.0, ash 2.6.	Alkaline, acid hydrolysis	Temperature: 30°C, pH: 5.5, initial inoculum: 0.5 g/L	5.0	[120]
Wheat bran	Beer, distillery	Solid waste generated after milling wheat	Cellulose 10.91, hemicellulose 38.99, lignin 5.08, protein 17.88, ash 0.04.	Acid hydrolysis	Temperature: 30°C, pH 5.5	7.3	[121]
Wheat stillage	Distillery	Liquid waste after centrifugation	Cellulose 18.59, hemicellulose 20.90, lignin 9.47	Microwave dilute acid pretreatment	Temperature: 35°C, pH: 5.5, initial inoculum: 1.25×10^9 CFU/mL	20.0	[122]

3.3 Bioethanol Production from Forestry-Based Industrial Lignocellulosic Wastes

Another type of lignocellulosic waste is forestry-based feedstocks, which consist of woody materials used to produce bioethanol. This can be categorized into softwood and hardwood. Softwoods have low density and can grow more frequently. Examples include spruce, fir, pine, cypress, etc. While hardwoods are angiosperms that grow slowly, they are generally deciduous. Examples include mahogany, beech, cottonwood, maple, oak, poplar, and others [123]. Unlike softwood, which is often used in construction and in the paper and manufacturing industries, hardwood cannot be recycled for use in these industries despite the huge amount of waste generated annually from forest thinning [99,123]. This is due to the presence of short fibers and knots in hardwood [99]. Hence, the need to recycle the discarded hardwood as a biomass source for the potential generation of bioethanol as a result of its high cellulose (45%–50%) and hemicellulose (25%–35%) contents, which increase its hydrolysis into fermentable sugars with fewer enzymes and also increase productivity [99].

Cottonwood is considered the most appropriate wood for bioethanol production because of its advantages, such as high productivity, potential for restoration, a large number of clones, and uniformity in planting material [124]. Several forestry-based feedstocks contain more lignin and less ash, making them attractive substrates for improved bioethanol conversion [125]. However, this high lignin content can make them more challenging to process than agricultural residues. New delignification procedures, such as deep eutectic solvents and ionic liquid pretreatment, have been shown to degrade lignin structure in forest-based residues [126,127].

The hydrogen peroxide-acetic acid (HPAC) pretreatment method has been reported to efficiently eliminate lignin from numerous biomasses by reacting to generate peracetic acid (PAA), which breaks down lignin into monomeric phenolic compounds, thereby converting the side chains of propane into either a carboxyl or hydroxyl group [128,129]. Research conducted by Song et al. [99] evaluated the enzyme hydrolysis of HPAC-pretreated fringe (*Chionanthus retusus*) wood and recorded a significant reduction in lignin, which improved the hydrolysis efficiency by about 2.8 times better than that of the raw resources, contributing to an amplification in the productivity of bioethanol at 14.8 mg/mL (Table 6).

Various forestry-based feedstocks offer several benefits as substrates and as renewable energy sources for bioethanol production. Palm wood was investigated for bioethanol generation. Pretreating this forestry biomass with hydrothermal and chemical methods provided a bioethanol yield of 22.90 g/L after hydrolysis by cellulase secreted by the fungus, *T. reesei*, with optimized conditions of Artificial Neural Network

at an agitation rate of 156 rpm, pH 5, temperature of 45°C, substrate concentration of 8% (v/v) and inoculum size of 3.2% (v/v) [130] (Table 6). Pine needle waste, another forest biomass, was hydrolyzed by a cellulase-producing *Bacillus subtilis* and a xylanase-producing *Bacillus pumilus*. This generated 25.64 ± 2.36 mg/mL of fermentable sugars with an ethanol concentration of 17.65 g/L and a fermentation efficiency of 91.22% [131] (Table 6). Another study investigated a mixed softwood sawdust feedstock as a substrate for bioethanol production. Enzymatic hydrolysis was conducted on the pretreated feedstock using the commercial enzyme Cellic Ctec2, and the hydrolysate was fermented with *S. cerevisiae*. The hydrolysis attained 80% saccharification yield, and about 80% theoretical conversion yield of glucose to ethanol [100].

Several other forestry-based lignocellulosic resources, such as poplar and willow wood, have been described for use in bioethanol production with fermentation efficiencies between 68% and 92% [132,133].

Table 6: Bioethanol production from forestry-based industrial lignocellulosic wastes.

Biomass Source	Pretreatment	Fermenting Microbe	Fermentation Conditions	Productivity	References
Pine needle waste	Milling and physico-chemical pre-treatments (1.0% NaOH + microwave)	*Schizosaccharomyces* sp. EF-3 and *Kluyveromyces marxianus*	The fermentation medium contained 3% pre-cultured inoculum of both yeast, 5 g of pre-treated pine needle waste biomass, peptone (0.25 g), $CaCl_2$ (1.00 g), KH_2PO_4 (0.10 g), K_2HPO_4 (0.012 g), $MgSO_4.7H_2O$ (0.012 g) at pH 4.5. It was incubated at 30°C for 24 h.	Total reducing sugar was 25.64 mg/mL, with ethanol production of 17.65 g/L, at a fermentation efficiency of 91.22%.	[131]
Willow wood	Steam explosion pretreatment	*S. cerevisiae*	The fermentation medium consists of 18.5 mL of the liquid, 0.5 mL of nutrients, and 1.0 mL of inoculum. The hydrolysate was fermented with yeast cells at 10 g of dry matter/L, and the flask was incubated at 30°C for 24 h, while stirring with a magnetic stirrer.	Cellulose-to-glucose conversion rate of ~80% with an ethanol yield of ~90%.	[132,134]
Palm wood	Acid and alkaline pretreatment	*K. marxianus* MTCC 1389	The fermentation medium containing hydrolysates was inoculated with *K. marxianus* and incubated in an orbital shaker for 96 h at optimal conditions: pH 5, 40°C, 170 rpm, and 4% (v/v) inoculum size.	The highest cellulose concentration (23.30 mg/g) and the highest reducing sugar content (1.75 mg/g) were attained, with a 22.90 g/L ethanol yield, under the artificial Neural Network (ANN) optimum conditions.	[130]
Fringe (*Chionanthus retusus*) wood	Hydrogen peroxide-acetic acid (HPAC) pretreatment	*S. cerevisiae*	Dry yeast (0.5 g) was added to the hydrolysate (100 mL) in a fermentation medium (150 mL) at 5.00% (w/v) substrate with 0.10% (w/v) yeast extract, 0.20% (w/v) peptone, and 0.05 M citrate buffer (pH 5.0) at an agitation of 190 rpm for 24 h at 30°C	Glucose production of 34.3 mg/mL was recorded with a 95.5% conversion rate, yielding 14.8 mg/mL ethanol at a theoretical ethanol yield of 80.7% after 24 h using SHF.	[99]

(Continued)

Table 6 (continued)

Biomass Source	Pretreatment	Fermenting Microbe	Fermentation Conditions	Productivity	References
Bamboo (*Phyllostachys edulis*) trees	Alkaline pre-extraction and acid-catalyzed steam pretreatment	*S. cerevisiae*	Serum bottles (125 mL) containing 50 mL hydrolysate were inoculated with an initial yeast cell concentration of 1.0 g/L at a pH of 5.5, and incubated at 35°C under anaerobic conditions at 180 rpm for up to 72 h.	The maximum yield of glucose was ~86.0%, and the hydrolysates produced a final ethanol concentration (50.1 g/L) at 68.9% yield of ethanol.	[135]

3.4 Bioethanol Production from Other Industrial Lignocellulosic Wastes

The paper and pulp industry is a lignocellulosic waste source that generates global waste (17%) [20,21]. They are linked to wood waste, including bark, unprocessed chips and sawdust. Another waste is the alkali compounds, a blend of sodium hydroxide, sodium sulphite and water-alkali-soluble lignin fragments, called black liquor, used to remove lignin. They are also linked to sludge from water treatment processes and to chemical solvents such as chlorine dioxide, ozone, chlorine and hydrogen peroxide for bleaching the pulp [20,21]. The major waste product from this industry is black liquor, amounting to about 170 million tonnes per year [21]. The composition of black liquor depends on wood type and pulping conditions, with an increased recovery rate of lignin for softwood [136].

Due to the physical and chemical properties of these woody wastes, which have lignocellulosic attributes, they can be widely used in several industries as a source of fuel or energy [137,138]. Some biofuel production using other industrial lignocellulosic biomass is presented in Table 7. Two types of pulp and paper sludge were evaluated for their potential as substrate in bioethanol production using a genetically modified cellulase-producing *S. cerevisiae* in an SSF process. The ethanol concentration was recorded as 52.7 ± 4.8 g/L for corrugated recycle paper sludge (CR-PS) and 101.8 ± 15.5 g/L for virgin pulp paper sludge (VP-PS). Ethanol yields of 12.0 ± 2.9 g ethanol/100 g dry PS for CR-PS and 22.7 ± 3.4 g ethanol/100 g dry PS for VP-PS were obtained, giving a percentage theoretical value of 89.6 ± 18.2% (CR-PS) and 111.3 ± 16.8% (VP-PS) [139] (Table 7). Farghaly et al. [140] also evaluated the ethanol-generating possibility of paperboard mill sludge using enzymes generated by *A. niger*, and *S. cerevisiae* as the fermenting microbe. The maximum glucose yield was noted at 33.2 ± 0.3 g/L, and the highest ethanol yield of 10.97 ± 0.55 g/L at 2% (v/v) HCl (Table 7).

Another industry that produces lignocellulosic waste is the textile industry. Wastes generated from textile industries include pigments, alkalis, textile residues, heavy metals, sludges, organic stabilizers, peroxides, and chemical solvents [20]. Only about 78%–90% of the solid waste is recyclable, yet only about 15%–20% of textile residues are presently being recycled [141]. Among other waste types, only the textile residues comprise lignocellulosic biomass [141]. Cotton gin waste and cotton gin dust, both being textile residues, were analyzed for their ethanol-producing potential using the commercial enzyme Cellic CTec2 and the microorganism, *S. cerevisiae*. The maximum fermentable sugar concentration was recorded at 47.8 and 42.5 g/L with an ethanol yield of 86.9 ± 1.6 and 87.5 ± 0.7% for cotton gin dust and cotton gin waste, respectively [142]. Another cotton-based textile waste [white T-shirt (WTS), linen and white towel (WT)] was evaluated for its ability to produce ethanol using cellulase produced by *T. reesei* [143]. Maximum ethanol yields were recorded at 537 mL/kg (WTS), 502 mL/kg (WT), and 487 mL/kg (linen), with glucose conversion yields of 83.5%, 82.3% and 75.7%, respectively, after 24 h of fermentation (Table 7).

Other industrial sources of lignocellulosic biomass, such as food and municipal waste generated from commercial and institutional sources, have also been implicated in bioethanol production. For example, Prasoulas et al. [144] showed that cellulase produced by *Fusarium oxysporum* F3 produced 33.7 g/L glucose, and when supplemented with commercial glucoamylase, showed an increase in glucose to ~45.0 g/L. The sugar produced was fermented with a blend of *S. cerevisiae* and *F. oxysporum* F3, yielding 20.0 g/L (with indigenous enzyme) and 30.0 g/L (with commercial glucoamylase) ethanol (Table 7). Another study on municipal waste using cellulase from *B. subtilis* gave the highest reducing sugar with biosurfactant addition at 9076.10 μg/mL, and the highest bioethanol production at 60.27 mL/L [145] (Table 7). These industrial lignocellulosic biomasses are considered easiest to use due to their high quality and are less likely to be mixed with other molecules [1].

Table 7: Industrial-based lignocellulosic biomass from other industrial sources.

Biomass Source	Pretreatment	Fermenting Microbe	Fermentation Conditions	Productivity	References
Paperboard mill sludge	Acid pretreatment	*S. cerevisiae*	The yeast cell (10 g/L) cultivated at 30°C for 24 h in a shaker bath was inoculated into the hydrolysate for fermentation.	The highest glucose yield was 33.20 g/L, and the highest ethanol yield was 10.97 g/L.	[140,146]
Corrugated recycled and virgin pulp paper sludge waste	Milling pretreatment	Genetically modified cellulase-producing *S. cerevisiae*	The fermenting vessel (~700 g, containing the hydrolysate, fermentation medium, and enzymes) was inoculated with 5% yeast cream (w/w) of the dry mass at pH 5-6. It was incubated at 37°C for 144 h, and the samples were centrifuged for 5 min at 14, 000 rpm	Ethanol concentration of 52.7 g/L for corrugated recycle paper sludge (CR-PS) and 101.8 g/L for virgin pulp paper sludge (VP-PS), with an ethanol yield of 12.0 g ethanol/100 g dry PS (CR-PS) and 22.7 g ethanol/100 g dry PS (VP-PS).	[139]
Cotton gin textile residue	NaOH pretreatment	*S. cerevisiae*	Fermentation was conducted with 42.7 g/L of hydrolysate supplemented with 1.0 g/L yeast extract, 0.5 g/L$(NH_4)_2PO_4$ and 0.025 g/L $MgSO_4 \cdot 7H_2O$, and then inoculated with yeast cells (4×10^6 cells/mL) at 37°C and 150 rpm. The aliquots were then filtered using a 0.45 μm filter	Maximum concentration of fermentable sugars 47.8 and 42.5 g/L for cotton gin dust (CGD) and cotton gin waste (CGW), respectively, was obtained. The ethanol yield was 86.9 ± 1.6% (CGD hydrolysate) and 87.5 ± 0.7% (CGW hydrolysate).	[142]
Cotton-based textile waste [white T-shirt (WTS), linen, and white towel (WT)]	NaOH pretreatment	*S. cerevisiae*	Fermentation was conducted using 0.1% (w/v) dry yeast, which was inoculated into 7.0 mL of hydrolysate and incubated at 32°C for 24 h	The maximum ethanol yield was 537 mL/kg (WTS), followed by WT at 502 mL/kg and linen at 487 mL/kg. With a glucose conversion yield of 83.5%, 82.3% and 75.7%, respectively.	[143]

(Continued)

Table 7 (continued)

Biomass Source	Pretreatment	Fermenting Microbe	Fermentation Conditions	Productivity	References
Food waste	Acid and autoclave heating pretreatment	Mixed cultures of *F. oxysporum* F3 and *S. cerevisiae*	Baker's yeast (15 mg/g), fungal mycelia and the *in situ* produced enzymes were inoculated into pretreated food waste (1/10 w/w) in a flask with a rubber stopper at 30°C and 80 rpm in a rotary shaker	The highest glucose yield was ~45 g/L with an ethanol concentration of 20 g/L, and when supplemented with commercial enzyme, it reached 30 g/L. This corresponds to a yield of ~8 g/100 g FW and 10 g/100 g FW, respectively.	[144]
Municipal waste	Dry milling	*S. cerevisiae*	Precultured yeast strain at a concentration of 10% (v/v) was inoculated into sterile refrigerated fermentation medium containing hydrolysate and peptone 10 g/L, KH_2PO_4 2 g/L and $MgSO_47H_2O$ 1 g/L in a self-sterilizer 10 L bioreactor. It was incubated at 30°C for 24, 48 and 72 h at 150 rpm under aerobic conditions, and thereafter centrifuged at 10,000× *g* for 10 min.	The highest total reducing sugar recorded with biosurfactant addition was at 9076.10 µg/mL and gave the maximum bioethanol production of 60.27 mL/L	[145]

4 Challenges and Prospects in the Use of Industrial Lignocellulosic Wastes for Enzymes and Bioethanol Production

One of the greatest challenges of using any lignocellulosic biomass (including those from industries) for bioethanol production, and the greatest drawback of second-generation bioethanol production, is the recalcitrant nature of the substrate and the need for pretreatments. The outer protective lignin and the crystalline hemicellulose layers usually shield the cellulose polymers in lignocellulose, which greatly hinders the applications of this biomass (Fig. 2) [1,14–16,147]. Several pretreatment methods, including physical, chemical and enzymatic approaches or combinations of them, have been demonstrated in some works to delignify biomass and aid the release of cellulose from various polymer shields. Pretreatment has also been reported to aid the access of hemicellulase enzymes to the hemicellulose constituents of the biomass [87,148,149].

However, these pretreatments come with their own challenges. Aside from the potential increase in production costs, chemical pretreatments often generate inhibitory by-products. These include furfural, formic and acetic acids, among others. At some concentrations, these compounds can be toxic and/or inhibit both microorganisms and their enzymes needed in the biorefinery process. Some studies have demonstrated that detoxification after pretreatment can remove inhibitory compounds, thereby facilitating better microbial hydrolysis and fermentation (Fig. 7). Notwithstanding, detoxification processes could also increase production costs, especially in large-scale bioethanol or lignocellulase enzyme production [150,151]. The use of pretreatment methods that release little or no inhibitory substances, or the isolation/selection and genetic modification of microorganisms that can withstand these inhibitory substances, or that hydrolyze or saccharify lignocellulosic biomass without pretreatment, is critical to addressing this challenge [4].

Such microbes are gaining relevance in bioethanol refinery technologies, including consolidated bioethanol process, where pretreatment and/or detoxification are not core necessities in bioethanol production from lignocellulose, thereby reducing production costs [4,43,152,153].

Another reason that may require detoxification is contamination of industrial lignocellulosic biomass with other chemicals. Since these waste biomasses are products of industrial processes, the possibility that they are laden with toxic industrial chemicals could pose another challenge. Detoxification of the lignocellulosic biomass could be done before use. However, getting or modifying microorganisms or enzymes that can thrive in such contamination is a more cost-effective approach than the costly detoxification [1,4].

The application of genetically engineered microbes for lignocellulase enzymes and bioethanol production from lignocellulosic waste biomass has not only helped develop microbes that can withstand inhibitory substances from pretreatment, but has also greatly improved enzyme and bioethanol yields. Several *Escherichia coli* strains (especially the BL21) have been transformed to express cellulases [154–156], xylanase [157] and other enzymes. Some other microorganisms that have been modified to improve lignocellulase enzyme production include: *B. subtilis* [158], *Neurospora crassa* [159], *Trichoderma afroharzianum* [160], among others. Furthermore, modified single organisms that can hydrolyze lignocellulosic biomass and ferment the resulting simple sugars (including hexoses and pentoses) have also been reported [8,40,43,46,98]. These have ultimately helped reduce the cost of producing lignocellulase enzymes and bioethanol.

Another serious challenge in lignocellulose applications in biofuel production is the cost of enzymatic hydrolysis. Emphasis on in-house crude enzyme production partly addresses this challenge. To further reduce cost, techniques that encourage enzyme and/or microbe reusability have been developed. One such approach is immobilization of enzymes and microbes. Studies have shown that immobilization in various carriers can enable the recycling of enzymes and microbes with retained efficacy for multiple cycles. This therefore, eliminates the time and cost of preparing fresh cultures [161]. However, this method may not be efficient for certain processes involving certain microbes, such as filamentous fungi. It could also affect mass transfer efficiency, and might easily introduce contamination into subsequent cycles. Estimating and standardizing immobilized microbes and enzymes can also be challenging. In microbial ecology, the fear that microbes may subtly evolve with different cycles is yet another consideration. Other technologies that could be adopted for reusability and optimized yield include applications of nanotechnology [52,162–164], genetic modifications of enzymes and microbes [155–157,159] and integrated biorefinery techniques [4,165].

5 Conclusion

No doubt, the increasing global population and industrialization will continue to increase the volume of industrial lignocellulosic waste generated. This increase in human population will also drive higher energy demand, which, if met solely from fossil fuels, would not be sustainable and eco-friendly. Converting these industrial lignocellulosic waste biomasses into essential enzymes and bioethanol, or other green biomass fuels, will simultaneously valorize the waste into commercial products while addressing waste management concerns. The role of microorganisms in this valorization process is sine qua non. As “living” biorefineries, microbes are paving the way for the production of essential bio-products, including fermentable sugars and bioethanol, from lignocellulosic waste biomass by circumventing conventional chemical pretreatment and/or detoxification processes. This will ultimately lead to the harnessing of green fuels and the achievement of several sustainable development goals.

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