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Review

Laccases as a Potential Tool for the Efficient Conversion of Lignocellulosic Biomass: A Review

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Abstract: The continuous increase in the world energy and chemicals demand requires the development of sustainable alternatives to non-renewable sources of energy. Biomass facilities and biorefineries represent interesting options to gradually replace the present industry based on fossil fuels. Lignocellulose is the most promising feedstock to be used in biorefineries. From a sugar platform perspective, a wide range of fuels and chemicals can be obtained via microbial fermentation processes, being ethanol the most significant lignocellulose-derived fuel. Before fermentation, lignocellulose must be pretreated to overcome its inherent recalcitrant structure and obtain the fermentable sugars. Usually, harsh conditions are required for pretreatment of lignocellulose, producing biomass degradation and releasing different compounds that are inhibitors of the hydrolytic enzymes and fermenting microorganisms. Moreover, the lignin polymer that remains in pretreated materials also affects biomass conversion by limiting the enzymatic hydrolysis. The use of laccases has been considered as a very powerful tool for delignification and detoxification of pretreated lignocellulosic materials, boosting subsequent saccharification and fermentation processes. This review compiles the latest studies about the application of laccases as useful and environmentally friendly delignification and detoxification technology, highlighting the main challenges and possible ways to make possible the integration of these enzymes in future lignocellulose-based industries.

Keywords: lignocellulosic biorefinery; delignification; detoxification; ethanol; fermentation; inhibitory compounds; laccase; lignin; pretreatment; saccharification

1. Introduction

Renewable fuels are considered promising alternatives to mitigate global warming and reduce our dependence on fossil fuels. In the particular case of transportation, ethanol is one of the few alternatives for the diversification of this sector in the short term, since it can be easily integrated into current fuel distribution systems [1]. Traditionally, certain food-related products including sugar crops and starch-based feedstocks have been used to produce ethanol. Alternatively, lignocellulosic biomass is an abundant and low-cost raw material that has no directly influence on food production [2]. Among them, forestry and agricultural residues (e.g., pine harvest forest, wheat straw, olive tree pruning, etc.), dedicated crops (e.g., elephant grass, forage sorghum, poplar, etc.), and municipal solid wastes are considered potential materials for ethanol production. Lignocellulosic biomass, in addition,

is expected to provide a wide range of different renewable products such as food and feed additives, chemicals and materials. This lignocellulose-based industry—also known as biorefinery—is likely to become increasingly important in the future society as a complement and/or alternative to the current petroleum-based industry.

Biochemical conversion of lignocellulose represents the most favorable route among all developed technologies [3]. It includes three major steps: pretreatment, enzymatic hydrolysis, and fermentation. Pretreatment increases the accessibility of lignocellulose to hydrolytic enzymes by removing or modifying lignin and hemicellulose polymers, and by altering cellulose structure. Enzymatic hydrolysis or saccharification breaks down carbohydrates into fermentable sugars by the combined action of different enzyme activities. Finally, microorganisms convert sugars into alcohols, organic acids, alkenes, lipids or other chemicals through fermentation processes.

Focusing on pretreatment processes, several physical and/or chemical technologies have been developed and optimized for improving the conversion of a high number of lignocellulosic feedstocks [4]. During pretreatment, high pressures and temperatures and/or the addition of chemicals and solvents are in general required. These harsh pretreatment conditions lead to biomass degradation and generation of different enzymatic (mainly phenolic compounds) and microbial inhibitors (weak acids, furan derivatives and phenols), which limits the subsequent saccharification and fermentation steps [5]. Another factor that limits enzymatic hydrolysis is the residual lignin that remains in pretreated materials. Lignin hampers the accessibility of carbohydrates to hydrolytic enzymes by acting as a physical barrier; but also, it promotes the non-specific adsorption of hydrolytic enzymes to the lignin polymer, lowering the number of enzymes available for hydrolyzing carbohydrates and therefore decreasing saccharification yields [6].

To overcome the effects of lignocellulose-derived inhibitors and lignin, different detoxification and delignification processes have been evaluated [7,8]. Among them, the utilization of laccase enzymes has been widely investigated, showing to be effective in removing and/or modifying the lignin polymer, and in reducing the phenolic content of pretreated lignocellulosic materials [9,10]. The present work focuses on review the use of laccases as delignification and detoxification agents for the efficient conversion of lignocellulosic biomass into value-added products, with special accent in the lignocellulosic ethanol production.

2. Lignocellulosic Biomass Conversion: The Sugar Platform

The implementation of a sugar platform offers the possibility to obtain a high number of fuel and chemical products (alcohols, organic acids, alkenes, lipids and other chemicals) via fermentation processes [11]. With a high carbohydrate content, lignocellulosic biomass represents a promising sugar source for such an aim. Lignocellulosic sugars can be obtained either by acidolysis or via enzymatic hydrolysis, being the latter a preferred choice since it is more selective, it requires less energy (lower temperatures are needed), and it releases no harmful by-products [3]. However, the recalcitrant structure of lignocellulose hinders the accessibility of carbohydrates to hydrolytic enzymes and prevents the release of fermentable sugars. In this context, a pretreatment process is therefore needed to alter the structure of lignocellulose and thus facilitate an efficient enzymatic hydrolysis of carbohydrates [12].

The effectiveness of pretreatment processes for improving enzymatic hydrolysis of lignocellulosic biomass has been attributed to (1) hemicellulose removal; (2) lignin removal and redistribution [13]; (3) a reduction in the degree of polymerization and crystallinity of cellulose [14]; and/or (4) an increment in the porosity of pretreated materials [15]. Over the years, many different pretreatment methods have been investigated on a wide variety of feedstocks, being classified into physical, chemical, physicochemical, and biological pretreatments [4,16]. It is important to highlight that there is no best pretreatment technology and that the choice of the pretreatment method depends very much on the type and composition of the feedstock to be processed [17]. Among pretreatment technologies, chemical and physicochemical pretreatments are the most effective and promising processes for

industrial applications [1]. Chemical methods, especially alkali- and acid-based pretreatments, are low cost processes and have shown to effectively remove hemicellulose and lignin from lignocellulosic feedstocks. Physicochemical pretreatments (e.g., steam explosion, liquid hot water, ammonia fiber explosion/expansion, wet oxidation, etc.), on the other hand, are also low cost technologies but with a lower environmental impact compared to chemical technologies [4]. These methods are capable of solubilizing hemicellulose, disrupting the structure of lignocellulose and increasing the accessible surface area of pretreated substrates. Other pretreatment technologies including milling, organosolv, and ionic liquids (ILs) can also significantly improve the digestibility of lignocellulosic materials [1]. Nevertheless, their high operational costs represent an important limitation for their commercial applications.

After pretreatment, enzymatic hydrolysis is responsible for breaking down lignocellulose-contained carbohydrates. It is in overall a crucial step that highly influences final process yields. Due to the complex structure and the heterogeneous composition of lignocellulose, a high number of enzymatic activities including cellulases, hemicellulases, and ligninases are needed for its complete hydrolysis [18]. Cellulases (endoglucanases, cellobiohydrolases, and β-glucosidases) hydrolyze cellulose into glucose monomers, while hemicellulases (e.g., xylanases, β-xilosidases, α-L-arabinofuranosidases, esterases, etc.) and ligninases (e.g., laccases, peroxidases, reductases, oxidases generating H_2O_2, etc.) depolymerize hemicellulose and lignin, respectively. Major limitations of the enzymatic hydrolysis are the costs for enzyme production and the necessity of providing the appropriate enzyme mixtures. Although significant advances have been achieved to overcome these limitations, the enzymatic mixtures and the enzyme production process still need to be optimized. This optimization involves the use of low-cost substrates and/or the inclusion of novel enzymatic activities, such as the non-hydrolytic proteins swollenins and expansins, and the polysaccharide monooxygenases (LPMOs) [19,20]. In addition, recent studies also aim at increasing the catalytic efficiency of hydrolytic enzymes, by screening and/or engineering of enzyme-producing microorganisms, while other studies aim at cost reduction by enzyme recycling [18].

The corresponding sugars obtained after enzymatic hydrolysis can be potentially converted to a large number of products via microbial fermentation processes. Among them, the sugar-to-ethanol conversion process has been the most widely studied. Three main process configurations have been described for ethanol production, including separate hydrolysis and fermentation (SHF), simultaneous saccharification and (co)fermentation (SSF/SSCF) and consolidating bioprocessing (CBP) [21]. SSF/SSCF processes integrate the enzymatic hydrolysis and the fermentation stages in a single step, which has shown to be beneficial for improving conversion efficiencies. During these processes, the introduction of a presaccharification step (PSSF/PSSCF) to liquefy the media prior yeast addition is especially suitable when working at high substrate loadings [18]. Several yeast, bacterial or fungal strains have been used for fermentation of lignocellulosic-based streams. Among them, the yeast *Saccharomyces cerevisiae* is the most commonly employed microorganism, especially in the alcohol industry. *S. cerevisiae* can utilize all kind of hexoses to produce ethanol, reaching conversion yields close to the theoretical. However, its inability to metabolize pentoses has led to the exploration and development of novel fermenting microorganisms with the capacity to convert all kind of sugars to ethanol [22]. Besides the capacity of utilizing a wide range of sugars, it is important that the fermenting microorganism also shows high tolerance to inhibitory compounds, temperatures, ethanol and/or mechanical and osmotic stress.

3. Inhibitors and Lignin in Pretreated Materials

Pretreatment of lignocellulose often involves side reactions resulting in the release of certain biomass-derived by-products that are inhibitors of downstream biochemical processes [5]. They mainly include furan derivatives, aliphatic acids, and phenolic and other aromatic compounds (Figure 1). Extractives (mainly terpenes, fats, waxes, and phenolics) and inorganic compounds may also promote inhibition of enzymes and microorganisms in the subsequent steps [1]. The nature and concentration

of all these inhibitory products is strongly dependent on the feedstock as well as the pretreatment process [23].

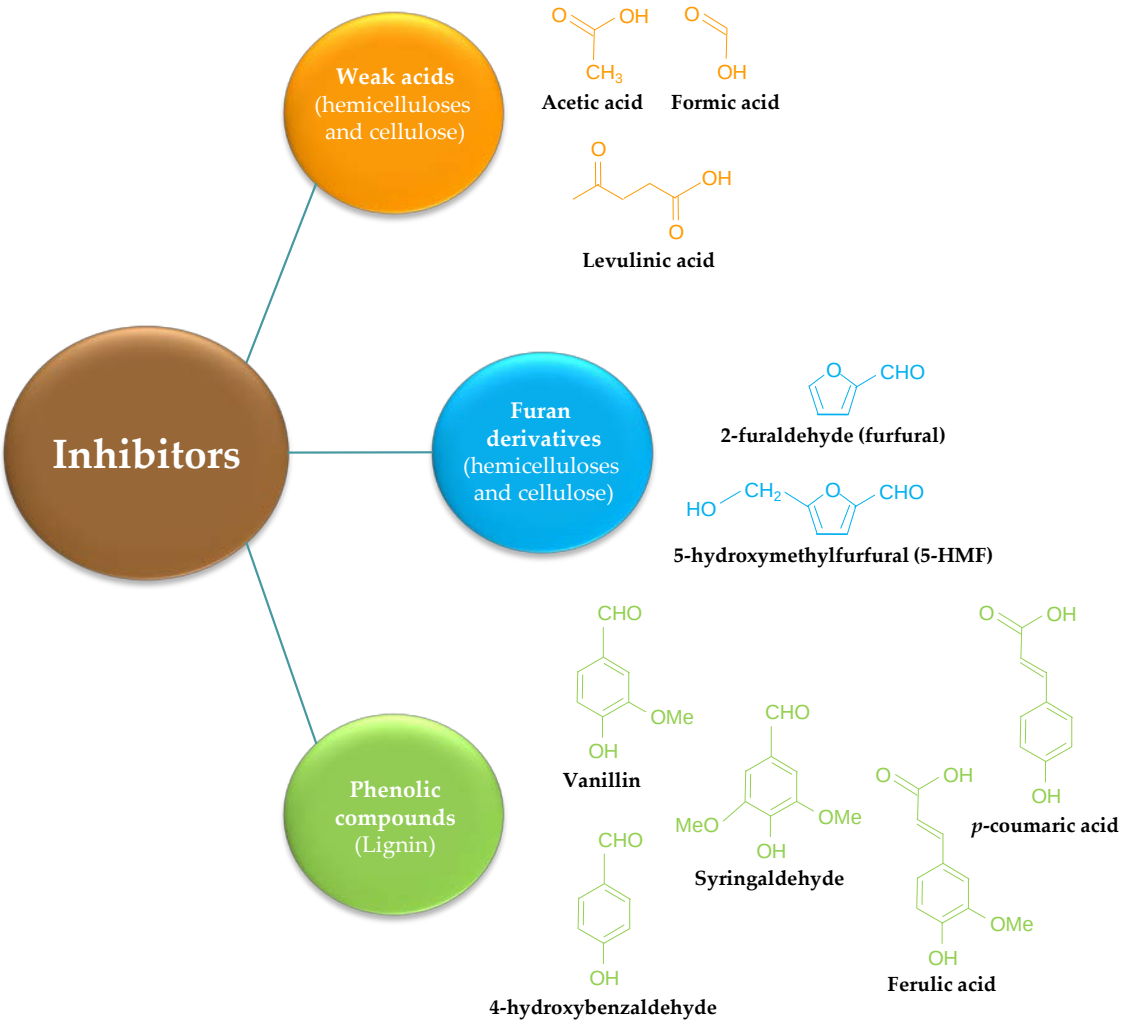

Figure 1. Common inhibitory compounds present in lignocellulosic pretreated materials, indicating main sources of its formation.

During pretreatment processes, the pentoses resulting from hemicellulose can undergo dehydration with formation of furfural, while hexoses can be dehydrated to 5-hydroxymethylfurfural (5-HMF). In addition, furan derivatives can be further degraded to form levulinic acid and formic acid, depending on the severity of the pretreatment process. From hemicelluloses, acetic acid can be also generated from the acetyl groups, while a large number of phenolic compounds, such as 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, vanillin, dihydro-coniferyl alcohol, coniferyl aldehyde, syringaldehyde, syringic acid, *p*-coumaric acid, ferulic acid, and Hibber's cetones, can be produced from lignin [5,24].

The inhibitory effects caused by degradation compounds can be observed in both hydrolytic enzymes and fermentative microorganisms [5,24–26]. Furan derivatives are one of the most important microbial inhibitors during fermentation. They affect cell viability and growth rates, extend the lag phase at the initial stage of the fermentation process, and lower ethanol yields and productivities. These effects derived from the inhibition of several intercellular enzymes (such as alcohol dehydrogenase and pyruvate dehydrogenase) and from the damage promoted to cell membranes and/or to genetic materials [5,24]. Carboxylic acids also affect biomass growth and ethanol production by mainly

promoting the intracellular accumulation of H^+ ions. Among the main biomass-derived carboxylic acids, formic acid has a greater inhibitory effect than levulinic acid, which in turn has shown to have a greater impact than acetic acid [5,24]. The undissociated form of carboxylic acids can diffuse through cell membranes and once inside the cell they are dissociated due to an increase in the pH (the pH increases from about 5 to 7). As a consequence, H^+ ions are accumulated, lowering the intracellular pH and causing an imbalance in the ATP/ADP ratio by the increase in the activity of ATP/H^+ pumps. At last, phenolic compounds have shown to affect microbial growth and reduce ethanol production rates, but not ethanol yield. Usually, this group of lignocellulosic-derived compounds causes loss of membrane integrity and affects specific intracellular enzymatic activities [5,24]. Regarding to hydrolytic enzymes, phenols are the main degradation compounds that inhibit and deactivate them, reducing both rates and yields during the saccharification step [25,26]. Thus, vanillin and syringaldehyde have shown to inhibit cellulases—and in particular β-glucosidases—, while ferulic acid and *p*-coumaric acid are capable of deactivate them. Nonetheless, cellobiose, glucose, and sugars from hemicellulose have been also shown to inhibit hydrolytic enzymes [18].

In addition to the inhibitory compounds, the residual lignin present in pretreated materials represents an important limiting factor during enzymatic hydrolysis of carbohydrates. Lignin constitutes a physical barrier that may unspecifically adsorb hydrolytic enzymes, decreasing the enzyme concentration during the saccharification process [6]. Lignin polymer is built up of *p*-hydroxyphenyl (H) (derived from *p*-coumaryl alcohol), guaiacyl (G) (derived from coniferyl alcohol), and syringyl (S) (derived from sinapyl alcohol) phenylpropanoid units and their acylated forms [27]. The G:S:H unit proportion varies depending on biomass feedstock. Softwood lignin is mainly composed of G units with small proportions of H units, whereas lignin in hardwood contains mainly S and G units. Lignin from non-woody plants, such as agricultural residues, also contains H units together with G and S units [27]. As can be observed in Figure 2, lignin units are linked through a variety of inter-unit linkages including C–C and ether bonds [28]. Among them, the most abundant inter-unit linkages are β-O-4′ (aryl ether), β-5′ (phenylcoumaran), and β-β′ (resinol) bonds. Other structural links such as β-1′ (spirodienone), 5-5′-O-4 (dibenzodioxocin), 5-5′ and 4-O-5′ bonds have been also described. In addition to the interaction between lignin units, lignin-carbohydrate complexes (LCC) are also formed in plant cell walls [28]. The main types of LCC linkages in lignocellulosic materials are phenyl glycoside, ether, or ester bonds.

It has been suggested that the chemical and physical structure of lignin plays an important role during enzymatic hydrolysis. Lignin structure is, in turn, highly dependent on biomass feedstock and/or on pretreatment conditions [29]. For instance, steam-explosion pretreatment produces great reductions in β-O-4′ linkages, resulting in partial lignin solubilization and the release of free phenolic groups [30,31]. Moreover, lignin repolymerization can also take place [32], increasing the number of aromatics substitutions at the C_6. Depending on pretreatment temperature and time, an increase of phenolic hydroxyl groups and a decrease in aliphatic hydroxyl groups can also be observed [33].

Different mechanisms including hydrophobic, electrostatic and hydrogen bonding interactions have been proposed to explain the inhibition of hydrolytic enzymes by lignin [15]. However, the actual mechanism by which hydrolytic enzymes interact with lignin and become inhibited has yet to be fully elucidated. One of the most common accepted explanations is related to an increase in lignin phenolic groups and hydrophobicity (resulted by a lower amount of carboxylic groups and aliphatic hydroxyl groups), which promotes enzyme adsorption to the lignin polymer [15]. This hypothesis is supported by Sewalt et al. [34], who reversed the inhibitory mechanism of organosolv-pretreated lignin by hydroxypropylation of the phenolic groups. Moreover, the addition of surfactants and certain polymers (e.g., tween, bovine serum albumin, polyethylene glycol, gelatin, etc.) has shown to reduce the unspecific adsorption of hydrolytic enzymes to lignin as they can bind to the adsorption sites, improving saccharification yields [34,35].

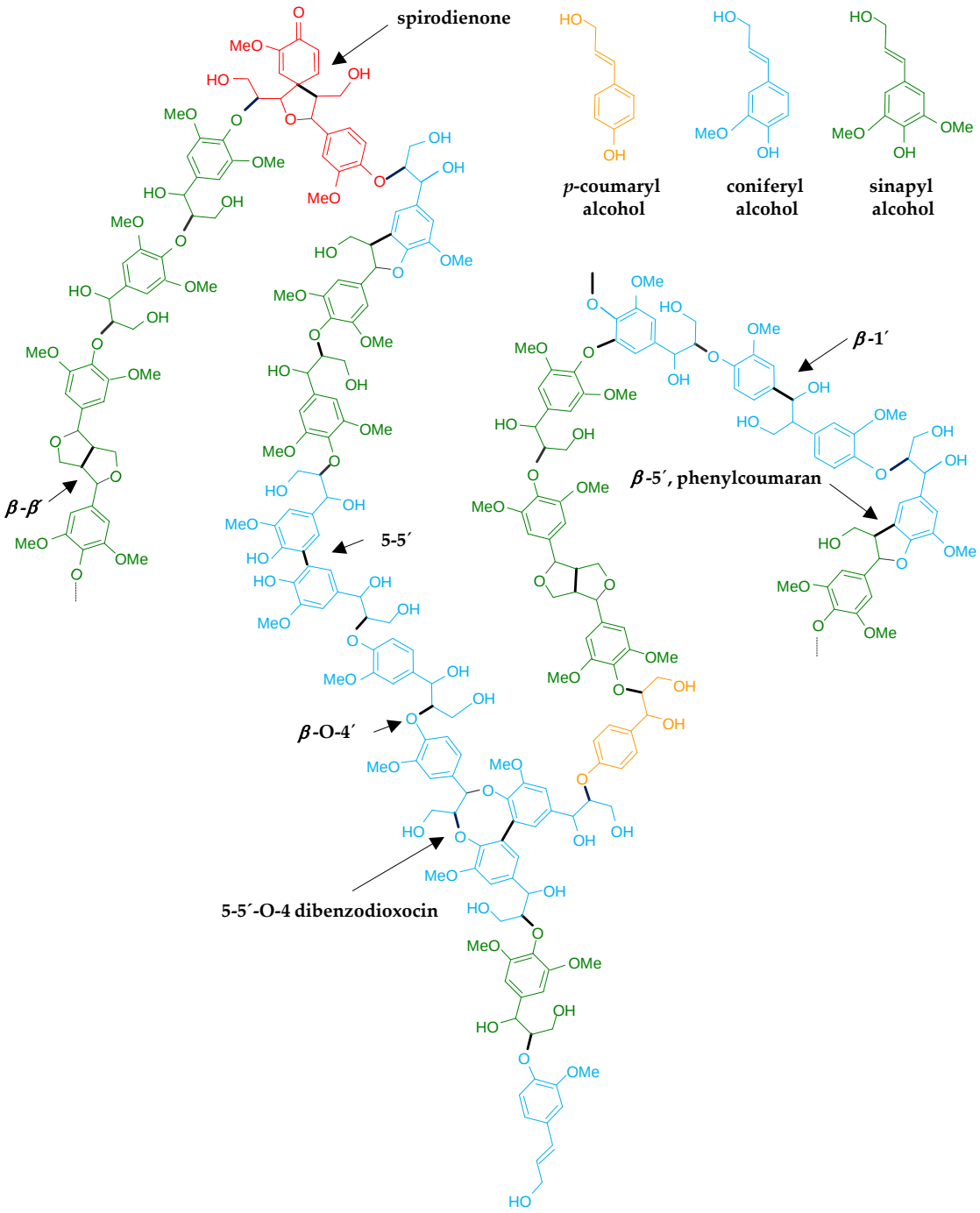

Figure 2. Schematic representation of lignin structure showing the main interunit linkages originated from *p*-coumaryl, coniferyl, and sinapyl alcohols.

The presence of inhibitors and residual lignin makes detoxification and delignification processes powerful tools for improving saccharification and fermentation of pretreated lignocellulosic biomass.

3.1. Detoxification of Pretreated Materials

A detoxification step prior to enzymatic hydrolysis and/or fermentation of pretreated materials may reduce the concentration of inhibitory compounds, enhancing saccharification and conversion yields. Filtration and washing processes have been widely used for this purpose. However, these methods involve additional and expensive steps, waste of water and loss of soluble sugars [36].

As alternative to filtration and washing, several detoxification technologies have been developed to overcome the effects of inhibitory compounds of pretreated materials [7–9]. Vacuum evaporation is capable of reducing volatile compounds such as furfural, acetic acid and vanillin [37]. Solvents (e.g., ethyl acetate) and active charcoal and/or ion-exchange resins reduce the concentration of inhibitors by extraction or adsorption, respectively [38–40]. Chemical transformation of inhibitors is also possible by addition of reducing agents (dithionite and sulfite) [41] and chemical catalysts, being overliming (treatment with $Ca(OH)_2$) the most efficient chemical detoxification method for removing phenols and furan derivatives [37].

Biological detoxification involves the use of microorganisms and/or their enzymes to decrease the inhibitory effects of degradation compounds. In comparison to physico-chemical detoxification processes, biological detoxification methods are advantageous as they have lower energy requirements, they take place at milder reaction conditions, they need no chemical addition and they have fewer side-reactions [9,10]. Among different microorganisms, fungi such as *Trichoderma reesei* have the ability to remove different inhibitory compounds. Larsson et al. [37] evaluated this fungus to detoxify a diluted-acid hydrolysate from spruce, observing an important removal of furans and a small proportion of phenols. Furthermore, *T. reesei* can produce hydrolytic enzymes while detoxification takes place. In this sense, Palmqvist et al. [42] used *T. reesei* to remove phenolic compounds, furan derivatives and aliphatic acids from acid-catalyzed steam-pretreated willow, simultaneously obtaining 0.2–0.6 IU/mL of cellulase activity. Besides fungi, several bacteria and yeasts have been also used for detoxification purposes [10]. For instance, the thermophilic bacterium *Ureibacillus thermophaercus* was employed to remove furfural and 5-HMF and phenolic compounds from a waste house wood hydrolysate [43], increasing markedly the ethanol production rate by *S. cerevisiae* in a subsequent fermentation stage. The yeast *S. cerevisiae* has also the natural ability to assimilate some of these inhibitory compounds –mainly furfural, 5-HMF and aromatic aldehydes such as vanillin, syringaldehyde or 4-hydroxybenzaldehyde– and convert them into less inhibitory forms [44,45]. Furthermore, this innate capacity can be improved by subjecting *S. cerevisiae* to evolutionary engineering in the presence of inhibitory compounds, boosting its fermentation performance in lignocellulosic pretreated materials [46]. Strategies such as genetic modification also offer the possibility to introduce a particular characteristic that is not present naturally in a certain microorganism. The yeast tolerance towards inhibitors has been improved by homologous or heterologous overexpression of certain genes. Larsson et al. [47] improved the tolerance of *S. cerevisiae* to phenylacrylic acids by overexpression of *Pad1p* gene (encoding a phenylacrylic acid decarboxylase). This genetically modified strain was capable of metabolizing different cinnamic acids from a spruce hydrolysate, showing higher growth rates and ethanol productivities. Similarly, Petersson et al. [48] overexpressed the gene *ADH6p* (which encodes an NADPH-dependent alcohol dehydrogenase enzyme with ability to reduce furfural and 5-HMF) on *S. cerevisiae*, increasing microbial conversion rates of 5-HMF in both aerobic and anaerobic cultures. Besides evolutionary or genetic engineering modifications, strategies such as cell retention, flocculation, and encapsulation of the fermenting microorganism have been also assessed to increase the intrinsic tolerance or the inherent detoxification capacity of some strains [9].

3.2. Delignification of Pretreated Materials

Together with detoxification processes, delignification is considered an important step for improving enzymatic saccharification of lignocellulosic biomass. Some traditional pretreatments methods such as alkaline, organosolv, and oxidative processes have been developed to target lignin removal. Biological delignification has also shown to be efficient in reducing the lignin content of lignocellulosic feedstocks. In contrast to physico/chemical delignification processes, biological methods are promising alternatives due to the lower environmental impact and the resulting higher product yield in the subsequent saccharification and fermentation steps. Biodelignification involves lignin removal/modification, the increase in the number of pores and the available surface area, and the reduction in the non-productive binding of hydrolytic enzymes. Wood-decaying fungi are

the sole organisms in nature capable of degrading the lignin polymer, making the carbohydrates of lignocellulose accessible to cellulolytic enzymes [49]. Microbial lignin attack is an extracellular and oxidative process that involves different oxidoreductase enzymes: ligninolytic peroxidases (lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase (VP), and dye-decolorizing peroxidase (DyP)), laccases, oxidases for the production of extracellular H_2O_2 (glyoxal oxidase, pyranose-2 oxidase, and aryl-alcohol oxidase), and dehydrogenases (aryl-alcohol dehydrogenase, and quinone reductase). Along with oxidoreductases, certain low molecular weight compounds play an important role, acting as mediators in some reactions [49]. Among peroxidases, LiP and MnP were first discovered in *Phanerochaete chrysosporium* and are capable of degrading non-phenolic (about 70–90%) and phenolic lignin units [49–51]. Regarding VP, it was first described in *Pleorotus* sp. [52,53], and combines properties from both LiP and MnP enzymes. DyP has been recently discovered during fungal pretreatment of wheat straw with *Irpex lacteus* [54], showing the ability to degrade non-phenolic lignin compounds. Finally, laccases can only address direct oxidation of phenolic compounds due to their lower redox potential [49]. However, in the presence of redox mediators, laccases can also degrade non-phenolic lignin units, as it is discussed in the following section.

Different wood-decaying fungi have been widely explored for biological delignification, being "white-rot" basidiomycetes (e.g., *P. chrysosporium*, *Trametes versicolor*, *Ceriporiopsis subvermispora*, *I. lacteus*, *Pleurotus ostreatus*, *Cyathus stercoreus*, etc.) the most efficient microorganisms for this purpose [9,10]. *T. versicolor* was grown on steam-exploded wheat straw for 40 days, resulting in 55.4% lignin degradation compared with the 20% obtained after steam-explosion treatment alone [55]. Salvachúa et al. [56] combined mild alkaline extraction with microbial delignification to reduce the lignin content of wheat straw. When using *C. subvermispora* and *I. lacteus*, 30% and 34% lower lignin content was measured, respectively, after 21 days of incubation. The lower lignin content increased the cellulose available for subsequent processing and conversion to around 66–69%, allowing to obtain 69% ethanol yields during the fermentation process. Microbial delignification was also studied with *P. ostreatus* on H_2O_2-pretreated rice hull [57]. This pretreatment combination increased the delignification range about two times, leading to 49.6% of glucose yield in the subsequent saccharification step. Although only "white-rot" basidiomycetes can degrade lignin extensively, certain ascomycetes can also colonize lignocellulosic biomass, showing to be beneficial for the subsequent saccharification step. Martín-Sampedro et al. [58] reported for the first time the ability of new endophytic fungi to enhance saccharification of autohydrolysis-pretreated eucalypt wood. Two of the evaluated fungi, *Ulocladium* sp. and *Hormonema* sp., produced a slight delignification in comparison to autohydrolysis pretreament alone, showing 8.5 and 8.0 times higher saccharification yields. Eventually, certain bacterial strains such as *Bacillus macerans*, *Cellulomonas cartae*, and *Zymomonas mobilis* are also capable of delignifying lignocellulosic feedstocks [59], yielding lignin degradation up to 50%.

In spite of the ability of ligninolytic microorganisms for delignification, treatment time as well as white-rot pattern must be taken into consideration for an efficient microbial delignification. Incubation time can vary from days to weeks, which depends on the strain used. An increment of lignin removal from 17% to 47% was reported when the residence time of wheat straw treatment with *Panus tigrinus* was increased from 7 days to 3 weeks (from 15% to 34% using *Coriolopsis rigida*) [56]. In terms of pattern lignocellulose deconstruction by microorganisms, selective delignification (sequential decay) should be favored against simultaneous cellulose and lignin degradation (simultaneous rot) to avoid carbohydrate consumption during microbial treatment [49]. These patterns vary among species and strains. Then, some fungi, such as *P. tigrinus* and *Phlebia radiata*, degraded lignin and sugars simultaneously in wheat straw; whereas *Pleurotus eryngii* was able to remove lignin selectively and faster than the carbohydrate components [56].

4. Outline of Laccase Enzymes

The use of ligninolytic enzymes, especially laccases, is an attractive method and an alternative to the use of microorganisms for detoxification and delignification of pretreated materials (Figure 3).

These enzymes are substrate specific and offer the possibility to increase conversion rates and yields during saccharification and fermentation processes, reducing detoxification and delignification times from weeks to hours and avoiding carbohydrate consumption [9]. Laccases enzyme was first isolated from sap of the Japanese lacquer tree *Rhus vernicifera* [60]. Afterwards, laccases have been widely described in higher plants, fungi, insects, and bacteria [61], being their production a characteristic distinctive of "white-rot" basidiomycetes [49], and some ascomycetes [62]. In plants, laccases are involved in the biosynthesis of lignin by inducing radical polymerization of the phenylpropanoid units. In contrast, in wood-decaying fungi laccases play a key role in lignin degradation [27].

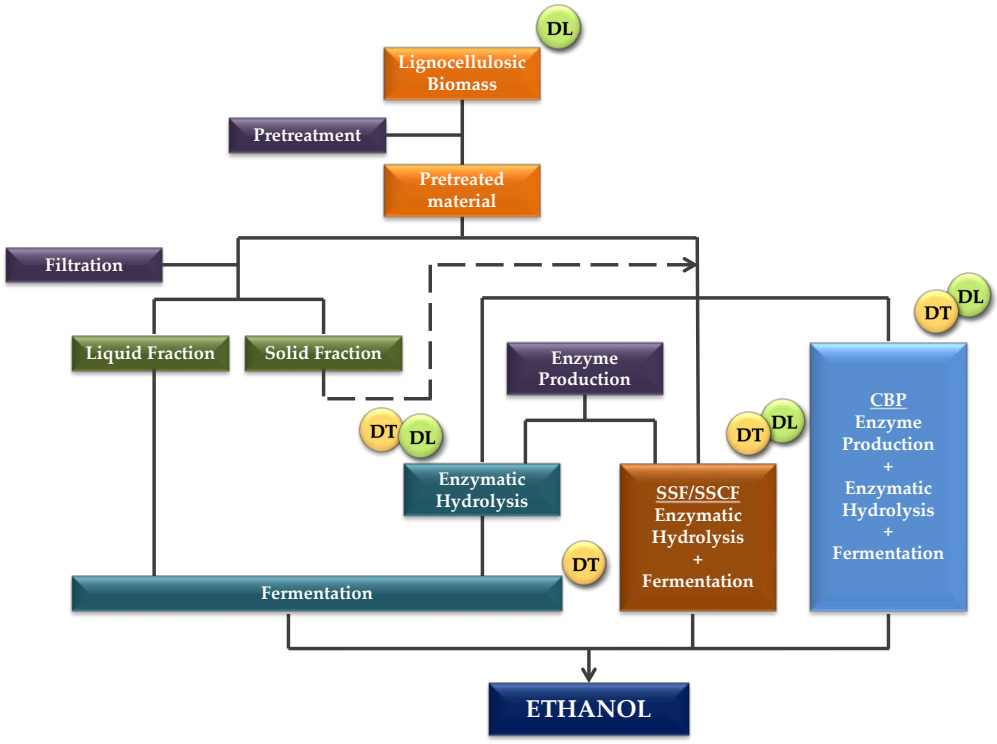

Figure 3. Schematic representation of lignocellulosic ethanol production showing (1) the different process configurations, and (2) the points where laccase delignification (DL) and laccase detoxification (DT) can be applied. The scheme can also be extended to the generation of several fermentation-based products including different alcohols, lipids, alkenes and other chemicals. SSF, simultaneous saccharification and fermentation; SSCF, simultaneous saccharification and co-fermentation; CBP, consolidated bioprocessing. Dashed line arrow represents the flow of the solid fraction after a water washing step.

Laccases (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) are multicopper-containing oxidases with phenoloxidase activity, which catalyze the oxidation of substituted phenols, anilines and aromatic thiols, at the expense of molecular oxygen [63]. The catalytic site of laccases involves four copper ions. Type-T1 copper (blue copper) is implicated in the oxidation of the reducing substrate, acting as the primary electron acceptor. Type-T2 copper together with two type-T3 coppers form a tri-nuclear copper cluster where the transferred electrons reduce the molecular oxygen to water. Electrochemical potential of type-T1 copper is one of the most significant features of laccases and might vary from 0.4 to 0.8 V [49]. Plant and bacterial laccases have comparatively low redox potential, whereas the highest values are generally reported for fungal laccases [64]. This redox potential allows the direct oxidation of some substrates by laccases, including the phenolic part of lignin (less than 20% of lignin polymer). However, potential substrates too large to enter the laccase catalytic site or with redox potential about 1.3 V cannot be oxidized directly by laccases.

Laccase-Mediator Systems (LMS)

The inability of laccases for the oxidation of complex lignocellulosic substrates or with high redox potential, such as non-phenolic lignin, can be overcome by using redox mediators in the so-called laccase-mediator systems (LMS). Certain low molecular compounds forming stable radicals that act as redox mediators, expand the catalytic activity of laccases towards more recalcitrant compounds which are not oxidized by laccase alone [65,66]. ABTS (2,2′-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid)) was the first chemical molecule described as laccase mediator for oxidation of non-phenolic lignin model compounds [66], following the electro transfer (ET) route for the oxidation of the target substrate [67]. Since then, new chemical mediators have been proposed for this purpose. Among them, the N–OH mediators such as 1-hydroxybenzotriazole (HBT), N-hydroxyphthalimide (HPI), violuric acid (VLA) or N-hydroxyacetanilide (NHA) have been described as the most efficient chemical mediators for the oxidation of recalcitrant compounds [68,69], performing the radical hydrogen atom transfer (HAT) route as oxidation mechanism [67]. These N–OH compounds have been successfully applied for delignification and bleaching of paper pulps, being the laccase-HBT system particularly effective in woody and non-woody pulp bleaching and delignification [70,71]. Moreover, decolorization of industrial dyes or detoxification of pollutants are another fields where the applicability potential of laccase-mediator systems has been comprehensively demonstrated [67,72].

Nevertheless, the high cost of chemical mediators and the generation of possible toxic species hamper the use of laccase-mediator systems at industrial scale. Consequently, the search of cheaper and environmental-friendly natural mediators has increased in the last years [67]. In this context, lignin-derived phenolic compounds obtained from lignocellulose biodegradation or as by-product or residue during the own industrial process of biomass conversion (e.g., from the black liquors of paper pulp industry) have been identified as potential natural mediators. A set of such compounds, including acetosyringone, syringaldehyde, vanillin, and *p*-hydroxycinnamic acids have been successfully applied in dye decolorization, delignification and bleaching of paper pulps, and removal of lipophilic extractives [73–75]. Similar to HBT, the HAT route is the mechanism by which the phenoxy radicals from these natural mediators oxidize the target substrate [67].

5. Application of Laccases for Detoxification of Pretreated Materials

5.1. Detoxification Mechanism

Laccases have been largely used to diminish the toxicity of different pretreated substrates (Table 1). These enzymes catalyze the selective oxidation of phenolic compounds generating unstable phenoxy radicals without affecting furan derivatives and aliphatic acids [37]. These phenoxy radicals further interact with each other and lead to the polymerization into aromatic compounds with lower inhibitory capacity [76]. It is important to highlight that not all phenolic compounds are susceptible to oxidation by laccase enzymes. Kolb et al. [77] described different catalytic activities for *T. versicolor* laccase when acting on phenolic compounds released from liquid hot water pretreatment of wheat straw. Thus, complete removal of syringaldehyde, *p*-coumaric acid and ferulic acid was achieved within 1-hour treatment, while vanillin was only removed after 24-h treatment, and 4-hydroxybenzaldehyde did not vary its concentration within 1-week reaction time in the presence of laccase. These reaction mechanisms are determined by the structure of the different phenolic compounds [67]. Laccase activity toward phenols is improved by the presence of electron-donating substituents in the ring and these substituents decrease the electrochemical potential of the corresponding phenols. Then, an additional methoxy group (the structural difference between vanillin and syringaldehyde) increases the affinity of the phenolic compounds toward laccase. Furthermore, the presence of ethylene groups in para-substituted phenols, such as *p*-coumaric and ferulic acids, also increases the activity of laccase [73,78].

Table 1. Application of laccase enzymes for detoxification of different pretreated materials.

Pretreated Material	Laccase Treatment	Effects Observed	Benefits Produced	Reference
Steam-exploded rice straw	*Coltricia perennis*	Removal of phenolic compounds by 76%	Increased saccharification yield by 48%	[79]
Steam-exploded wheat straw	*Pycnoporus cinnabarinus* or *Trametes villosa*	Removal of phenols identified (vanillin, syringaldehyde, ferulic acid and *p*-coumaric acid) by 93–95% with both laccases	Improved the fermentation performance of *Kluyveromyces marxianus* CECT 10875, shortening its lag phase and enhancing the ethanol yields	[80]
SO_2 steam-pretreated willow	*Trametes versicolor*	Removal of phenolic compounds (93–95%), revealing an oxidative polymerization mechanism by SEC analysis	Higher yeast growth, glucose consumption rate, ethanol productivity and ethanol yield using *Saccharomyces cerevisiae*	[81]
Dilute acid steam-pretreated spruce	*T. versicolor*	Removal of phenolic compounds by 93–95%	Ethanol yield produced by *S. cerevisiae* comparable with that obtained after detoxification with anion exchange chromatography at pH 10	[37]
Steam-exploded wheat straw	Commercial bacterial laccase MetZyme®	Phenol reduction of 18% (laccase alone) and 21% (simultaneous laccase and presaccharification)	Improved the fermentation performance of *K. marxianus* CECT 10875 during SSF and PSSF processes, shortening the adaptation phases and the overall fermentation times	[82]
Water and acid-impregnated steam-exploded wheat straw	*T. versicolor* or *Coriolopsis rigida*	Removal of phenolic compounds by 93–95% with both laccases	Reduction of the toxic effects on *S. cerevisiae*, resulting in higher yeast growth and improved ethanol production	[76]
Steam-exploded wheat straw	*P. cinnabarinus*	Phenol reduction around 67% (laccase alone) and 73% (simultaneous laccase and presaccharification)	Laccase detoxification allowed to obtain ethanol concentrations and yields with *K. marxianus* CECT 10875 comparable to those obtained with *S. cerevisiae*	[83]
Steam-exploded wheat straw	*P. cinnabarinus*	Removal of phenolic compounds by 95%	Improvement of cell growth and ethanol production of *S. cerevisiae* during SSF process	[84]
Steam-exploded sugarcane bagasse	*T. versicolor*	Approximately 80% of the phenolic compounds removal	Improvements in ethanol yield and ethanol volumetric using a xylose-utilizing *S. cerevisiae*	[85]
Steam-exploded sugarcane bagasse	*Ganoderma lucidum* 77002	84% of the phenolic compounds in prehydrolysate	Ethanol yield was improved when *S. cerevisiae* was used on detoxified prehydrolysate	[86]
Alkali-extracted sugarcane bagasse	*Aspergillus oryzae*	Not observed	Laccase improved the fermentation efficiency by 6.8% for one-pot SSF and 5.7% for SSF	[87]
Acid hydrolyzed from sugarcane bagasse	*Cyathus stercoreus*	Reduction of 77.5% of total phenols	Improvements in the performance of *Candida shehatae* NCIM 3501	[88]
Steam-exploded wheat straw	*P. cinnabarinus*	Phenol reduction around 44% (laccase alone) and 95% (simultaneous laccase and presaccharification) at 12% (*w*/*v*) of substrate loading	Laccase detoxification triggered the fermentation by *K. marxianus* of steam-exploded material at 12% (*w*/*v*), resulting in an ethanol concentration of 16.7 g/L during SSF process	[89]
Steam-exploded wheat straw	*P. cinnabarinus*	Reduction of total phenolic compounds by 50–80%	Laccase detoxification allowed the fermentation of pretreated material at 20% (*w*/*v*) of substrate loading using the evolved xylose-consuming yeast *S. cerevisiae* F12, producing more than 22 g/L during SSCF process	[90]
Steam-exploded wheat straw	*P. cinnabarinus*	Approximately 73–81% of the phenolic compounds removal	Laccase detoxification improved cell viability of the evolved xylose-recombinant *S. cerevisiae* KE6-12, and increased the ethanol production up to 32 g/L when fed-batch SSCF process was used at 16% (*w*/*v*) of substrate loading	[91]
Steam-exploded wheat straw	*P. cinnabarinus*	Phenols removal by 53% during simultaneous laccase and presaccharification at 25% (*w*/*v*) of substrate loading	Ethanol production of 58.6 g/L at 48 h with detoxified material at 25% (*w*/*v*) of substrate loading during PSSF process with *S. cerevisiae*	[92]

Table 1. *Cont.*

Pretreated Material	Laccase Treatment	Effects Observed	Benefits Produced	Reference
Dilute-acid spruce hydrolysate	*T. versicolor* expressed in a recombinant *S. cerevisiae* strain	Reduction of low-molecular of phenolic compounds	Laccase-producing transformant was able to ferment at a faster rate than the control transformant	[93]
Organosolv pretreated wheat straw	*T. versicolor* immobilized on both active epoxide and amino carriers	Higher phenols removal (82%) efficiency with laccase immobilized on active amino carrier	Better performance of *Pichia stipitis* during fermentation and reusability of immobilized laccase	[94]
Steam-exploded wheat straw	*T. villosa* or a bacterial laccase from *Streptomyces ipomoeae*	Phenol content reduction of 29% and 90% with bacterial and fungal laccases, respectively	Improvement performance of *S. cerevisiae* during SSF and PSSF process	[95]

SEC, Size exclusion chromatography; SSF, simultaneous saccharification and fermentation process; PSSF, presaccharification and simultaneous saccharification and fermentation process; SSCF, simultaneous saccharification and co-fermentation process. Generally, laccases source is fungal, except in those cases where it is indicated.

Incomplete phenols removal has been widely described with different high redox fungal laccases. Kalyani et al. [79] achieved a phenol removal of 76% when steam-exploded whole slurry from rice straw was treated with *Coltricia perennis* laccase. Moreno et al. [80] reported higher phenol reductions (93–95%) when *Pycnoporus cinnabarinus* and *Trametes villosa* laccases were used to detoxify steam-exploded wheat straw. Similar ranges were observed by Jönsson et al. [81] with acid steam-pretreated willow and *T. versicolor* laccase, and by Jurado et al. [76] with both water and acid-impregnated steam-exploded wheat straw and *T. versicolor* and *C. rigida* laccases. Together with the structure of phenols, the redox potential of laccases also determines the grade of action toward them. Then, low redox potential laccases, a particular property of bacterial laccases [96], show minor reactivities on phenols [97]. In this sense, Moreno et al. [82] described a lower phenol reduction of 21% when a commercial bacterial laccase (MetZyme®, Kaarina, Finland) was used to reduce the toxicity of a whole slurry from steam-exploded wheat straw. Finally, other factors, such as the viscosity of the medium in which the laccase detoxification is implemented also affects the laccase efficiency. Higher viscosity when higher solids content is used difficult the blending of laccase with the pretreated material, consequently reducing the laccase efficiency [90].

Laccase detoxification is usually performed either by using a partially purified laccase [88], or with a totally purified enzyme [83]. Nevertheless, culture enriched in laccase activity has been also successfully proved [79]. The treatments can be carried out at a wide range of optimal pH and temperature depending of laccases source. Then, the treatment of steam-exploded wheat straw with a fungal laccase from *T. villosa* at optimal pH 4 removed 90% of phenols, while a reduction in the phenol content of 29% was achieved with a bacterial laccase from *S. ipomoea* at optimal pH 8 [95]. Regarding to temperature, Moreno et al. [80] reported phenols reduction around of 94% when steam-exploded wheat straw was treated with laccases from *P. cinnabarinus* and *T. villosa* at their optimal temperatures of 50 and 30 °C, respectively. The treatment time and the enzyme loading at which the detoxification is carried out are also two important factors. Moreno et al. [92] obtained similar phenols reduction, 65% and 53%, in steam-exploded wheat straw using *P. cinnabarinus* laccase after 3 h and 12 h of treatment, respectively. In terms of enzyme loading, laccase can be added at low or high loadings, depending on process optimization and material type. Then, only 1.5 U/mL of a laccase from *C. perennis* was enough to remove 77.5% of total phenols from acid steam-exploded rice straw [79]; whereas a higher enzyme loading (100 times more) of *C. stercoreus* laccase was necessary to remove the same phenols range from sugarcane bagasse hydrolysate [88].

5.2. Detoxification and Fermentation

S. cerevisiae, the most commonly employed microorganism for ethanol production, has been also largely used to evaluate the effects generated by laccase detoxification. Jönsson et al. [81] and

Larsson et al. [37] reported higher yeast growth together with higher glucose consumption rate, ethanol productivity, and ethanol yield when liquid fractions from acid steam-exploded wood were detoxified by *T. versicolor* laccase. Similarly, Moreno et al. [83,84] used *P. cinnabarinus* laccase to detoxify steam-exploded wheat straw, observing higher cell viability and shorter lag phase during SSF and PSSF processes. Jurado et al. [76] also described a greater influence on ethanol concentration and yeast growth when both enzymatic hydrolyzed from water and acid-impregnated steam-exploded wheat straw were treated with *T. versicolor* and *C. rigida* laccases. On the other hand, Martín et al. [85] explored the use of *T. versicolor* laccase to detoxify a steam-exploded sugarcane bagasse hydrolysate, resulting in improved ethanol yield and ethanol volumetric productivity by using a recombinant xylose-utilizing *S. cerevisiae* strain. Steam-exploded sugar cane bagasse prehydrolysate was also detoxified by Fang et al. [86] with *Ganoderma lucidum* laccase, resulting in improved yeast growth and ethanol yield. Finally, one-pot SSF process with alkali-extracted sugar cane bagasse was carried out with *Aspergillus oryzae* laccase, improving the fermentation efficiency by 6.8% [87].

In addition to *S. cerevisiae*, similar effects derived from laccase detoxification have been also reported in other fermenting yeasts. Chandel et al. [88] observed an improvement in the performance of *Candida shehatae* during the fermentation of an acid hydrolysate from sugarcane bagasse treated with *C. stercoreus* laccase. Moreno et al. [83] described similar ethanol concentrations and yields comparable to those obtained by *S. cerevisiae* when steam-exploded wheat straw was detoxified by *P. cinnabarinus* laccase and fermented with the thermotolerant yeast *Kluyveromyces marxianus* CECT 10875. This thermotolerant yeast was also used by Moreno et al. [82] during both SSF and PSSF processes of steam-exploded wheat straw detoxified with the bacterial laccase MetZyme®. In this case, a shorter adaptation phase and an increase in cell viability could be observed in laccase-treated samples. This result is of special relevance, since the use of thermotolerant yeasts lead to a better integration of both saccharification and fermentation processes. Saccharification has an optimum temperature around of 50 °C, whereas most fermenting yeasts have an optimum temperature ranging from 30 to 37 °C [98]. The use of thermotolerant microorganisms such as *K. marxianus*, with capacity of growing and fermenting at temperature above 40 °C, represents therefore an advantage to obtain higher saccharification and fermentation yields [99]. In addition, the use of thermotolerant strains has shown to reduce overall process costs due to the reduction cooling costs.

Another strategy to reach higher ethanol concentrations and make the process more economically viable is to operate saccharification and fermentation processes at high-substrate consistencies. This approach offers possibilities to reduce freshwater consumption and downstream processing, and minimize energy consumption during subsequent distillation—due to the higher ethanol concentrations after fermentation—and evaporation stages [100]. Nevertheless, increasing the substrate consistency presents some disadvantages such as accumulation of glucose and cellobiose (that inhibits hydrolytic enzymes), mixing and mass transfer limitations, and larger concentration of inhibitors in the fermentation medium [101]. In this context, laccase detoxification enables the fermentation of inhibitory hydrolysates at higher substrate consistencies, improving final ethanol concentrations and yields. Moreno et al. [89] used laccase from *P. cinnabarinus* to detoxify steam-exploded wheat straw at 12% (*w*/*v*) substrate loadings, triggering its fermentation by *K. marxianus* CECT 10875 during SSF processes and yielding an ethanol concentration of 16.7 g/L. These authors also described the fermentability of steam-exploded wheat straw at 20% (*w*/*v*) substrate loadings. At this consistency, the evolved xylose-consuming yeast *S. cerevisiae* F12 was unable to growth. However, this inhibition was overcome by *P. cinnabarinus* laccase, allowing *S. cerevisiae* F12 to produce more than 22 g/L of ethanol during a SSCF process [90]. The evolved xylose-recombinant *S. cerevisiae* KE6-12 was also explored to produce ethanol from steam-exploded wheat straw at 16% (*w*/*v*) of substrate loading. In this case, *P. cinnabarinus* laccase reduced the toxicity of this media improving cell viability and increasing the ethanol production up to 32 g/L during a fed-batch SSCF process [91]. Finally, a water insoluble solids (WIS) fraction from steam-exploded wheat straw was used at 25% (*w*/*v*) of substrate loading for

ethanol production. This material, detoxified by *P. cinnabarinus* laccase, was then subjected to PSSF processes with *S. cerevisiae*, obtaining an ethanol production of 58.6 g/L [92].

5.3. Detoxification and Saccharification

Laccase detoxification processes have been also evaluated in terms of enzymatic hydrolysis, showing contradictory effects. Kalyani et al. [79] observed an enhancement in the saccharification yield by 48% of acid-pretreated rice straw due to a phenols reduction by *C. perennis* laccase. Contrary, Tabka et al. [102], Jurado et al. [76] and Moreno et al. [80,89] described lower glucose concentration after enzymatic hydrolysis of steam-exploded wheat straw treated with *P. cinnabarinus*, *T. villosa* and *C. rigida* laccases. This negative phenomenon was attributed to the formation of laccase-derived compounds from phenols that inhibit cellulolytic enzymes. In this sense, Oliva-Taravilla et al. [103] showed a strong inhibition due to oligomeric products derived from the oxidative polymerization of vanillin and syringaldehyde by *Myceliophthora thermophila* laccase. The presence of these resulting oligomers caused a decrement on enzymatic hydrolysis yield of a model cellulosic substrate (Sigmacell) of 46.6% and 32.6%, respectively. Moreover, a decrease in more than 50% of cellulase and β-glucosidase activities was observed in presence of laccase and vanillin. Negative effects on xylose production has been also reported by phenolic oligomers formed from vanillin, syringaldehyde and ferulic acid, as was observed by Oliva-Taravilla et al. [104] with a WIS fraction from steam-exploded wheat straw treated with *M. thermophila* laccase in the presence of the mentioned phenols. Finally, an increase in the competition of cellulose binding sites between hydrolytic enzymes and laccases has been also suggested as a reason for the reduction in glucose recovery [105].

5.4. Other Comments

Although significant advances have been demonstrated about the use of laccases for detoxification, the high enzyme production cost is one of the most important limitations for its application at industrial scale. An alternative approach to adding directly laccase to pretreated materials could be the genetic engineering of fermenting yeast for laccase production. This would allow detoxification and ethanolic fermentation processes simultaneously, thus reducing the cost and time associated with laccase production and detoxification step, respectively. In this matter, Larsson et al. [93] designed a recombinant *S. cerevisiae* strain carrying the laccase gene from the white-rot fungus *T. versicolor*. This strain had the ability to decrease the content of low-molecular phenolic compounds and ferment a dilute-acid spruce hydrolysate, showing higher ethanol productivity compared to control. On the other hand, laccase recycling by enzyme immobilization or co-immobilization could also represent a cost effective approach. Ludwig et al. [94] immobilized a laccase from *T. versicolor* on both active epoxide and amino carriers (Sepabeads® EC-EP and EC-EA, respectively) for detoxification of a wheat straw organosolv fraction. With the immobilized laccase phenolic compounds could be efficiently removed (higher with EC-EA), observing a better performance of *Pichia stipitis* during the fermentation of the detoxified fraction. Additionally, reusability of the immobilized laccase was demonstrated.

6. Application of Laccases for Delignification of Pretreated Materials

The modification or partial removal of lignin by laccases has been shown to be effective for improving enzymatic hydrolysis of different lignocellulosic materials. Different strategies have been assayed with this purpose, either using laccases alone or in combination with mediators (LMS). Consequently, lignin oxidation is produced leading to the formation of aromatic lignin radicals that give rise to a variety of reactions, such as ether and C–C bonds degradation, and aromatic ring cleavage, and finally resulting in lignin degradation [49].

6.1. Delignification by Laccase Alone

Although the direct action of laccases on lignin is, in principle, restricted to phenolic units—which only represent a small percentage of the total polymer—, different studies have showed the ability

of laccase alone for delignifying different pretreated materials, improving the subsequent enzymatic hydrolysis (Table 2). Kuila et al. [106,107] explored the use of a laccase from *Pleurotus* sp. to treat milled materials from Indian Thorny bamboo (*Bambusa bambos*) and Spanish flag (*Lantana camara*). A range of delignification between 84–89% was obtained for both materials, observing an increment of the saccharification performance because of the better accessibility of hydrolytic enzymes. The same laccase was used by Mukhopadhyay et al. [108] to treat a milled material from *Ricinus communis*, reporting a delignification yield of about 86%, which increased the yields on reducing sugars by 2.68-fold. Similar lignin removal (81.6%) was achieved by Rajak and Banerjee [109] using a laccase produced by *Lentinus squarrosulus* MR13 to delignify karn grass (*Saccharum spontaneum*), resulting in a sugar production increase by 7.03 fold. On the other hand, lower lignin loss (18%) was obtained when milled material from wheat straw was treated with *P. cinnabarinus* laccase followed by an alkaline peroxide extraction [110]. Then, 24–25% increase in glucose and xylose release was produced. In the same way, Rico et al. [111] compared laccases from *M. thermophila* and *P. cinnabarinus* to treat milled eucalypt wood followed by an alkaline peroxide extraction in a multistage sequence (four cycles of enzyme-alkaline extraction). Whereas the treatment with *M. thermophila* decreased the lignin content of about 20%, *P. cinnabarinus* laccase did not affect the lignin content. Concerning glucose release, the treatment with *M. thermophila* and *P. cinnabarinus* laccases produced an increase of glucose liberation of 9% and 4%, respectively. Finally, Singh et al. [112] has recently described the use of a small bacterial laccase from *Amycolatopsis* sp. to delignify steam-pretreated poplar, obtaining a 6-fold increase in terms of the release of acid insoluble lignin. Then, glucose production from laccase-treated sample was increased by 8%.

Table 2. Application of laccase alone for delignification of different pretreated materials.

Pretreated Material	Laccase Treatment	Effects Observed	Benefits Produced	Reference
Milled material from Thorny bamboo and Spanish flag	*Pleurotus* sp.	Range of delignification between 84–89%, revealing the lignin removal by FTIR, XRD, and SEM analysis	Better accessibility of hydrolytic enzymes	[106,107]
Milled material from *Ricinus communis*	*Pleurotus* sp.	86% of lignin loss, resulting in a degradation of the surface tissues (SEM analysis)	Reducing sugar yields increased 2.68-fold	[108]
Milled material from karn grass	*Lentinus squarrosulus* MR13	Lignin removal of 81.6%. Porosity analysis evidenced the specific action of laccase on lignin	Increase of sugar production of 7.03 fold	[109]
Milled material from wheat straw	*P. cinnabarinus* laccase followed by alkaline peroxide extraction	18% decrease in lignin after sequential treatment	24–25% increase in glucose and xylose production	[110]
Milled wood from *Eucalyptus globulus*	Four cycles of *Myceliophthora thermophila* laccase -alkaline extraction	Up to 20% of lignin loss after four cycles treatment	Increase of glucose production by 9%	[111]
Steam-pretreated poplar	Bacterial laccase from *Amycolatopsis* sp.	Increment of acid insoluble lignin release by 6 fold, observing a reduction of molar mass lignin (approx. 50%) by SEC analysis	8% increment of glucose production	[112]
Alkali-extracted corn straw	*Trametes hirsuta*	Increment of porositiy and surface area in laccase-treated samples	2-fold increment in sugar production	[113]
Alkali-extracted straw from *Brassica campestris*	*Ganoderma lucidum*	Higher number and density of holes with greater width and depth after laccase treatment	Saccharification yield increased 1.7-fold	[114]
Steam-exploded wheat straw	*Sclerotium* sp.	Loosening of lignin-carbohydrate complex	16.8% increase in cellulose hydrolysis	[115]
Acid steam-pretreated spruce	*T. hirsuta*	Reduction of lignin hydrophobicity and enrichment of carboxylic groups revealed by ESCA (electron spectroscopy for chemical analysis)	13% increase in sugar yield	[116]
Acid steam-pretreated spruce	*Cerrena unicolor* and *T. hirsuta* laccases	Reduced binding of hydrolytic enzymes by lignin modification	Improvement of hydrolysis yield by 12%	[117,118]
Steam-exploded sugarcane bagasse	*G. lucidum*	Delignification	75% increase in glucose production	[119]
Corncob residue	*Trametes* sp. AH28-2 heterologously expressed in *Trichoderma reesei*	Not investigated	Up to 71.6% increase in reducing sugar yields	[120]

Table 2. *Cont.*

Pretreated Material	Laccase Treatment	Effects Observed	Benefits Produced	Reference
Milled wheat straw	Bacterial laccase from *Thermobifida fusca* incorporated into a designer cellulosome including two cellulases and xylanase	Not investigated	Reducing sugar yields increased 2.0-fold	[121]
Milled sugarcane bagasse	Bacterial laccase from *T. fusca*	SEM analysis of laccase-treated sample shows smaller shatters	2-fold increment in sugar production	[122]
Steam-exploded wheat straw	Alkaline extraction followed by a commercial bacterial laccase MetZyme®	Slight delignification (2%) after alkaline extraction-laccase sequence	Increment of glucose and xylose production by 21% and 30%, respectively	[82]
Steam-exploded wheat straw	Alkaline extraction followed by *Trametes villosa* laccase or bacterial laccase from *Streptomyces ipomoeae* treatment	Slight delignification (4%) after alkaline extraction-laccase sequence. No delignification observed by *T. villosa*	Increment of glucose and xylose production by 16% and 6%, respectively. No positive effects observed by *T. villosa*	[95]

FTIR, Fourier transform infrared spectroscopy; XRD, X-ray diffraction; SEM, Scanning electron microscopy; SEC, Size exclusion chromatography; Generally, laccases source is fungal, except in those cases where it is indicated.

In addition to lignin removal, the improvement of enzymatic hydrolysis due to lignin and/or microfiber structure modification by laccase has been also reported. Properties such as porosity, surface area, and hydrophobicity can be altered, resulting in the reduction of unproductive binding of hydrolases. Li et al. [113] observed an increment in the porosity and surface area of alkali-extracted corn straw after a treatment with *Trametes hirsuta* laccase, doubling the sugar production. The same effect was observed on alkali-extracted straw from *Brassica campestris* [114]. Then, the treatment of this material with a laccase from *Ganoderma lucidum* increased saccharification yields 1.7-fold. Regarding steam-exploded materials, laccase treatment has shown contradictory results. Qiu and Chen [115] explored the use of a laccase from *Sclerotium* sp. to treat steam-exploded wheat straw. Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) analysis indicated that laccase oxidized lignin, which contributed to loose the compact wrap of lignin-carbohydrate complexes and consequently enhancing the cellulose hydrolysis. Palonen and Viikari [116] also reported lignin modification of acid steam-pretreated spruce (*Picea abies*) by treatment with *T. hirsuta* laccase. This modification consisted in a reduction of lignin hydrophobicity together with an enrichment of carboxylic groups, which reduced the unproductive binding of hydrolytic enzymes to lignin. Consequently, an enhancement of saccharification yield by 13% was observed during the subsequent enzymatic hydrolysis. Similar results were attained by Moilanen et al. [117,118] when acid steam-pretreated spruce was treated with *C. unicolor* and *T. hirsuta* laccases. However, using acid steam-pretreated giant read (*Arundo donax*), *C. unicolor* laccase reduced the hydrolysis yield by 17% [117]. In this case, the lower sugar production was explained by an increase of the unproductive adsorption of hydrolytic enzymes onto the lignocellulosic fibers and a major strengthening of lignin-carbohydrate complexes. Moreno et al. [84] also reported a reduction of glucose recovery by almost 6–7% after 72 h of enzymatic hydrolysis of steam-exploded wheat straw treated with *P. cinnabarinus* laccase. These authors observed a slight lignin content increment after laccase treatment due to a grafting phenomenon. Grafting takes place when the lignin-derived phenols resulting from steam explosion pretreatment are oxidized by laccase to phenoxy radicals, which can undergo polymerization by radical coupling or being grafted onto steam-exploded material (via radical coupling to lignin residues) [123]. This lignin content increment by grafting phenomenon might prevent the accessibility of hydrolytic enzymes to cellulose, either by reducing the number and/or the size pores or hindering the processivity of cellulases. Moreover, the grafting process could also lead to an increase of the lignin surface area, thereby limiting the accessibility of hydrolytic enzymes to cellulose, and consequently reducing sugar recovery yields. Oliva et al. [105] also suggested the grafting effect to support the lower sugar recovery obtained after treatment of steam-exploded wheat straw with *P. cinnabarinus* laccase. For the first time, these authors observed by FTIR spectroscopy the incorporation of *p*-hydroxycinnamic acids into the fibers of laccase-treated samples.

6.2. Delignification by Laccase-Mediator System (LMS)

Compared to laccase alone, laccase in the form of LMS can oxidize both phenolic and non-phenolic component of lignin moieties, producing an extensive cleavage of covalent bonds in lignin. Different pretreated materials have been subjected to the LMS action for delignification in order to improve the enzymatic hydrolysis, being chemical mediators mainly used (Table 3). Milled material from oil palm empty fruit bunch (OPEFB) was treated with an enzymatic crude extract from *Pycnoporus sanguineus* and HBT and ABTS as mediators [124]. This process leads to lignin removal of 8% and 8.7% when using HBT and ABTS as mediators, respectively. As a consequence, the LMS treatment resulted in a fermentable sugars production of 30 g/L, in comparison to the crude ligninolytic extract without mediator, which showed a maximum concentration of fermentable sugars of 19.1 g/L. Higher delignification range (up to 97%) was reported in liquid hot water pretreated wheat straw and corn stover when using *P. sanguineus* laccase and violuric acid (VIO) as mediator [125]. Al-Zuhair et al. [126] treated milled materials from palm trees fronds and seaweed with a laccase from *T. versicolor* and using HBT as mediator, achieving 9% and 24% of lignin removal, respectively. Consequently, the subsequent enzymatic hydrolysis was improved from 0.04% to 3.1%. Furthermore, when combining laccase-HBT system with the ionic liquid [C_2 mim] [OAc] (1-ethyl-3-methylimidazolium acetate), saccharification yields increased up to 13%. Moniruzzaman and Ono [127] also combined LMS treatment with ionic liquids. These authors reported 50% delignification yields when wood chips from hinoki cypress (*Chamaecyparis obtusa*) pretreated with [C_2 mim] [OAc] (1-ethyl-3-methylimidazolium acetate) were treated with the commercial laccase Y120 (*Trametes* sp.) and HBT as mediator. The same laccase-mediator system was also applied on OPEFB biomass pretreated with the hydrophilic ionic liquid [EMIM] [DEP] (1-ethyl-3-methylimidazolium diethyl phosphate) [128], resulting in a delignification range of 35%. On the other hand, a sequential combination of ultrasonication, liquid hot water and a commercial LMS (PrimaGreen® EcoFade LT100 composed principally by a laccase from modified strains of *C. unicolor* and the mediator 3,5-dimethoxy-4-hydroxybenzonitrile) was performed on cotton gin trash [129]. This process led up to 15% lignin removal, increasing glucose and ethanol yields by 23% and 31%, respectively. A new sequential pretreatment combines an alkaline ultrasonication with liquid hot water and the commercial LMS PrimaGreen® EcoFade LT100, was again evaluated [130]. When applied to cotton gin trash, the delignification range was increased to 27%, resulting in increments of 41% and 64% of glucose and ethanol yields, respectively. Ultrasound pretreatment was also applied on elephant grass (*Pennisetum purpureum*) by Nagula and Pandit [131]. The pretreated material was then subjected to a LMS treatment consisting of *T. hirsuta* crude laccase supernatant and ABTS as mediator, resulting in a delignification range of 69%. In another study, Gutiérrez et al. [132] evaluated the ability of *T. villosa* laccase, together with HBT as mediator and a subsequent alkaline extraction, to remove lignin from milled eucalypt wood and elephant grass. 48% and 32% of the eucalypt and elephant grass lignin were removed, respectively. Consequently, the glucose yield was increased by 61% and 12% from both lignocellulosic materials, respectively, as compared to those without LMS treatment. Additionally, lignin structural changes were observed by two-dimensional nuclear magnetic resonance (2D NMR), as a result of the laccase-HBT system. A significant decrease of aromatic lignin units (with preferential degradation of guaiacyl over syringyl units) and aliphatic (mainly β-O-4′-linked) side-chains of lignin after LMS treatment was showed, leading to residual lignin with mainly oxidized syringyl units. These authors also described similar lignin structural changes when four cycles of a sequential treatment of LMS (including *P. cinnabarinus* laccase-HBT) followed by an alkaline peroxide extraction were applied on milled eucalypt wood [111]. Rencoret et al. [110] also reported lignin structural variations in milled wheat straw treated with the same laccase-mediator system. Moreover, a substantial lignin removal (37%) was produced by *P. cinnabarinus* laccase in the presence of HBT, which was increased up to 48% when a subsequent alkaline peroxide extraction was applied. This LMS treatment increased glucose yields by 60% after enzymatic hydrolysis.

Table 3. Application of laccase-mediator systems for delignification of different pretreated materials.

Pretreated Material	LMS Treatment	Effects Observed	Benefits Produced	Reference
Oil palm empty fruit bunch milled	*Pycnoporus sanguineus* laccase with HBT and ABTS as mediators	Klason lignin reduction of 8% and 8.7% for HBT and ABTS, respectively	Increment of sugar yield by 16–17% compared to laccase alone	[124]
Wheat straw and corn stover pretreated with liquid hot water	*P. sanguineus* H275 laccase with VIO as mediator	Up to 97% lignin loss	19.98% increase in sugar production	[125]
Milled material from palm trees and seaweed	*Trametes versicolor* laccase with HBT as mediator	Lignin removal of 9% and 24% for palm trees and seaweed, respectively	Better enzymatic hydrolysis with a ionic liquid [C_2 mim] [OAc] (1-ethyl-3-methylimidazolium acetate) treatment prior to laccase-HBT	[126]
Wood chips swollen with ionic liquid [C_2 mim] [OAc] (1-ethyl-3-methylimidazolium acetate)	*Trametes* sp. Y120 laccase with HBT as mediator	50% delignification, revealing structural lignin changes by SEM and FTIR analysis	Pretreated material with cellulose more accessible	[127]
Oil palm empty fruit bunch pre-treated with ionic liquid [EMIM] [DEP] (1-ethyl-3-methylimidazolium diethyl phosphate)	*Trametes* sp. Y120 laccase with HBT as mediator	35% decrease in lignin	Cellulose rich-material	[128]
Cotton gin trash pretreated with a sequential combination of ultrasonication and liquid hot water	*Cerrena unicolor* laccase with 3,5-dimethoxy-4-hydroxybenzonitrile as mediator	Up to 15% lignin loss	Up to 23% and 31% increase in glucose and ethanol yields, respectively	[129]
Cotton gin trash pretreated with a sequential combination of alkaline ultrasonication and liquid hot water	*C. unicolor* laccase with 3,5-dimethoxy-4-hydroxybenzonitrile as mediator	27% reduction in lignin, observing lignin aromatic change structure by FTIR	41% and 64% increase in glucose and ethanol yields, respectively	[130]
Elephant grass pretreated with ultrasond	*Trametes hirsuta* laccase with ABTS as mediator	Delignification range of 69%	Better accessibility of cellulose	[131]
Milled materials from eucalypt wood and elephant grass	*Trametes villosa* laccase with HBT as mediator and a subsequent alkaline extraction	Up to 48% and 32% lignin removal for eucalypt and elephant grass, respectively	Increase in glucose yield (61% and 12% for eucalypt and elephant grass, respectively) and ethanol production (over 4 g/L in eucalypt and 2 g/L in elephant)	[132]
Eucalypt wood milled	Four cycles of *Myceliophthora thermophila* laccase with methyl syringate as mediator and a subsequent alkaline peroxide extraction	50% delignification, observing by Py/GC-MS and 2D NMR analysis a significant reduction of both aromatic and aliphatic lignin with high presence of oxidized syringyl units	Increases (approximately 40%) in glucose and xylose yields after enzymatic hydrolysis	[133]
Eucalypt wood milled	Comparing four cycles of *Pycnoporus cinnabarinus* laccase with HBT as mediator (or *M. thermophila* laccase with methyl syringate as mediator) and a subsequent alkaline peroxide extraction	50% decrease in lignin with both LMS after four cycles, Slight delignification observed after the first cycle with *P. cinnabarinus* laccase and HBT, but not after *M. thermophila* laccase and methyl syringate	Increased glucose yield (30%) with both LMS after four cycles Saccharification increment of 10% after the first cycle with *P. cinnabarinus* laccase and HBT, but not after *M. thermophila* laccase and methyl syringate	[111]

Table 3. *Cont.*

Pretreated Material	LMS Treatment	Effects Observed	Benefits Produced	Reference
Acid steam-pretreated spruce	*T. hirsuta* laccase with acetosyringone as mediator	Reduction of unproductive hydrolases adsorption due to an increment of syringyl/guaiacyl ratio	Downstream cellulose hydrolysis was improved 36%	[118]
Acid steam-pretreated spruce	*T. hirsuta* laccase with ABTS, HBT, and TEMPO as mediators	Lignin modification resulting in a decrease of unproductive cellulases adsorption, except with HBT. TEMPO also oxidized cellulose	Increment of enzymatic hydrolysis by 54% and 49% with ABTS and TEMPO, respectively. No positive effects with HBT	[118]
Milled material from date palm waste	*T. versicolor* laccase with HBT as mediator	Reduced binding of hydrolytic enzymes by lignin modification	Improvement of sugar production 8 times	[134]
Ensiled corn stover	*T. versicolor* laccase with HBT as mediator	Lignin side chain oxidation	Downstream cellulose hydrolysis was improved 7%	[135]
Acid steam-exploded wheat straw	*T. versicolor* laccase with HBT as mediator followed by alkaline peroxide extraction	Lignin oxidation revealed by Py/GC-MS TMAH	Increment of glucose release by up to 2.3 g/L	[136]
Acid steam-pretreated spruce	*T. hirsuta* laccase with NHA as mediator	Lignin modification showing both modified hydrophobicity and surface charge	Enzymatic hydrolysis yield increased 1.61-fold compared to laccase alone	[116]
Steam-exploded eucalypt wood	*M. thermophila* laccase and HBT as mediator	Lignin oxidation let to an increment of both secondary OH groups and degree condensation	Slightly increase of sugar production	[137,138]

HBT, 1-hydroxybenzotriazole; VIO, violuric acid; ABTS, 2,2′-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid; TEMPO, (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl; FTIR, Fourier transform infrared spectroscopy; SEM, Scanning electron microscopy; Py/GC–MS, Pyrolysis/gas chromatography-mass spectrometry; TMAH, tetramethylammonium hydroxide; Generally, laccases source is fungal, except in those cases where it is indicated.

The use of lignin-derived soluble phenols, such as vanillin, acetosyringone, *p*-hydroxycinnamic acids, etc., as natural mediators for ethanol production would offer environmental and economic advantages compared to chemical mediators. Although their use could be compromised by laccase-mediated coupling reactions, several studies have also shown their potential for improving delignification and cellulose hydrolysis. Rico et al. [111] evaluated the use of methyl syringate as natural mediator in the presence of *M. thermophila* laccase to delignify milled eucalypt wood. Four cycles of LMS-alkaline peroxide extraction were performed, resulting in a lignin content reduction of about 50%, and an increase in glucose yields of 30%. These results were comparable to those obtained with *P. cinnabarinus* laccase-HBT as LMS. Moilanen et al. [118], in contrast, observed an increase in the lignin content of acid-steam pretreated spruce when using acetosyringone mediator together with *T. hirsuta* laccase. In spite of this effect, laccase-acetosyringone treatment improved the hydrolysis yield by 36%. This result was explained by an increment of the syringyl/guaiacyl ratio promoted by the enzymatic treatment, which let to reduce the unproductive adsorption of cellulases.

In addition to the reduction in the lignin content, LMS has been also reported for improving enzymatic hydrolysis by lignin modification. By using ABTS and TEMPO as mediators of *T. hirsuta* laccase, Moilanen et al. [118] increased the hydrolysis yields of acid steam-exploded spruce by 54% and 49%, respectively. These improvements were explained to be based on the reduction of the unspecific adsorption of hydrolases on enzyme-treated lignin. Similar results were obtained by Al-Zuhair et al. [134], which showed an increment of sugar production from 5.6% to 45.6% after treatment of a milled material from date palm lignocellulosic waste with *T. versicolor* laccase and the mediator HBT. Using the same LMS and the pyrolysis/gas chromatography-mass spectrometry (Py/GC–MS) with tetramethylammonium hydroxide (TMAH) thermochemolysis analysis, Chen et al. [135] described a significant lignin modification (lignin side chain oxidation) after treatment of ensiled corn stover. This resulted in an increment of the subsequent hydrolysis yield of 7%. The *T. versicolor* laccase-HBT system was also used by Heap et al. [136] for improving the saccharification yield of acid steam-exploded wheat straw. In a first assay, LMS impaired the enzymatic hydrolysis of acid steam-exploded material. However, when a subsequent alkaline peroxide extraction was carried out after LMS, the released glucose concentration increased by up to 2.3 g/L (35%) compared to untreated control. Py/GC–MS with TMAH analysis also revealed lignin oxidation via C_α–C_β sidechain cleavage at the C_α position. In another study, the use of *N*-hydroxy-*N*-phenylacetamide (NHA) as mediator of *T. hirsuta* laccase increased the saccharification yield of acid steam-exploded spruce from 13% to 21% compared to the treatment with laccase alone [116]. Nevertheless, a filtration and washing step had to be performed between laccase-mediator treatment and enzymatic hydrolysis due to inhibitory effect of oxidized NHA on cellulases. In this sense, Moreno et al. [84] also observed a direct inhibition on hydrolytic enzymes activities of different oxidized radicals generated by *P. cinnabarinus* laccase from HBT, VIO, and ABTS mediators. A decrease of about 34% was observed for overall cellulase activity in the presence of the different chemical mediators. However, enzymatic deactivation was even more remarkable in the case of β-glucosidase activity, showing a reduction of about 50%. Martín-Sampedro et al. [137] also observed lignin changes after treatment with LMS (*M. thermophila* laccase and the mediator HBT) of steam-exploded eucalyptus wood chips. By using 2D NMR and ^{13}C NMR, these authors reported an increase in the amount of secondary OH groups and in the degree of lignin condensation. In a subsequent enzymatic hydrolysis, this LMS-treated material showed an increase in the glucose yield from 24.7% to 27.1% [138].

6.3. Other Comments

As previously discussed for laccase detoxification, the genetic engineering of microorganisms for the simultaneous production of laccase and hydrolytic enzymes would allow better processes integration for delignification and saccharification of lignocellulose biomass, and thus reducing the cost and time associated with laccase production and delignification step, respectively. Zhang et al. [120] observed higher saccharification yields during the hydrolysis of corn residue by the heterologous

expression of *Trametes* sp. AH28-2 laccase in *T. reesei*. With a similar concept, Davidi et al. [121] have recently incorporated laccase activity into a cellulase- and xylanase-containing cellulosome. For that, authors designed a dockerin-fused variant of a recently characterized laccase from the aerobic bacterium *Thermobifida fusca* [122]. The resulting cellulosome complex yielded a 2-fold increase in the amount of reducing sugars released from wheat straw compared with the same system lacking laccase activity.

7. Laccases for Detoxification and Delignification in a Lignocellulose-based Biorefinery

On the basis of the current review, laccase enzymes have been largely evaluated as specific, effective and environmental friendly tools for detoxification and delignification of lignocellulosic feedstocks. After laccase treatment, higher saccharification and fermentation yields are usually observed, which offer high potential to reduce overall process costs. For instance, by modifying or partially removing lignin, the unspecific adsorption of hydrolases is reduced and lower enzymes loadings are therefore required for the enzymatic hydrolysis of lignocellulose. This fact represents an important breakthrough, since the costs of hydrolytic enzymes is one of the major economical bottlenecks in the conversion of lignocellulosic biomass. The cost of laccase and/or of mediators should also take into account. Another relevant advantage is the possibility of having a better water economy. After detoxification with laccase, pretreated material contains lower inhibitory compounds, avoiding the necessity of including a filtration and washing step and therefore saving freshwater and reducing the amount of wastewater. Also, by having less inhibitory pretreated materials, conversion processes can be performed at higher substrate loadings, giving the possibility of reaching higher product concentrations with shorter fermentation times.

With the aim of implementing laccases in the current conversion processes, in situ laccase treatment with saccharification and/or fermentation offers some advantages as they do not require extra equipment and thus generates benefits in terms of lower capital and operating costs. Simultaneous delignification and detoxification with laccase is another interesting strategy to consider. However, little is known about the existence of laccases with capacity for simultaneous delignification and detoxification. Furthermore, it should be noted that lower sugar yields are usually observed during saccharification of detoxified feedstocks [76,82,89,102]. Searching for novel laccases with ability to delignify and detoxify simultaneously or designing new ones with the required properties need to be further explored. In this sense, Moreno et al. [82] has recently evaluated the commercial bacterial laccase MetZyme® for enhancing saccharification and ethanol fermentation of steam-exploded wheat straw. When the pretreated material was subjected to laccase action, a modest increase of about 5% in the sugar recovery yield was observed. In contrast, when performing an alkaline extraction prior to laccase treatment, the glucose and xylose recovery increased by 15% and 23%, respectively, compared to alkaline treatment alone. A modest phenols removal could be also observed during treatment of steam-exploded wheat straw with Metzyme® laccase. The lower phenolic content allowed to improve the fermentation performance of the thermotolerant yeast *K. marxianus* CECT 10875 during SSF processes, shortening its adaptation phase and reducing fermentation times. Similarly, De La Torre et al. [95] compared the use of both bacterial *Streptomyces ipomoeae* and fungal *T. villosa* laccases for delignification and detoxification of steam-exploded wheat straw. When using the bacterial laccase, no significant effects were observed on delignification or saccharification of laccase-treated biomass. However, the use of fungal laccase resulted in higher lignin content and lower sugar recoveries. By combining an alkali extraction with *S. ipomoeae* laccase, a 4% reduction in the lignin content was observed compared to alkaline treatment alone, increasing the glucose and xylose concentrations in the resulting hydrolysate by 16% and 6%, respectively. These positive effects were however not observed when using *T. villosa* laccase. In addition to delignification, the capacity of these bacterial and fungal laccases for detoxification of pretreated material was also evaluated. A reduction in the phenol content of 29% and 90% were achieved with the bacterial and fungal laccases,

respectively. This reduction resulted in an improved fermentation performance of *S. cerevisiae* during SSF processes.

Cost-effectiveness in future biorefineries goes through valorization of all components of lignocellulosic biomass. In this context, biorefineries have to deal with producing not only high-volume and low-cost fuels but also low-volume and high-value compounds, minimizing downstream wastes. With this purpose, in addition to carbohydrate fermentation processes such as ethanol and/or organic acids production, alternative value-added products and chemicals can be also obtained from lignin. Laccases can also contribute to such an aim, assisting in certain processes during the manufacture of new value-added products. For instance, laccases have been typically applied in the pulp and paper industry (1) for pulp bleaching, removing the residual lignin responsible of pulp color [71], (2) for controlling pitch deposits that reduce pulp quality [75], or (3) for detoxification of bleaching effluents rich in phenolic compounds [139]. Laccases have been also evaluated for the synthesis of new materials and products from lignocellulosic feedstocks. Laccases can limit and/or avoid the use of toxic synthetic adhesives (such as formaldehyde-based resins) during production of fiberboards and other materials, by catalyzing the cross-linking reactions of phenolic residues in lignin based-materials [140]. Tailoring of lignocellulosic materials by laccase-assisted biografting of phenols and other compounds is another emerging area. Also, laccase-assisted functionalization of wood and non-wood fibers to modify different properties has been achieved, obtaining new physico-mechanical, optical and antimicrobial properties [141–143]. Finally, laccases are also a promising approach to decompose the lignin polymer into several phenolic and aromatic compounds that are currently produced from fossil fuels [144].

8. Conclusions

In the current biorefinery concept, laccases constitute a powerful biotechnological tool for the complete utilization of lignocellulosic biomass to new added-value products and fuels, with lower energy demand, better economy and less environmental impact. Laccases act selectively to remove lignin-derived phenolic compounds released from biomass pretreatment, diminishing the impact of these inhibitors on the subsequent saccharification and ethanol fermentation stages. Then, a reduction of phenols by laccase-aided polymerization promotes microbial growth, glucose consumption and increase notably the ethanol production. Laccases and laccase-mediator systems can also be effective in oxidative modification and/or partially depolymerization of lignin, increasing the final hydrolysis yields of different pretreated materials. Nevertheless, the costs for enzyme production and the use of expensive synthetic mediators are current challenges to overcome for the successful implementation of laccases in these lignocellulose-based industries. Screening of microorganism cultures and genomes for novel laccases or engineering of existing ones by direct evolution and related approaches are solutions to consider. Moreover, the search of new, cheap and environmentally friendly mediators can also push these biocatalysts toward their application on an industrial level.

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