fermentation

Review

Improve Enzymatic Hydrolysis of Lignocellulosic Biomass by Modifying Lignin Structure via Sulfite Pretreatment and Using Lignin Blockers

Caoxing Huang [1], Ruolin Li [1], Wei Tang [2], Yayue Zheng [1] and Xianzhi Meng [3,*]

[1] Co-Innovation Center for Efficient Processing and Utilization of Forest Resources, College of Chemical Engineering, Nanjing Forestry University, Nanjing 210037, China
[2] School of Pharmacy & School of Biological and Food Engineering, Changzhou University, Changzhou 213164, China
[3] Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, TN 37996, USA
* Correspondence: xmeng5@utk.edu

Abstract: Even traditional pretreatments can partially remove or degrade lignin and hemicellulose from lignocellulosic biomass for enhancing its enzymatic digestibility, the remaining lignin in pretreated biomass still restricts its enzymatic hydrolysis by limiting cellulose accessibility and lignin-enzyme nonproductive interaction. Therefore, many pretreatments that can modify lignin structure in a unique way and approaches to block the lignin's adverse impact have been proposed to directly improve the enzymatic digestibility of pretreated biomass. In this review, recent development in sulfite pretreatment that can transform the native lignin into lignosulfonate and subsequently enhance saccharification of pretreated biomass under certain conditions was summarized. In addition, we also reviewed the approaches of the addition of reactive agents to block the lignin's reactive sites and limit the cellulase-enzyme adsorption during hydrolysis. It is our hope that this summary can provide a guideline for workers engaged in biorefining for the goal of reaching high enzymatic digestibility of lignocellulose.

Keywords: biomass; pretreatment; enzymatic hydrolysis; lignin modification; lignin blocker

check for updates

Citation: Huang, C.; Li, R.; Tang, W.; Zheng, Y.; Meng, X. Improve Enzymatic Hydrolysis of Lignocellulosic Biomass by Modifying Lignin Structure via Sulfite Pretreatment and Using Lignin Blockers. *Fermentation* **2022**, *8*, 558. https://doi.org/10.3390/fermentation8100558

Academic Editor: Xiaoqing Lin

Received: 21 September 2022
Accepted: 19 October 2022
Published: 20 October 2022

1. Introduction

With the development and progress of society, human beings face more and more severe energy issues, such as decreased energy storage and increased environmental pollution caused by fossil energy burning [1]. The conventional energy reserves on earth, such as petroleum, natural gas, and high-carbon energy coal are increasingly consumed. At the current rate of consumption, it has been reported that the approximate lifetime of the world's petroleum and natural gas reserves is only 50 years [2]. Therefore, it is essential to look for and develop renewable and clean energy for energy security and economic development. At present, clean and renewable energy has been widely studied, and bioethanol derived from carbohydrates by biological fermentation represents one of the best alternatives to replace traditional energy sources and reduce the dependence on other fossil fuels [3,4]. Specifically, the first generation of bioethanol is mostly created by hydrolysis and fermentation of precious food crops, but the consumption of grain is not in line with the long-term goals of human development, especially in countries with a food crisis. After 20 years of development, abundant lignocellulosic biomass has attracted much attention from many researchers. The second-generation bioethanol that could be directly produced from non-food lignocellulosics has the advantage of utilizing biomass and agricultural waste as the feedstock, thus could avoid the food vs. fuel debate. However, the processes of producing bioethanol from lignocellulosic are complicated and require multiple steps (e.g., pretreatment and hydrolysis), and the high cost related to these processes is the main reason that restricts its commercialization process [5,6]. Therefore, many researchers started to investigate the technical method of improving the efficiency of the bioethanol production process in the past few decades.

Currently, many biorefineries for the production of bioethanol have not been commercialized, which is due to poor economic returns from final products. Hence, various technologies have been proposed to co-produce fermentable sugars, xylo-oligosaccharides (XOS) from hemicellulose, and valuable products from lignin [7]. For XOS, it is a type of prebiotics that can inhibit the growth of other harmful bacteria and promote the growth of probiotics for improving intestinal motility [8,9]. It has been reported that XOS can be applied as nutraceuticals, feed, food additives, and pharmaceuticals, which has rendered the XOS with high price ($25,000–50,000/t) for widespread application [10,11]. For the biorefinery lignin, it can be used as the active substance in different tissue engineering fields [12], as the precursor to prepare lignin-based nanomaterials in different smart fields [13], and as the precursor to fabricate functional fertilizer in the agricultural field [14]. Hence, the production of value-added products from hemicellulose and lignin via sustainable routes in the concept of biorefineries will benefit the transition of world industrial matrix.

The refining process of bioethanol mainly includes feedstock collection, pretreatment, enzymatic hydrolysis, fermentation, and product isolation, which is shown in Figure 1 [15]. Pretreatment technology is a crucial step aforehand to enzymatic hydrolysis, which aims to increase the accessibility of carbohydrates by destroying the cell wall of lignocellulose [16,17]. As shown in Figure 2, the commonly used pretreatment technologies mainly include chemical pretreatment (e.g., dilute acid, alkali, organosolv, deep eutectic solvent, ionic liquid, etc.), physical pretreatment (e.g., ball milling and extrusion), and their combinations (e.g., supercritical fluids and ammonia fiber explosion) [18]. In addition, microbial pretreatment has also been considered as the alternatives technology for improving the enzymatic hydrolysis of lignocellulose, which is due to its ability to degrade the lignin via fungus (such as white rot fungi, brown rot fungi, and soft-rot fungi) [19]. Regardless of the applied pretreatment methods, their objective is always to crack natural physical barriers and expose more carbohydrates for cellulase degradation for the downstream hydrolysis process [20]. The action of various cellulases enzymes on the surface layer of cellulose is shown in Figure 3 [21]. During the enzymatic hydrolysis, the exoglucanase attacks the end of the cellulose chain, and the endoglucanase is cut off the cellulose chain in the middle, both of which reduce the degree of cellulose polymerization [22]. The β-glucosidase works to break the 1,4-glucoside bond that connects glucoses. After pretreatment, not only cellulose but also a fraction of hemicellulose remains in the solid fraction and represents a valuable source of sugar. Hence, the hemicellulases should also be explored to hydrolysis the hemicellulose into monosaccharides [23]. Finally, under the synergy of each individual enzymes (cellulase and hemicelluase) or enzyme cocktail (containing cellulase and hemicelluase), the carbohydrate from the lignocellulose can be degraded into fermentable sugar such as glucose and xylose, which are the raw material for microbial fermentation [24,25]. Bioethanol could then be produced from this fermentable sugar by yeast in a suitable environment. In actual industrial applications, since the yeast fermentation broth is not a pure ethanol solution, it is often necessary to further obtain pure bioethanol by purifying the fermentation solution.

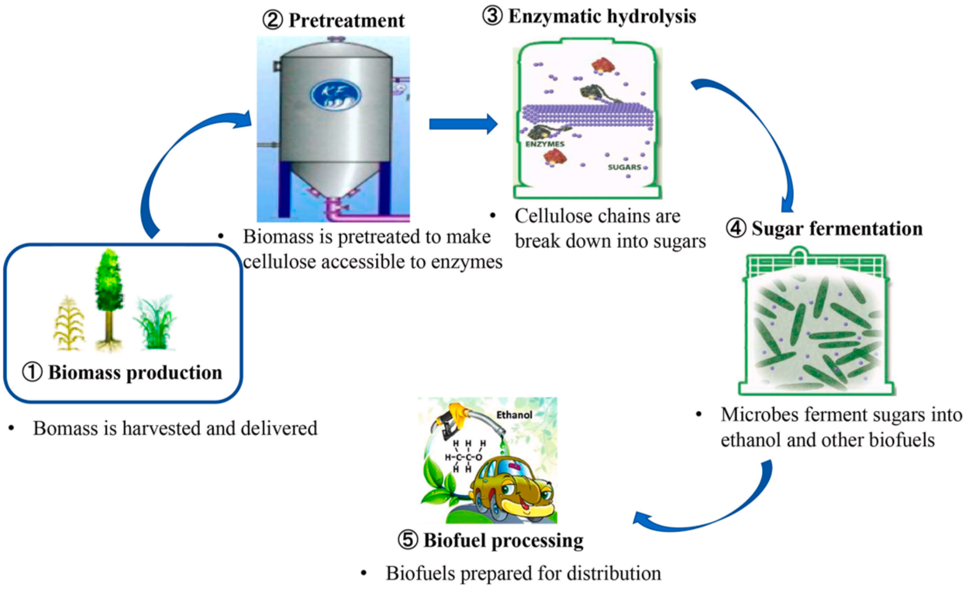

Figure 1. Bioconversion of lignocellulosic biomass to biofuel via feedstock production, biomass pretreatment, enzymatic hydrolysis, sugar fermentation, and biofuel processing [15].

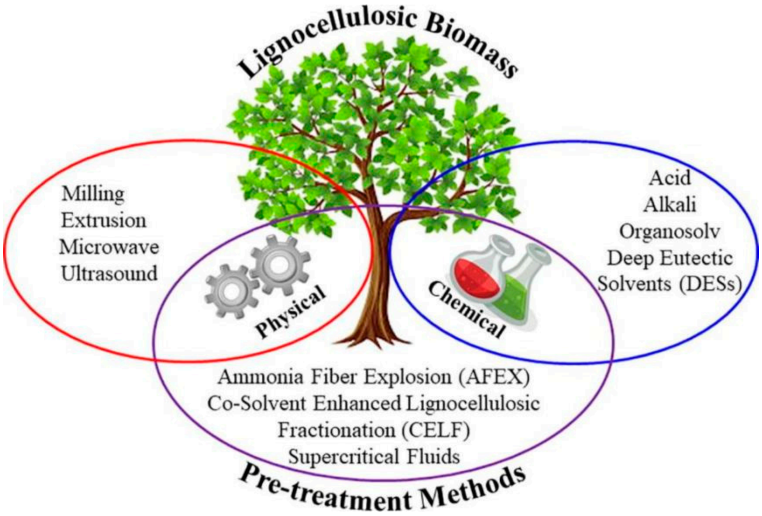

Figure 2. Various physical and chemical pretreatment technologies [18].

As a class of aromatic polymers, lignin in the lignocellulosic raw materials is often considered the most recalcitrant component in the plant cell wall. Native lignin contributes to biomass recalcitrance mainly via two mechanisms [26,27]. On one hand, lignin can inhibit the enzyme hydrolysis of lignocellulosic by physically limiting the access of cellulose to cellulases. On the other hand, it can also bind to cellulase through electrostatic adsorption, hydrophobic effect, hydrogen bonding, etc., thus limiting the availability of cellulases [3]. During the pretreatment process, especially for the lignin-target pretreatment, the residue lignin often had significant diffident chemical structures compared to the native lignin when a portion of lignin was partially removed from the cell wall of biomass. It is expected that the remaining lignin in pretreated biomass still restricts its enzymatic hydrolysis by limiting cellulose accessibility and lignin-enzyme nonproductive interaction. While majority of those lignin plays a negative role, some studies have indicated that the modified lignin with specific structures or from a certain location of the pretreated lignocellulose actually increases the enzymatic hydrolysis efficiency, and such lignin substrates include but are not limited to sulfonated lignin, organosolv lignin, and some water-soluble lignin. For example, it has been reported that the non-productive adsorption between cellulase and lignin could be intervened by the addition of sulfonated lignin [28–30]. In addition, Lai et al. reported that the ethanol organosolv lignin from sweetgum could facilitate the enzymatic hydrolysis [31]. The water-soluble sulfate lignin polyoxyethylene ether (LS-PEG) from the modified lignin has also been proposed to improve cellulase stability and enzymatic digestibility of dilute acid pretreated hardwood [32]. Therefore, lignin's exact impact on enzymatic hydrolysis remains unclear, and how to restrain the negative and boost the positive effect of lignin still requires further study.

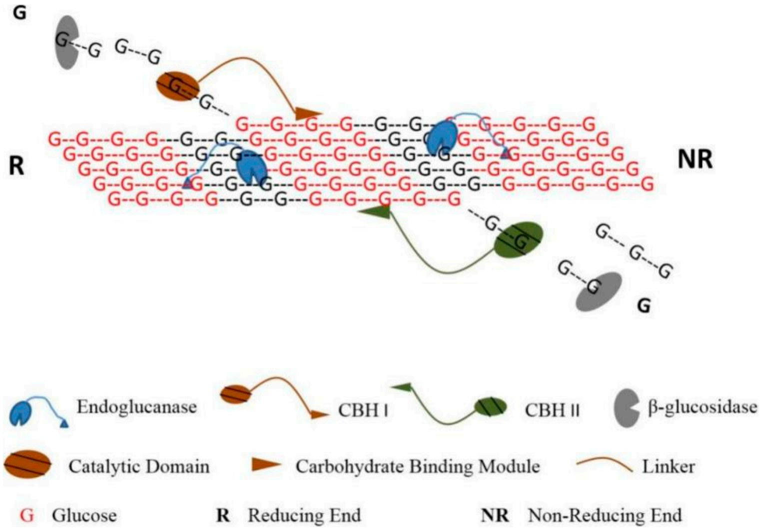

Figure 3. The action of various cellulases enzymes on surface layer of cellulose [21].

Actually, many reviews have summarized the typically acid and alkaline pretreatments to improve the enzymatic digestibility by removing lignin and hemicellulose [33–35]. In addition, there are also some published reviews focusing in investigating the interaction between lignin and cellulase, which is aimed to understand how lignin inhibit the enzymatic hydrolysis efficiency of cellulose [3,36]. For example, in the work of Yao et al., [37], they reviewed the works how lignin is transformed during various pretreatment methods as well as how these changes impact the cellulases inhibition, while they did not focus on the modification of lignin during pretreatment for improving the enzymatic hydrolysis. Actually, some special pretreatments showed ability to alter lignin's structure during the pretreatment process, which can enhance the enzymatic hydrolysis efficiency of lignocellulosic materials. Hence, the main goal of this review is to summarize the latest special pretreatment methods with the ability to in situ reduce lignin's hydrophobicity (sulfite pretreatments and additives-aided pretreatments) and the utilization of "lignin blockers" (synthetic and natural polymeric nonionic surfactant, lignin-based surfactants, and non-catalytic proteins) to improve enzymatic hydrolysis of lignocellulosic biomass. Especially, the recent technologies of genetic modification of lignin and enzyme resources for improving the enzymatic digestibility of biomass have also been showed in the paper, which has not be systematically reviewed before. It is our hope this review will provide a new guideline for workers engaged in biorefining for the goal of reaching high enzymatic digestibility of lignocellulose.

2. Effect of Lignin on Enzymatic Digestibility of Biomass

After the pretreatment process, the remained lignin and lignin-derived phenolic molecules (to a lesser degree) left in the pretreated lignocellulose surface can all decrease the stability and activity of cellulases, which shows a negative effect on the enzymatic hydrolysis digestibility of pretreated biomass (Figure 4) [38–41]. The lignin dissolved in the solution can be attached to the surface of biomass, leading to a decrease in the accessibility for enzymes to cellulose (Figure 4B). Lignin can also cause the inhibition of activity for cellulase due to the non-productive adsorption through hydrophobic, electrostatic, and hydrogen bonding interactions (Figure 4A) [3,27]. In this section, these mechanisms will be discussed and reviewed in detail.

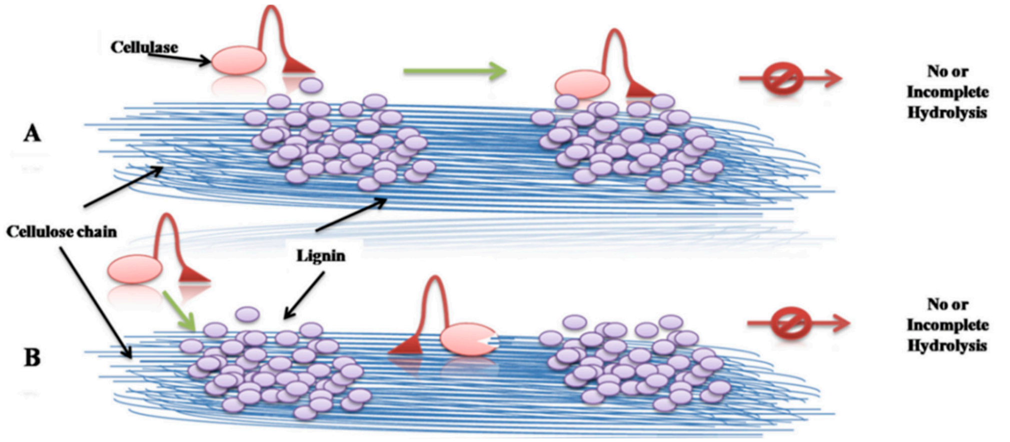

Figure 4. Lignin inhibition on cellulase. (**A**). Non-productive adsorption of cellulase onto lignin; (**B**) Physical blockage of cellulase by lignin [41].

2.1. Steric Hindrance to Hinder Reaction between Cellulose and Cellulase

Generally, the solubilized lignin from the cell wall of biomass during the pretreatment process can be re-deposited on the surface of biomass as droplets upon cooling, and the lignin in biomass can also be redistributed to the surface of biomass [42,43], which may cause an adverse effect on the enzymatic hydrolysis of substrates. While most studies suggest that pretreatment typically possesses a positive effect on the enzymatic hydrolysis process, high pretreatment severity is not always necessary and could occasionally cause a negative effect. For instance, Pappas et al. reported that the amount of released sugars was positively correlated with the dilute acid pretreatment severity [44]. On the other hand, Donaldson et al. reported that alkali pretreatment using NaOH could cause lignin to be attached to the porous cell wall and ultimately decrease the biomass porosity and reduce the digestibility [45]. In addition, the lignin-carbohydrate complex (LCC), a hybrid structure formed by hydrogen and covalent bonds between lignin and carbohydrate (mainly hemicellulose), also showed negative impacts on cellulase hydrolysis [46]. Balakshin et al. showed that the existence of different types of LCC significantly affects the purification and extraction process for carbohydrates and lignin [47]. It has been proposed and shown that the degradation of LCC plays a critical role in the removal of hemicellulose and lignin from biomass [48,49]. Accordingly, the presented LCC in pretreated biomass can also decrease the enzymatic hydrolysis efficiency of various kinds of pretreated lignocellulosics [50–53]. Hence, removing the physical barrier of lignin is the crucial approach to improve the enzymatic digestibility of biomass. Now, various pretreatment processes have been developed to reduce the lignin content in cell wall of biomass or remove the lignin droplets from the surface of pretreated biomass, aiming to increase the diameter and volume of the pores and the accessible surface area of cellulose for cellulase for an efficient enzymatic hydrolysis [54,55].

2.2. Non-Productive Adsorption between Lignin and Cellulase

As shown in Figure 5, the main non-covalent interactions between cellulase and lignin are hypothesized by hydrophobic, electrostatic, aromatic π-π, charge-π, and hydrogen bonding interactions [41,56,57]. Hydrophobic interaction is mainly entailed by the ring stacking between hydrophobic surface of lignin and the hydrophobic amino acids residues of cellulase enzymes [24]. Lignin and enzymes both contain multiple hydroxyl groups, and their dissociation and association in an aqueous environment could result in different charges on their surface, causing either pH-dependent electrostatic attractive or repulsive interactions between them [56,58]. Last but not least, many of these functional groups from lignin and enzymes contain hydrogen atoms bonded to a strongly electronegative atom such as oxygen, thus offering great opportunities for them to interact with each other via hydrogen bonding. For a more elaborate discussion on how these driving forces affect the non-productive lignin-enzyme adsorption, these dedicated reviews can be referred [3,57,58]. Based on the atomic-detail molecular dynamics simulation study, lignin not only binds to specific residues preferentially on the cellulose-binding module of the cellulase, which is critical for cellulose binding, but also to the hydrophobic faces of cellulose where the cellulases preferentially bind. As a result, it binds exactly where it is least needed for industrial use. Two recent studies using NMR titration analysis (i.e., chemical shift perturbation) studied the lignin/cellulose-enzyme adsorption mechanism at a molecular level and results showed that milled wood lignin was absorbed onto lignin at multiple binding sites, including the subsites at a much higher affinity than cellohexaose, and aromatic rings in the lignin models are the major sites for interactions [59,60]. The contributions of each individual factors for adsorption performance between lignin and cellulase remains controversial, as it significantly depends on sources of lignin, type of cellulase enzymes as well as the experiment conditions. Some studies concluded that hydrophobic interaction was the main driving force responsible for the adsorption of enzymes onto lignin surfaces [61,62], while others have emphasized the importance of electrostatic [63,64], and hydrogen bonding (to a lesser degree) interactions [65]. The combined effects of individual factors on lignin-enzyme adsorption were also assessed. Huang et al. concluded that both hydrophobic interactions and electrostatic repulsions influence the enzymatic hydrolysis of lignin, and lignin stimulation is controlled by electrostatic interaction (e.g., the negative zeta potential) while the inhibition is largely governed by the lignin hydrophobicity [66].

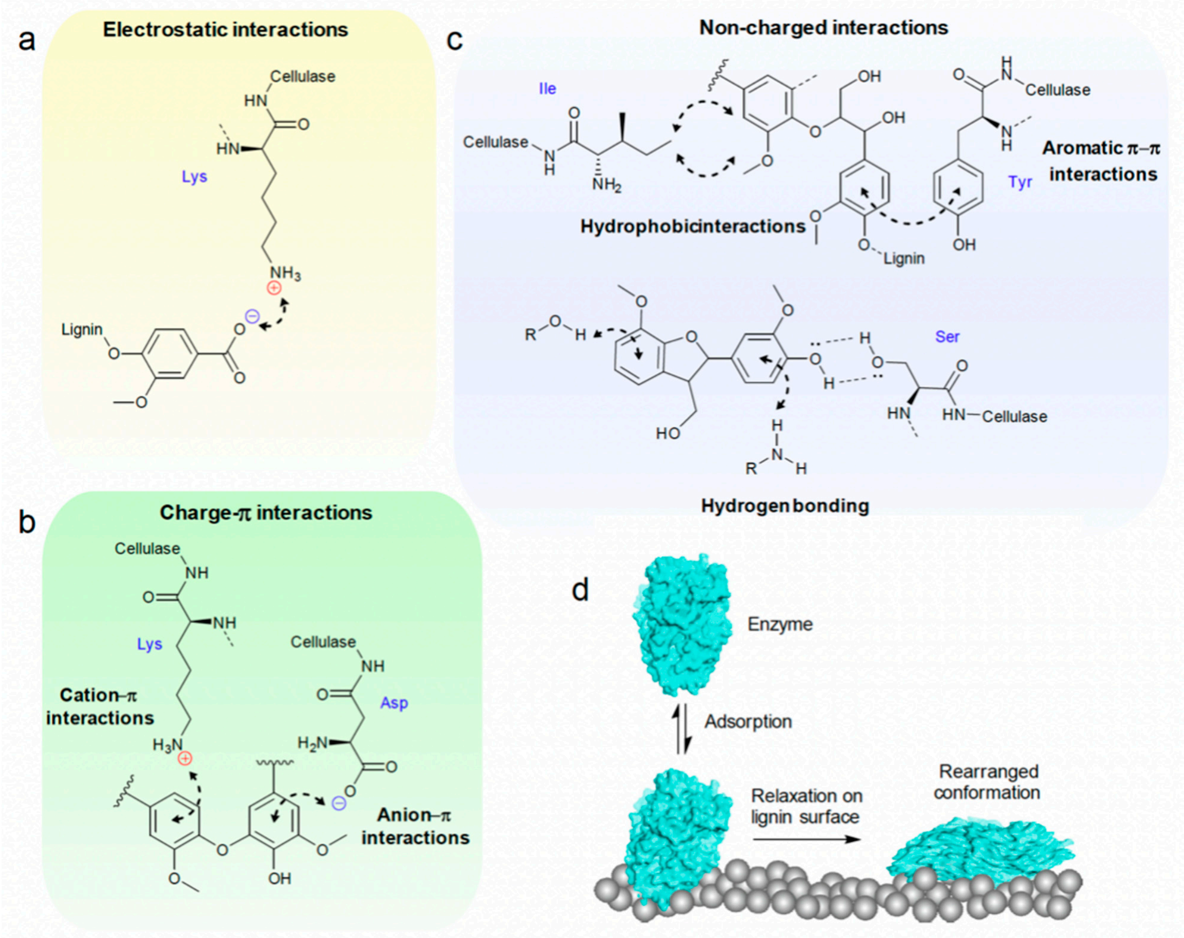

Figure 5. The main interactions between cellulose enzyme and lignin (**a**–**c**) that lead to the adsorption, structural rearrangements, and irreversible binding of enzymes on the surface of lignin (**d**) [57].

3. Methods for Enhancing the Enzymatic Digestibility of Pretreated Biomass

In order to enhance the enzymatic digestibility of pretreated biomass, various pretreatment technologies and post-treatment methods have been developed and proposed. For the pretreatment, it is aimed to remove or modify lignin in the cell wall of biomass to reduce its non-productive binding ability for cellulase [67,68]. For post-treatment methods, such as the addition of synthetic polymers, surfactants, and non-catalytic proteins, it is aimed to improve the enzymatic digestibility of pretreated biomass by obstructing the exposed lignin surfaces.

3.1. Special Pretreatment Method

Due to the natural biomass recalcitrance, it is necessary to perform pretreatment to increase the accessibility of cellulose and improve the subsequent enzymatic hydrolysis efficiency [69–71]. Conventional organosolv, alkaline, novel ionic liquid, and deep eutectic solvent pretreatment could remove majority of the lignin from the plant cell wall, thus facilitating the subsequent enzymatic hydrolysis. These kinds of lignin-target pretreatments have been reviewed several times and thus will not be covered in this manuscript [72–75]. Besides being removed from the plant cell wall, native lignin's structure could be severely modified by biomass pretreatment [6], and these structural changes could lead to either beneficial or detrimental impacts on the enzymatic hydrolysis. For example, lignin sulfonation [76] and alkoxylation of the aliphatic side chains have all shown positive effects on the enzymatic hydrolysis process [77], while the formation of phenolic OH [78] and condensed lignin units all had a detrimental effect on the enzymatic digestibility of pretreated biomass [62]. The changes in lignin functionalities from different types of biomass as a result of various types of pretreatment as well as their impact on the enzymatic hydrolysis process have been reviewed [57]. The net effect of lignin on the enzymatic digestibility of pretreated biomass is a function of the amount and chemical structure of residual lignin left in pretreated biomass. Hence, this section mainly focuses on some special pretreatment means that could alter the structure of lignin in a way that alleviates its negative effect with cellulase enzymes, thus improving the enzymatic hydrolysis of pretreated biomass.

3.1.1. Sulfite Pretreatment

As a byproduct of sulfite pulp, the dilute sulfite waste liquid produced from the pulp and paper mill is a valuable lignin resource [79]. Sulfite pretreatment, a novel lignocellulosic biomass pretreatment, was firstly proposed by Zhu and coworkers for bioethanol production [80]. The proposed schematic process flow diagram of the sulfite pretreatment can be seen in Figure 6. This pretreatment technology utilizes various concentrations of sulfite or bisulfite solution at different pH to effectively remove hemicellulose and lignin from the substrate to facilitate the enzyme hydrolysis of cellulose [80]. During sulfite pretreatment, the active reagents mainly include SO_3^{2-}, HSO_3^{-} or the combination of the two depending on the pH of the pretreatment liquor. Sulfite pretreatment improves the enzymatic hydrolysis yield of pretreated lignocellulosic substrates by solubilizing hemicellulose/lignin, lowering the hydrophobicity of lignin by sulfonation, thus decreasing its binding affinity toward cellulase enzymes. Table 1 summarizes the application of sulfite pretreatment on various biomass substrates at different pH to overcome biomass recalcitrance.

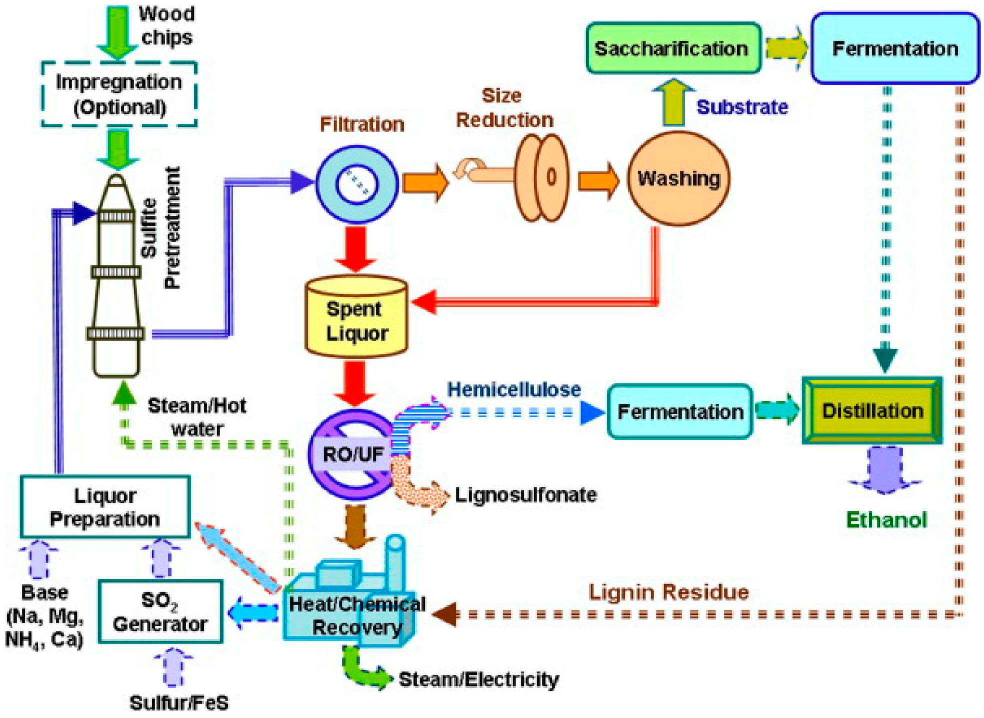

Figure 6. Schematic process flow diagram of the sulfite pretreatment [80].

Acid Sulfite Pretreatment

Acid sulfite pretreatment is one of the most effective sulfite pretreatments [81,82]. Although it is effective in solubilizing hemicellulose, many enzymatic hydrolysis and fermentation inhibitors, such as hydroxymethylfurfural (HMF) and furfural (FF), could be produced during the pretreatment process under severe conditions, which could reduce the yield of ethanol after fermentation [83]. Furthermore, the acid treatment also has the disadvantage of equipment corrosion problems and requires a large amount of alkali neutralization for the downstream process. Noparat et al. studied the effect of acid sulfite pretreatment on enzymatic hydrolysis of oil palm stem (OPT) [84]. When the cellulase dosage of 15 FPU/g cellulose was applied for the pretreated biomass at the optimized conditions, a high enzymatic hydrolysis efficiency with 92% for cellulose could be achieved at 48 h. Jaisamut et al. investigated the effects of sulfite pretreatment conditions on the changes of biomass composition, formation of furan derivatives, the enzymatic hydrolysis yield, and the production of ethanol by fermentation [83]. Results showed that a high ethanol yield with 17.3 g/100 g dry biomass could be achieved, which represents 75% of the theoretical yield of glucose to ethanol. In addition, it is found that pretreatment temperature beyond 160 °C had a limited impact on lignin removal for the biomass of Douglas-fir due to lignin condensation, which agrees with another study showing that the degree of delignification was irrelative to temperature in the acid sulfite pretreatment of Douglas-fir at 165–180 °C [85]. Tan et al. pretreated corn straw in sodium bisulfite solution composed of 7% sodium bisulfite and 1% H_2SO_4 at 170 °C for 30 min, and the enzymatic hydrolysis showed that the transforms of glucan and xylan increased to 80% and 86% by acidic sulfite pretreatment, which were 220% and 200% higher than that of untreated corn stalk, respectively [86].

Table 1. The over review of sulfite pretreatment to increase enzyme hydrolysis efficiency of lignocellulose.

Pretreatment	Reagent	Lignocellulose	Cellulase Loading	Results	Advantage	Disadvantage
Acid sulfite pretreatment	7% H_2SO_4 and 6% Na_2SO_3	Oil palm stem	15 FPU/g-cellulose	Improved enzymatic efficiency into 92%	Effective in overcoming biomass recalcitrance; low environmental and technological barriers and risks for commercialization	Possibility of formation of inhibitory by-products under acid condition; potentially need high pretreatment temperature
Acid sulfite pretreatment	1% H_2SO_4 and 2.4% Na_2SO_3	Wheat straw	35 FPU/g-cellulose	172.5 kg per ton ethanol was generated higher than 38.3 kg of the raw wheat straw		
Acid sulfite pretreatment	1% H_2SO_4 and 7% Na_2SO_3	Corn straw	10 FPU/g-cellulose	The conversion of glucan and xylan increased to 80% and 86%		
Alkaline sulfite pretreatment	1% NaOH and 4% Na_2SO_3	Hybrid pennisetum	20 FPU/g of cellulase and xylanase	The glucose released from alkaline sulfite pretreated HP was 734 mg/g greater than that of sodium hydroxide pretreated HP (483 mg/g)	Only need mild pretreatment temperature; various uronic acid substitutes from lignin and hemicellulose can be effectively removed and the polysaccharide loss is relatively low	Alkaline wastewater is harmful to the environment; high cost of alkaline catalyst; long pretreatment time
Alkaline sulfite pretreatment	5% NaOH and 20% Na_2SO_3	Delignated hybrid pennisetum	5 FPU/g-cellulose	The yield of glucose and xylose ranged from 74.8% to 90.8% and 65.9 to 79.5%		
Alkaline sulfite pretreatment	5% NaOH and 10% Na_2SO_3	Bagasse	10 FPU/g-cellulose	The highest sugar yields of the glucose and xylose were 76.8% and 61.2%		
Alkaline sulfite pretreatment	12% NaOH and 10% Na_2SO_3	Corn straw	20 FPU/g-cellulose	The total sugar yield was 74.73% after 48 h enzymatic hydrolysis		
Neutral sulfite pretreatment	5.27% $Mg(HSO_3)_2$	Corn straw	15 FPU/g-cellulose	The enzymatic hydrolysis yield of corn straw was increased from 31.02% to 90.44%	Relatively mild reaction conditions; no post-treatment required; low environmental impact	Low delignification efficiency; low degree of lignin sulfonation
Neutral sulfite pretreatment	12% Na_2SO_3	Corn cob residue	5 FPU/g-substrate	The enzymatic hydrolysis efficiency and glucose yield was increased by 28.80% and 20.10%		

Alkaline Sulfite Pretreatment

To minimize the loss of carbohydrate fraction and prevent the formation of fermentation inhibitors such as FF and HMF, the alkaline version of sulfite pretreatment was also proposed. The temperature of alkaline sulfites pretreatment is typically lower than that of acidic sulfites pretreatment, which reduces energy consumption accordingly. In addition, alkaline sulfites pretreatment can effectively remove various uronic acid substitutes from lignin and hemicellulose, and the polysaccharide loss is relatively low compared with acid-catalyzed pretreatment [87,88]. The other advantage of this type of alkaline sulfite pretreatment is the possibility of using the recovered lignosulfonates as lignin derivatives [89,90]. During the alkaline sulfite pretreatment, the nucleophilic sulfite leads to the cleavage of -alkyl ether and -benzyl ether linkages on phenolic lignin as well as lignin sulfonation [91]. Yang et al. showed that after alkaline sulfite pretreatment, cellulase enzymes were preferably adsorbed on the carbohydrate fraction of the pretreated Pennisetum since lignin sulfonation reduced the unproductive adsorption on cellulase [92]. As a result, the glucose production after enzymatic hydrolysis for pretreated Pennisetum reached near 100%. Similarly, Wang et al. showed that the reduction of unproductive binding of the enzymes on the residual lignin left in the alkaline sulfite pretreated Pennisetum was mainly attributed to the presence of sulfonic acid groups in lignin and the enhancement of hydrophilicity [29].

Neutral Sulfite Pretreatment

Sulfite pretreatment could also be performed in neutral, which could achieve a similar level of delignification, prevent the excessive degradation of fermentable sugars, and avoid the corrosion of equipment in acid and base sulfite pretreatment, thus it has a high industrial potential [93,94]. Yu et al. developed a neutral type of magnesium bisulfate pretreatment for bioethanol production. ~90% of lignin could be removed from cell wall of corn stover and consequently over 90% of cellulose could be hydrolyzed to glucose [28]. Chen et al. studied the effect of pH on enzymatic hydrolysis of sulfite pretreated corncob residue, and the results showed that a pH value of 7 led to a delignification rate of 77.45%, a sulfonation degree of 0.677 mmol/g, along with the best cellulose conversion yield (>90%) [95].

3.1.2. Other Lignin Sulfonation Pretreatment and Its Impact on Enzymatic Hydrolysis

It has been shown that sulfonated lignin can promote the enzymatic hydrolysis process by altering lignin's hydrophobicity [96,97]. Chandra et al. confirmed that pre-sulfonation of steam-pretreated substrates could increase hydrolysis yield by ~10% [98]. Yang and Pan modified lignin by sulfonation and carboxylation, which reduced lignin's hydrophobicity by 22–30%, thus eliminating 76–96% of lignin inhibition [65]. Lai et al. showed that the addition of 2-naphthol-7-sulfonate could suppress lignin condensation and introduce the sulfonyl group onto the lignin sub-units could promote the enzymatic digestibility of pretreated larch [99]. Huang et al. pretreated bamboo residue with SO_2-ethanol-water (SEW) co-solvent and found that during the pretreatment process, ethanol promoted the transformation of SO_2 to biomass and also served as a solvent to eliminate the generated sulfonated lignin [100]. A positive relationship between lignin degree of sulfonation and enzymatic digestibility was found due to the change of substrates hydrophobicity. Ying et al. developed a successive Fenton oxidation and sulfonated methylation pretreatment (FSP) to further decrease the surface lignin hydrophobicity [96]. Results showed that Fenton oxidative as a pre-step could further facilitate the introduction of sulfomethyl group onto the lignin sub-structure, thereby improving the removal yield of lignin and alleviating the interaction between lignin and enzyme.

In conclusion, certain chemical groups could be introduced into lignin during these pretreatments to increase the hydrophilicity of lignin, inhibit the condensation of lignin and change the surface charge of lignin [99], all of which could intervene the lignin-enzyme interaction [66]. Sulfonation pretreatment could also be relevant to other pretreatment technologies as a post-treatment step [98,99,101].

3.2. Utilization of "Lignin Blockers" during Hydrolysis to Reduce Adsorption between Lignin and Enzymes

Besides changing the chemical structure of lignin by pretreatment, "lignin blockers" including proteins, surfactants, polymers, peptides, and metal ions, can be added during enzymatic hydrolysis to block the lignin, therefore minimizing the interaction between lignin and enzyme. This has become a hot topic because of its operational feasibility. Table 2 summarizes some recent advances in utilizing these lignin blockers to improve the enzymatic digestibility of pretreated biomass.

3.2.1. Lignin Blockers including Surfactant and Non-Catalytic Proteins

Surfactants are a kind of substance that can significantly reduce the surface tension of liquids even at a very low concentration [102]. Many surfactants are amphiphilic—having both hydrophilic and hydrophobic groups. These surfactants (e.g., synthetic and natural) usually have no influence on the charge of the hydrolysis system, thus will not cause cellulase flocculation, and their utilization as lignin blockers has been demonstrated in several studies [103–105].

Synthetic and Natural Polymeric Nonionic Surfactant

The nonionic surfactant is a type of surfactant that has been widely used to enhance the enzymatic digestibility of pretreated biomass. It has the advantages of high stability, good compatibility, and high solubility in the aqueous enzymatic hydrolysis system [106]. At present, the major mode of action for nonionic surfactants depends on promoting the hydrolysis of lignocellulosic substrate, mainly including (1) reducing non-productive enzyme adsorption [107,108]; (2) increasing the stability and activity of the enzyme and protect it from denaturing when heated and affected by shear force [109,110]; (3) lower the surface tension of lignocellulose and increase its cellulose accessibility [111] and (4) facilitate enzyme recycling [112]. Although a clear mechanism that can consistently explain how nonionic surfactants enhance hydrolysis has yet to be developed, various types of synthetic and natural polymeric nonionic surfactants, including PEG 4000/6000/8000 [113,114] and Tween 20/80 [107,115,116] have been proposed, and all repeatedly showed enhanced enzymatic hydrolysis performance in the past decades (Table 2). Additives mainly affect the hydrolysis system by blocking the non-productive adsorption sites of lignin. Non-ionic surfactants could have the interaction with enzymes via hydrophobic interaction and modify the architecture of micellar interface, resulting in more efficient conformations [117,118]. They could take up the surface areas of the liquid phase and decrease the exposure of activated enzymes to air, thus increasing its activity [119]. In addition, the presence of nonionic surfactants also increased the thermal stability of cellulase enzymes and prevented the deactivation of enzymes by mechanical stress [120]. As a result, the activities of enzymes including β-glucosidases and endoglucanases were reported to be increased by the addition of surfactant (e.g., Tween 80) [121,122]. Addition of surfactants such as PEG in the pretreatment process also facilitated the delignification thus consequently increasing the enzymatic hydrolysis yield [123]. Finally, the benefits of the utilization of surfactants such as Tween 20 and 80 during the simultaneous saccharification and fermentation (SSF) process were also demonstrated in several studies, but a more systematic evaluation should be carried out to evaluate the exact effects of these surfactants on fermentation bacteria as well as the techno-economic evaluation is still needed for large-scale industrial application [124,125].

Table 2. The over reviews of application of reaction agent to increase enzymatic hydrolysis efficiency of lignocellulose.

Reactive Agent		**Lignocellulose**	**Result**
Types	**Reagent**		
Synthetic surfactant	1.5% PEG 4000	Coffee scrap waste	The reducing sugar yield was higher than that of the control group by 3.4%
Synthetic surfactant	1% PEG 4000/8000	Poplar	The glucose production was increased by 19.2% (PEG 4000) and 14.1% (PEG 8000) higher than the control group after 96 h
Synthetic surfactant	2% PEG 6000	Wood	The enzyme digestion rate increased from 75.2% to 91%
Synthetic surfactant	1% PEG 6000 and Tween 80	Bamboo shoots	The glucose yield is increased from 65.5% to 98.3%, and the cellulose is almost all hydrolyzed
Synthetic surfactant	1% PEG 6000	Reed	The loading of enzyme is reduced by 88% than that of without PEG
Synthetic surfactant	5 g/L Tween 20	Wheat straw	Increased the glucan transformation of the diluted acid pretreatment substrate for 4–31%
Synthetic surfactant	1.5% Tween 80	Wheat straw	The concentration of bioethanol was increased by 9.62% and the maximum bioethanol concentration was 24.84 g/L
Synthetic surfactant	2% Tween 80	Bagasse	Increased the enzymatic digestibility of pretreated bagasse by 36.20%
Synthetic surfactant	1%Tween 80	Sugarcane bagasse	The time of enzymatic hydrolysis after the addition of Tween 80 was shortened from 72 h to 6 h, and the enzyme load was reduced by 50% to achieve the same glucose yield level (91.7%)
Synthetic surfactant	0.25% and 0.5% Tween 80	Palm fruit skewers and palm fruit rough	The amount of reductant sugar can be increased by 50.5% and 38.8% after addition of 0.25%, 0.5% of Tween 80
Natural surfactants	0.5% rhamnolipid	Rice straw	The reducing sugar yield was increased by 15.9% compared with the control group under the optimal supplemental level
Natural surfactants	1% sophorolipid	Agricultural residue	The enzymatic hydrolysis can be increased by 20.00%.
Protein	0.5 g/L BSA	Acidification straw	The conversion rate was increased from 45.5% to 52.8%
Protein	0.6 g/L BSA	Poplar	Increase the sugar yield of poplar pretreated by extremely dilute acid from 59% to 68%
Protein	1 g/L BSA	Hardwood	Increase the enzymatic hydrolysis efficiency from 17.4% to 71.9%
Protein	1 g/L casein, BSA, collagen	-	The activity of cellulase was retained from 64.9% to 74.6%, 73.3%, and 71.8%
Sulfonated lignin	0.5 g/L lignosulfonate	Microcrystalline cellulose	Increased the sugar yield of 72-h hydrolysis from 44.5% to 49.4%
Sulfonated lignin	0–6% sodium lignosulfonate	Corn stover	The total sugar yield after enzymatic hydrolysis increased from 41.0% to 56.8%
Sulfonated lignin	0.75 g/L lignosulfonate	Poplar	The conversion rate of total sugar in enzymatic hydrolysis was 83.3% and 91.2%
Sulfonated lignin	1% lignosulfonate	Microcrystalline cellulose	The enzyme digestibility of substrate increased significantly from 30% to 46%
Ethanol-soluble lignin	1% Ethanol-soluble lignin	Microcrystalline cellulose	The 72-h hydrolysis efficiency of cellulose were increased by 5–8%
Ethanol-soluble lignin	1% Ethanol-soluble lignin	Poplar	The glucose yield of ethanol pretreated material increased by 15.17% for 72 h
Ethanol-soluble lignin	0.8% Ethanol-soluble lignin	Microcrystalline cellulose	The enzymatic hydrolysis efficiency at 72 h was increased from 69.0% to 88.4
Water-soluble lignin	0.1 g/g-substrate kraft lignin	Poplar	The maximum total sugar conversion reached from 65% to 83.1%
Water-soluble lignin	kraft lignin-based PEG	Eucalyptus	Promoted enzymatic hydrolysis efficiency from 58.3% to 93.8%
Water-soluble lignin	Alkaline lignin	Wheat straw	The total sugar recovery increased from 66.8% to 76.9%

Lignin-Based Surfactants

Generally, cationic surfactants should be avoided due to their ability to combine with cellulase and make it wrap in the micelles [126]. On the other hand, anionic surfactants such as sodium dodecyl sulfate and lignosulfonate could promote enzymatic efficiency [127,128]. The presence of lignin-based surfactant as an enzymatic hydrolysis enhancing factor was reported in several studies and has become a topic of great interest.

Sulfonated lignin (lignosulfonate) has been successfully applied in enzymatic hydrolysis as a surfactant to increase the cellulose conversion [129–132]. Lignosulfonate could bind to cellulase as evidenced by its noticeable inhibition on pure cellulose's hydrolysis. However, it enhanced the enzymatic digestibility of acid pretreated biomass because it could increase the electrostatic repulsion between the lignin residue in pretreated biomass and cellulase [133]. Thus, lignosulfonate could be used as surfactant to enhance the hydrolysis yield only at high concentrations when its positive effect offsets the typically inhibitive effect from its binding to cellulase [128]. It has been reported that lignosulfonate with low molecular weight and a high degree of sulfonation enhances the enzymatic hydrolysis of pure cellulose [129,134]. Lignosulfonate could also be incorporated into polyoxyethylene ether (PEG) to increase its binding rates on lignin surfaces and make cellulase enzyme aggregates dispersive, accordingly reducing the binding strength between lignin residue and enzymes [135–137]. Some lignin with low molecular weight obtained from pretreatment of lignocellulosic materials could also promote enzymatic hydrolysis [30,138]. These types of lignin could be easily obtained by a simple organic solvent extraction, which is more depolymerized and typically less condensed [139]. For example, Lai et al. found that the promoting mechanism of ethanol organosolv lignin for improving cellulase hydrolysis efficiency was related to pH, and higher pH could intensify the suppression of interaction between residual lignins and enzymes [140]. Recent studies also showed that adding an appropriate amount of water-soluble lignin to the enzymatic hydrolysis system can effectively increase the cellulose conversion of pretreated biomass. For example, Jiang et al. proposed a strategy to use water-soluble alkaline lignin to promote the enzymatic digestibility of wheat straw by reducing the non-productive adsorption ability of lignin for cellulase [51]. The results revealed that the enzymatic hydrolysis yield of alkaline pretreated substrate could be enhanced from 66.8% to 76.9% when the soluble alkali lignin was added.

Non-Catalytic Proteins

Non-catalytic proteins such as bovine serum albumin (BSA), soybean, tea seed waste, and corn steep liquor have also been evaluated as lignin blockers during enzymatic saccharification of lignin-rich pretreated lignocellulosic. BSA could bind to lignin via hydrophobic and hydrogen bonding and thus could be used to enhance the enzymatic digestibility of pretreated biomass [141,142]. For example, Kumar et al. found that the cellulose conversion of steam pretreated Douglas fir increased from 16 to 66% due to adding BSA as additives [26]. It can be realized that a nearly complete conversion of cellulose from liquid hot water pretreated lignocellulosic substrates to glucose by adding inexpensive and biocompatible soy protein (Figure 7) [55]. Similarly, Florencio and coworkers reported the onsite enzyme cocktails with soybean protein led to 2 times higher glucose release [143]. Lai et al. showed that addition of 0.075 g/g glucan of tea saponin enhanced the 72-h glucose yield of organosolv pretreated poplar by nearly 50% [144]. Last but not least, it is found that addition of casein polypeptides with dosage of 0.5g/g glucan could improve the cellulose conversion of dilute acid, alkali, lime, extrusion, and AFEX pretreated corn stover by 32, 23, 17, 30, and 17%, respectively, while no positive impact was found on pure cellulose Avicel [145].

3.3. Genetic Modification of Lignin and Enzyme Resources

Besides removing or modifying lignin structure via biomass pretreatment and utilizing lignin blockers during hydrolysis, lignocellulosic biomass could be genetically modified to produce lignin with unique composition, distribution, and structure to increase its digestibility [146–148]. Many efforts have been made to modify lignin biosynthesis gene expression to reduce the native biomass recalcitrance without exhibiting physiological irregularities or negatively impacting the plant productivity. For example, downregulating 4-coumarate:CoA ligase (4CL) family is certified to be an efficient way to reduce the lignin content in hardwood Populus tremuloides by 40–45% [149]. Following appropriate pretreatment, it is fully expected that an increase in sugar digestibility could be achieved from these low lignin, 4CL downregulated biomass samples [150]. The increase in enzymatic hydrolysis yield (e.g., up to 103% sugar yield increase) was also demonstrated in the case of untreated 4CL downregulated Populus with lower lignin content [151]. Besides lowering lignin content, other genes such as 4-coumarate-3-hydroxylase (C3H) and cinnamyl alcohol dehydrogenase (CAD) could also be modified to alter the chemical structure of lignin (e.g., S/G ratio) and increase the hydrolysis yield of biomass [152,153]. Wilkerson and coworkers reported a way to incorporate ester bonds into the lignin to produce "zip lignin" [154], and subsequent studies showed that the engineered plant is more amenable to degradation after a variety of pretreatment methods [155,156].

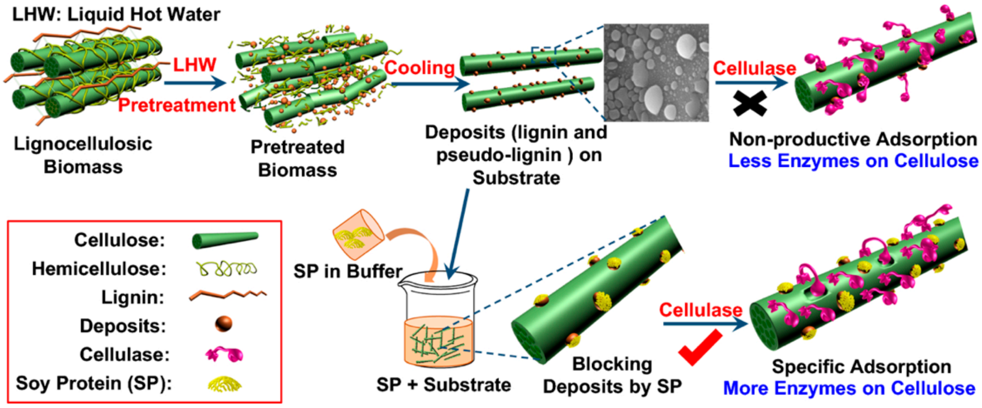

Figure 7. A proposed mechanism of the soy protein enhanced enzymatic hydrolysis of liquid hot water pretreated lignocellulosic biomass [45].

Cellulase enzymes could also be genetically or chemically modified to reduce their binding affinity toward lignins [157,158]. The carbohydrate-binding module (CBM) of cellulase enzymes shows a critical role for its interaction with cellulose and lignin, which has been evidenced by the fact that fungal cellulases expressed without the CBM exhibited much lower lignin affinity. Thus, one way to alleviate the lignin-enzyme interaction is to use the CBM-less enzymes [159,160]. The CBM of enzymes could be mutated in a way that the aromatic tyrosine was replaced with a less hydrophobic alanine, and the resulting mutant CBM showed a much lower affinity to lignin than the unmodified CBM [161]. Enzyme charge could be engineered to lower the lignin inhibition [132]. For example, Nordwald et al. performed acetylation and succinylation on the positively charged amine groups of the Trichoderma reesei cellulase, changing them to neutral acetyl and negatively charged acid groups, respectively [162]. Through mixing the modified enzyme with 1% lignin, the hydrolysis rate of Avicel increased by more than 2 times due to the increased repulsion between the modified enzyme and lignin.

4. Conclusions

The traditional pretreatment that carried out at high severity could inevitably cause the repolymerization or condensation of lignin, which shows a negative effect on the enzymatic digestibility of pretreated biomass. The sulfite and other types of sulfonation pretreatments can modify the existed lignin into lignosulfonate, which not only can achieve a high degree of delignification but also increases lignin's hydrophilicity for higher enzymatic efficiency. To further promote hydrolysis efficiency, additives including synthetic and natural surfactants, modified lignin, and non-catalytic proteins could all be used as lignin blockers to intervene in the interaction between lignin and enzymes.

Author Contributions: C.H.: Writing—original draft; Conceptualization R.L.: Writing—original draft; W.T.: Writing—original draft; Y.Z.: Writing—original draft; X.M.: Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work was sponsored by National Natural Science Foundation of China (32271796), the Jiangsu Qing Lan Project and the Young Elite Scientists Sponsorship Program by CAST for Caoxing Huang.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: X.M.'s efforts were supported by the University of Tennessee.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Jiang, J.; Ye, B.; Liu, J. Peak of CO_2 emissions in various sectors and provinces of China: Recent progress and avenues for further research. *Renew. Sustain. Energy Rev.* **2019**, *112*, 813–833. [CrossRef]
2. Dhillon, R.S.; von Wuehlisch, G. Mitigation of global warming through renewable biomass. *Biomass Bioenergy* **2013**, *48*, 75–89. [CrossRef]
3. Huang, C.; Jiang, X.; Shen, X.; Hu, J.; Tang, W.; Wu, X.; Ragauskas, A.; Jameel, H.; Meng, X.; Yong, Q. Lignin-enzyme interaction: A roadblock for efficient enzymatic hydrolysis of lignocellulosics. *Renew. Sustain. Energy Rev.* **2022**, *154*, 111822. [CrossRef]
4. Yin, X.; Cai, T.; Liu, C.; Huang, C.; Wang, J.; Hu, J.; Li, N.; Jiang, J.; Wang, K. A mild biomass pretreatment process with efficiency and specificity in co-solvent of γ-valerolactone and aqueous p-toluenesulfonic acid. *Chem. Eng. J.* **2022**, *437*, 135408. [CrossRef]
5. Patel, A.; Shah, A.R. Integrated lignocellulosic biorefinery: Gateway for production of second generation ethanol and value added products. *J. Bioresour. Bioprod.* **2021**, *6*, 108–128. [CrossRef]
6. Huang, C.; Lin, W.; Zheng, Y.; Zhao, X.; Ragauskas, A.; Meng, X. Evaluating the mechanism of milk protein as an efficient lignin blocker for boosting the enzymatic hydrolysis of lignocellulosic substrates. *Green Chem.* **2022**, *24*, 5263–5279. [CrossRef]
7. Huang, C.; Jeuck, B.; Du, J.; Yong, Q.; Chang, H.-M.; Jameel, H.; Phillips, R. Novel process for the coproduction of xylo-oligosaccharides, fermentable sugars, and lignosulfonates from hardwood. *Bioresour. Technol.* **2016**, *219*, 600–607. [CrossRef]
8. Huang, C.; Yu, Y.; Li, Z.; Yan, B.; Pei, W.; Wu, H. The preparation technology and application of xylo-oligosaccharide as prebiotics in different fields: A review. *Front. Nutr.* **2022**, *9*, 996811. [CrossRef]
9. You, S.; Ma, Y.; Yan, B.; Pei, W.; Wu, Q.; Ding, C.; Huang, C. The promotion mechanism of prebiotics for probiotics: A review. *Front. Nutr.* **2022**, *9*, 1000517. [CrossRef]
10. Santibáñez, L.; Henríquez, C.; Corro-Tejeda, R.; Bernal, S.; Armijo, B.; Salazar, O. Xylooligosaccharides from lignocellulosic biomass: A comprehensive review. *Carbohydr. Polym.* **2021**, *251*, 117118. [CrossRef]
11. Yan, B.; Huang, C.; Lai, C.; Ling, Z.; Yong, Q. Production of prebiotic xylooligosaccharides from industrial-derived xylan residue by organic acid treatment. *Carbohydr. Polym.* **2022**, *292*, 119641. [CrossRef] [PubMed]
12. Liu, Y.; Wang, X.; Wu, Q.; Pei, W.; Teo, M.J.; Chen, Z.S.; Huang, C. Application of lignin and lignin-based composites in different tissue engineering fields. *Int. J. Biol. Macromol.* **2022**, *222*, 994–1006. [CrossRef]
13. Liu, K.; Du, H.; Zheng, T.; Liu, W.; Zhang, M.; Liu, H.; Zhang, X.; Si, C. Lignin-containing cellulose nanomaterials: Preparation and applications. *Green Chem.* **2021**, *23*, 9723–9746. [CrossRef]
14. Chen, X.J.; Guo, T.; Yang, H.C.; Zhang, L.D.; Xue, Y.N.; Wang, R.F.; Fan, X.L.; Sun, S.L. Environmentally friendly preparation of lignin/paraffin/epoxy resin composite-coated urea and evaluation for nitrogen efficiency in lettuce. *Int. J. Biol. Macromol.* **2022**, *221*, 1130–1141. [CrossRef]
15. Zhang, H.; Han, L.; Dong, H. An insight to pretreatment, enzyme adsorption and enzymatic hydrolysis of lignocellulosic biomass: Experimental and modeling studies. *Renew. Sustain. Energy Rev.* **2021**, *140*, 110758. [CrossRef]
16. Orejuela-Escobar, L.M.; Landázuri, A.C.; Goodell, B. Second generation biorefining in Ecuador: Circular bioeconomy, zero waste technology, environment and sustainable development: The nexus. *J. Bioresour. Bioprod.* **2021**, *6*, 83–107. [CrossRef]
17. Wang, Z.-K.; Huang, C.; Zhong, J.-L.; Wang, Y.; Tang, L.; Li, B.; Sheng, J.-J.; Chen, L.; Sun, S.; Shen, X. Valorization of Chinese hickory shell as novel sources for the efficient production of xylooligosaccharides. *Biotechnol. Biofuels* **2021**, *14*, 226. [CrossRef]
18. Mankar, A.R.; Pandey, A.; Modak, A.; Pant, K.K. Pretreatment of lignocellulosic biomass: A review on recent advances. *Bioresour. Technol.* **2021**, *334*, 125235. [CrossRef]
19. Kameshwar, A.K.S.; Barber, R.; Qin, W. Comparative modeling and molecular docking analysis of white, brown and soft rot fungal laccases using lignin model compounds for understanding the structural and functional properties of laccases. *J. Mol. Graph. Model.* **2018**, *79*, 15–26. [CrossRef]
20. Wiman, M.; Dienes, D.; Hansen, M.A.T.; van der Meulen, T.; Zacchi, G.; Lidén, G. Cellulose accessibility determines the rate of enzymatic hydrolysis of steam-pretreated spruce. *Bioresour. Technol.* **2012**, *126*, 208–215. [CrossRef]

21. Kumar, D.; Murthy, G.S. Stochastic molecular model of enzymatic hydrolysis of cellulose for ethanol production. *Biotechnol. Biofuels* **2013**, *6*, 63. [CrossRef] [PubMed]
22. Van Dyk, J.S.; Pletschke, B.I. A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes—Factors affecting enzymes, conversion and synergy. *Biotechnol. Adv.* **2012**, *30*, 1458–1480. [CrossRef] [PubMed]
23. Ramos, M.D.N.; Milessi, T.S.; Candido, R.G.; Mendes, A.A.; Aguiar, A. Enzymatic catalysis as a tool in biofuels production in Brazil: Current status and perspectives. *Energy Sustain. Dev.* **2022**, *68*, 103–119. [CrossRef]
24. Lin, W.; Yang, J.; Zheng, Y.; Huang, C.; Yong, Q. Understanding the effects of different residual lignin fractions in acid-pretreated bamboo residues on its enzymatic digestibility. *Biotechnol. Biofuels* **2021**, *14*, 143. [CrossRef] [PubMed]
25. Zheng, Y.; Yu, Y.; Lin, W.; Jin, Y.; Yong, Q.; Huang, C. Enhancing the enzymatic digestibility of bamboo residues by biphasic phenoxyethanol-acid pretreatment. *Bioresour. Technol.* **2021**, *325*, 124691. [CrossRef]
26. Kumar, L.; Arantes, V.; Chandra, R.; Saddler, J. The lignin present in steam pretreated softwood binds enzymes and limits cellulose accessibility. *Bioresour. Technol.* **2012**, *103*, 201–208. [CrossRef]
27. Zhao, X.; Meng, X.; Ragauskas, A.J.; Lai, C.; Ling, Z.; Huang, C.; Yong, Q. Unlocking the secret of lignin-enzyme interactions: Recent advances in developing state-of-the-art analytical techniques. *Biotechnol. Adv.* **2022**, *54*, 107830. [CrossRef] [PubMed]
28. Yu, H.; Ren, J.; Liu, L.; Zheng, Z.; Zhu, J.; Yong, Q.; Ouyang, J. A new magnesium bisulfite pretreatment (MBSP) development for bio-ethanol production from corn stover. *Bioresour. Technol.* **2016**, *199*, 188–193. [CrossRef]
29. Wang, J.; Hao, X.; Yang, M.; Qin, Y.; Jia, L.; Chu, J.; Zhang, J. Impact of lignin content on alkaline-sulfite pretreatment of Hybrid Pennisetum. *Bioresour. Technol.* **2018**, *267*, 793–796. [CrossRef]
30. Lai, C.; Tu, M.; Xia, C.; Shi, Z.; Sun, S.; Yong, Q.; Yu, S. Lignin Alkylation Enhances Enzymatic Hydrolysis of Lignocellulosic Biomass. *Energy Fuels* **2017**, *31*, 12317–12326. [CrossRef]
31. Lai, C.; Tu, M.; Shi, Z.; Zheng, K.; Olmos, L.G.; Yu, S. Contrasting effects of hardwood and softwood organosolv lignins on enzymatic hydrolysis of lignocellulose. *Bioresour. Technol.* **2014**, *163*, 320–327. [CrossRef]
32. Lin, X.; Qiu, X.; Lou, H.; Li, Z.; Zhan, N.; Huang, J.; Pang, Y. Enhancement of lignosulfonate-based polyoxyethylene ether on enzymatic hydrolysis of lignocelluloses. *Ind. Crops Prod.* **2016**, *80*, 86–92. [CrossRef]
33. Kim, J.S.; Lee, Y.Y.; Kim, T.H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour. Technol.* **2016**, *199*, 42–48. [CrossRef] [PubMed]
34. Rezania, S.; Oryani, B.; Cho, J.; Talaiekhozani, A.; Sabbagh, F.; Hashemi, B.; Rupani, P.F.; Mohammadi, A.A. Different pretreatment technologies of lignocellulosic biomass for bioethanol production: An overview. *Energy* **2020**, *199*, 117457. [CrossRef]
35. Woiciechowski, A.L.; Neto, C.J.D.; de Souza Vandenberghe, L.P.; de Carvalho Neto, D.P.; Sydney, A.C.N.; Letti, L.A.J.; Karp, S.G.; Torres, L.A.Z.; Soccol, C.R. Lignocellulosic biomass: Acid and alkaline pretreatments and their effects on biomass recalcitrance—Conventional processing and recent advances. *Bioresour. Technol.* **2020**, *304*, 122848. [CrossRef]
36. Zhai, R.; Hu, J.; Jin, M. Towards efficient enzymatic saccharification of pretreated lignocellulose: Enzyme inhibition by lignin-derived phenolics and recent trends in mitigation strategies. *Biotechnol. Adv.* **2022**, *61*, 108044. [CrossRef]
37. Yao, L.; Yang, H.; Meng, X.; Ragauskas, A.J. Toward a Fundamental Understanding of the Role of Lignin in the Biorefinery Process. *Front. Energy Res.* **2022**, *9*, 895. [CrossRef]
38. Lin, W.; Chen, D.; Yong, Q.; Huang, C.; Huang, S. Improving enzymatic hydrolysis of acid-pretreated bamboo residues using amphiphilic surfactant derived from dehydroabietic acid. *Bioresour. Technol.* **2019**, *293*, 122055. [CrossRef]
39. Li, M.; Yi, L.; Bin, L.; Zhang, Q.; Song, J.; Jiang, H.; Chen, C.; Wang, S.; Min, D. Comparison of nonproductive adsorption of cellulase onto lignin isolated from pretreated lignocellulose. *Cellulose* **2020**, *27*, 7911–7927. [CrossRef]
40. Zhang, Q.; Wan, G.; Li, M.; Jiang, H.; Wang, S.; Min, D. Impact of bagasse lignin-carbohydrate complexes structural changes on cellulase adsorption behavior. *Int. J. Biol. Macromol.* **2020**, *162*, 236–245. [CrossRef]
41. Saini, J.K.; Patel, A.K.; Adsul, M.; Singhania, R.R. Cellulase adsorption on lignin: A roadblock for economic hydrolysis of biomass. *Renew. Energy* **2016**, *98*, 29–42. [CrossRef]
42. Li, H.; Pu, Y.; Kumar, R.; Ragauskas, A.J.; Wyman, C.E. Investigation of lignin deposition on cellulose during hydrothermal pretreatment, its effect on cellulose hydrolysis, and underlying mechanisms. *Biotechnol. Bioeng.* **2014**, *111*, 485–492. [CrossRef]
43. Selig, M.J.; Viamajala, S.; Decker, S.R.; Tucker, M.P.; Himmel, M.E.; Vinzant, T.B. Deposition of Lignin Droplets Produced During Dilute Acid Pretreatment of Maize Stems Retards Enzymatic Hydrolysis of Cellulose. *Biotechnol. Prog.* **2007**, *23*, 1333–1339. [CrossRef]
44. Pappas, I.A.; Koukoura, Z.; Tananaki, C.; Goulas, C. Effect of dilute acid pretreatment severity on the bioconversion efficiency of Phalaris aquatica L. lignocellulosic biomass into fermentable sugars. *Bioresour. Technol.* **2014**, *166*, 395–402. [CrossRef] [PubMed]
45. Donaldson, L.A.; Wong, K.K.Y.; Mackie, K.L. Ultrastructure of steam-exploded wood. *Wood Sci. Technol.* **1988**, *22*, 103–114. [CrossRef]
46. Jeong, S.-Y.; Lee, E.-J.; Ban, S.-E.; Lee, J.-W. Structural characterization of the lignin-carbohydrate complex in biomass pretreated with Fenton oxidation and hydrothermal treatment and consequences on enzymatic hydrolysis efficiency. *Carbohydr. Polym.* **2021**, *270*, 118375. [CrossRef]
47. Balakshin, M.; Capanema, E.; Gracz, H.; Chang, H.-M.; Jameel, H. Quantification of lignin–carbohydrate linkages with high-resolution NMR spectroscopy. *Planta* **2011**, *233*, 1097–1110. [CrossRef]
48. Liu, K.-X.; Li, H.-Q.; Zhang, J.; Zhang, Z.-G.; Xu, J. The effect of non-structural components and lignin on hemicellulose extraction. *Bioresour. Technol.* **2016**, *214*, 755–760. [CrossRef]

49. Liu, Y.; Chen, W.; Xia, Q.; Guo, B.; Wang, Q.; Liu, S.; Liu, Y.; Li, J.; Yu, H. Efficient Cleavage of Lignin-Carbohydrate Complexes and Ultrafast Extraction of Lignin Oligomers from Wood Biomass by Microwave-Assisted Treatment with Deep Eutectic Solvent. *ChemSusChem* **2017**, *10*, 1692–1700. [CrossRef]
50. Huang, C.; He, J.; Li, X.; Min, D.; Yong, Q. Facilitating the enzymatic saccharification of pulped bamboo residues by degrading the remained xylan and lignin–carbohydrates complexes. *Bioresour. Technol.* **2015**, *192*, 471–477. [CrossRef]
51. Jiang, B.; Yu, J.; Luo, X.; Zhu, Y.; Jin, Y. A strategy to improve enzymatic saccharification of wheat straw by adding water-soluble lignin prepared from alkali pretreatment spent liquor. *Process Biochem.* **2018**, *71*, 147–151. [CrossRef]
52. Min, D.Y.; Li, Q.; Chiang, V.; Jameel, H.; Chang, H.M.; Lucia, L. The influence of lignin–carbohydrate complexes on the cellulase-mediated saccharification I: Transgenic black cottonwood (western balsam poplar, California poplar) P. trichocarpa including the xylan down-regulated and the lignin down-regulated lines. *Fuel* **2014**, *119*, 207–213. [CrossRef]
53. Min, D.Y.; Yang, C.; Chiang, V.; Jameel, H.; Chang, H.M. The influence of lignin–carbohydrate complexes on the cellulase-mediated saccharification II: Transgenic hybrid poplars (*Populus nigra* L. and *Populus maximowiczii* A.). *Fuel* **2014**, *116*, 56–62. [CrossRef]
54. Nnaemeka, I.C.; Maxwell, O.; Christain, A.O. Optimization and kinetic studies for enzymatic hydrolysis and fermentation of colocynthis vulgaris Shrad seeds shell for bioethanol production. *J. Bioresour. Bioprod.* **2021**, *6*, 45–64. [CrossRef]
55. Luo, X.; Liu, J.; Zheng, P.; Li, M.; Zhou, Y.; Huang, L.; Chen, L.; Shuai, L. Promoting enzymatic hydrolysis of lignocellulosic biomass by inexpensive soy protein. *Biotechnol. Biofuels* **2019**, *12*, 51. [CrossRef] [PubMed]
56. Li, X.; Zheng, Y. Lignin-enzyme interaction: Mechanism, mitigation approach, modeling, and research prospects. *Biotechnol. Adv.* **2017**, *35*, 466–489. [CrossRef]
57. Sipponen, M.H.; Rahikainen, J.; Leskinen, T.; Pihlajaniemi, V.; Mattinen, M.-L.; Lange, H.; Crestini, C.; Österberg, M.Ö. Structural changes of lignin in biorefinery pretreatments and consequences to enzyme-lignin interactions—OPEN ACCESS. *Nord. Pulp Pap. Res. J.* **2017**, *32*, 550–571. [CrossRef]
58. Liu, H.; Sun, J.; Leu, S.-Y.; Chen, S. Toward a fundamental understanding of cellulase-lignin interactions in the whole slurry enzymatic saccharification process. *Biofuels Bioprod. Biorefin.* **2016**, *10*, 648–663. [CrossRef]
59. Tokunaga, Y.; Nagata, T.; Kondo, K.; Katahira, M.; Watanabe, T. NMR elucidation of nonproductive binding sites of lignin models with carbohydrate-binding module of cellobiohydrolase I. *Biotechnol. Biofuels* **2020**, *13*, 164. [CrossRef]
60. Tokunaga, Y.; Nagata, T.; Suetomi, T.; Oshiro, S.; Kondo, K.; Katahira, M.; Watanabe, T. NMR Analysis on Molecular Interaction of Lignin with Amino Acid Residues of Carbohydrate-Binding Module from Trichoderma reesei Cel7A. *Sci. Rep.* **2019**, *9*, 1977. [CrossRef]
61. Sammond, D.W.; Yarbrough, J.M.; Mansfield, E.; Bomble, Y.J.; Hobdey, S.E.; Decker, S.R.; Taylor, L.E.; Resch, M.G.; Bozell, J.J.; Himmel, M.E.; et al. Predicting Enzyme Adsorption to Lignin Films by Calculating Enzyme Surface Hydrophobicity. *J. Biol. Chem.* **2014**, *289*, 20960–20969. [CrossRef] [PubMed]
62. Sun, S.; Huang, Y.; Sun, R.; Tu, M. The strong association of condensed phenolic moieties in isolated lignins with their inhibition of enzymatic hydrolysis. *Green Chem.* **2016**, *18*, 4276–4286. [CrossRef]
63. Rahikainen, J.L.; Evans, J.D.; Mikander, S.; Kalliola, A.; Puranen, T.; Tamminen, T.; Marjamaa, K.; Kruus, K. Cellulase–lignin interactions—The role of carbohydrate-binding module and pH in non-productive binding. *Enzym. Microb. Technol.* **2013**, *53*, 315–321. [CrossRef] [PubMed]
64. Zhu, Y.; Wang, W.; Wang, Y.; Jin, Y. Effects of pH and Sulfonated Lignin on the Enzymatic Saccharification of Acid Bisulfite- and Green Liquor-pretreated Poplar Wood. *BioResources* **2015**, *10*, 11. [CrossRef]
65. Yang, Q.; Pan, X. Correlation between lignin physicochemical properties and inhibition to enzymatic hydrolysis of cellulose. *Biotechnol. Bioeng.* **2016**, *113*, 1213–1224. [CrossRef] [PubMed]
66. Huang, Y.; Sun, S.; Huang, C.; Yong, Q.; Elder, T.; Tu, M. Stimulation and inhibition of enzymatic hydrolysis by organosolv lignins as determined by zeta potential and hydrophobicity. *Biotechnol. Biofuels* **2017**, *10*, 162. [CrossRef] [PubMed]
67. Xie, Z.; Tian, Z.; Liu, S.; Ma, H.; Ji, X.-X.; Si, C. Effects of different amounts of cellulase on the microstructure and soluble substances of cotton stalk bark. *Adv. Compos. Hybrid Mater.* **2022**, *5*, 1294–1306. [CrossRef]
68. Wang, Y.; Ji, X.-X.; Liu, S.; Tian, Z.; Si, C.; Wang, R.; Yang, G.; Wang, D. Effects of two different enzyme treatments on the microstructure of outer surface of wheat straw. *Adv. Compos. Hybrid Mater.* **2022**, *5*, 934–947. [CrossRef]
69. Dai, L.; Gu, Y.; Xu, J.; Guo, J.; Jiang, K.; Zhou, X.; Xu, Y. Toward green production of xylooligosaccharides and glucose from sorghum straw biowaste by sequential acidic and enzymatic hydrolysis. *Ind. Crops Prod.* **2022**, *179*, 114662. [CrossRef]
70. Lian, Z.; Zhang, Q.; Xu, Y.; Zhou, X.; Jiang, K. Biorefinery Cascade Processing for Converting Corncob to Xylooligosaccharides and Glucose by Maleic Acid Pretreatment. *Appl. Biochem. Biotechnol.* **2022**, *194*, 4946–4958. [CrossRef]
71. Dai, L.; Huang, T.; Jiang, K.; Zhou, X.; Xu, Y. A novel recyclable furoic acid-assisted pretreatment for sugarcane bagasse biorefinery in co-production of xylooligosaccharides and glucose. *Biotechnol. Biofuels* **2021**, *14*, 35. [CrossRef] [PubMed]
72. Satari, B.; Karimi, K.; Kumar, R. Cellulose solvent-based pretreatment for enhanced second-generation biofuel production: A review. *Sustain. Energy Fuels* **2019**, *3*, 11–62. [CrossRef]
73. Satlewal, A.; Agrawal, R.; Bhagia, S.; Sangoro, J.; Ragauskas, A.J. Natural deep eutectic solvents for lignocellulosic biomass pretreatment: Recent developments, challenges and novel opportunities. *Biotechnol. Adv.* **2018**, *36*, 2032–2050. [CrossRef]
74. Yoo, C.G.; Pu, Y.; Ragauskas, A.J. Ionic liquids: Promising green solvents for lignocellulosic biomass utilization. *Curr. Opin. Green Sustain. Chem.* **2017**, *5*, 5–11. [CrossRef]

75. Chin, D.W.K.; Lim, S.; Pang, Y.L.; Lam, M.K. Fundamental review of organosolv pretreatment and its challenges in emerging consolidated bioprocessing. *Biofuels Bioprod. Biorefin.* **2020**, *14*, 808–829. [CrossRef]
76. Wu, J.; Chandra, R.P.; Takada, M.; Liu, L.-Y.; Renneckar, S.; Kim, K.H.; Kim, C.S.; Saddler, J.N. Enhancing Enzyme-Mediated Cellulose Hydrolysis by Incorporating Acid Groups Onto the Lignin During Biomass Pretreatment. *Front. Bioeng. Biotechnol.* **2020**, *8*, 608835. [CrossRef]
77. Lancefield, C.S.; Panovic, I.; Deuss, P.J.; Barta, K.; Westwood, N.J. Pre-treatment of lignocellulosic feedstocks using biorenewable alcohols: Towards complete biomass valorisation. *Green Chem.* **2017**, *19*, 202–214. [CrossRef]
78. Huang, C.; He, J.; Min, D.; Lai, C.; Yong, Q. Understanding the Nonproductive Enzyme Adsorption and Physicochemical Properties of Residual Lignins in Moso Bamboo Pretreated with Sulfuric Acid and Kraft Pulping. *Appl. Biochem. Biotechnol.* **2016**, *180*, 1508–1523. [CrossRef]
79. Restolho, J.A.; Prates, A.; De Pinho, M.N.; Afonso, M.D. Sugars and lignosulphonates recovery from eucalyptus spent sulphite liquor by membrane processes. *Biomass Bioenergy* **2009**, *33*, 1558–1566. [CrossRef]
80. Zhu, J.Y.; Pan, X.J.; Wang, G.S.; Gleisner, R. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresour. Technol.* **2009**, *100*, 2411–2418. [CrossRef]
81. Li, Z.; Yang, Q.; Jiang, Z.; Fei, B.; Cai, Z.; Pan, X. Comparative Study of Sulfite (SPORL), Dilute Acid and NaOH Pretreatments of Bamboo for Enzymatic Saccharification. *J. Biobased Mater. Bioenergy* **2012**, *6*, 544–551. [CrossRef]
82. Qin, S.; Wang, Y.; Xing, Y.; Zhao, P.; Bu, L.; Sun, D.; Jiang, J. Comparison of Enzymatic Hydrolysis of Bamboo Using Steam Explosion and Acid Sulfite, Alkali, and Alkaline Sulfite Pretreatments. *BioResources* **2015**, *10*, 7580–7590. [CrossRef]
83. Jaisamut, K.; Paulová, L.; Patáková, P.; Kotúčová, S.; Rychtera, M. Effect of sodium sulfite on acid pretreatment of wheat straw with respect to its final conversion to ethanol. *Biomass Bioenergy* **2016**, *95*, 1–7. [CrossRef]
84. Noparat, P.; Prasertsan, P.; O-Thong, S.; Pan, X. Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose for Enzymatic Hydrolysis of Oil Palm trunk. *Energy Procedia* **2017**, *138*, 1122–1127. [CrossRef]
85. Zhang, C.; Houtman, C.J.; Zhu, J.Y. Using low temperature to balance enzymatic saccharification and furan formation during SPORL pretreatment of Douglas-fir. *Process Biochem.* **2014**, *49*, 466–473. [CrossRef]
86. Tan, L.; Liu, Z.; Liu, T.; Wang, F. Efficient fractionation of corn stover by bisulfite pretreatment for the production of bioethanol and high value products. *BioResources* **2019**, *14*, 15. [CrossRef]
87. Yang, L.; Cao, J.; Mao, J.; Jin, Y. Sodium carbonate–sodium sulfite pretreatment for improving the enzymatic hydrolysis of rice straw. *Ind. Crops Prod.* **2013**, *43*, 711–717. [CrossRef]
88. Saady, N.M.C.; Rezaeitavabe, F.; Espinoza, J.E.R. Chemical Methods for Hydrolyzing Dairy Manure Fiber: A Concise Review. *Energies* **2021**, *14*, 6159. [CrossRef]
89. Yan, M.; Yang, D.; Deng, Y.; Chen, P.; Zhou, H.; Qiu, X. Influence of pH on the behavior of lignosulfonate macromolecules in aqueous solution. *Colloids Surf. A Physicochem. Eng. Asp.* **2010**, *371*, 50–58. [CrossRef]
90. Tavares, J.; Łukasik, R.M.; de Paiva, T.; da Silva, F. Hydrothermal alkaline sulfite pretreatment in the delivery of fermentable sugars from sugarcane bagasse. *New J. Chem.* **2018**, *42*, 4474–4484. [CrossRef]
91. Liu, H.; Pang, B.; Wang, H.; Li, H.; Lu, J.; Niu, M. Optimization of Alkaline Sulfite Pretreatment and Comparative Study with Sodium Hydroxide Pretreatment for Improving Enzymatic Digestibility of Corn Stover. *J. Agric. Food Chem.* **2015**, *63*, 3229–3234. [CrossRef] [PubMed]
92. Yang, M.; Wang, J.; Hou, X.; Wu, J.; Fan, X.; Jiang, F.; Tao, P.; Wang, F.; Peng, P.; Yang, F.; et al. Exploring surface characterization and electrostatic property of Hybrid Pennisetum during alkaline sulfite pretreatment for enhanced enzymatic hydrolysability. *Bioresour. Technol.* **2017**, *244*, 1166–1172. [CrossRef] [PubMed]
93. Bu, L.; Xing, Y.; Yu, H.; Gao, Y.; Jiang, J. Comparative study of sulfite pretreatments for robust enzymatic saccharification of corn cob residue. *Biotechnol. Biofuels* **2012**, *5*, 87. [CrossRef] [PubMed]
94. Zhong, N.; Chandra, R.; Yamamoto, M.; Leskinen, T.; Granström, T.; Saddler, J. Sulphite addition during steam pretreatment enhanced both enzyme-mediated cellulose hydrolysis and ethanol production. *Bioresour. Bioprocess.* **2022**, *9*, 71. [CrossRef]
95. Chen, H.; Jiang, L.; Cheng, Y.; Lu, J.; Lv, Y.; Yan, J.; Wang, H. Improving enzymatic hydrolysis efficiency of corncob residue through sodium sulfite pretreatment. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 7795–7804. [CrossRef]
96. Yuan, Y.; Jiang, B.; Chen, H.; Wu, W.; Wu, S.; Jin, Y.; Xiao, H. Recent advances in understanding the effects of lignin structural characteristics on enzymatic hydrolysis. *Biotechnol. Biofuels* **2021**, *14*, 205. [CrossRef]
97. Song, Y.; Chandra, R.P.; Zhang, X.; Saddler, J.N. Non-productive celluase binding onto deep eutectic solvent (DES) extracted lignin from willow and corn stover with inhibitory effects on enzymatic hydrolysis of cellulose. *Carbohydr. Polym.* **2020**, *250*, 116956. [CrossRef]
98. Chandra, R.P.; Chu, Q.; Hu, J.; Zhong, N.; Lin, M.; Lee, J.-S.; Saddler, J. The influence of lignin on steam pretreatment and mechanical pulping of poplar to achieve high sugar recovery and ease of enzymatic hydrolysis. *Bioresour. Technol.* **2016**, *199*, 135–141. [CrossRef]
99. Lai, C.; Yang, B.; He, J.; Huang, C.; Li, X.; Song, X.; Yong, Q. Enhanced enzymatic digestibility of mixed wood sawdust by lignin modification with naphthol derivatives during dilute acid pretreatment. *Bioresour. Technol.* **2018**, *269*, 18–24. [CrossRef]
100. Huang, C.; Ma, J.; Liang, C.; Li, X.; Yong, Q. Influence of sulfur dioxide-ethanol-water pretreatment on the physicochemical properties and enzymatic digestibility of bamboo residues. *Bioresour. Technol.* **2018**, *263*, 17–24. [CrossRef]

101. Ying, W.; Xu, G.; Yang, H.; Shi, Z.; Yang, J. The sequential Fenton oxidation and sulfomethylation pretreatment for alleviating the negative effects of lignin in enzymatic saccharification of sugarcane bagasse. *Bioresour. Technol.* **2019**, *286*, 121392. [CrossRef] [PubMed]
102. Belhaj, A.F.; Elraies, K.A.; Mahmood, S.M.; Zulkifli, N.N.; Akbari, S.; Hussien, O.S. The effect of surfactant concentration, salinity, temperature, and pH on surfactant adsorption for chemical enhanced oil recovery: A review. *J. Pet. Explor. Prod. Technol.* **2020**, *10*, 125–137. [CrossRef]
103. Wang, J.; Fan, Y.; Wang, H.; Yin, J.; Tan, W.; Li, X.; Shen, Y.; Wang, Y. Promoting efficacy and environmental safety of photosensitive agrochemical stabilizer via lignin/surfactant coacervates. *Chem. Eng. J.* **2022**, *430*, 132920. [CrossRef]
104. Li, Y.; Yang, D.; Lu, S.; Lao, S.; Qiu, X. Modified Lignin with Anionic Surfactant and Its Application in Controlled Release of Avermectin. *J. Agric. Food Chem.* **2018**, *66*, 3457–3464. [CrossRef] [PubMed]
105. Li, S.-X.; Li, M.-F.; Bian, J.; Wu, X.-F.; Peng, F.; Ma, M.-G. Preparation of organic acid lignin submicrometer particle as a natural broad-spectrum photo-protection agent. *Int. J. Biol. Macromol.* **2019**, *132*, 836–843. [CrossRef]
106. Lou, H.; Zeng, M.; Hu, Q.; Cai, C.; Lin, X.; Qiu, X.; Yang, D.; Pang, Y. Nonionic surfactants enhanced enzymatic hydrolysis of cellulose by reducing cellulase deactivation caused by shear force and air-liquid interface. *Bioresour. Technol.* **2018**, *249*, 1–8. [CrossRef]
107. Cui, M.-J.; Hu, Y.-P.; Li, B.-Y.; Wang, R.-X. Effects of non-ionic surfactants on bioethanol production using extracted cellulose from wheat straw and kinetic studies. *Biomass Convers. Biorefinery* **2021**, 1–8. [CrossRef]
108. Eriksson, T.; Börjesson, J.; Tjerneld, F. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. *Enzym. Microb. Technol.* **2002**, *31*, 353–364. [CrossRef]
109. Börjesson, J.; Peterson, R.; Tjerneld, F. Enhanced enzymatic conversion of softwood lignocellulose by poly(ethylene glycol) addition. *Enzym. Microb. Technol.* **2007**, *40*, 754–762. [CrossRef]
110. Kaar, W.E.; Holtzapple, M.T. Benefits from Tween during enzymic hydrolysis of corn stover. *Biotechnol. Bioeng.* **1998**, *59*, 419–427. [CrossRef]
111. Mizutani, C.; Sethumadhavan, K.; Howley, P.; Bertoniere, N. Effect of a nonionic surfactant on Trichoderma cellulase treatments of regenerated cellulose and cotton yarns. *Cellulose* **2002**, *9*, 83–89. [CrossRef]
112. Eckard, A.D.; Muthukumarappan, K.; Gibbons, W. Enzyme recycling in a simultaneous and separate saccharification and fermentation of corn stover: A comparison between the effect of polymeric micelles of surfactants and polypeptides. *Bioresour. Technol.* **2013**, *132*, 202–209. [CrossRef] [PubMed]
113. Alhammad, A.; Adewale, P.; Kuttiraja, M.; Christopher, L.P. Enhancing enzyme-aided production of fermentable sugars from poplar pulp in the presence of non-ionic surfactants. *Bioprocess Biosyst. Eng.* **2018**, *41*, 1133–1142. [CrossRef]
114. Goshadrou, A.; Lefsrud, M. Synergistic surfactant-assisted [EMIM] OAc pretreatment of lignocellulosic waste for enhanced cellulose accessibility to cellulase. *Carbohydr. Polym.* **2017**, *166*, 104–113. [CrossRef] [PubMed]
115. Zhang, H.; Zhang, S.; Yuan, H.; Lyu, G.; Xie, J. $FeCl_3$-catalyzed ethanol pretreatment of sugarcane bagasse boosts sugar yields with low enzyme loadings and short hydrolysis time. *Bioresour. Technol.* **2017**, *249*, 395–401. [CrossRef] [PubMed]
116. Oladi, S.; Aita, G.M. Interactive effect of enzymes and surfactant on the cellulose digestibility of un-washed and washed dilute ammonia pretreated energy cane bagasse. *Biomass Bioenergy* **2018**, *109*, 221–230.
117. Shome, A.; Roy, S.; Das, P.K. Nonionic Surfactants: A Key to Enhance the Enzyme Activity at Cationic Reverse Micellar Interface. *Langmuir* **2007**, *23*, 4130–4136. [CrossRef]
118. Eckard, A.D.; Muthukumarappan, K.; Gibbons, W.R. The Role of Polymeric Micelles on Chemical Changes of Pretreated Corn Stover, Cellulase Structure, and Adsorption. *BioEnergy Res.* **2013**, *7*, 389–407. [CrossRef]
119. Bhagia, S.; Wyman, C.E.; Kumar, R. Impacts of cellulase deactivation at the moving air–liquid interface on cellulose conversions at low enzyme loadings. *Biotechnol. Biofuels* **2019**, *12*, 96. [CrossRef]
120. Lee, R.C.; Despa, F.; Guo, L.; Betala, P.; Kuo, A.; Thiyagarajan, P. Surfactant Copolymers Prevent Aggregation of Heat Denatured Lysozyme. *Ann. Biomed. Eng.* **2006**, *34*, 1190–1200. [CrossRef]
121. Zhou, Y.; Chen, H.; Qi, F.; Zhao, X.; Liu, D. Non-ionic surfactants do not consistently improve the enzymatic hydrolysis of pure cellulose. *Bioresour. Technol.* **2015**, *182*, 136–143. [CrossRef] [PubMed]
122. Shi, W.; Zhang, Z.; Shun, Q.; Liu, X.; Ding, C.; Zheng, H.; Wang, F. Protective effects of five surfactants on cellulase in the saccharification of corn stover based on the impeded Michaelis-Menten model. *BioResources* **2020**, *15*, 4089–4109. [CrossRef]
123. Sindhu, R.; Binod, P.; Mathew, A.K.; Abraham, A.; Pandey, A.; Gnansounou, E.; Castro, G.E. An effective surfactant-assisted hydrothermal pretreatment strategy for bioethanol production from chili post-harvest residue by separate hydrolysis and fermentation. *Bioprocess Biosyst. Eng.* **2018**, *41*, 565–571. [CrossRef] [PubMed]
124. Liu, Z.-H.; Qin, L.; Zhu, J.-Q.; Li, B.-Z.; Yuan, Y.-J. Simultaneous saccharification and fermentation of steam-exploded corn stover at high glucan loading and high temperature. *Biotechnol. Biofuels* **2014**, *7*, 167. [CrossRef]
125. Alkasrawi, M.; Eriksson, T.; Börjesson, J.; Wingren, A.; Galbe, M.; Tjerneld, F.; Zacchi, G. The effect of Tween-20 on simultaneous saccharification and fermentation of softwood to ethanol. *Enzym. Microb. Technol.* **2003**, *33*, 71–78. [CrossRef]
126. Zheng, T.; Jiang, J.; Yao, J. Surfactant-promoted hydrolysis of lignocellulose for ethanol production. *Fuel Process. Technol.* **2021**, *213*, 106660. [CrossRef]

127. Xin, F.; Geng, A.; Chen, M.L.; Gum, M.J.M. Enzymatic Hydrolysis of Sodium Dodecyl Sulphate (SDS)—Pretreated Newspaper for Cellulosic Ethanol Production by Saccharomyces cerevisiae and Pichia stipitis. *Appl. Biochem. Biotechnol.* **2010**, *162*, 1052–1064. [CrossRef]
128. Wang, Z.J.; Lan, T.Q.; Zhu, J.Y. Lignosulfonate and elevated pH can enhance enzymatic saccharification of lignocelluloses. *Biotechnol. Biofuels* **2013**, *6*, 9. [CrossRef]
129. Lou, H.; Wang, M.; Lai, H.; Lin, X.; Zhou, M.; Yang, D.; Qiu, X. Reducing non-productive adsorption of cellulase and enhancing enzymatic hydrolysis of lignocelluloses by noncovalent modification of lignin with lignosulfonate. *Bioresour. Technol.* **2013**, *146*, 478–484. [CrossRef]
130. Wang, W.; Zhu, Y.; Du, J.; Yang, Y.; Jin, Y. Influence of lignin addition on the enzymatic digestibility of pretreated lignocellulosic biomasses. *Bioresour. Technol.* **2015**, *181*, 7–12. [CrossRef]
131. Xu, H.; Yu, G.; Mu, X.; Zhang, C.; DeRoussel, P.; Liu, C.; Li, B.; Wang, H. Effect and characterization of sodium lignosulfonate on alkali pretreatment for enhancing enzymatic saccharification of corn stover. *Ind. Crops Prod.* **2015**, *76*, 638–646. [CrossRef]
132. Ying, W.; Shi, Z.; Yang, H.; Xu, G.; Zheng, Z.; Yang, J. Effect of alkaline lignin modification on cellulase–lignin interactions and enzymatic saccharification yield. *Biotechnol. Biofuels* **2018**, *11*, 214. [CrossRef] [PubMed]
133. Zheng, W.; Lan, T.; Li, H.; Yue, G.; Zhou, H. Exploring why sodium lignosulfonate influenced enzymatic hydrolysis efficiency of cellulose from the perspective of substrate–enzyme adsorption. *Biotechnol. Biofuels* **2020**, *13*, 19. [CrossRef]
134. Lou, H.; Zhou, H.; Li, X.; Wang, M.; Zhu, J.Y.; Qiu, X. Understanding the effects of lignosulfonate on enzymatic saccharification of pure cellulose. *Cellulose* **2014**, *21*, 1351–1359. [CrossRef]
135. Lai, C.; Jia, Y.; Yang, C.; Chen, L.; Shi, H.; Yong, Q. Incorporating Lignin into Polyethylene Glycol Enhanced Its Performance for Promoting Enzymatic Hydrolysis of Hardwood. *ACS Sustain. Chem. Eng.* **2020**, *8*, 1797–1804. [CrossRef]
136. Lin, X.; Qiu, X.; Yuan, L.; Li, Z.; Lou, H.; Zhou, M.; Yang, D. Lignin-based polyoxyethylene ether enhanced enzymatic hydrolysis of lignocelluloses by dispersing cellulase aggregates. *Bioresour. Technol.* **2015**, *185*, 165–170. [CrossRef]
137. Huang, C.; Zhao, X.; Zheng, Y.; Lin, W.; Lai, C.; Yong, Q.; Ragauskas, A.J.; Meng, X. Revealing the mechanism of surfactant-promoted enzymatic hydrolysis of dilute acid pretreated bamboo. *Bioresour. Technol.* **2022**, *360*, 127524. [CrossRef]
138. Lai, C.; Tang, S.; Yang, B.; Gao, Z.; Li, X.; Yong, Q. Enhanced enzymatic saccharification of corn stover by in situ modification of lignin with poly (ethylene glycol) ether during low temperature alkali pretreatment. *Bioresour. Technol.* **2017**, *244*, 92–99. [CrossRef]
139. Lai, C.; Yang, B.; Lin, Z.; Jia, Y.; Huang, C.; Li, X.; Song, X.; Yong, Q. New strategy to elucidate the positive effects of extractable lignin on enzymatic hydrolysis by quartz crystal microbalance with dissipation. *Biotechnol. Biofuels* **2019**, *12*, 57. [CrossRef]
140. Lai, C.; Tu, M.; Yong, Q.; Yu, S. Synergistic effects of pH and organosolv lignin addition on the enzymatic hydrolysis of organosolv-pretreated loblolly pine. *RSC Adv.* **2018**, *8*, 13835–13841. [CrossRef]
141. Feng, Z.; Liu, Q.; Zhang, H.; Xu, D.; Zhai, H.; Ren, H. Adsorption of bovine serum albumin on the surfaces of poplar lignophenols. *Int. J. Biol. Macromol.* **2020**, *158*, 290–302. [CrossRef] [PubMed]
142. Bhagia, S.; Kumar, R.; Wyman, C.E. Effects of dilute acid and flowthrough pretreatments and BSA supplementation on enzymatic deconstruction of poplar by cellulase and xylanase. *Carbohydr. Polym.* **2017**, *157*, 1940–1948. [CrossRef] [PubMed]
143. Florencio, C.; Badino, A.C.; Farinas, C.S. Soybean protein as a cost-effective lignin-blocking additive for the saccharification of sugarcane bagasse. *Bioresour. Technol.* **2016**, *221*, 172–180. [CrossRef] [PubMed]
144. Lai, C.; Yang, C.; Zhao, Y.; Jia, Y.; Chen, L.; Zhou, C.; Yong, Q. Promoting enzymatic saccharification of organosolv-pretreated poplar sawdust by saponin-rich tea seed waste. *Bioprocess Biosyst. Eng.* **2020**, *43*, 1999–2007. [CrossRef]
145. Eckard, A.D.; Muthukumarappan, K.; Gibbons, W. Enhanced bioethanol production from pretreated corn stover via multi-positive effect of casein micelles. *Bioresour. Technol.* **2013**, *135*, 93–102. [CrossRef]
146. Yadav, M.; Paritosh, K.; Chawade, A.; Pareek, N.; Vivekanand, V. Genetic Engineering of Energy Crops to Reduce Recalcitrance and Enhance Biomass Digestibility. *Agriculture* **2018**, *8*, 76. [CrossRef]
147. Rocha-Meneses, L.; Ferreira, J.A.; Mushtaq, M.; Karimi, S.; Orupõld, K.; Kikas, T. Genetic modification of cereal plants: A strategy to enhance bioethanol yields from agricultural waste. *Ind. Crops Prod.* **2020**, *150*, 112408. [CrossRef]
148. Saravanan, A.; Kumar, P.S.; Jeevanantham, S.; Karishma, S.; Vo, D.-V.N. Recent advances and sustainable development of biofuels production from lignocellulosic biomass. *Bioresour. Technol.* **2022**, *344*, 126203. [CrossRef]
149. Hu, W.-J.; Harding, S.A.; Lung, J.; Popko, J.L.; Ralph, J.; Stokke, D.D.; Tsai, C.-J.; Chiang, V.L. Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. *Nat. Biotechnol.* **1999**, *17*, 808–812. [CrossRef]
150. Min, D.; Li, Q.; Jameel, H.; Chiang, V.; Chang, H.-M. The Cellulase-Mediated Saccharification on Wood Derived from Transgenic Low-Lignin Lines of Black Cottonwood (*Populus trichocarpa*). *Appl. Biochem. Biotechnol.* **2012**, *168*, 947–955. [CrossRef]
151. Xiang, Z.; Sen, S.K.; Min, D.; Savithri, D.; Lu, F.; Jameel, H.; Chiang, V.; Chang, H.-M. Field-Grown Transgenic Hybrid Poplar with Modified Lignin Biosynthesis to Improve Enzymatic Saccharification Efficiency. *ACS Sustain. Chem. Eng.* **2017**, *5*, 2407–2414. [CrossRef]
152. Ralph, J.; Akiyama, T.; Coleman, H.D.; Mansfield, S.D. Effects on Lignin Structure of Coumarate 3-Hydroxylase Downregulation in Poplar. *BioEnergy Res.* **2012**, *5*, 1009–1019. [CrossRef] [PubMed]
153. Van Acker, R.; Déjardin, A.; Desmet, S.; Hoengenaert, L.; Vanholme, R.; Morreel, K.; Laurans, F.; Kim, H.; Santoro, N.; Foster, C.; et al. Different Routes for Conifer- and Sinapaldehyde and Higher Saccharification upon Deficiency in the Dehydrogenase CAD1. *Plant Physiol.* **2017**, *175*, 1018–1039. [CrossRef] [PubMed]

154. Wilkerson, C.G.; Mansfield, S.D.; Lu, F.; Withers, S.; Park, J.-Y.; Karlen, S.D.; Gonzales-Vigil, E.; Padmakshan, D.; Unda, F.; Rencoret, J.; et al. Monolignol Ferulate Transferase Introduces Chemically Labile Linkages into the Lignin Backbone. *Science* **2014**, *344*, 90–93. [CrossRef] [PubMed]
155. Bhalla, A.; Bansal, N.; Pattathil, S.; Li, M.; Shen, W.; Particka, C.A.; Karlen, S.D.; Phongpreecha, T.; Semaan, R.R.; Gonzales-Vigil, E.; et al. Engineered Lignin in Poplar Biomass Facilitates Cu-Catalyzed Alkaline-Oxidative Pretreatment. *ACS Sustain. Chem. Eng.* **2018**, *6*, 2932–2941. [CrossRef]
156. Kim, K.H.; Dutta, T.; Ralph, J.; Mansfield, S.D.; Simmons, B.A.; Singh, S. Impact of lignin polymer backbone esters on ionic liquid pretreatment of poplar. *Biotechnol. Biofuels* **2017**, *10*, 101. [CrossRef]
157. Paul, M.; Mohapatra, S.; Das Mohapatra, P.K.; Thatoi, H. Microbial cellulases—An update towards its surface chemistry, genetic engineering and recovery for its biotechnological potential. *Bioresour. Technol.* **2021**, *340*, 125710. [CrossRef]
158. Pedersen, J.N.; Zhou, Y.; Guo, Z.; Pérez, B. Genetic and chemical approaches for surface charge engineering of enzymes and their applicability in biocatalysis: A review. *Biotechnol. Bioeng.* **2019**, *116*, 1795–1812. [CrossRef]
159. Várnai, A.; Siika-Aho, M.; Viikari, L. Carbohydrate-binding modules (CBMs) revisited: Reduced amount of water counterbalances the need for CBMs. *Biotechnol. Biofuels* **2013**, *6*, 30. [CrossRef]
160. Pakarinen, A.; Haven, M.; Djajadi, D.T.; Várnai, A.; Puranen, T.; Viikari, L. Cellulases without carbohydrate-binding modules in high consistency ethanol production process. *Biotechnol. Biofuels* **2014**, *7*, 27. [CrossRef]
161. Rahikainen, J.L.; Martin-Sampedro, R.; Heikkinen, H.; Rovio, S.; Marjamaa, K.; Tamminen, T.; Rojas, O.J.; Kruus, K. Inhibitory effect of lignin during cellulose bioconversion: The effect of lignin chemistry on non-productive enzyme adsorption. *Bioresour. Technol.* **2013**, *133*, 270–278. [CrossRef] [PubMed]
162. Nordwald, E.M.; Brunecky, R.; Himmel, M.E.; Beckham, G.T.; Kaar, J.L. Charge engineering of cellulases improves ionic liquid tolerance and reduces lignin inhibition. *Biotechnol. Bioeng.* **2014**, *111*, 1541–1549. [CrossRef] [PubMed]