Novel and Efficient Lignin Fractionation Processes for Tailing Lignin-Based Materials

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Emerging Technologies for Biorefineries, Biofuels, and Value-Added

Commodities

Novel and efficient lignin fractionation processes for tailing lignin-based materials

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Abstract: As the most abundant aromatic biopolymer in nature, lignin has attracted great attention due to the complexity and yet richness of its functional groups for value-added applications. The yield of production of lignin and the reactivity of prepared lignin are very important to guarantee the feasibility of lignin-based material utilization. Various fractionation techniques have been developed to obtain high yield and relative high-purity lignin as well as carbohydrates (hemicelluloses and celluloses), and to reduce the condensed and degraded nature of conventional biorefinery lignin. In this chapter, novel and efficient lignin fractionation for tailing lignin-based material preparation are summarized and discussed.

Keywords: Fractionation; Lignocelluloses; Lignin; Material

1. Introduction

Due to resource shortage and the energy crisis, biorefinery has attracted much attention. Lignin is the most abundant aromatic renewable resource in nature, accounting for 15% to 30% by weight and 40% by the energy of lignocellulosic biomass[1]. However, in the pulp and paper industry, lignin has long been treated as a waste byproduct and commonly burned to supply heat and energy due to its chemical recalcitrance and structural complexity. In recent years, with the better understanding of lignin physics and chemistry, more and more high performance materials are being developed from lignin resources, which would open not only a new design for functional materials but also a promising way for biorefinery[2].

The efficient fractionation strategies for lignin are the key and basis for biorefinery[3]. The intricate relationship among lignin, cellulose, and hemicellulose and the similarity between some components of lignin and high glycan increases the difficulty of separation[4]. So, the harsh treatment was put to issue this question, which causes an irreversible degradation and condensation reactions of lignin during traditional carbohydrate first conception of lignocellulose biorefinery. Generally speaking, lignin presents the heterogeneous structure and broad molecular weight distribution which

would severely affect its potential applications in materials[5]. In addition, the phenolic hydroxyl (PhOH) group content of lignin is one of the most important factors for chemical modification and physical interaction. Many studies showed that the β-O-4 content closely related to the reactivity of lignin[6]. However, during the cleavage of ether bonds of lignin, the stable carbon-carbon (C-C) bonds instead of the hydroxyl are formed, thus the lignin exhibits lower reactivity than native lignin. That has brought up the difficulty of catalytic depolymerization of lignin in order to modify or functionalize lignin for downstream application.

Therefore, the single target product, the serious degradation of lignin, and some other disadvantages of the traditional fractionation method need to be overcome urgently. Moreover, the presence of sulfur in lignin makes them more problematic for chemical modification and further application. Although sulfite lignin is the most widely used industrial lignin, sulfur-free lignin has greater potential applications due to it is more environmentally friendly and odor-free[7]. According to this philosophy, it is very important to obtain high purity lignin with commendable chemical reactivity. In addition, the exploitation of a lignin-based coproduct also depends on the inherent structures and chemical reactivity of the lignin polymers.

Hence, numerous researches have been made to develop novel fractionation approaches to isolate lignin from biomass and to obtain chemicals economically valuable for several applications. Based on concept to utilize the entire lignocellulose substrate, alkali-based fractionation, organosolv fractionation, ionic liquids (ILs) fractionation, and deep eutectic solvents (DESs) fractionation approaches are recently gradually developed and have received the attention among academia and industries. Enzymes can also be used to assist the separation of lignin by e.g. hydrolyzing carbohydrates. What's more, several methods for further fractionation of lignin has been studied to obtain specific molecular lignin fractions with defined properties.

The main objectives of the lignin fractionation process are to obtain relatively high-purity lignin besides carbohydrates (hemicelluloses and celluloses), and to reduce the condensed and degraded nature of conventional biorefinery lignin. Importantly,

fractionation should meet the following requirements:

- Based on all-component separation

- Avoid the further condensation of lignin

- Increased the stability and uniformity of lignin

2. Lignin Properties

2.1 The basic structural features of lignin

Lignin, as one of the three major components of plant raw materials, has a complex structure. Numerous studies have confirmed that the chemical structure of lignin consists of phenylpropane units, originating from three aromatic alcohol precursors (monolignols), p-coumaryl, coniferyl, and sinapyl alcohols. The phenolic substructures that originate from these monolignols are called p-hydroxyphenyl (H, from coumaryl alcohol), guaiacyl (G, from coniferyl alcohol), and syringyl (S, from sinapylalcohol) moieties, as shown in Fig. 1[8]. Softwood lignins mainly consist of G units whereas hardwood lignins are mainly composed of G and S units. The lignins from non-wood such as wheat straw are mainly composed of G, S and H units.
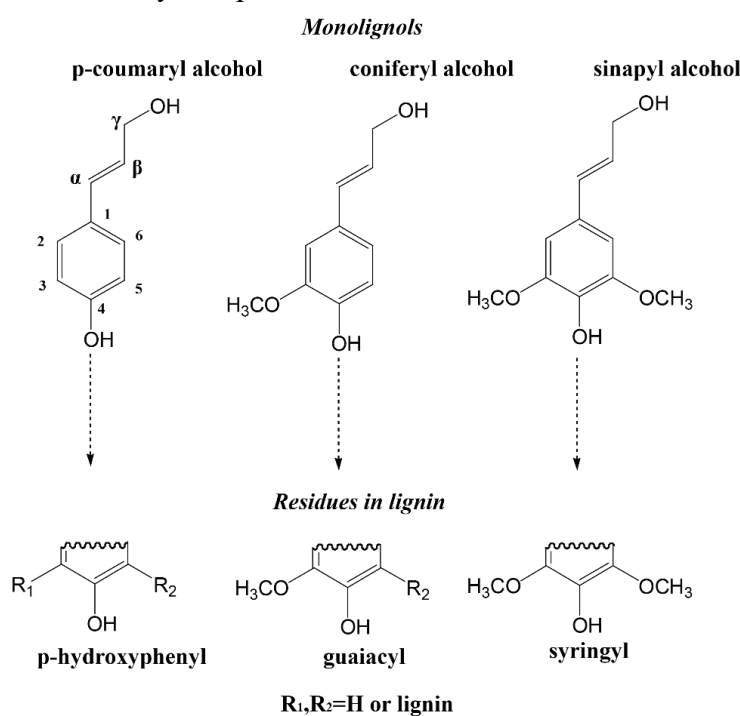
Monolignols

p-coumaryl alcohol   coniferyl alcohol   sinapyl alcohol

Residues in lignin

p-hydroxyphenyl   guaiacyl   syringyl

R₁,R₂=H or lignin

2.2 Functional groups of lignin

In the complex structure of lignin, there are aromatic, phenolic, alcohol, carbonyl, methoxy, carboxyl, conjugated double bond and other active groups, which can carry out many chemical reactions such as oxidation, reduction, hydrolysis, alcoholysis, acidolysis, photolysis, acylation, sulfonation, alkylation, halogenation, nitration, polycondensation or graft copolymerization.

The typical C-O links are β-O-4, α-O-4 and 4-O-5; and C-C links are β-5, 5-5, β-1 and β-β linkage (Fig.2). Especially, β-O-4 plays an important role in the internal connection of lignin, accounting for 45% ~ 60% of the total number of all linkages. In addition, there are also LCC (lignin-carbohydrate complexes) in lignin[9].
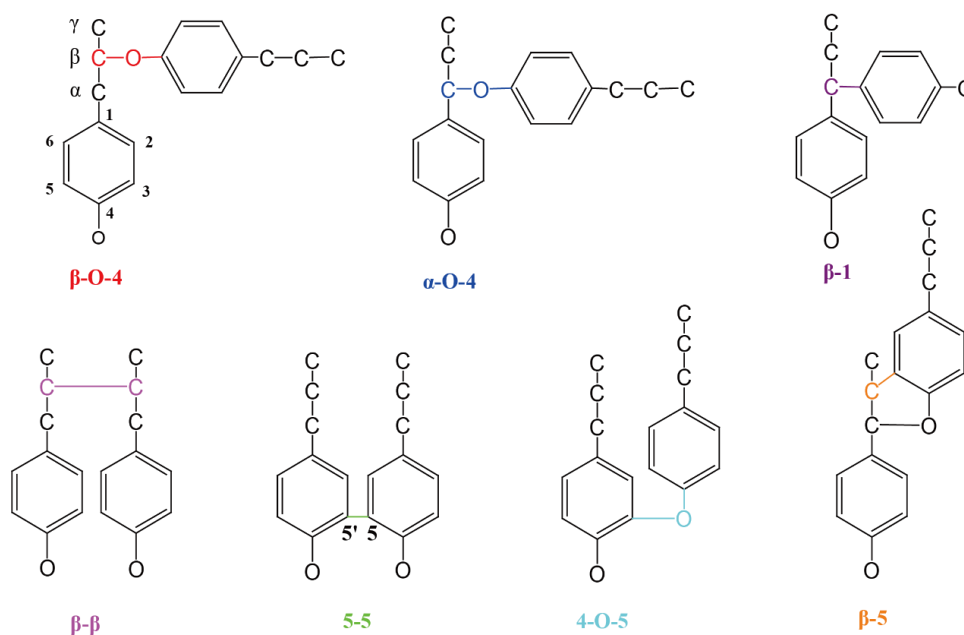
Fig. 2. Characteristic linkages in lignin[10]

3. Lignin Fractionation Process

3.1 Alkali-based fractionation

The deprotonation of phenolic OH-groups makes the lignin easily dissolved in alkaline media[11]. Therefore, the treatment of lignocellulosic biomass is usually in alkaline conditions. For example, nowadays, the well-known traditional pulping processes are kraft pulping, sulfite pulping, and soda pulping. But the lignin obtained by the kraft and sulfite pulping process containing the sulfur and the structure of the lignin was severely damaged after soda pulping, which can complicate lignin downstream valorization.

Alkali-based pretreatment was investigated, which utilizes various kinds of alkaline (e.g., NaOH, $Ca(OH)_2$, ammonia, etc.). Typically, it can efficient to treat the graminaceous feedstocks due to its lower severity of the treatment compared to traditional soda pulping[12]. Obtaining the lignin from wood after alkali-based pretreatment may need harsher conditions that risk altering the lignin structure[13]. The common reagent used in alkali-based fractionation was the NaOH, followed by was ammonia which with several well-known benefits, such as the easy recovery of solvent, the preserve of lignin structure and alters cellulose crystalline structure[14]. Up to now, some ammonia based on extraction and isolation procedures are available, mainly including ammonia fiber explosion (AFEX)[15], anhydrous ammonia pretreatment (AAP) [16]and recycled aqueous ammonia expansion (RAAE)[17].

Ammonia fiber expansion (AFEX) is a promising thermochemical pretreatment which only makes the cell walls changed to enhance cellulase accessibility without the lignin and hemicelluloses removal. The mechanism of AFEX was discussed by Chundawat et al., they found the Ammonia penetrates the cell walls from the outer walls facing the lumen and middle lamella which would facilitate various ester linkages at first, then decomposition products could be allowed onto outer wall surfaces with the rapid pressure release and hence results in increased wall porosity (white spaces) (Fig.3).

During the process, the biomass matrix cannot fractionate to the lignin fraction, but the following lignin extraction were benefiting a lot from AFEX, which can up to 50% removal of the AFEX-pretreated feedstocks by organic or alkaline solution[18]. Subsequently, the mechanism and the causes for improved digestibility of AFEX pretreated materials were further studied, the results show that it is the disruption of lignin-carbohydrate linkages of mainly polymeric lignin that contribute to the efficiency of AFEX pretreatment, and the process shows minimum change to feedstocks which can preserve the lignin structure[19].

Another method is anhydrous ammonia pretreatment (AAP). Mittal et al. use this method followed by a mild NaOH extraction (0.1 M NaOH, 25 °C) to treat corn stover with the result of more than 65% lignin removal[16]. The extracts of lignin show considerable antimicrobial and antioxidant activities, when corn stover treated by low-moisture anhydrous ammonia (LMAA), subsequently extracted its lignin by 4% (w/v) sodium hydroxide [20].

Besides, a novel technology named recycled aqueous ammonia expansion (RAAE) was investigated with the object to get high biomass digestibility under relatively a lower temperature and shorter pretreatment time. After RAAE pretreatment, about 50-75% lignin was removed while most of the carbohydrates were preserved. Specifically, only by 11 min treatment at 85 °C, 80% water to dry corn stalks loading and 1.5 L/min ammonia flow rate, up to 68.3% lignin was removed and 85.69% of glucan digestibility was achieved[17].
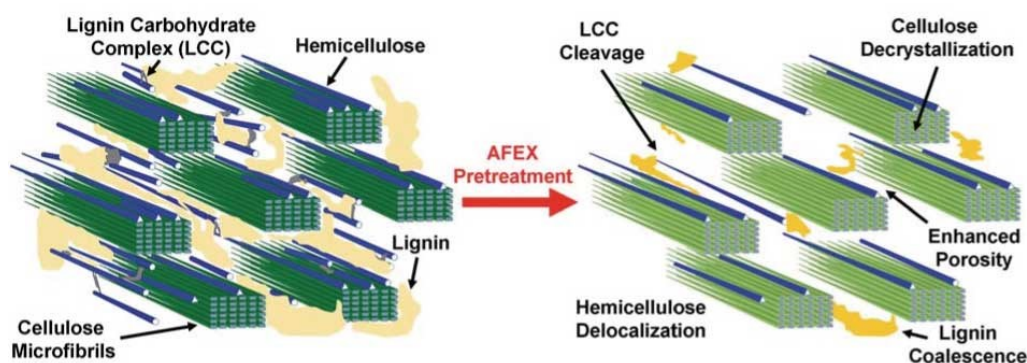

Fig. 3 Schematic models for lignocellulosic cell walls depicting overall nanoscale ultrastructural modifications as a

result of ammonia based pretreatments. Different components of the cell wall depicted are: cellulose (green lines), hemicelluloses (blue strings), lignin (yellow matrix). [18].

In addition, a few modified aqueous ammonia methods were used in the pretreatment process of biomass. For example, the sugarcane bagasse treat by aqueous ammonia with glycerol (AAWG) at 120°C~180°C with 49.34%~77.46% delignification rates, which indicates that the level of delignification is directly related to temperature[21]. A modified aqueous ammonia soaking (AAS) method was used to treat the eucalyptus, and the results show that the addition of H_2O_2 to the AAS could further promote the delignification of the eucalyptus[22].

The ammonia-based fractionation process is sustainable for large-scale applications without containing carbon or release greenhouse gas (GHG) contain[23]. Although the ammonia-based fractionation is more preferable to limit ether bond cleavage due to milder treatment conditions, ammonia lignin residue showed higher cellulase adsorption affinity compared to organosolv lignin, which reduced cellulose hydrolysis[24].

3.2 Organosolv fractionation

Organosolv fractionation is a promising process using an organic solvent to dissolve lignin from the plant cell walls for effective separation of lignin. The lignin was separated in the recovery process to achieve the purpose of the comprehensive utilization of components, which fits well into the concept of sustainable biorefinery (Fig. 4). Unlike impure lignin obtained by the traditional pulping process, e.g. kraft and sulfite process, the lignin obtained from organosolv fractionation is free of sulfur and the molecular weight is low compared with other lignin production methods. Moreover, nontoxic reagent organic solvent can be recovered during organosolv fractionation. A wide range of organic solvents, mainly including organic alcohols (i.e., methanol, ethanol), organic acids (i.e., Acetic acid, formic acid), esters, and combined solvents with and without acid or alkaline catalysts are used in biomass fractionation. The catalysts used are acid, a base, or a salt.

These solvents providing selective solubilization of lignin from various lignocellulosic biomass including poplar wood, wheat straw, and eucalyptus, etc. The excellent solubility to dissolve lignin results in slight chemical modification and the mechanisms are considered as the cleavage of aryl ether bonds. During the organosolv delignification, the β-O-aryl ether bonds are more hardly broken than the α-O-aryl ether bonds which normally need more severe conditions, especially at elevated acid concentrations[25].

Despite organosolv fractionation or pulping has been utilized for more than 100 years, the application is still not widely used in industrial production due to considering high equipment requirements and the dangers, toxic and costs-increase posed by the use of solvents[26]. In addition, organosolv fractionation for non-wood feedstocks (e.g., corn stover, wheat straw, rice straw, bamboo, etc.) exhibit better effect relative to woody feedstocks. These feedstocks represent a small fraction of the global chemical pulping market but may represent a significant feedstock for biorefining processes. Up to now, several organosolv fractionations based on extraction and isolation procedures are available. The main fractionations including alcohols based fractionation and organic acid-based fractionation.
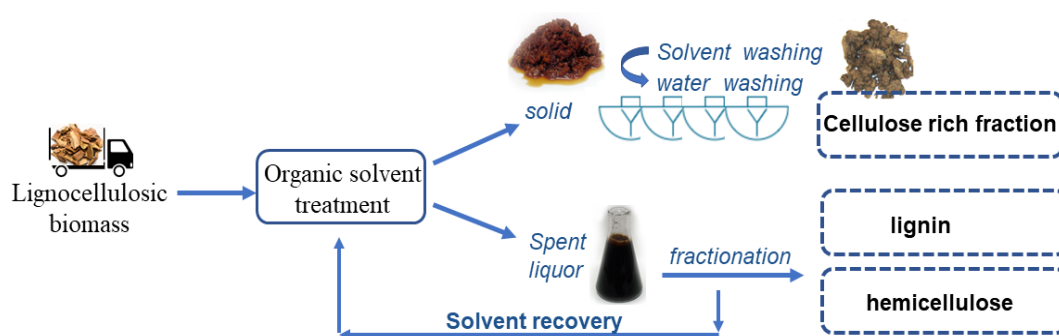

Fig. 4. Flowchart of organic solvent treatment of lignocellulosic biomass.

3.2.1 Alcohols based fractionation

For alcohols-based fractionation, ethanol pulping is the earliest and has the most

reports. Methanol is cheaper and yields higher pulp than ethanol, but it is toxic. So, the ethanol has been considered one potential solvent to treat lignocellulosic. Generally, the ethanol fractionation process is carried out under elevated temperatures without or with the addition of the acidic or alkaline catalysts.

Temperatures used in the process can be as high as 200 °C, but lower temperatures can also be used depending on the type of biomass and the use of a catalyst. The process produces three main fractions including high purity lignin, a relatively pure cellulose fraction, and hemicellulose-derived products (mainly xylose).

Wheat straw is fractionated by using autocatalytic and acid-catalyzed ethanol organosolv and the lignin yield of 84% was reached without the use of a catalyst (organosolv at 210 °C, 50% w/w aqueous EtOH). Similar results were obtained at 190 °C using 30 mM H_2SO_4 as the catalyst and 60% w/w aqueous EtOH[27].

Lignin condensation is unavoidable reaction during acidic or alkaline ethanol fractionation process. It is formed via formation of the carbocation since it easily form bond with an electron-rich carbon atom in the aromatic ring of another lignin units.[28] The obtained solid residues are separated by filtration and washed with warm organosolv solvent avoiding dissolved lignin redeposition. Then, the lignin was obtained by precipitation with water. The treatment of willow use 2-PrOH/H_2O (7:3) at 190 °C for 10 h which show that the obtained lignin yield can reach 61% with high purity (93%)[29].

Obviously, ethanol fractionation yields relatively clean and impurity-free lignin that can be directly used for the production of value-added materials. But there are some factors limiting the application, such as limited delignification (high kappa number), safety problems caused by high temperature and pressure.

Based on flow-through fractionation development, the flow-through setup provided lignin with a β-O-4 content in the range of 45–60 β-O-4 linking motifs, whereas in the batch system the β-O-4 content decreased to 8–30 β-O-4 linking motifs for the extractions performed with over 80% EtOH[30].

3.2.2 Organic acid-based fractionation

Because of effective hydrolysis and extensive delignification organic acids, primarily acetic acid, and formic acid, have been extensively employed in the biomass fractionation process. Moreover, the ash content is lower than traditional pulping, for example, approximately 64% of ash or 83% of silica in wheat straw remained in the pulp after atmospheric acetic acid (AcOH) pulping [31], lower impurities (ash 0–0.1%) in formic acid lignins[32]. Due to a lower boiling point than acetic acid, formic acid had been recognized as a promising agent for organosolv pulping and fractionation. As a volatile weak organic acid, formic acid can be converted directly from the fraction of lignocelluloses or directly recycled for delignification[33].

Chempolis and CIMV processes involve using formic acid for the treatment of non-wood fibre sources in a single-stage process. DapíA et al. demonstrated that beech wood could be fractionated using 80% formic acid at a temperature from 110 °C to 130 °C[34]. Zhang et al. obtained the FA-lignin (FAL) with high guaiacyl content by using one-step mild formic acid pretreatment, and FAL fraction exhibited a loose structure which is prominent for its further catalytic conversion into chemicals and energy[35]. Similarly, after one-step formic acid hydrolysis (85 °C, 5 h), about 80% of lignin was removed from corn husk, and the obtained lignin with high-purity (>99%) would have potential for phenol-formaldehyde resin and concrete water-reducer applications[36].

In order to realize delignification, severe experimental conditions were needed such as increasing temperature and pressure. So, the β-O-4 bonds are broken during the formic acid fractionation, which resulting the increase of phenol hydroxyl content, the formation of C-C condensation structure and the side chain formylation of lignin. Although the yield of lignin separated by this method is high, the high value potential use of lignin is greatly limited due to a large number of β-O-4 broken.

For example, bamboo was fractionated using formic acid under high pressure (at 145 °C for 45 min, 0.3 MPa), presents a quick and efficient delignification method by enhancing the cleavage of interunitary bonds in lignin (β-O-4′, β-β, and β-5′), and

dissolved lignin also occurred condensation reaction [37].

Formic acid fractionation can be conducted under low temperatures and at atmospheric pressure[33], but it still needs more treatment or long reaction time to avoid lignin condensation, e.g. 60 °C for 8 h[38], 105 °C for 3 h[39]. Li et al. reported bamboo using the Milox method at 101 °C for 2 h achieved the highest delignification (88.9%)[32]. After acetic and formic acid treatment, M. x giganteus showed a low S/G ratio (0.7) and the β-O-4 linkages lost during organosolv fractionation up to 21% and 32%, respectively[40]. It can conclude that extensive degradation of lignin occurred as a result of long reaction time as indicated by a significant reduction of aryl-ether linkages.

These fractionations using formic acid-based organosolv processes are in a batch operation. In order to develop organosolv processes for commercialization, developing a continuous treatment system suitable for flexible biomass feedstocks should be considered[41]. Recently, the flow-through strategy was successively applied in lignin-first biorefinery [42] and hemicelluloses extraction [43]. The flow-through strategy had been verified to be an alternative for effective biomass fractionation with insignificant degradation of lignin and carbohydrate. Wang et al. use 72wt% aqueous formic acid by flow-through fractionation of biomass which shows similar lignin yields compared to batch extractions but retains high β-O-4 ether bond[44,45].

3.3 Ionic liquid (IL)-assisted fractionation

Ionic liquids (ILs) have received considerable attention and have been extensively exploited for biomass fractionation due to their excellent ability to destroy the crystalline structure of cellulose or remove lignin/hemicelluloses, the low equipment and energy costs requirement. ILs are mainly composed of cations (generally organic) and anions (organic or inorganic)[46]. The use of ILs as biomass processing solvents started with the discovery of cellulose dissolving ILs[47].

Ionic liquids (ILs) can selectively break the bond between cellulose, hemicellulose, and lignin. According to selectivity of ILs, the methods of lignin extraction from

lignocellulosic biomass can be classfied into partial and total dissolution systems (Fig.5). The former IL-based system, only lignin and hemicellulose can be extracted by ILs while the cellulose fraction remains in the form of a solid pulp, then the lignin can be precipitated by antisolvent [48].In the latter system, the entire lignocellulose substrate can be dissolved, then cellulose and lignin were precipitated respectively from the product mixture by the addition of an antisolvent (organic or aqueous–organic solution) [49]. Recent researches on lignin fractionation in ionic liquids have been listed in Table 1.
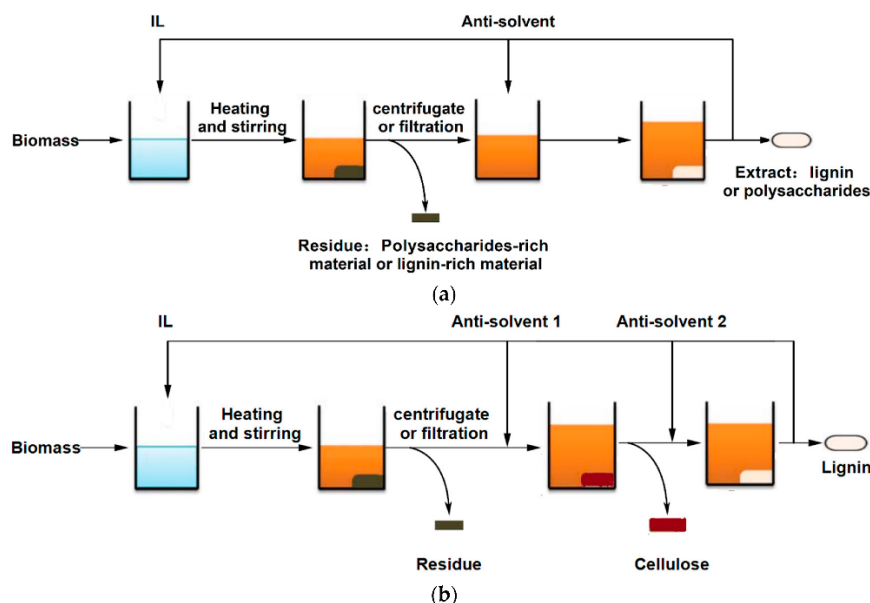
Fig. 5. Fractionation procedure of lignocellulose with ILs: (a) extraction of lignin or polysaccharide using selective ILs（partial dissolution systems) and (b) separation of cellulose and lignin with non-selective ILs (full dissolution systems)[50].

Table 1: Ionic liquids used for lignin fractionation.

Substrate	Ionic liquids[a]	Conditions	Results	Ref.
Corn stover	[HMIM]Cl	70°C, 3 h	lignin yield 60.48%	[48]
Wheat straw	[emim][HSO$_4$]	131.0°C, 58.7 wt.% H$_2$O and 88.0 min, followed by alkaline extraction and acidified	lignin yield 42.6 wt.%	[51]

Miscanthus x giganteus	[TEA][HSO₄]	120°C, 8 h, 1:10	88% delignification	[52]
Poplar	[Hpy]Cl and [Hmim]Cl	100°C for 30 min	lignin yield 61.0% and 60.4%	[53]
Bamboo	[Hpy]Cl and [Hmim]Cl	100°C for 30 min	lignin yield 51.7% and 50.3%	[53]
Eucalyptus	[bmim]OAc	120 °C, 4 h	29.7−43.3% delignification	[54]
Alkaline lignin	[C₂mim]-[OAc]	110-170°C, 1-16 h	lignin yield (60%-93%)	[55]

[a]Ionic liquids: [HMIM]Cl: 1-*H*-3-methy-limidazolium chloride, [emim][HSO₄]:1-ethyl-3-methylimidazolium hydrogen sulfate, [TEA][HSO₄]:triethylammonium hydrogen sulfate, [Hpy]Cl: Pyridinium chloride, [bmim]OAc:1-butyl-3-methylimidazolium acetate, [C₂mim]-[OAc]: 1-ethyl-3-methylimidazolium acetate.

Among numerous ILs, 1-ethyl-3-methylimidazolium acetate ([C₂mim]-[OAc]) was demonstrated to effectively extract lignin from lignocellulose raw material, such as wood[56], wheat straw, etc. Bogel-Łukasik et al. investigated the fractionation process of the wheat straw in 1-ethyl-3-methylimidazolium acetate ([emim][CH₃COO]) and reported that 87wt% purity of the carbohydrate-free lignin-rich fraction can be recovered[57]. And the chemical transformations involved in the [C₂mim]-[OAc] pretreatment was more deeply investigated[58].

Various process parameters would affect the dissolution of lignocellulosic material，such as the effect of IL anion or cation, solvation properties of ILs, and viscosity, etc. These relevant parameters were summarized by Badgujar and Bhanage [59], who suggested that IL should in the context of green sustainable chemistry and IL properties should be optimized for biomass processing. A key example is the high viscosity of most ionic liquids, so several studies try to solve this issue. For example, increasing the temperature may be a method to reduce IL viscosity[55]. In addition, the currently popular ionic liquids in lignocellulosic biomass treatment such as the imidazolium

based ionic liquids are likely to remain expensive and their preparation is often very difficult. Therefore, the recyclability of ionic liquids is necessary. Up to now, numerous investigations of the use of IL have focused on the cost and recyclable problems. Up to 85% of the lignin can be solubilized into the IL solution 80% lignin recovery can be achieved by using the low-cost ionic liquid triethylammonium hydrogen sulfate [TEA][HSO$_4$] at mild temperature (120 °C)[52]. The lignin-rich solid fractions were obtained with distinct purities and yields by aqueous ionic liquid solution ([emim][HSO$_4$]/H$_2$O), and the IL was successful separation, recovery and reused [51].

3.4 Deep eutectic solvents (DESs) fractionation

Issues such as toxicity, poor biodegradability, and high cost have nevertheless restricted the implementation of ILs. In order to overcome these problems, a new solvent-deep eutectic solvent (DES, also known as the third generation of ionic liquids) has entered the field of vision of researchers. The term deep eutectic solvents (DESs) was coined by Abbott and co-workers in 2003[60]. It has important significance as a substitute for traditional ionic liquids for the isolation and extraction of lignin. Deep eutectic solvents (DESs) are solvents that are synthesized in a liquid state at room temperature by at least one hydrogen bond donor (HBD) usually quaternary ammonium salts and one hydrogen bond acceptor (HBA) such as alcohols or carboxylic acids.

Francisco et al. find DESs show high lignin solubility and very poor or negligible cellulose solubility[61], and the properties of DESs can be easily tuned by changing the HBDs and HBAs[62]. Mechanism investigation revealed that the functional groups in the DESs significantly affected their ability to dissolve lignin**[63]**, and it can evaluate the potential of DES for lignin processing more closely. In addition, the parameters such as temperature, pH and DES viscosity play a role in the fractionation of lignocellulosic components by DES. Chen et al. found that the molar ratio of ChCl–PCA (1:1) would allow the formation of more hydrogen bonds which is helpful for the stability of the solvents and lignin removal.[64].

Several notable studies have highlighted the benefits of DESs for the preparation of

lignin. Yu et al. have demonstrated that both the hydrogen bonds and ether bonds in lignin–carbohydrate complexes could be cleaved during DESs pretreatment, thereby facilitating the selective extraction of lignin[65]. The lignin yield by ChCl and HBD (acetic acid, lactic acid, levulinic acid, and glycerin) could reach 78% and 58% from poplar and D. fir respectively, and the extracted lignin has high purity (95%) with unique structural properties[66]. Although the main delignification mechanism employed by DES treatment is the cleavage of ether linkages, the re-condensation of lignin fragments was little occurred (Fig. 6). Singh et al. use lignin-derived compounds as potential raw materials for DES preparation to biomass processes and the lignin removal (60.8%) provided by ChCl-PCA. Higher lignin yield (up to 80%) could obtain from prairie cordgrass (PCG) and switchgrass (SWG) biomass[67].
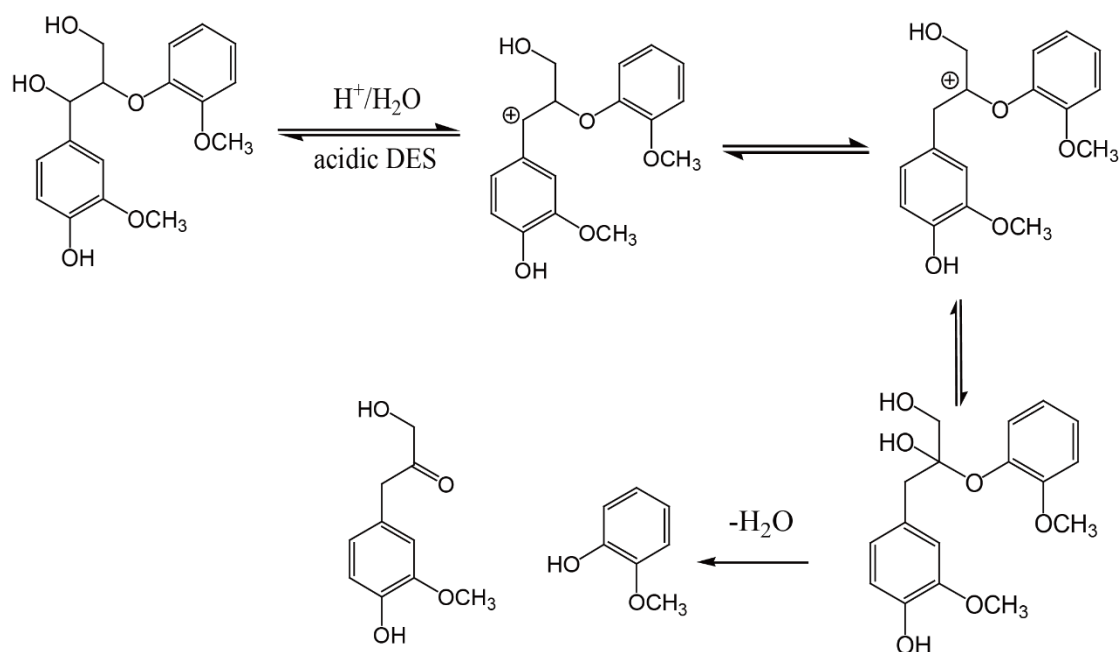
Fig. 6. Main mechanisms of β-O-4 linkage cleavage reaction during DES treatment of lignin based on study of model compound guaiacylglycerol-β-guaiacyl ether[66].

Generally speaking, there is still much room for improvement in the fractionation of lignocellulosic biomass and the extraction of lignin using DES. The integration of DES pretreatment with other technologies show good results. For example, the lignin removal can reach 65–80% using acidic ChCl:lactic acid pretreatment with ultrafast 45 s microwave heating at 800 W and the purity reach 85–87%[68]. Recently, the green

processing of lignocellulosic biomass and its derivatives in the DESs system has been comprehensively and critically reviewed. The authors pointed out that DESs are attractive solvents for the fractionation of lignocelluloses and the valorization of lignin[69]. Another review stressed three key parts: performance of varying types of DESs and pretreatment schemes for biopolymer fractionation, properties and conversion of fractionated saccharides, as well as DES, extracted lignin[70].

In spite of the rapid development in the past ten years, some drawbacks still restricted the practical applications of these solvent systems, such as the thermal instability, susceptibility to contaminants[69]. In another aspect, the ideal DES type for biomass conversion is still uncertain, and the studies about the application using the DES lignin still not enough. There is still much research to be done for the DES fractionation technology.

3.5 Enzyme assisted fractionation

Usually, Milled wood lignin (MWL) was prepared for the lignin structural characterization research, which was considered to be the most similar to native lignin. The isolation of lignin has a detailed introduction[71]. The obtained lignin has low yield, which maybe half that of the crude preparation and its residual carbohydrate content is about 4%. Subsequently, enzyme assisted extraction to obtain lignin was developed which mainly uses cellulase to hydrolyze cellulose aiming to remove carbohydrates. The assistant function of enzyme improves the yield of lignin and the obtained lignin was named as cellulolytic enzyme lignin (CEL).

The addition of enzymes into lignin fractionation would reduce the use of toxic chemicals with economic and environmental protection advantages. But it may extend treat time, so enzymatic mild acidolysis lignin (EMAL), one modified method was investigated to improve lignin recovery and purity[72]. This strategy is usually used in laboratory research. Li et al compare the pyrolysis of wood and herbaceous EMAL find that high phenolics yield can obtain at mild pyrolysis temperature of 450 °C to 650 °C[73].

There is no reason not to acknowledge that the enzymatic treatment has successfully assisting in lignin extraction, such as mild reaction conditions, the use of renewable and inexpensive biocatalysts, reduction in the use of toxic chemicals. Enzymatic hydrolysis was applied after mild alkaline treatments in situ to the ball-milled swollen cell wall and the yield of swollen residual enzyme lignin (SREL) can reach 95%[74]. The novel method combined the alkaline treatment and enzymatic hydrolysis, which not only has little change on the lignin structures but also obtain the syringyl-rich lignin macromolecules as compared to CEL.

The enzyme also use in further fractionation of the lignin. For example, two common lignin (alkaline lignin: AL, and hydrolysis lignin: HL) were treated with laccase, and the results show that the structure, especially the Ph-OH contents and molecular weight of lignin could be effectively controlled which benefits for the antioxidant application[75].

3.6 Strategies to quench reaction during lignin fractionation

During lignin fractionation, ether bonds are cleaved and a new stable carbon-carbon (C-C) bonds are formed, which is an undesirable reaction, limiting the lignin activity for added-value using. It is well acknowledged that the use of acid and/or high temperatures during lignin fractionation leads to severe and irreversible condensation, so the mild treatment conditions can avoid condensation to some extent. Wang et al. obtained the lignin (up to 90% of original lignin removal) using one-pot method for poplar fractionation by acidic water/phenol pretreatment at mild temperature (120 °C), and the phenol phase, which contains depolymerized and phenolated lignin, is directly used to prepare lignin-based phenolic foam, with satisfactory properties[76]. In order to develop a new solvent with mild treatment conditions, the separation of lignin with high yield and high activity can also be achieved by appropriate physical and chemical quenching methods.

In order to avoid condensation, strategies to prevent structural degradation is the direct hydrogenolysis of native lignin in biomass. Currently, A "lignin-first" biorefinery

concept was proposed, which mainly including tandem depolymerization–stabilization of native lignin and active preservation of β-O-4 bonds[77-79]. However, there are still several important obstacles, such as catalyst recovery and mass transfer limitations, which have nevertheless restricted the large-scale implementation of this method. So, Shuai et al.[80] reported a lignin stabilization strategy by forming 1,3-dioxane structure using formaldehyde, which can block reactive positions without extra catalysts.

However, these yields uncondensed or less condensed strategies require extra chemicals or catalysts. Different from these strategies, a simple but effective strategy to preserve lignin structures by employing rapid flow-through fractionation was applied over the last few decades. The diffusive flux remains high in a flow-through setup and the lignin concentration is relatively low, as a fresh solvent is constantly added to the system. In a batch system, the lignin concentration is increased during the extraction, resulting in a decrease in diffusive flux as a function of time. In another word, when operating in flow-through mode, the dissolved lignin fragments are removed from the heating zone, which limits the extent of structural alteration and redeposition. Wang et al. use 72wt% aqueous formic acid by flow-through fractionation of biomass which shows similar lignin yields compared to batch extractions but retains high β-O-4 ether bond[81] (Fig. 7A). Using acidic alcohol-water mixtures (120 °C) by flow-through fractionation show the similar results that extraction efficiencies of over 55% were achieved, yielding lignin with good structural quality in terms of β-O-4 linking motifs (typically over 60 per 100 aromatic units from Fig. 7B)[30]. In addition, sequential extraction (SF) of hemicellulose and lignin to avoid excessive degradation of them have been described[82,83]. The second step of SF was characterized by mild conditions of 100 °C, 40wt% p-TsOH, and one hour, and achieved 83 % lignin dissolution with a well-preserved structure.
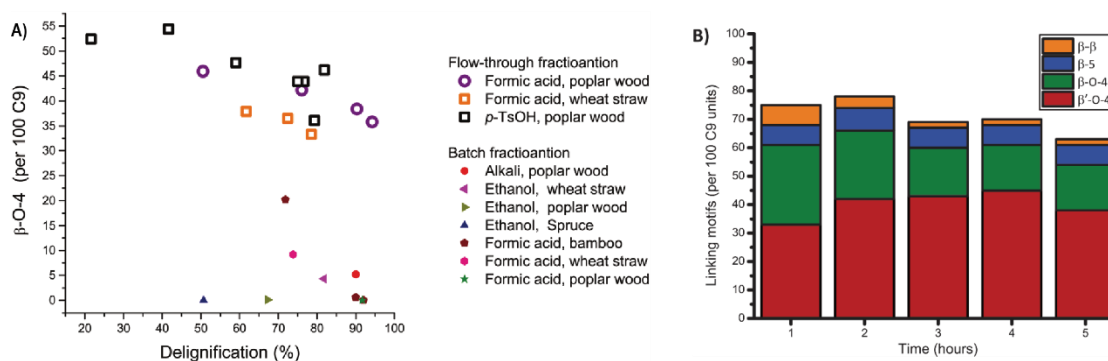
Fig. 7. A) Correlation between delignification and β-O-4 linkages during fractionation[44]. B) Linking motifs distribution during the mild organosolv extraction (80:20 EtOH/H$_2$O, 0.18 M H$_2$SO$_4$, 120˚C, 5 h)[30]

The above discussion showed that even if the cleavage of β-O-4 ether bond could not be prevented during the lignin fractionation, further C-C bonds condensation reaction could be prevented by appropriate quenching methods for improving the reactivity of lignin after fractionation.

4. Further Fractionation of Lignin

Although numerous studies have showed to obtain the lignin for further application, the lignin presents a broad molecular weight distribution that limits the comprehensive and efficient utilization. Therefore, the further fractionation of lignin seems to be a preferential way as it can achieve specific molecular lignin fractions with defined properties.

Until now, many studies have reported about the methods to fractionate lignin. Table 1 highlights the major fractionation conditions, molecular weights distribution, and potential applications. After further fractionation of lignin, the differences in their chemical structure were investigated. The lignin obtained by ultrafiltration present narrow molecular weight distribution in the results of the size exclusion chromatography (Table 1). After the ultrafiltration, the obtained lignin can be used as a bio-based components in blends with polyethylene due to low molecular weight (Mw) and a high quantity of phenolic hydroxyl groups[84]. As mature technology is widely used in industry, but there are some disadvantages, for example, the existence of partial polysaccharides, fouling of the membranes and expensive instrumentation. Compare to

ultrafiltration, differential precipitation was an easier and simpler technique and less energy consuming. Labidi et al. divided kraft lignin into three fractions by precipitation at different pH conditions with various acids (sulphuric acid and hydrochloric acid), and the effect of the process and the precipitation technique on lignin composition were analyzed. The fraction of lignin with higher Mw were precipitated at higher pH due to this fraction is easier to destabilize (Table 2)[85]. Subsequently, sequential acid precipitation method showed the similar results that lignins with a higher molecular mass were obtained at higher pH values[86].

Solvent fractionation is based on partial solubility of polymers into solvents. Jiang et al. using the solvent assisted fractionation method of kraft lignin yielded four fractions with varied molecular weights and polydispersities[87]. These solvents are inexpensive, but toxic. GVL is arousing the interest of researchers, due to its better stability, harmlessness, biodegradability, and recyclability. Wang et al. fractionated enzymatic hydrolysis lignin (EHL) into three parts only by GVL/water solvent (GWS), the GVL subsequent reuse for lignin fractionation, and they found that the obtained fractions presented significantly low molecular weight polydispersity and structural heterogeneity[88]. Another renewable solvent, ethanol-water solution, was successfully used to fractionate EHL. The high-molecular-weight lignin obtained by 80% ethanol-water (v/v) fractionation of EHL, and its antioxidant activity improved followed by depolymerization[89]. Also, the antimicrobial activity of EHL was improved after ethanol-water fractionation[90]. Based on the concept of green environmental protection, glycerol was applied with ethanol for lignin fractionation and the results showed that this approach could realize the efficient fractionation of EHL with relatively narrow polydispersity[91]. In addition, Dai et al. developed a novel hydrogel-assisted fractionation approach using lignin-containing cellulose (Cell-AL) hydrogel to fractionate lignin to produce uniform lignins[92]. Depending on the future use of the lignin, the right technique to obtain the fractions has to be chosen.

Table 2 A list of major methods for further fractionation of lignin.

Lignin type	Fractionation conditions	Molecular weight distribution	Ref.

			$\bar{M}w$	$\bar{M}n$	D($\bar{M}w/\bar{M}n$)	
Enzymatic hydrolysis lignin (EHL)	gradient precipitation 40%, 30%, and 5% aqueous GVL solutions	F1	11135	7131	1.56	[88]
		F2	8310	4704	1.77	
		F3	4235	2277	1.86	
		EHL	8930	4420	2.02	
Bamboo kraft lignin (BKL)	95% ethanol, solid-liquid ratio of 1:20, ambient temperature	Fs	2518		1.79	[93]
		Fi	5216		1.65	
		BKL	4032		2.04	
Black liquor (alkaline pulping)	ultrafiltration	Rough	5654	1879	3.01	[94]
		>15kDa	6300	2032	3.10	
		15kDa	3544	1891	1.87	
		10kDa	20221	946	2.14	
		5kDa	1806	940	1.92	
Black liquor (kraft pulp)	acid precipitation	LKS 2	3522	695	5.06	[85]
		LKS 4	5741	852	6.73	
		LKS 6	4575	750	6.10	
		LKH 2	4993	866	5.76	
		LKH 4	5798	895	6.47	
		LKH 6	6760	1054	6.41	

5. Lignin-based Material and Applications

Based on the richness of its functional groups and high carbon contents, lignin has been known as a very useful macromolecule in material industries[95]. Generally, lignin can be utilized as a replacement in different polymeric materials by modification or not [96], such as polyurethane products that including rigid and flexible foams, adhesives, coatings, and elastomers[96,97]. A variety of polymers can be derived from lignin by simple chemical modification in order to overcome the brittle nature and its

incompatibility with other polymer systems, e.g. the esterification of lignin[98] Dai et al. developed a novel functional lignin-based filler and found that lignin-graft-poly(D-lactic acid) (LG-g-PDLA) could accelerate crystallization in PLLA systems[99]. The hydroxyl group acts as a reaction site for the above chemical reaction. But there are several factors limited to its application including some technical lignin may contain sulfur leading to products yellowing[100]; polydisperse lignin show low solubility may lead to the difficult application; low reactivity leads to the poor incorporation into a polymer matrix. So, the obtaining of relative purity and high reactivity lignin is essential for lignin valorization.

Up to now, the application of lignin-based functional materials has changed from the direct physical blending and incorporate into other polymer materials to as a new functional material for high value-added applications. There are two main ways to prepare lignin-based functional materials including preparing lignin nanostructured functional materials and lignin carbon functional materials. The former materials are based on its own structure and performance characteristics. The latter materials are based on high carbon contents (up to 60%) and aromatic monomers[101].

Currently, a variety of nanomaterials would be prepared by different methods in different fields. Therefore, it is worthwhile to prepare nanomaterials combined with the characteristics of lignin. Lignin based nanomaterials have been used in adsorption[102,103], catalysis[104], packaging[105], drug delivery and sustained release system[106] to name a few. In the biomedical field, Dai et al. created a lignin-containing self-nanoemulsifying drug delivery system (SNEDDS) that can effectively improve the stability of trans-RSV[107]. The delivery and storage of trans-RSV also could be achieved via Pickering emulsion stabilized by lignin-based nanoparticles[108]. In wastewater treatment field, Wang and his co-workers evaluated the behavior of dye adsorption from wastewater using lignin-based Fe_3O_4@lignosulfonate/phenolic core-shell microspheres and their results demonstrated that it could be very efficient in removing methylene blue from aqueous solutions[109]. However, in the aquatic environment, the aggregation and deactivation of nanomaterials have hindered its

development and practical application. Currently, using organosolv lignin for the modification of nanoscale zero-valent iron (nZVI) was investigated to issue this problem[102]. Ma et al. acquired super long-term stable lignin nanoparticles via an acid precipitation method directly from black liquor which also offer the possible to overcome the poor stability problem[110].

In addition, many studies have reported to use lignin as carbon materials[111,112] including activated carbons[113], carbon fibers[101], and graphitic carbon or carbon dots[114], etc. It has brought forth a wide array of promising applications, ranging from advance materials, energy storage to as adsorbents for organic pollutants or heavy metals.

Approaches to preparing carbon sorbents derived from lignin are summarized by Chistyakov[111]. Zhu et al. summarized the applications of lignin-derived materials in rechargeable batteries and supercapacitors including their use as binders and electrodes for rechargeable batteries, and electrodes and electrolytes for supercapacitors with a focus on the mechanisms behind their operation[70]. Lawoko et al. reviewed both the topics of the direct use of lignin and of the chemical modifications of lignin toward micro- and nanostructured materials. But there are various factors that limit its application such as the heterogeneity of lignin molecule, ash content, and thermoplastic foaming behavior[12].

In the last decade, lignin-based materials development has picked significant momentum due to the bio-refinery concept as aging pulp and paper mills need to diversify their product portfolio to maintain their vitality. However, most of these studies have not yet reached an industrial scale. So far, there are many studies needed to do about lignin-based materials including the major lignin-derived materials, functional products, and their potential applications.

6. Summary and Outlook

Lignin is the only renewable aromatic native biopolymer in nature, and lignin-based

materials avoiding the waste of resources become one of the hot topics recently. The application can lead to higher profits for pulp and paper industries and second-generation biorefineries as well as better environmental performance. Because the fractionation conditions, such as temperature and robust solvent can destroy the structure of lignin and affect the purity of lignin, accordingly, the main challenge in the development of lignin for materials is that the stable carbon-carbon (C-C) bonds are formed once β-O-4 linkages break which can influence the activities of lignin. That gives the opportunities to investigate novel effective fractionation technologies for the next application of lignin.

It has now been widely demonstrated that a higher quantity of phenolic hydroxyl, higher β-O-4 linkages, narrow polydispersity, and purity of lignin often determines the lignin activities for downstream application. With this in mind, we sought to further improve the quality of fractionated lignin by using novel solvents, mild conditions or novel technologies, such as IL-assisted fractionation, EDS-assisted fractionation, flow-through fractionation etc. In view of the huge varieties of fractionation technologies available, most focus on lignin yield and purity. However, the ideal fractionation for lignin obtained is still uncertain. Aside from the ideal fractionation, the fractioned lignin can be integrated into a variety of applications as it can also be treated as raw materials for further use. But there is a lack of studies in the downstream application of fractionated lignin.

In addition, consideration has to be taken on the prospect of applying the fractionation technologies on a large scale and eventually achieving the commercial utilization process. Techno-economic analysis is also needed to constantly evaluate the progress of the developed technology and scale-up production and application.

Acknowledgments

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