Review

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

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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

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 beforehand 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 cellulas 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 glucose. 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.
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 different 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 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].

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](image-url). 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.
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 irrelevant 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% H₂SO₄ 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 overview of sulfite pretreatment to increase enzyme hydrolysis efficiency of lignocellulose.

| Pretreatment           | Reagent                        | Lignocellulose          | Cellulase Loading | Results                                                      | Advantage                                                                 | Disadvantage                                                                 |
|------------------------|--------------------------------|-------------------------|-------------------|--------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Acid sulfite pretreatment | 7% H₂SO₄ and 6% Na₂SO₃ | Oil palm stem          | 15 FPU/g-cellulose | Improved enzymatic efficiency into 92%                      | Effective in overcoming biomass recalcitrance; low environmental and     | Possibility of formation of inhibitory by-products under acid condition;   |
|                        |                                |                         |                   |                                                              | technological barriers and risks for commercialization                   | potentially need high pretreatment temperature                            |
| Acid sulfite pretreatment | 1% H₂SO₄ and 2.4% Na₂SO₃ | 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% H₂SO₄ and 7% Na₂SO₃ | Corn straw              | 10 FPU/g-cellulose | The conversion of glucan and xylan increased to 80% and 86% |                                                                            |                                                                            |
| Alkaline sulfite pretreatment | 1% NaOH and 4% Na₂SO₃ | 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% Na₂SO₃ | 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% Na₂SO₃ | Bagasse                 | 10 FPU/g-cellulose | The highest sugar yields of the glucose and xylan were 76.8% and 61.2% |                                                                            |                                                                            |
| Alkaline sulfite pretreatment | 12% NaOH and 10% Na₂SO₃ | Corn straw              | 20 FPU/g-cellulose | The total sugar yield was 74.73% after 48 h enzymatic hydrolysis |                                                                            |                                                                            |
| Neutral sulfite pretreatment | 5.27% Mg(HSO₃)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 |                                                                            |
| Neutral sulfite pretreatment | 12% Na₂SO₃ | Corn cob residue        | 5 FPU/g-substrate | The enzymatic hydrolysis efficiency and glucose yield was increased by 28.80% and 20.10% | Low delignification conditions; no lignin sulfonation                    |                                                                            |
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 SO2-ethanol-water (SEW) co-solvent and found that during the pretreatment process, ethanol promoted the transformation of SO2 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 non-ionic 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.

| Types               | Reactive Agent | Lignocellulose           | Result                                                                 |
|---------------------|----------------|--------------------------|------------------------------------------------------------------------|
| 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| 2% 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 | Microcrystalline cellulose | 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, alkal, 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](image_url)

**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.

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