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Review

Biological Activities and Emerging Roles of Lignin and Lignin-Based Products—A Review

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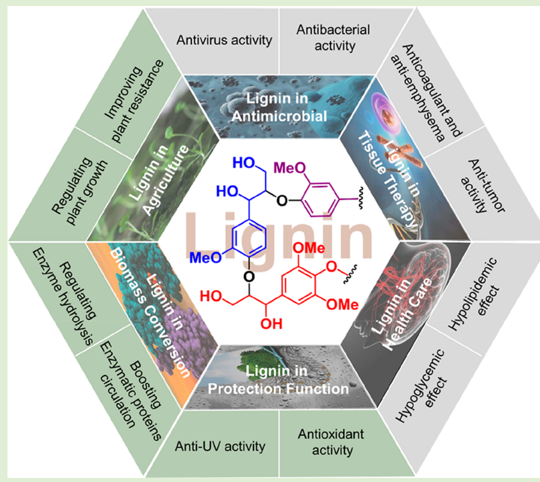
ABSTRACT: Bioactive substances, displaying excellent biocompatibility, chemical stability, and processability, could be extensively applied in biomedicine and tissue engineering. In recent years, plant-based bioactive substances such as flavonoids, vitamins, terpenes, and lignin have received considerable attention due to their human health benefits and pharmaceutical/medical applications. Among them is lignin, an amorphous biomacromolecule mainly derived from the combinatorial radical coupling of three phenylpropane units (*p*-hydroxypenyl, guaiacyl, and syringyl) during lignification. Lignin possesses intrinsic bioactivities (antioxidative, antibacterial, anti-UV activities, etc.) against phytopathogens. Lignin also enhances the plant resistance (adaptability) against environmental stresses. The abundant structural features of lignin offer other significant bioactivities including antitumor and antivirus bioactivities, regulation of plant growth, and enzymatic hydrolysis of cellulose. This Review reports the latest research results on the bioactive potential of lignin and lignin-based substances in biomedicine, agriculture, and biomass conversion. Moreover, the interfacial reactions and bonding mechanisms of lignin with biotissue/cells and other constituents were also discussed, aiming at promoting the conversion or evolution of lignin from industrial wastes to value-added bioactive materials.



1. INTRODUCTION

Bioactive substances typically found in small amounts in plants and certain foods have a physiological correlation and influence on the phenomena of life.[1] Bioactive substances have been extensively used as antioxidant, antiviral, and anticancer materials or agents in biomedicine and tissue engineering owing to their excellent biocompatibility, chemical stability, and good processability.[2] They can directly trigger the reaction of living cells, tissues, or organisms and remain stable, showing special advantages over inert biomaterials that bind to tissues in a mechanically embedded way.[3] In recent years, natural bioactive compounds originating from animals, plants, marine life, and microbes have received increasing attention due to their renewability, environmental friendliness, and biodegradable nature.[4−7] Some examples of bioactive compounds derived from plants are polysaccharides, terpenoids, flavonoids, tannins, sterols, amino acid, and lignin.

Among these well-known natural bioactive compounds, lignin attracts much attention due to its considerable quantity in plants (15−30%), favorable renewability, and some significant biological activities,[8−10] as summarized in Figure 1. Lignin is an amorphous biomacromolecule with a highly complex structure. It is composed of three phenylpropane units of guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H).[11−14] These structural units of lignin are cross-linked mainly via aryl ether linkages viz. β−O−4′ and carbon−carbon linkages such as β−5′, β−β′, and 5−5′.[15,16] From a biosynthesis perspective, deaminase converts the starting material, phenylalanine and tyrosine, into cinnamic acid and *p*-coumaric acid, respectively. The two carboxylic acid compounds are reduced to synthesize hydroxycinnamyl alcohol and a methoxylated aromatic ring, which then form coniferyl alcohol and sinapyl alcohol. The three monomers finally form an amorphous lignin structure through a combinatorial radical coupling reaction.[16] In addition, other phenolic compounds also participate in the radical coupling reaction during lignification and are fully integrated into the lignin polymer.[17] Protolignin promotes the strength and hardness of plant cell walls while maintaining the transport of water and salt in the vascular system to ensure stable plant growth. More importantly, the abundant functional

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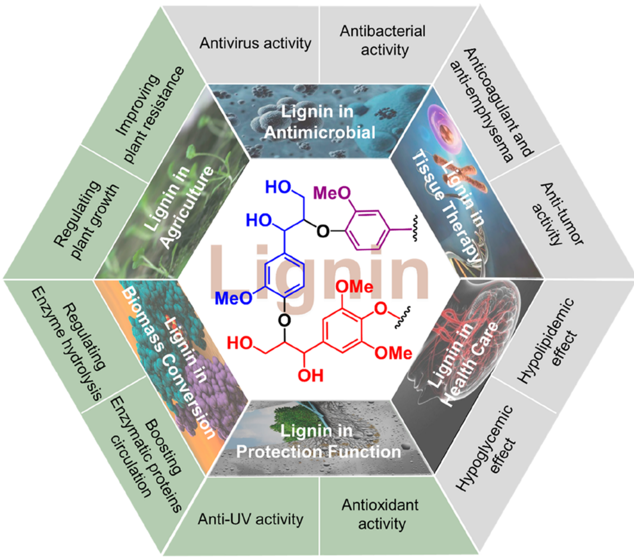

Figure 1. Biological activities and effective utilizations of lignin.

groups in lignin impart special structural characteristics and biological activities.[18,19] For example, phenolic hydroxyl groups not only have excellent antioxidative, antibacterial, and anti-UV functions but also serve as modifiable active sites in lignin, thereby allowing an improvement of biological activity through modification.[20−22] In addition, it has been reported that lignin has a variety of other biological activities, such as antitumor and antiviral activities in medical treatment. It also confers health benefits, regulates enzymatic hydrolysis in biorefineries, promotes plant growth, and improves plant stress resistance in agricultural production. All of these functional properties open the possibility of lignin to be considered an advanced bioactive material.[23−25] Meanwhile, a lignin−carbohydrate complex (LCC) formed by chemical bonds also possesses biological activities such as antioxidative and antivirus activities, which further expands the diverse applications.

Among the various functional groups of lignin, the phenolic hydroxyl group prevents the oxidation reaction by providing hydrogen atoms and reducing the auto-oxidation of polymers. The π-electronic system of the aromatic ring makes the phenolic hydroxyl group acidic, so the deprotonation of the phenolic hydroxyl groups occurs more likely than the aliphatic ones.[26,27] Kai et al.[28] reported that lignin/poly-β-hydroxybutyrate nanofibers promoted cartilage tissue regeneration and neutralized free radicals produced by osteoarthritis. In recent years, the development of food additives such as food gums, antioxidants, and preservatives of vegetables and plant lignin as the main raw materials has also received extensive attention.[29]

During the free-radical polymerization of lignin monomers, the electron pairs of the para vinyl and phenolic hydroxyl are lost, resulting in the generation of UV chromophores at the coupling site.[30] Lignin shows a strong absorption capacity in the UV/vis light region; therefore, it has been largely applied in many fields as an anti-UV screening agent. For example, as a natural polymer sunscreen agent, lignin has a great effect on the UV protection of the human skin.[31,32] In addition, the potential application of lignin in maintaining food safety and prolonging the shelf life of food has been thoroughly

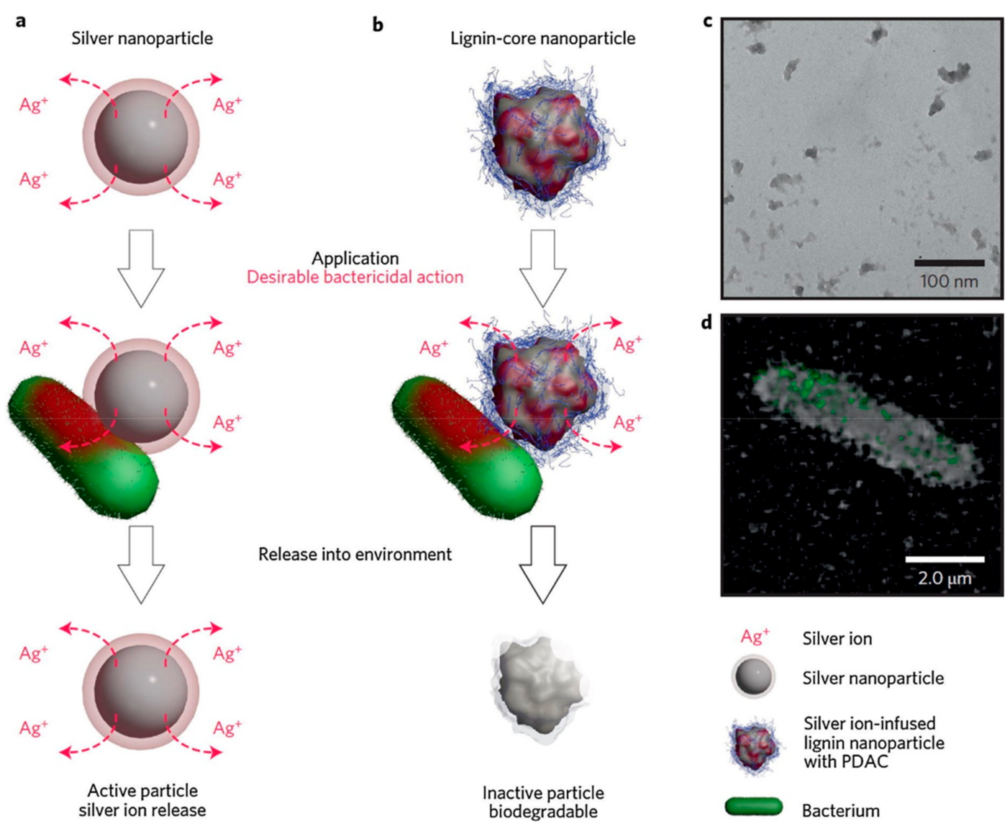

Figure 2. Schematic representation of the bactericidal action of lignin-core nanoparticles and silver nanoparticles.[39] Reprinted with permission from ref 39. Copyright 2016 Springer Nature.

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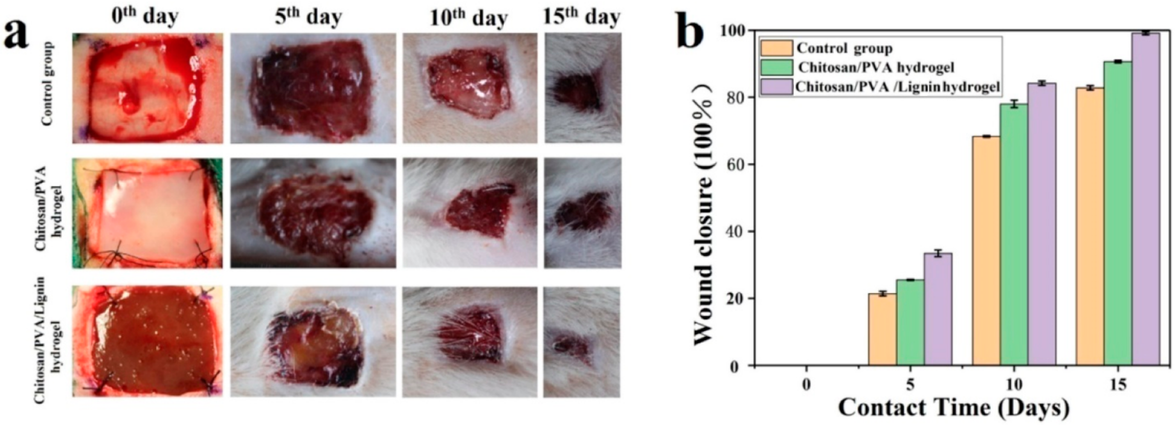

Figure 3. (a) Wound healing status of a blank control group, chitosan−PVA hydrogel, and chitosan−PVA−lignin hydrogel (lignin 10 wt %) at days 0, 5, 10, and 15. (b) Recovery rate of the wound treated with a blank control group, chitosan−PVA hydrogel, and chitosan−PVA−lignin hydrogel (lignin 10 wt %).[48] Reprinted with permission from ref 48. Copyright 2019 Elsevier.

explored.[33−35] As mentioned above, the antioxidative and anti-UV activities of lignin have been well-documented and will not be discussed in this Review.

Aimed at promoting the conversion of lignin from industrial wastes to bioactive materials, the biological activities of lignin, such as antibacterial activity, antiviral activity, plant growth regulation, and an enzymatic hydrolysis improvement, and its application as active additives in medicine, agriculture, and biomass conversion were particularly summarized in the current Review. Meanwhile, the structure−activity relationship in terms of the biological activity of lignin was also discussed, while the activities associated with the antioxidative and anti-UV properties were excluded. The multiple activities of lignin as functional additives were covered. Furthermore, we also addressed the value and biological potential of lignin and lignin-based products in a variety of research fields. Importantly, lignin in this Review includes protolignin, extracted and/or isolated lignin, lignin derivatives, and degraded and/or low-molecular-weight (LMW) lignin. This Review is organized according to the application aspects.

2. LIGNIN AS ANTIMICROBIAL AGENTS

2.1. Antibacterial Activity. Protolignin can prevent the decomposition of carbohydrates by inhibiting the bacteria and fungi during the process of plant growth.[36] Lignin extracted from plants has been directly applied in medicine and biology as an antibacterial agent or additive.[37] The antibacterial effect of the lignin structure was mainly associated with phenolic hydroxyl and methoxy groups.[38]

In addition to acting as an antimicrobial additive alone, lignin can also be incorporated with other antimicrobial materials for synergetic effects. For example, when lignin replaces the core of silver nanoparticles, it shows excellent antibacterial properties without producing silver ions which tend to cause adverse ecological effects (Figure 2).[39] Silver-coated lignin nanoparticles directly synthesized in water showed 10%, 12%, and 80% inhibition against the human pathogens *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger*, respectively.[40] Poly(vinyl alcohol) (PVA)−lignin nanofiber mats loaded with silver nanoparticles were prepared via electrospinning, which also exhibited apparent antibacterial effects on *Bacillus circulans* (MTCC 7906) and *Escherichia coli* (MTCC 739).[41]

In recent years, lignin composite materials have been thoroughly applied as potential wound dressings because of their antibacterial, nontoxic, and water absorption capabilities.[42,43] Wound healing is the natural process of the regeneration of human skin and epidermal tissue after an injury.[12,44] An ideal wound dressing should absorb exudate from the wound while keeping the surface moist, protect the wound from infection and secondary injury, and subsequently relieve the pain of the wound.[45,46] From the *in vivo* wound healing study conducted by Reesi et al.[42] on hydrogels prepared with arginine surface modified lignin nanofibers, the results showed that the hydrogels can promote wound closure, re-epithelialization, and angiogenesis. It was found that chitosan−lignin hydrogels with good biocompatibility created a favorable environment for cell adhesion and growth, without cytotoxicity. From the wound healing test using NIH 3T3 mouse fibroblasts, the wound was adequately covered with cells after 24 h. This suggests that hydrogel serves as a suitable scaffold for promoting wound healing and skin regeneration.[47] The introduction of lignin effectively improved the mechanical strength, protein adsorption capacity, and wound environmental regulation of hydrogels. In a murine wound model, the lignin−chitosan−PVA composite hydrogel not only maintained a moist healing environment but also significantly promoted cell growth and migration and promoted wound healing (Figure 3), thereby confirming the application value of lignin in wound dressing.[48]

Dragica et al.[49] studied the antibacterial activity of a lignin model polymer, i.e., dehydrogenated polymer (DHP) in alginate brine gel (Alg). DHP−Alg showed strong antibacterial activity without any cytotoxic effect. These results suggested the effectiveness of DHP−Alg as a wound healing agent for healthcare applications. Subsequently, a composite hydrogel consisting of bacterial cellulose (BC) and cypress alcohol dehydrogenation polymer (DHP) showed a promising result of accelerating the wound healing.[50] Although the antibacterial effects of lignin have been well studied, the mechanisms of lignin promoting cell growth and wound healing need to be further elucidated. More in-depth cellular experiments, such as cell structure or sequence changes, could be used to reveal the mechanism of wound healing recovery linked with lignin.

2.2. Antiviral Activity. Lignin showed significant resistance against human immunodeficiency virus (HIV), herpes

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simplex virus (HSV), and syphilis.[51] Furthermore, it was confirmed that lignin at low concentration showed antiviral activity against HIV-1.[51] HIV-1 is a highly variable virus and the main cause of acquired immunodeficiency syndrome (AIDS) and other associated diseases. The activation of NF-κB (nuclear factor kappa-B) increases the HIV-1 replication. Mitsuhashi et al.[52] demonstrated that lignin can achieve anti-HIV-1 effects by inhibiting promoter LTR (5′-long terminal repeat) and NF-κB-mediated transcription. It should be noted that LMW lignin showed a better inhibition effect in comparison to high-molecular-weight (HMW) lignin. Additionally, the synthetic lignin dimerlike compounds with a $\beta-5'$ structure displayed a stronger inhibitory activity, while the compounds with $\beta-O-4'$ showed a moderate inhibitory activity, and the compounds with $\beta-\beta'$ selectively inhibit viral transcription efficiently. From the above discussion, it can be concluded that the LMW lignin is expected to serve as a new drug for the treatment of AIDS.

As early as 1991, Harada et al.[53] reported that alkaline lignin could inactivate the influenza virus by infecting MDCK cells and could inhibit RNA synthesis. No antiviral activity was found against the influenza virus after using the degraded components of lignin, synthetic polyphenols (nonlignin components) and natural or chemically modified dextran. Therefore, the authors concluded that the polymerized phenolic structure of the lignin component was crucial for anti-influenza virus activity. The therapeutic retroviral approach has been shown to be safe and effective in preventing the sexual transmission of HIV-1;[54] lignosulfonic acid has demonstrated synergistic effects with a variety of reverse transcriptase inhibitors. The treatment of peripheral blood mononuclear cells (PBMCs) with lignosulfonic acid neither increased the expression level of cell activation markers nor enhanced HIV-1 replication. Overall, these results related to the inhibition of HIV highlight the role of lignosulfonic acid as a potential and unique low-cost antiviral agent.[55]

Lignosulfonic acid also showed a broad-spectrum antiherpes simplex virus activity; however, it does not interfere with the growth of the beneficial lactobacillus flora. Recently, Srisapoome et al.[56] reported the role of kraft lignin (KL) as a regulator of the antiviral immune response in black tiger shrimp. It was also reported that the use of 1−20 mg/L KL as feed had the potential to control and reduce the risk of infection of the invading virus. Matsuhisa et al.[57] explored the antihepatitis C virus (HCV) activity of *Lentinus edodes* mycelium solid culture extract (MSCE) and LMW lignin. The results showed that both MSCE and LMW lignin prevented the two HCV pseudoviruses (HCVpv) from entering Huh7.5.1 cells. In comparison with MSCE, a lower concentration of LMW lignin exerts a significant antiviral activity. It was the first demonstration of the anti-HCV effect of plant-derived long-acting lignin. Additionally, lignin has successfully undergone a clinical trial as an antiviral drug. The patients infected with herpes simplex virus type 1 (HSV-1) were fed with pineal lignin and ascorbic acid within 48 h after the onset of the disease. The results indicated that the therapeutic effect was obvious and the onset time shortened, and the symptoms were alleviated.[58] The therapeutic effect seemed to be dependent on the antioxidant and immune enhancing activities of lignin and ascorbic acid. The results of clinical trials assisted in the evaluation of the true effectiveness of lignin's antiviral activity.

Simultaneously, lignin-containing compounds such as LCC have also shown antiviral effects.[59,60] It has been reported that LCC from *Prunella vulgaris* showed an antiherpes virus activity by inhibiting virus binding and infiltration.[60] LCC prepared from cacao husk also has an outstanding antiviral and macrophage stimulating activity and possesses a higher anti-HIV activity compared to lipopolysaccharides.[61] Lignin has great potential as an antiviral additive despite facing challenges in clinical trials.

3. LIGNIN IN TISSUE THERAPY

3.1. Antitumor Activity. Plenty of *in vitro* studies have shown that the free phenolic hydroxyl groups lay the foundation for an antitumor effect in lignin.[62−64] As early as 1998, it was reported that lignin might be involved in fiber−colon cancer interactions. It was suggested that the defense of dietary fiber against colon cancer might be dependent in part on the amount of lignin in dietary fiber and the ability of lignin to trap the free radicals.[62]

From the literature base, Jeyaraj et al.[63] modified natural lignin with methacrylate to increase the antitumor efficacy. *Acacia* lignin extracted from Barapatre et al.[64] showed high anticytotoxicity and anticancer potential against breast cancer cells, while it was ineffective versus hepatic stellate cells. These findings indicated that lignin had great potential as a natural anticancer drug in preventing the diseases caused by the excessive production of free radicals. Figueiredo et al.[65] compared the growth inhibition effect of pure benzazulene (BZL) and pure lignin nanoparticles loaded with BZL (BZL-PLNPs) on breast cancer cells. It was found that pure BZL did not show any inhibitory effect on the multiplication of breast cancer cells. However, the antiproliferation effect of BZL-PLNPs was very significant, which reduced the viability of cancer cells from about 90% to 0%. Therefore, it was concluded that lignin not only strengthens the protection of drugs but also improves the anticancer activity, thereby leading to a new generation of natural anticancer drugs with an enhanced performance.

LCC extracted from plants has been shown to have a potent antitumor activity. For example, two LCCs extracted from *Inonotus obliquus* could inhibit the activation of the nuclear transcription factor (NF-κB) in cancer cells.[66] Huang et al.[67] reported that LCCs isolated from bamboo might reduce the incidence of breast tumors by inhibiting the growth of breast tumor cells (MCF-7) and might stimulate the growth of macrophage cells (RAW 264.7).

The antitumor biological activity of lignin has set the foundation for modern drug research. Due to the poor water solubility of lignin, the bioavailability of lignin in animals is at a low level. Thus, the modification of water solubility could lead to the development of lignin as a medicinal substance. For instance, a novel water-soluble lignin derivative, benzene poly(carboxylic acid) compound (BP-Cx-1), formed from polyphepan, a lignin-based enterosorbent, had multiple pharmacological effects on neurotransmitter receptors, ligand ion channels, and transporters. It could also assist in treating multifactorial diseases, such as disease associated with cancer and type 2 diabetes.[68]

Lubna et al.[69] used lignin as an important ingredient in an innovative and robust treatment of colon cancer. Sudheer et al.[70] successfully utilized the bagass- based KL in the preparation of beneficial materials for cancer treatment. Through the free-radical reaction induced by the ascorbic

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acid/H_2O_2 redox pair, a desired amount of KL was combined with dextran aldehyde (Dex-ald) and bovine serum albumin (BSA) for the synthesis of new conjugated materials Dex-ald−KL and BSA−KL with enhanced solubility. In comparison with Dex-ald, KL, and BSA alone, Dex-ald−KL and BSA−KL reduced the activity of colon cancer cells (SW480) by 55−61% within 48 h, demonstrating the reasonably high antitumor activity. It was also found that even a small amount of lignin exhibited a significant cytotoxicity against colon cancer cells. It was concluded that the combination of lignin with other biopolymers improved the ideal repair effect, thus becoming alternative chemotherapeutic agents for the treatment of malignant tumors.

3.2. Anticoagulant and Antiemphysema Activity of LMW Lignin. Sulfonated lignin is reported to have an anticoagulant activity.[71] The anticoagulant mechanism of sulfonated lignin involves the allosteric inhibition of thrombin mediated by allosteric exosite II.[72,73] Henry et al.[73] designed sulfate dehydropolymers (DHPs) of 4-hydroxycinnamic acid with excellent anticoagulant properties. They selectively inhibit thrombin and coagulation cascade factor Xa (a trypsin-like serine protease) through different anticoagulant mechanisms in comparison with current clinical anticoagulants. Further research[74] found that sulfated LMW lignins (LMWLs) bind at the heparin active site and inhibit the activities of thrombin and factor Xa. From the structural−functional characteristics, lignin can be transformed into sulfated LMWLs by appropriate methods. LMWLs can be utilized in treatments involving anticoagulation and antiemphysema. These results can promote the high-value utilization of lignin in the healthcare industry. The effects of three sulfated LMWLs ($CDSO_3$, $FDSO_3$, and $SDSO_3$) were further studied. LMWLs are complex three-dimensional oligomers prepared from the 4-hydroxycinnamic acid monomer (caffeic acid, ferulic acid, and erucic acid) by a two-step chemical enzymatic process. These sulfated phenolic acids are joined through β−O−4′, β−5′, and β−β′ linkages (Figure 4). It was found that LMWLs uniformly

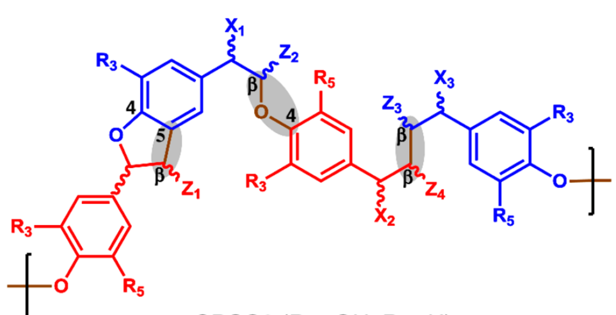

Figure 4. LMWLs are complex three-dimensional oligomers prepared from a 4-hydroxycinnamic acid monomer. X_1−X_3 are substituents at Cα and may be H, OH, or SO_3Na. Z1−Z3 may be H or COONa.[74] Reprinted with permission from ref 74. Copyright 2014 Elsevier.

inhibited the activity of thrombin and factor Xa, thereby providing new opportunities for the design of novel agents to regulate the complex pathology.[75]

Based on the inhibition of sulfated LMWLs on serine protease, factor Xa, and thrombin, they has been used in the treatment of emphysema which is one of the main pathological manifestations of chronic obstructive pulmonary disease. Elastic decomposition, oxidative stress, and inflammation are the three main pathogenic mechanisms causing emphysema.[76] According to literature reports, the sulfated LMWLs had an effective inhibitory effect on neutrophil elastase, oxidation, and *in vitro* inflammation.[77] Mehta et al.[78] reported that sulfated β−O−4′ lignin could effectively reduce the contractile force of platelet clots and prevent artery occlusion by interfering with thrombin and binding to target substances. The above results showed the possibility of utilizing lignin in novel drugs for antiemphysema treatments.

4. LIGNIN IN HEALTH CARE

4.1. Hypoglycemic Effect. Diabetes is a troublesome disease that endangers public health, often causing other complications and shortening life expectancy. The development of new therapeutic drugs is of great significance for the control and prevention of the disease. It was found that lignin and its derivatives can decrease blood glucose by reducing intestinal glucose absorption and promoting insulin release.

Quesille et al.[79] showed that the modified alkaline lignin has a strong inhibitory activity on α-amylase *in vitro*. Barapatre et al.[80] reported that the formation of hydrogen bonds between alkali-lignin and α-amylase significantly inhibited the activity of α-amylase, indicating its potential antihyperglycemia activity. Alkaline lignin alters the binding efficiency of α-amylase to glucose molecules, thereby affecting their movement across cell membranes and improving glycemic control by limiting glucose absorption after meals. This property of lignin provides a potential treatment for diabetes as demonstrated by Medina et al.[38]

Hasegawa et al.[81] investigated the effect of lignin sulfonic acid on intestinal glucose absorption. The results showed that lignin sulfonic acid is a reversible noncompetitive inhibitor of α-glucosidase, which can bind to enzymes and enzyme substrate complexes to enhance the inhibition of α-glucosidase activity. The lignin extracted from *Canna edulis* residue, reported by Xie et al.,[82] is mainly composed of G and S monomers. In comparison with acarbose, it yielded a stronger inhibitory effect on α-D-glucosidase activity. A molecular docking analysis showed that there is a single binding site for lignin on glucosidase, and the main binding forces between lignin and α-D-glucosidase are hydrophobic interaction, hydrogen bonding, and van der Waals forces. Lignin and its derivatives can achieve a hypoglycemic effect by inhibiting α-glucosidase activity and the intestinal absorption of glucose, which are potentially used in functional foods and medical fields. Therefore, it is of great importance to pay attention to the application of lignin as a safe food additive, as well as the mechanism of lignin transformation *in vivo*.

4.2. Hypolipidemic Effect. LMW lignin can lower cholesterol and triglyceride levels in the blood. Lignin is reported to be the strongest bile acid adsorbent among dietary fiber components because of the presence of methoxy and β-carbonyl groups. Lignin binds to the bile acid formed by cholesterol in the liver and consequently eliminates it from the body.[83,84] This could be an effective strategy for lowering blood lipids in obese individuals and individuals with atherosclerosis.

Rodríguez-Gutiérrez et al.[85] studied the adsorption of bile acids by lignin in olive nuclei *in vitro* and found that lignin has a strong adsorption capacity for bile acids in the form of sodium salt, enabling the binding of cholic acid of the

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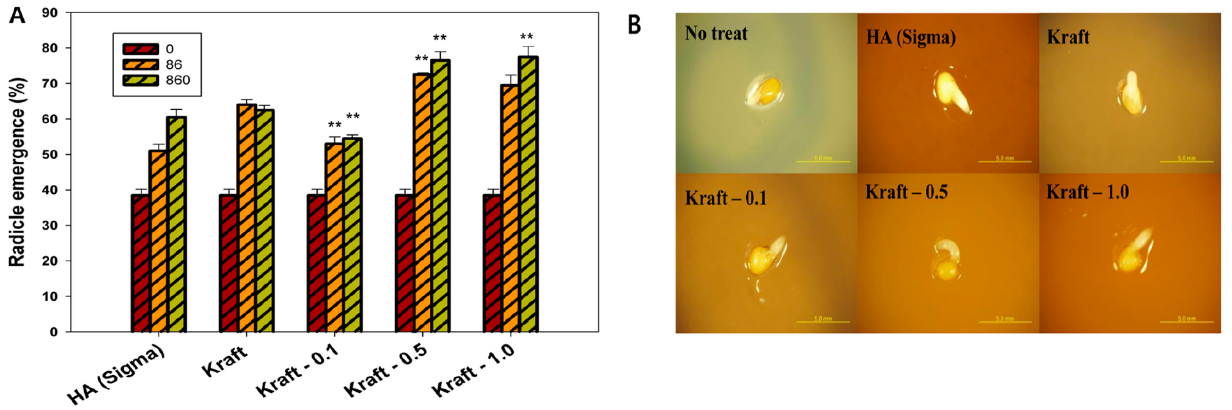

Figure 5. (A) Growth rate of roots. (B) Seed germination of *Arabidopsis* seeds in the presence of commercial humic acids, kraft lignins, and their Fenton variants (86 or 860 mg/L). HA, commercial humic acids; Kraft, kraft lignins; Kraft-0.1, Kraft-0.5, and Kraft-1.0, kraft lignins treated with 0.05, 0.25, and 0.5 g of $FeSO_4 \cdot 7H_2O$. Reprinted with permission from ref 103. Copyright 2018 ACS.

cholesterol-lowering drug cholestyramine. Norikura et al.[86] found that the lignophenol treatment of HepG2 cells reduced the secretion of the carrier protein apo-B. This in turn will inhibit the expression of cell triglyceride transfer protein mRNA, thereby reducing the formation of cholesterol. Lignin and its derivatives are biologically active substances that potentially promote human health, but the effect of lignin on blood lipid metabolism, especially the mechanism of diet-induced obesity, needs further research. Furthermore, the mechanisms through which lignin acts on gastrointestinal hormones and affects satiety and obesity are worthy to be explored.

5. LIGNIN IN AGRICULTURE

5.1. Regulating Plant Growth. Lignin being an important macromolecule plays a vital role in the normal growth and development of plants. The inhibition of the lignin biosynthesis gene will interfere with the biosynthesis process, resulting in the abnormal development of plants, such as xylem collapse, stem bending, and growth retardation.[87,88] In the study of Liang et al.,[89] the lower lignin content of the *Arabidopsis thaliana* mutant seed coat in comparison with the wild type has been linked with the significant reduction of the germination rate in the seeds. In *Arabidopsis* cinnamic acid 4-hydroxylase (C4H) mutants, the decrease in lignin content severely inhibited growth and led to the loss of apical dominance.[90] In addition, lignin is also closely related to cell expansion and cell growth.[91] For example, the overexpression and downregulation of the rice expansin-like gene-*OsPEX1* in rice could reduce lignin content, affect cell expansion, and cause decreased plant growth,[92] and on the other hand, it is hypothesized that increasing lignin content could promote cell expansion and plant growth.

In addition to the protolignin content of the plant which can regulate the growth, lignin has been found to have humic substancelike biological activities for promoting plant growth.[93,94] Humic acid (HA) as a phytohormone regulates plant growth by fine-tuning the plant physiological metabolism. The biological activity of HA is related to its molecular structure and supramolecular conformation.[95,96] Some of the functional groups of HA are also present in the lignin structure, such as an aromatic unit system, phenolic hydroxyl groups, carboxyl groups, methoxy groups, etc. Exploring the structural similarity between lignin and common plant growth regulators is beneficial to the directional modification of lignin, thus allowing a more efficient regulation of plant growth.[97] Recently, the essence of phenolic structural units derived from lignin regulated the endogenous plant hormones, which in turn has been linked with the promotion of growth in *Euglena gracilis*. It has been reported that phenylpropanoid compounds can affect the biosynthesis of L-tryptophan and the transformation of indole-3-acetic acid (IAA), thus affecting the growth and development of plants.[98]

Balas et al.[99] applied flax lignin to tomato plants and found that lignin promoted the growth of the tomato seedling leaf area. Savy et al.[100] isolated lignin from four kinds of nonfood crops using alkaline oxidation and studied the effects of different concentrations of lignin on maize seedling development. ^{31}P NMR and ^{13}C-CPMAS-NMR spectroscopies were used to assess the structure−activity relationship. The results showed that the hydrophobicity of lignin extract and the content of aliphatic OH groups and S and G molecules affected the biological activity. Hydrophilicity and conformational instability promote the release of bioactive molecules under the action of exudated organic acids. The discovery of the HA-like bioactivity of lignin has generated an interest to recover lignocellulosic residues as plant growth regulators; this could increase the economic benefits and sustainability of nonfood crops. Further research revealed the effect of humus-like lignin isolated from giant reeds on tomato, watercress, and chicory seeds. The results showed that humus-like lignin could act directly on the development of plants and seeds as gibberellin (GA) and can also actively disrupt the hormonal balance associated with GA.[101] This affected the physiological mechanisms mediated by GA and improved the development of plants and seeds.[101] These findings suggested that humic-like lignin extracted from energy crops may play an important role in enhancing sustainable agriculture as seed germination promoters and biological stimulants for plant growth. As mentioned in Savy's research study, lignin exhibiting good water solubility was obtained through alkaline oxidation.[100,101] Water-soluble lignin enhances the activities of plant cells due to their chemical composition and supramolecular arrangement. Based on previous work, Savy et al.[102] used ^{13}C-CPMAS-NMR and ^{31}P NMR spectra to reveal the characteristics of the humic-like substance (HLS) isolated from agricultural residues with high lignin content and proposed that aromatic molecules and *O*-alkyl from lignin sources had

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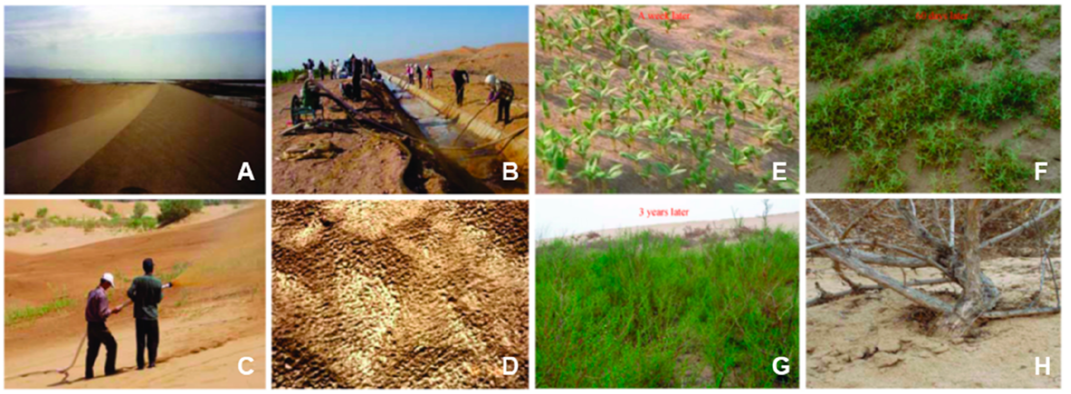

Figure 6. Dual function of sand fixation and vegetation restoration of lignin-based sand stabilizing material (LSSM). (A) Original fugitive dune. (B) dissolution of LSSM. (C) Spraying of LSSM aqueous solution on a fugitive dune after the seeding of arenaceous plants. (D) Formed crust after LSSM solution was sprayed on the sand surface. (E, F, G) Fugitive dune stabilized and greened gradually after arenaceous plants were seeded with LSSM spraying. (H) Largest individual plant of a 3 year-old *Artemisia desertorum* Spreng.[110] Reprinted with permission from ref 110. Copyright 2009 Elsevier.

positive effects on root and coleoptile elongation. To further study the relationship between the HLS structure and plant characteristics, chemical techniques could be applied to control the molecular composition of HLS in order to improve its biological activity. It is beneficial for the development of lignin-based biological regulators with a high efficiency and sustainable biostimulation ability to increase crop yield.

In nature, fungus-driven nonspecific oxidation is the key to the decomposition of refractory plant lignin, leading to its humification. In actual production, lignin sulfate can be effectively converted into humus-like plant fertilizer by Fenton oxidation. The lignin variant obtained by the Fenton reaction can successfully promote the germination of *Arabidopsis* seeds and improve the tolerance of the plant to abiotic stress. More importantly, these lignin variants stimulate plant growth in a similar fashion to that of commercial HA or even better, as shown in Figure 5.[103]

Lignin not only affects the balance of endogenous hormones but also regulates plant growth and development through plant photosynthesis, the meristematic ability of plant tissue, and nutrient absorption via plant roots. Daniele et al.[104] confirmed that lignin stimulated chlorophyll biosynthesis in plants. The chlorophyll content of maize treated with lignin nanoparticles increased significantly, which directly enhanced photosynthesis. In addition, lignin may have the ability to directly act on the mitotic activity of seeds, thereby promoting seed germination.[105] Furthermore, the lignin added to the pot experiment of *Malus hupehensis* var. *pingyiensis*[106] promotes the absorption of P by plant roots and directly promotes plant growth and development.

Bioactive lignin has been added to mulching film for various purposes. It was noticed that lignin mulching film not only maintains soil moisture but also promotes plant growth. Lignosulfonates have been widely used in sand-fixing materials to effectively improve the strength, humidity, and fertility of sand.[107] Liang et al.[108] utilized water-borne polyurethane, HA, chitosan, and sodium lignosulfonate for the preparation of environmentally friendly liquid mulching film, which has an excellent antierosion performance and an obvious dust-suppressing and sand-fixing effect in desert areas. Technical lignin derived from pulping spent liquor can be used as a sand stabilizing material after cross-linking modification with urea and formaldehyde (Figure 6).[109,110] This kind of lignin-based material has the dual function of sand fixation and vegetation restoration which could be applied to stabilize sand dunes and improve the growth of arenaceous plants effectively in an extremely dry climate condition. The organic matter and total nitrogen in sand were increased significantly due to the formation of the community.

5.2. Improving Plant Resistance. Stress resistance refers to some traits possessed by plants that must resist adverse environments, such as cold resistance, drought resistance, salt resistance, insect resistance, and so on. The cell walls of plants play an essential role in resisting external hazards, whereas protolignin accumulation increases under biological and abiotic stresses.[25,111]

Lignin metabolism is related to plant stress resistance. Climate change affects the growth of plants. At low temperatures, plant cell membranes are damaged and photosynthesis and respiration are reduced, thus inhibiting their growth and development severely.[112] In contrast, high temperatures destroy macromolecules such as proteins and nucleic acids, disrupting normal plant metabolism.[113] When the rhododendron is in a cold environment, its lignin content increases significantly with respect to the increased gene expression of *p*-coumarate 3-hydroxylase (C3H). Moreover, C3H might affect the stiffness and permeability of cell walls by regulating the S/G ratio, thereby increasing the cold resistance.[114] Similarly, under high temperature, the increase of lignin content could improve the ability of Satsuma mandarin to resist environmental stress.[115]

Abiotic stresses such as drought and salinity are the main threats to plants and often occur simultaneously, causing osmotic imbalances that lead to severe crop losses and even death.[116] Lignin regulates the osmotic balance of the cell by reducing the permeability of the cell wall and transpiration. It was found that the lignin content in the stem base of *Eucalyptus globulus* and the stem apex of *Eucalyptus globulus* increased significantly under drought conditions.[117] Srivastava et al.[118] found that, under the stress of drought and salinization, the increase of lignin content in the stem of *Leucaena* seedlings may play an important role in drought resistance.

Lignin content is an important index to evaluate the lodging resistance of plants. Liu et al.[119] explored the effect of shade stress on lignin metabolism in the soybean stem. The results

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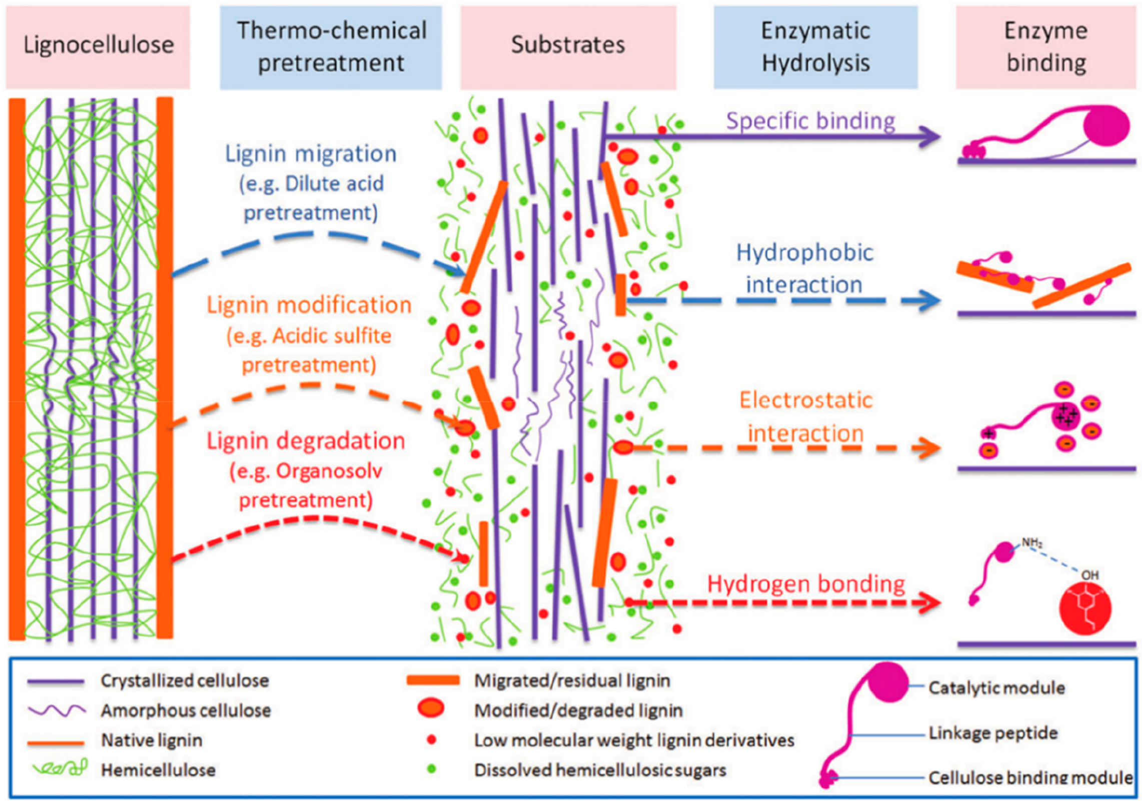

Figure 7. Schematic illustration of the cellulase−lignin interaction dependent on lignin. Reprinted with permission from ref 129. Copyright 2016 John Wiley and Sons.

showed that lignin content was negatively correlated with the actual lodging rate.

Lignin plays a pivotal role in plant pest resistance.[120] For example, Wang et al.[121] found that the transcription of *CmMYB19* was induced by aphid infection in chrysanthemums. The overexpression of *CmMYB19* increases the lignin content, thereby limiting aphid proliferation on the host. An insect-specific toxin peptide *LqhIT2* activates the phenylpropane pathway via jasmonate-mediated priming, which subsequently increases the lignin content and enhances the insect resistance of rice.[122] Sclareol induced genes related to signaling and phenylpropanoid metabolism in *Arabidopsis* roots. The resistance to the root knot nematode increases with the increase of lignin content.[123] In summary, protolignin can act as a barrier to increase plant insect resistance, either directly or through related hormone signaling pathways.

The above improvement of plant stress resistance occurs due to the accumulation of protolignin. The lignin regulator added in the process of plant development has been proved to promote plant growth. Whether it can improve plant stress resistance and its mechanism are worth exploring. Finally, in theory, plants with strong roots and well-grown plants will also have appropriate stress resistance, so whether there is a connection between plant growth and stress resistance is worthy of further discussion and refinement.

6. LIGNIN IN BIOMASS CONVERSION

6.1. Regulating the Hydrolysis of Carbohydrates. The conversion of polysaccharides to monosaccharides by enzymatic hydrolysis is an effective strategy to refine lignocellulosic biomass. From the traditional viewpoint, lignin can hinder the hydrolysis of cellulose in lignocellulosic feedstock during enzymatic hydrolysis.[124] The hindrance is mainly reflected in two aspects: one is that lignin prevents cellulase from accessing cellulose, and the other is that lignin adversely affects enzymatic hydrolysis by the unproductive adsorption of cellulase.[125−127] A large amount of research has shown that there are three noncovalent interactions between the substrate lignin and cellulase, namely, hydrophobic interaction, electrostatic association, and hydrogen bonding, which make nonproductive or ineffective adsorption between the substrate lignin and cellulase (Figure 7).[128,129] However, it must be emphasized that the addition of water-soluble lignin effectively resolves the above problems and promotes enzymatic hydrolysis. In other words, as an additive, lignin has great potential to promote enzymatic hydrolysis.

Li et al.[130] clarified that a variety of lignin inhibited enzymatic hydrolysis by different mechanisms and revealed that the structural characteristics of lignin, such as functional groups, S/G ratio, etc., affected the adsorption activity of lignin in enzymatic hydrolysis. Lignin with a high aliphatic hydroxyl group content and low carboxyl group content had high surface hydrophobicity, which increased the adsorption between enzymes and lignin and reduced the hydrolysis efficiency of enzymes. Lan et al.[131] proved that the increase of pH in the enzymatic hydrolysis process would increase the negative charge on the surface of lignin; this increases the coulomb repulsion between lignin and cellulase, which ultimately weakened the nonproductive adsorption of lignin on cellulase. This is line with the fact that the adsorption activity of lignin is affected by charge modulation of the substrate, thereby regulating the enzymatic hydrolysis.

Although the irreversible adsorption of substrate lignin to cellulase reduces the efficient enzymatic hydrolysis, water-

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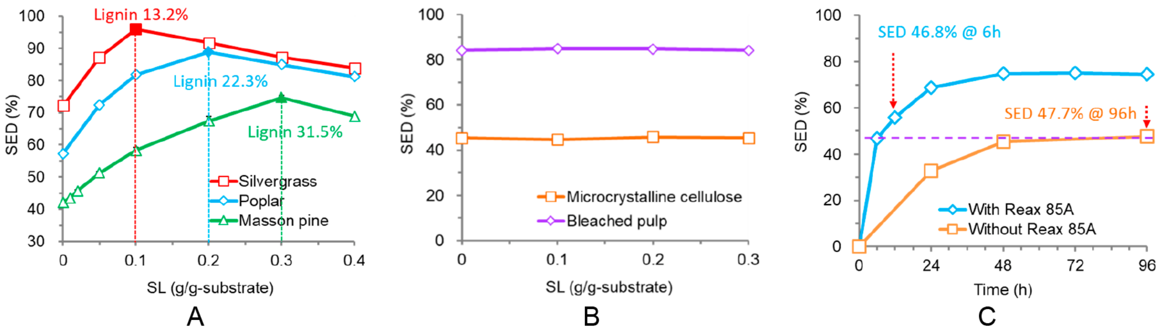

Figure 8. (A) Effect of SL addition on SED of different materials pretreated with GL; (B) Effect of SL addition on SED of other materials (without substrate lignin); (C) Effect of SL addition on the enzymatic hydrolysis time of masson pine pretreated with GL.[137] Reprinted with permission from ref 137. Copyright 2015 Elsevier.

soluble lignin improves the accessibility of cellulase and enhances enzymatic saccharification under certain conditions. Wang et al.[132] proposed that water-soluble lignosulfonate in combination with cellulase yielded the "lignin−cellulase complex" in the hydrolysis system, thus significantly reducing the nonproductive adsorption between substrate lignin and cellulase. Jiang et al.[133] improved the enzymatic hydrolysis efficiency by adding the soluble fraction of alkaline lignin in the enzymatic hydrolysis system of pretreated wheat straw. The effect of water-soluble lignin on enzymatic hydrolysis depends on the presence of substrate lignin. The molecular weight of water-soluble lignin is relatively low, and it binds to the adsorption domain of cellulase to form the "lignin−cellulase complex" to block the adsorption domain. This blockage is not conducive for the binding of cellulase to cellulose and does not promote the enzymatic hydrolysis of pure cellulose substrates. However, if lignin enriched substrate take part in the reaction, then both water-soluble lignin and substrate lignin undergo "competitive" adsorption for cellulase; this reduces the nonproductive adsorption of cellulase and substrate lignin and significantly improves the efficiency of enzymatic hydrolysis.[134,135] This explains the key mechanism of enhancing cellulase hydrolysis with the aid of water-soluble lignin.[125]

The modification of lignin to obtain polymers with specific properties or functional groups is also an effective way to promote enzymatic hydrolysis. The sulfonation of lignin can increase its surface activity, reduce the hydrophobic interaction between lignin and cellulase, and reduce the ineffective adsorption of lignin to cellulase. In recent years, some researchers have found that lignosulfonate as anionic surfactants can effectively improve the enzymatic hydrolysis of lignin fiber feedstock.[136,137] Wang et al.[137] demonstrated that sulfonated lignin (SL) promoted enzymatic hydrolysis, which was related to the substrate lignin. As shown in Figure 8, the lignin content of masson pine, poplar, and silvergrass pretreated with green liquor (GL) was 31.5%, 22.3%, and 13.2%, respectively. The substrate enzyme digestibility (SED) was improved most when the SL addition was 0.3, 0.2, and 0.1 g/g substrate, respectively. The results showed that the optimal ratio of SL to substrate lignin was around 1:1, and excessive SL caused the significant decrease of SED. Meanwhile, the addition of SL did not produce any significant effect on substrate microcrystalline cellulose and bleached pulp. The existence of substrate lignin is a necessary condition for water-soluble lignin to play its role. In addition, the addition of SL (Reax 85A) could significantly shorten the enzymatic hydrolysis time. The enzymatic hydrolysis time of masson pine reduced from 96 to 6 h after the pretreatment with SL.

The modification of the interaction between substrate lignin and cellulase is achieved by adding an appropriate amount of structurally regulated lignin to the hydrolysis system. This process has important theoretical significance for reducing the saccharification cost of the pretreatment substrate.

A further understanding of the interaction between the molecular structure of lignin (such as functional groups, structural units, bonding types, etc.) and cellulase is expected to fundamentally reduce the ineffective adsorption of lignin in the substrate and cellulase. This could ultimately improve the efficiency of enzymatic hydrolysis.

6.2. Boosting Enzymatic Protein Circulation. In addition to solving the problem of low enzymatic hydrolysis efficiency, in recent years, some research has been focused on how to improve the recovery and recycling efficiency of cellulase. Cellulase can be immobilized on lignin-based carriers through the adsorption process.[138] For example, Nonaka et al.[139] immobilized cellulase on lignophenols, and Zdarta et al.[140] immobilized cellulase on the TiO_2−lignin complex. These immobilized cellulases gained high stability and recyclability, but the hydrolysis ability tends to be weakened.

Qiu's team prepared a pH-responsive lignin-based carrier (pH-LC) by the sulfonation and quaternization of lignin.[141] pH-LC not only increased the enzymatic hydrolysis efficiency of corn cob residue to 93% but also recovered the cellulase components in the solution through electrostatic interaction after adjusting the pH to 3.2, which subsequently reduced the cost of the production process. At the same time, they also grafted a small amount of monomethoxy polyethylene glycol (MPEG) on enzymatic hydrolysis lignin (EHL) to synthesize pH-responsive surfactant EHL-MPEG and promote the enzymolysis and recovery of lignocellulosic biomass.[142] When compared with PH-LC, EHL-MPEG is more efficient and pH responsive and has a better ability to recover cellulase. Recently, Qiu et al.[143] also prepared pH-responsive lignin magnetic nanoparticles (Fe_3O_4/LSQA) for the immobilization and recovery of cellulase. By adjusting the pH of the system, cellulase was highly immobilized and desorbed on Fe_3O_4/LSQA. The immobilization rate of cellulase was 55.52%, while the desorption rate was 68.27%. The desorbed cellulase retained 31.79% of the relative activity after five cycles. From the above studies, lignin-based materials were used as additives in the enzymatic hydrolysis process, which not only effectively recycled cellulase but also highlighted the application of lignin.

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7. CONCLUSIONS AND FUTURE PERSPECTIVES

In recent years, great progress has been made in the research area linked with lignin bioactivity, which has provided a theoretical basis for the applications of lignin in medicine, health care products, food, biomass resource utilization, and agricultural production. As a renewable and biodegradable polymer, lignin displays natural bioactivities such as antioxidant, antibacterial, antiviral, and antitumor bioactivities. In addition, lignin has shown great potential in the applications associated with wound dressings, new drugs, and other medical treatments. In the food industry, researchers are no longer only concerned about the antioxidant and anti-UV properties of lignin and the corresponding food packaging materials. As a major dietary insoluble fiber, lignin is involved in the biological activity of lowering blood glucose and blood lipids. Lignin has also been shown to have an emerging role in acting as a special functional food additive. In the case of the lignocellulosic hydrolysis system, the sustainable and efficient utilization of biomass energy could be achieved by using lignin as an enzymatic hydrolysis regulator and enzyme protein recycler. In agriculture, protolignin is indispensable for the process of plant growth. Lignin also plays an important role in preventing wind, fixing sand, and restoring vegetation in desert areas.

Although the biological potential of lignin has become a hot research topic, the complex nature and undefined chemical structure of lignin limit its applications in the field of biomedicine and the food industry. Due to the inability to accurately simulate human systems, the obstacles between theory and application restrict the application of lignin in different biological fields. Some mechanisms of lignin for maximizing its application in medicine and food still need to be verified; in particular, the action mechanism and metabolic pathway of lignin in the human body should be further explored. At present, there is an urgent need for plant-based antioxidants, antibacterial agents, and drugs in pharmaceutical and medicinal industries. Additionally, the fundamental research on lignin as a growth regulator has not been accomplished sufficiently; this needs to be sorted out in order to increase its industrial applications. The research findings of the structure−activity relationship will enable the ubiquitous use of lignin in agricultural production.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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