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Effluent of biomass cooking with active oxygen and solid alkali (CAOSA): component separation, recovery and characterization†

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Effluent from biomass pretreatment industries affects a substantial proportion of industrial wastewater and can be troublesome to treat. In a new environmentally benign biomass pretreatment approach based on cooking with active oxygen and solid alkali (CAOSA), some precipitates were found to be produced while the effluent (yellow liquor) was treated with alcohol or acid. Based on the precipitation approaches, component contents in CAOSA yellow liquor were successfully made clear, consisting of 57.19 wt% low molecular weight acid salts, 15.47 wt% lignin, 4.30 wt% saccharides and 23.04 wt% inorganic salts. Different organic solvents were tested as the precipitants, and when ethanol was used, over 81 wt% of the compounds in the yellow liquor could be precipitated. Meanwhile, 89.2% of the solid alkali consumed in CAOSA pretreatment could be recovered, showing prospects for primary effluent treatment. Furthermore, CAOSA lignin (CL) was recovered by acid precipitation from yellow liquor. By characterization, CL was found to have lower molecular weight and higher β-O-4 bond (60.05%) ratio than milled wood lignin (MWL, 48.64%), and thus, it might be a more ideal feedstock for further valorization.

1. Introduction

Biomass refining, a concept that has arisen since the 1980s, has attracted increasing attention from practitioners and researchers.[1] Countless chemicals are obtained from biomass, especially lignocellulosics.[2,3] Due to the natural recalcitrance of biomass, employing efficient pretreatment technologies to separate their components is necessary for further utilization.[4–6] However, effluent from the biorefinery industry, which makes quite a large proportion of industrial wastewater, has been particularly challenging to address because of its high total organic carbon (TOC) content, strong alkalinity and even toxicity.[7–9] Current disposal methods mainly include coagulation, precipitation, biological aeration or the introduction of oxidizing agents such as Fenton's reagent, ozone and peroxide.[10,11] By the method of oxygen delignification, 51, 57 and 43% of BOD_3, COD and color were reduced of the soda-anthraquinone pulping effluent.[12] And in Garg's work, with addition of H_2O_2 and $FeCl_3$, the maximum lignin and TOC removals reached 85% and 48% from the Kraft pulping

effluent.[13] However, the complex disposal process will undoubtedly increase the treatment cost. Further, plenty of biomass resources are abandoned into effluent, which is used for supplying heat or discharged directly, thus also leading to the waste of biomass resources.[10]

In current biomass pretreatment processes, lignin is often treated as a by-product in the effluent without high-value utilization.[14,15] However, lignin is truly not waste without any application value; in contrast, it has great potential for use as feedstock to produce aromatic monomers.[16] Lignin from different biomass species is considered to have different structures and application values.[15] Lignin from gymnosperms is thought to lack a syringyl unit and, thus, shows a higher branching degree and a less active β-O-4 ether bond, resulting in more difficulties to be degraded.[17] C-lignin has been found in plant seeds with a simple linear catechol structure; therefore, C-lignin is recognized as a better raw material for lignin valorization.[18] Extraction methods can also influence the structure of lignin. Milled wood lignin (MWL) and cellulosic enzymatic lignin (CEL) are thought to have a structure that is most close to the structure of native lignin.[14] Many different extraction methods have been put forward to obtain modified lignin, which is more suitable for further valorization.[19] However, until now, technical lignin has only been applied to prepare low-value materials such as sulfonate, with industrial lignin use rarely reported for the large-scale production of lignin monomers.[20–22]

Previously, a new environmentally benign technology for biomass pretreatment based on cooking with active oxygen and solid alkali, short for CAOSA, has been exploited and developed

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by our group.[23,24] By CAOSA, pulp with a desirable brightness and low kappa value can be obtained.[25] Further, the viscosity, alkalinity, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of the yellow liquor are found to show lower values than black liquor in the traditional process, thus meaning easier treatment.[26] However, even though the treatment difficulty of yellow liquor is much less than black liquor, there have also been some problems involving effluent treatment and compound re-utilization. Jiang and co-workers have tried to recover the solid alkali by calcium causticization. However, the calcium salts are always co-precipitated together with the magnesium alkali, resulting in the difficulty of the re-utilization of the solid alkali.[24]

To find an effective pathway for effluent organic separation and catalyst recovery, in this work, a precipitation approach utilizing alcohol and acid was proposed. Surprisingly, with such a precipitation method, qualitative and quantitative analysis of all constituents in yellow liquor was determined. This work provided a simple method to recover the solid alkali and reduce the TOC value of yellow liquor. Furthermore, CAOSA lignin, which was acid-precipitated from yellow liquor, was determined to contain a higher β-O-4 ether bond proportion (60.05%) compared with that of MWL (48.64%), showing great potential for further valorization to prepare vanillin or methyl vanillate. In a word, the developed precipitation approach can undoubtedly improve the efficiency of biomass utilization and provide preferable economic benefits for biomass refineries.

2. Experimental

2.1 Materials

Spruce wood was provided by YST Group Co. Ltd. (Dongguan, Guangdong, China). Deuterated water (D_2O) and Deuterated dimethyl sulfoxide (DMSO-d6) with a purity of over 99.9% were purchased from Sigma–Aldrich (Shanghai, China). Other reagents were of analytical grade and purchased from Shanghai Aladdin Bio-Chem Technology Co. Ltd.

2.2. Methods

2.2.1 Obtaining of yellow liquor (YL). Spruce wood powder was milled to the size of 40 mesh and cooked with a ball-shaped digester with oxygen and solid alkali (MgO).[26] 1.0 kg of spruce wood powder (dry basis) was cooked with 4.0 kg of water and 150 g of MgO under initial oxygen pressure of 2.0 MPa. The digester was heated and then the temperature remained at 160 °C for 3 h. After cooking, the muddy product, which was a mixture of pulp and YL, was filtered with a 100 meshes filter cloth. After settling, a little mass of suspended particles was sedimented and the supernatant was YL.

2.2.2 MWL preparation. Spruce wood MWL was prepared by the procedures described below.[27] Firstly, spruce wood powder with sizes of 40 mesh was Soxhlet extracted using a mixture of benzene and ethanol (2 : 1) over 12 h, then dried and ball-milled for 48 h. The resulting powder was extracted with a mixture of dioxane and water (96 : 4) at 30 °C for over 24 h. This extraction process was repeated 3 times, and the

extracts were combined and concentrated using a rotary evaporator. Then the concentrated liquid was dropped into ethanol solvent (10 times the volume of the liquid) to remove saccharides. The ethanol solvent was filtered and concentrated again. And the concentrated ethanol solvent was then dropped into a mixture (5 times the volume of the liquid) of acetic acid and water (9 : 1) to obtain the lignin precipitate. Finally, the precipitate was washed and lyophilized to obtain the MWL powder.

2.2.3 Preparation of series of precipitates. In a typical organic solvent precipitation experiment, yellow liquor was dropped into the organic solvent (v/v = 1 : 5, methanol, ethanol, acetone, 1,4-dioxane and tetrahydrofuran). Immediately the solid precipitate or the separated liquid phase was produced.

In a typical acid precipitation experiment, yellow liquor was acidified by HCl solution to pH about 1.0, and the precipitate was produced.

In two groups of cross precipitation experiments, ethanol was used as the organic precipitant. In the first group, YL was firstly added into ethanol solvent (v/v = 1 : 5). The obtained precipitate was separated by filtration and washed by ethanol, then stored as Pre-E. Afterward, the filtrate was evaporated to recover ethanol solvent. And the concentrated liquid was acidified by HCl solution to pH about 1.0. Precipitate in this step was then washed by deionized water and stored as Pre-E–A.

In the other group, the order of precipitants was exchanged. Yellow liquor was firstly added into HCl solution and Pre-A was obtained firstly. Subsequently, the liquid was evaporated to remove excess acid and then to be precipitated by ethanol (v/v = 1 : 5), the precipitate labeled as Pre-A–E.

2.3 Determination and characterization

2.3.1 Determination of dry weight and ash content. 50.00 g YL sample was loaded in a flask and the initial weight was determined. The sample was stored overnight at −20 °C and the frozen sample was dried by lyophilization, resulting in the formation of a yellow powder. The dry weight and water content could be calculated from the difference between the initial and final weight of the sample. The precipitates were also lyophilized to determine the dry weight. YL lyophilization and all the precipitation processes were repeated for 5 times. An average dry weight of YL and those precipitates was used in this article.

Additionally, ash contents of YL dry matter and precipitates were determined by thermogravimetric analysis (TG) for 3 times according to the following program: 25–20 °C, 20 °C min^{-1}; 120 °C, 10 min; 120 °C −800 °C, 10 °C min^{-1}; 800 °C, 10 min. The average ash content and the representative thermogravimetric curve was displayed.

2.3.2 High performance liquid chromatography (HPLC). YL or Pre-E were acidified or dissolved by H_2SO_4 solvent to adjust pH around 1.0. Then the supernatant was taken and diluted to an appropriate concentration for analysis. Low-molecular-weight acids (LMWA) in acidified yellow liquor were determined using HPLC (WATERS Alliance e4695) with a Bio-Rad Aminex HPX-87H column and a refractive index detector (RID).[26] The column temperature was set at 60 °C; 5 mM H_2SO_4

was used as mobile phase with a flow rate of 0.6 mL min^{-1}, and detection time was set 30 min. Main components were determined by external standard method.

2.3.3 Heteronuclear single quantum coherence nuclear magnetic resonance (HSQC NMR). In this study, samples were characterized by HSQC NMR for obtaining the structural information, including MWL, CAOSA lignin (CL), YL and precipitates.[28] DMSO-d6 was used as the NMR solvent for MWL and CL, while D$_2$O was used to dissolve YL, Pre-E and Pre-A–E. When DMSO-d6 was used, the chemical shifts were referenced to the residual DMSO peak at δ_C/δ_H, 40.0/2.50 ppm. All the 2D HSQC NMR experiments were recorded on a Bruker AV 600 MHz NMR spectrometer (Germany). Semi-quantification was performed by integral of each peak, and proportion of each unit was calculated by the ratio of the integral to the sum.

2.3.4 ^{31}P NMR. The ^{31}P NMR spectra were performed according to a published method.[29,30] Solvent A was prepared as a mixture of pyridine/CDCl$_3$ (1.6 : 1, v/v). 20 mg lignin (MWL or CL) was dissolved into 600 μL solvent A. 100 μL cyclohexanol solution (10.85 mg mL^{-1} in A) was added as an internal standard. 100 μL 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) was added into above solution and the mixture was kept for 10 min. The final phosphorylated sample was transferred into a 5 mm NMR tube for subsequent determination. Semi-quantification was performed referred to the last item and all the proportion was normalized by the internal standard.

2.3.5 Fourier transform infrared spectroscopy (FTIR). FTIR spectra were obtained on an attenuated total reflectance (ATR) FTIR spectrophotometer (Thermo Nicolet Is5) using KBr disks containing 1% (w/w) of the finely ground lignin samples (MWL and CL).[23] The spectra were recorded over a frequency range of 4000–400 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode.

2.3.6 Gel permeation chromatography (GPC). The molecular weights of YL and its precipitates were determined by Agilent 1260 Infinity GPC with RID. For yellow liquor, Pre-E and Pre-A–E, 1.0 mg sample was dissolved into 1.0 mL deionized water and then subjected to GPC equipped with series-wound Ultrahydrogel-1000 (7.8 × 300 mm) and Ultrahydrogel-120 (7.8 × 300 mm) column. And for Pre-E–A and Pre-A, 1.0 mg sample was dissolved into 1.0 mL tetrahydrofuran (THF) and then analyzed by GPC equipped with series-wound Stragel-HR-5E (7.8 × 300 mm) and Stragel-HR-2 (7.8 × 300 mm) column. Calibration curves were established with standard glucan and polystyrene samples respectively.

3. Results and discussion

3.1 Yellow liquor precipitation by organic solvents

The yellow liquor used in this work was obtained from the CAOSA pretreatment process at 160 °C for 3 hours with spruce wood. Under such conditions, alkali or salts were dissolve in yellow liquor together with the organics. A lot of efforts had been put on recovering the components in YL, especially the solid alkali used in CAOSA process. In the meanwhile, once the
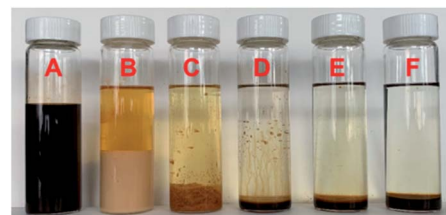

Fig. 1 Yellow liquor (A) and its mixture with methanol (B)/ethanol (C)/acetone (D)/ 1,4-dioxane (E)/tetrahydrofuran (F).

Table 1 Water-soluble LMWA salts in yellow liquor and precipitates

Components[a]		YL/%	Pre-ethanol/%	Pre-methanol/%
Proportion to YL dry weight		—	81.9	64.8
Ash content		25.35	27.61	32.33
LMWA	Formic acid	15.60	3.28	3.38
	Acetic acid	12.49	3.75	4.14
	Glycolic acid	6.90	4.36	5.17
	3-Hydracrylic acid	2.69	1.40	1.32
	Oxalic acid	4.27	4.86	5.38
	Malonic acid	1.21	1.44	1.73
	Succinic acid	3.09	3.79	4.39
	Maleic acid	1.32	1.43	1.73
	Fumaric acid	1.29	1.32	1.63

[a] The acid contents were calculated in the corresponding acid form.

alkali, salts and organics were recovered, the treatment difficulty of the effluent would also be reduced.

Considering the low solubility of the salts and alkali in organic solvents, some organic solvents, ordered by their polarity, were selected as precipitants, including methanol, ethanol, acetone, 1,4-dioxane and tetrahydrofuran. Once the yellow liquor was added into methanol or ethanol, faint yellow sediment was immediately observed (Fig. 1). Compared with the original yellow liquor, the supernatant of the sample looked much more limpid and brighter, inferring that most of the color components were precipitated. However, when acetone, 1,4-dioxane or tetrahydrofuran was used as precipitant, the salt solution and organic solvents were separated into two liquid layers without any solid precipitate generated. The reason for the layer separation was perhaps that with the solvents' polarity decreased, the inter-solubility between the solvents and salt solution is not as good as that with pure water. Therefore, among the selected solvents, alcohols (methanol and ethanol) were verified as the better precipitants for yellow liquor.

Interestingly, a larger precipitate ratio of 81.9 wt% (based on the total dry weight of yellow liquor, Table S1†) was achieved with ethanol precipitant than methanol (64.8 wt%, Table 1). When using ethanol as precipitant, a darker solid precipitate and brighter supernatant were obtained (Fig. 1). This difference indicated that the color compounds, such as oxidized lignin, had lower solubility in ethanol solvent. In Zhang's work, Kraft lignin was treated by a mixture of methanol and water and separated into the dissolved part and undissolved part.[31] The
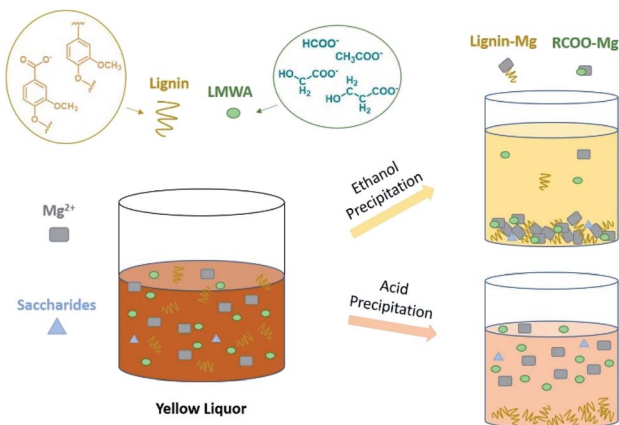
Fig. 2 Schematic diagram about YL ethanol and acid precipitation.

undissolved Kraft lignin was proved with a higher molecular weight than the dissolved ones. The undissolved lignin occupied 47.51% of the total lignin sample while the methanol ratio was 70%. While the methanol ratio reached 90%, the undissolved lignin decreased to 37.73. Yet, in this work, ethanol, which has a lower polarity, could separate more lignin from yellow liquor than methanol, implying that the lignin derivates were not be precipitated as the lignin molecule. The ethanol precipitation scheme was similar to the YL alkali precipitation process described in Jiang's work.[24] Therefore, in the organic solvent precipitation process, lignin was more likely to be co-precipitated from the CAOSA yellow liquor with magnesium ion as the salt form (Fig. 2).

As revealed by all the precipitation cases, the precipitation ability of the precipitant could be concluded. The amount of precipitates would reduce both with the increment of the precipitant's magnesium salt solubility and decrement of its polarity. Based on the precipitation results, ethanol was suggested to be the best precipitant among the applied solvents and was therefore used in the following research.

3.2 Constituent distribution of yellow liquor

To clarify the constituents of yellow liquor, two different precipitation routes were designed, by which four kinds of
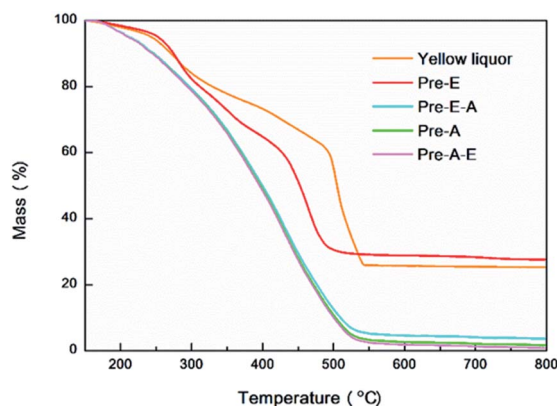

Fig. 3 Typical TG curves of yellow liquor and precipitates.

precipitates were collected as Pre-A, Pre-A–E, Pre-E and Pre-E–A. In the CAOSA process, only biomass raw material, alkali, oxygen and water were brought into the pretreatment system, yellow liquor product could be reasonably classified into four parts: degraded lignin, degraded saccharides, low-molecular-weight degraded products and inorganic alkali/salts.

Salts (organic and inorganic) in yellow liquor could be determined by TG analysis. The ash content of the dry component of yellow liquor was measured as an average of 25.34 wt% (Fig. 3, Table S2†). And the ash content of Pre-E was a little higher than YL and reached 27.61 wt%, meaning the magnesium salts were enriched into Pre-E during the ethanol precipitation process. Additionally, as for another three other kinds of precipitates (Pre-E–A, Pre-A and Pre-A–E), almost no solid residue was collected after the calcination at 800 °C, implying that these three precipitates were made up of organic compounds.

Considering the different ash contents and solubilities of the four precipitates, it was reasonable to assume that the precipitates were composed of different constituents. HSQC NMR was adopted to analyze the lignin and its linkage with saccharides, such as the lignin–carbohydrate complexes (LCC).[14] Signals in the lignin NMR spectra were classified into two regions: side chain region and aromatic region.[32]

From the comparison of the HSQC NMR spectra, Pre-E–A and Pre-A were proven to show almost the same chemical shift, indicating that they had almost the same structure. Moreover, most of the signals in the Pre-E–A and Pre-A spectra were found in the MWL spectra for spruce wood. Thus, they were reasonably seen as the lignin fragment without complete degradation (Fig. 4). According to the signal assignment, some typical structures in lignin were disappeared after CAOSA pretreatment (Table S3,† Fig. 5). The regulation of the structural changes in spruce lignin was parallel with the bamboo lignin during the CAOSA process.[26] The phenylcoumarans structure was degraded rapidly, while the β-O-4 structure and ferulates structure remained stable after the pretreatment process. Although both Pre-E–A and Pre-A showed the similarity to MWL, the amounts of precipitate from Pre-E–A (1.72 wt%) and Pre-A (15.47 wt%) were different. This difference could be explained by the fact that after CAOSA treatment, the oxygen-containing groups in lignin were oxidized. In alkaline conditions, salts could be formed once hydroxyl groups are oxidized to carboxyl groups, then the solubility of the salts in alcohol was decreased. Thus, after ethanol precipitation, most of the oxidized lignin was separated, and only a small amount of which was obtained in the following acid precipitation.

It was worth mentioning that many signals in Pre-A–E could be assigned to saccharides and LCC linkage, indicating that Pre-A–E, which could be precipitated by alcohol and without any signal in aromatic region, was probably the product of incompletely degraded cellulose and hemicellulose.[33]

As reported previously, LMWA salts hold a majority part in yellow liquor.[26] In an oxidation–delignification process such as CAOSA, aromatic rings are considered to be broken by active oxygen groups.[34] LMWA products are mainly obtained from the ring-opening reaction of lignin aromatics. The LMWA salts,
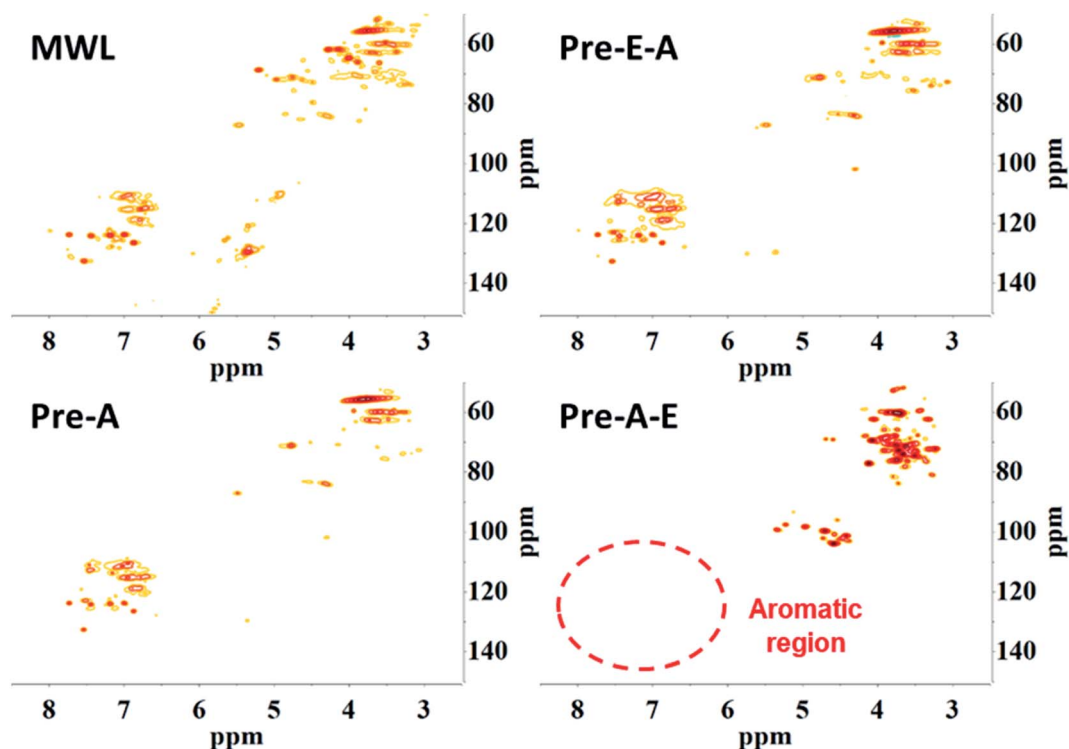
Fig. 4 HSQC NMR spectra of MWL and precipitates.

mainly including formate, acetate, glycolate and hydracrylate, were acidified and then determined by HPLC (Table 1). It was calculated that over half of the dry components of yellow liquor was made up of LMWA salts, evidencing the occurrence of severe delignification and a ring-opening reaction alongside the CAOSA process.

Pre-E had an ash content of 27.61 wt% (Table S2†), and the LMWA contents of Pre-E and Pre-Methanol were determined by HPLC (Table 1). By comparison of the contents, LWMA salts, especially the formic and acetic salts, showed a better solubility in methanol than ethanol. And with the polarity of the organic groups in the salts became lower, more proportion of which was
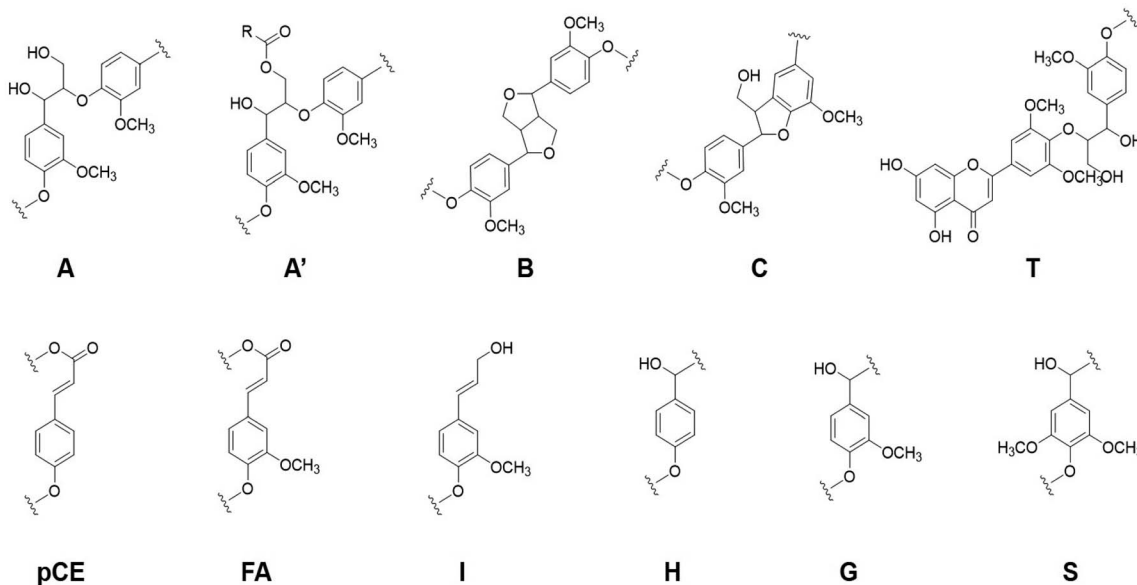

Fig. 5 Main structure present in the bamboo lignin: (A) β-O-4 alkyl-aryl ethers; (A′) β-O-4 alkyl-aryl ethers with acylated γ-OH with *p*-coumaric acid; (B) resinols; (C) phenylcoumarans; (T) a likely incorporation of tricin into the lignin polymer through a G-type β-O-4 linkage; (PCE) *p*-coumarates; (FA) ferulates; (I) *p*-hydrocinnamyl alcohol end-groups (H) *p*-hydroxyphenyl units; (G) guaiacyl units; (S) syringyl units.

Table 2 Components distribution in yellow liquor

Entry	Component	Content[a]/%
1	Lignin salts	15.47
2	Saccharides	4.30
3	Inorganic salts	23.04
4	LMWA salts	57.19

[a] Content was calculated based on dry component of yellow liquor.

precipitated, such as the salts combined with the oxidized lignin. Only a few proportions of the formic and acetic salts could be precipitated by ethanol or methanol, remaining the possibility to recover formic and acetic acids from YL for further fine utilizations.[22] The amount of the precipitation proved that more LWMA salts would be separated with the polarity of the organic solvent decreased.

Apparently, the precipitates by organic solvent were not only composed of LMWA salts. Some lignin, saccharides and inorganic salts were perhaps precipitated together with the LMWA salts in the precipitation process. And the content of inorganic salts dissolved in yellow liquor could be calculated from the mass difference between the dry component of yellow liquor and other constituents. Inorganic salts were identified as magnesium salts including $MgCO_3$, $Mg(HCO_3)_2$, Mg_2OCO_3, MgO and $Mg(OH)_2$.[24] These inorganic salts can be transformed again into solid alkali MgO by calcination, providing a potential means for catalyst recovery.

After analysis of the precipitates, the complete composition of the yellow liquor was finally made clear (Table 2).

3.3 Effluent treatment and catalyst recovery

By understanding the overall component distribution, the alcohol and acid precipitation approach can be substantially developed.

Currently, alkali used in the traditional pulping process is usually recovered by evaporation-calcination or causticization by calcium oxide. However, different shortcomings have been exposed by such methods. In the evaporation–calcination process, effluent is firstly concentrated and evaporated, and then, the solid residue is collected to recover heat and alkali by calcination. The most obvious disadvantage of the evaporation–

calcination method is the huge energy cost consumed by evaporation and the serious harm to equipment brought about by foaming and carbonization problems. For calcium oxide causticization method, effluent can be re-adjusted to a proper alkaline condition and re-used in the cooking process. However, while the calcium oxide is used to treat CAOSA yellow liquor, magnesium will be precipitated together with calcium. After that, separation of calcium and magnesium will be the subsequent difficulty.[24] Removal of the organic compounds from yellow liquor is the other question. By usage of the activated sludge system, the COD removal is reported a decrease of 67–76%.[35] And when nano-Fe_2O_3/H_2O_2/UV is used to treat the Kraft pulping effluent, the maximum aromatic compounds and COD reduction reach 68.4% and 89.8%.[36] However, it's difficult to achieve the re-utilization of the recovered organic compounds from the effluent through those degradation processes.

Compared with the pathways mentioned above, alcohol precipitation, by which over 80% of the constituents can be simply separated, is indeed an effective approach for treating CAOSA yellow liquor (Fig. 6A). After alcohol precipitation, the secondary effluent would contain fewer organics and could be easier-treated. Especially, by this effluent treatment approach, the solid alkali could also be contemporarily recovered, avoiding an extra complicated catalyst recovery process. Based on the contents of each constituents, the solid alkali recovery, lignin removal and organics removal reached 89.2%, 88.9% and 76.2% respectively.

3.4 CL analysis

The acid precipitation approach affords more options for lignin utilization (Fig. 6B). Pre-A, the lignin obtained by acidification from yellow liquor, is named as CAOSA lignin, short for CL. Further structural analysis of CL will deepen the understanding of the CAOSA delignification mechanism and make better use of the undegraded component in biomass pretreatment effluent.

3.4.1 GPC. Molecular weights of lignin extracted by different methods were found different. MWL is thought to have the most similar molecular structure to native lignin; however, its molecular weight is found to be lower than that of native lignin. The Molecular weight of spruce MWL was determined to be approximately 3700 for reference. By performing GPC analysis, the molecular weight of CL was found distributed from about 200 to 2000. In the GPC results, there were two obvious
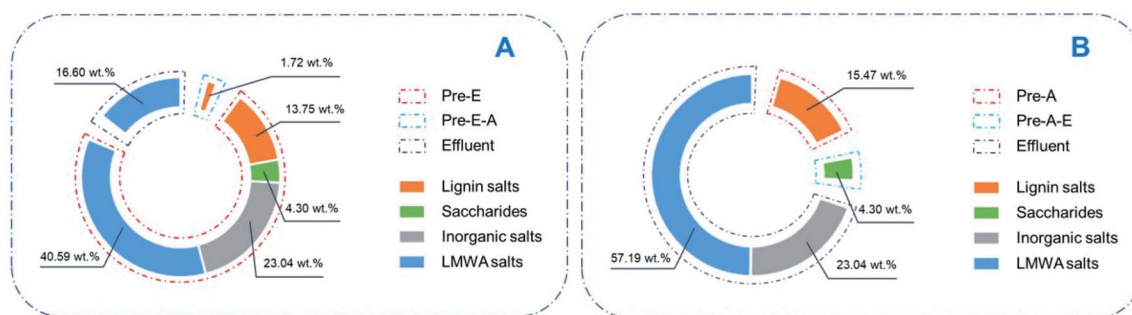

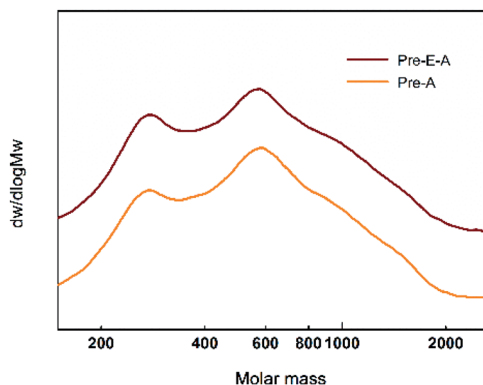Fig. 6 Components content in different precipitates (A: ethanol added first; B: acid added first).

Fig. 7 GPC results of Pre-E−A and Pre-A.

peaks appeared about 300 and 600 (Fig. 7), and the corresponding M_w and M_n were calculated as 210.7, 194.7, 718.9 and 601.3. And the polydispersity was calculated as 1.08 and 1.19 respectively. The polydispersity was such close to 1.0, indicating that the molecular weight dispersion was highly concentrated around the two peaks, and the compounds had similar side chain structures. GPC results verified it again that Pre-E–A and Pre-A were similar in their composition. Compared with the M_w of MWL, the much lower M_w of CL also illustrated the occurrence of severe broken within the CAOSA delignification process. Referring to Huang's classification, the vast majority of the CL could be regarded as lignin monomers, dimers, trimers or tetramers.[37]

Additionally, GPC results for yellow liquor presented three peaks: 600, 750 and 1100 (Fig. S1†). By the acid precipitation method, it was found that the GPC result for Pre-A–E showed almost no signal at 750, and the signal of M_w 600 also decreased, meaning lignin was precipitated by the acid precipitation process.

3.4.2   HSQC NMR. Different linkage contents in MWL and CL were semi-quantified from HSQC NMR spectra (Fig. 4, Table 3).

Notably, the proportion of β-O-4 linkages in the lignin side chain, which attracted great attention from researchers investigating lignin valorization, was increased from 48.64% to 60.05% after the CAOSA process. The increase of β-O-4 linkage was caused by its relatively lower reactivity under the weak alkaline conditions.[26] Hence, CL has the potential to be a better feedstock to produce aromatic monomers through lignin valorization methods.

The other significant difference appeared around δ_C/δ_H 4.09/61.2 ppm, where belongs to the cinnamyl alcohol end-groups (I). The disappearance of that signal indicated the thorough degradation of the I structure. The conjugated $C_\alpha{=}C_\beta$ structure with aromatic ring was easy to be attacked by the electrophilic active oxygen groups, as a result, the $C_\alpha{=}C_\beta$ bond was then cleaved.[23]

The content of methoxyl groups in CL was calculated to be 25.51%, which remained at the same level as the content in the MWL (26.56%). In one piece of our previous study, the aryl-ether bonds were found degraded under CAOSA conditions, including the methoxyl groups at lignin side chain. Cleavage of the aryl-ether bonds would decrease the methoxyl group content in lignin. But the hydroxyl group generated in the aryl bond cleavage process would further trigger the ring-opening reaction of the lignin aromatic ring. Because of that, the aromatic ring with a phenol hydroxyl group would be fast broken under CAOSA condition, and the methoxyl group was therefore remained at a similar value with the original one.

3.4.3   ^{31}P NMR. More oxygen-contained functional groups can lead lignin to be more active in chemical reactions.[38] ^{31}P NMR is used to determine the lignin hydroxyl group.[29,30] Generally, in lignin side chains, a large proportion of α-C is linked with hydroxyl groups. However, there are also some lignin structures featuring a C=C bond between their α-C and β-C, such as cinnamyl alcohol end-groups (I), cinnamyl aldehyde (pCE) or ferulates (FA) structure, as shown in the HSQC NMR results obtained for spruce MWL and CL (Fig. 4).

By semi-quantification with ^{31}P NMR, content of α-OH was found decreased after CAOSA treatment, which was probably a result of C_α–C_β bond cleavage under the oxidation conditions (Fig. 8, Table 4). In an alkaline condition, the phenol hydroxyl group tended to transform into the quinone structure, and then, β-C at the side chain became a stronger negative charge center. Followed by the charge center transformed, the β-C became easy to be attacked by active oxygen group (Scheme 1).[23] Afterward, the other ending of the oxygen free radical was attracted by α-C, which had a lower electronegativity, and an extremely unstable tetra-atomic ring was formed. As the result of the crack of the unstable ring, the C_α–C_β bond of the lignin side chain was broken and two molecules of carbonyl compounds were produced. Subsequently, benzoic structure

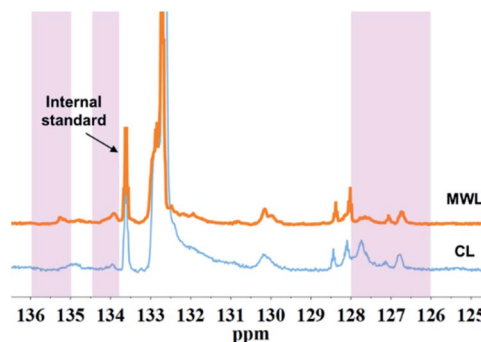**Table 3**   Content of lignin linkages in MWL and CL (side chain)

Entry	Structures	MWL	CL
1	β-O-4 ether bond (A)	48.64	60.05
2	Resinols (B)	13.97	11.85
3	Phenylcoumarans (C)	20.90	26.42
4	Cinnamyl alcohol end-groups (I)	16.49	1.67
5	S/G ratio	0.89	0.74
6	–OCH$_3$	26.56	25.51

Fig. 8   ^{31}P NMR spectra of MWL and CL.

Table 4 Normalization content of methoxyl group in MWL and CL

Entry	Signal range/ppm	Structures	MWL	CL
1	136.0–135.0	α-OH, E	0.11	0
2	134.5–133.8	α-OH, T	0.32	0.04
3	133.8–133.3	Internal standard	—	—
4	133.3–132.0	Primary –OH	6.39	15.22
5	132.0–131.5	S –OH	0.33	0.99
6	131.5–129.5	G –OH	0.43	0.57
7	128.8–128.0	pCE –OH	0.58	0.75
8	128.0–126.0	–COOH	0.54	1.31
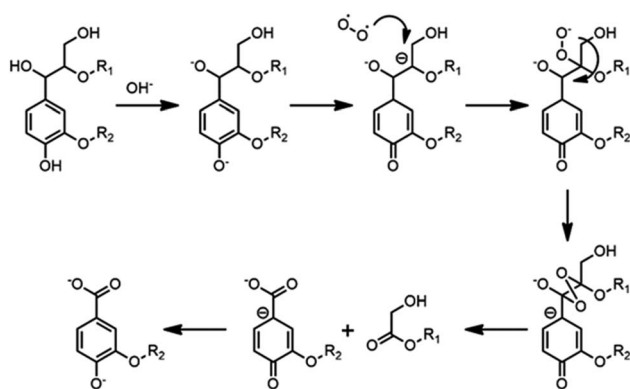
Scheme 1 Possible routine of C_α–C_β bond cleavage in CAOSA process.

and hydroxyl acid ester was generated and could be found out as the products in yellow liquor. Therefore, it is easy to understand that in ^{31}P NMR spectra of CL, the signal of –COOH was much higher than that of MWL.

Moreover, as indicated by the difference in the α-OH content, the unconjugated C_α–C_β bond was almost completely broken under CAOSA conditions. In contrast with the unconjugated bond, the conjugated C_α–C_β structure in pCE and FA could be detected both before and after CAOSA. The relative stability of pCE and FA structure might because of that, in their conjugated structure, electronic density was diluted among the whole conjugated structure instead of being concentrated at the β-C.
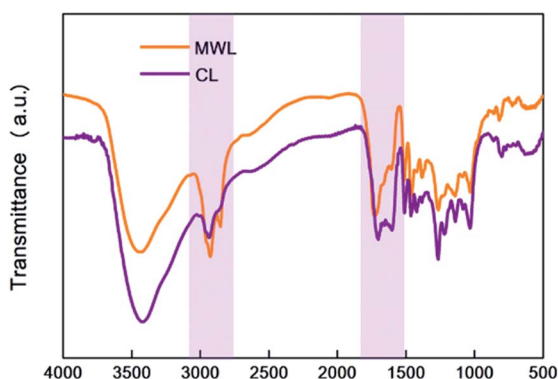
Fig. 9 FTIR spectra of MWL and CL.

Therefore, the negative charge was no longer concentrated at the β-C. As a result, the β-C would not tend to attract active oxygen groups and the C–C bond would not be cleaved.

3.4.4 FTIR. With FTIR analysis, band assignment for each functional group in lignin referred to Faix's work (Table S4†).[39,40]

First of all, it should be noted that the main difference between MWL and CL was found for the peaks between 1750–1510 cm^{-1}, which represented the stretching and vibration of C–O bonds, typically for the C=O functional group. The band at 1710 cm^{-1}, which was assigned to C=O stretching in unconjugated ketone and carbonyl groups, was notably reduced after CAOSA pretreatment, indicating the severely broken of that structures in lignin during CAOSA process. Band around 1600 cm^{-1} was the signal for aromatic skeletal vibrations plus C=O stretch. And in CL, the band around 1600 cm^{-1} was even stronger than MWL, meaning more C=O bonds conjugated with the aromatic ring were detected in CL sample. This was consistent with the conclusion drawn from the ^{31}P NMR results, which showed that under alkaline oxidation conditions, lots of carbonyl or carboxyl groups were generated.

Next, the decrease in the intensity of bands at 2925 cm^{-1} and 2850 cm^{-1}, which were assigned to C–H stretching in methyl groups and methylene groups. Methyl and Methylene groups were mainly existing in lignin side chain structure, therefore, the decrease of that after CAOSA pretreatment might be caused by the degradation of lignin side chain structures, such as the C_α–C_β bond cleavage. Furthermore, as the nature of spruce lignin, whether in MWL or CL samples, only the guaiacyl (G) units could be detected with the band range from 925–817 cm^{-1} (Fig. 9).

Hence, CL, the acid precipitate of CAOSA yellow liquor with a higher β-O-4 bond ratio, shows better prospects for application on preparing substituted benzoic acid or its ester.

4. Conclusions

In summary, a preferable chemical precipitation method to treat yellow liquor, the effluent of the CAOSA process, was put forward. By use of this precipitation method, the content of the constituents in yellow liquor was determined. Alcohol precipitation showed a great potential to serve as an efficient approach to recover the solid alkali and reduce the TOC value of the CAOSA yellow liquor. While ethanol used as precipitant, over 81 wt% components could be separated from the liquid and solid alkali recovery and lignin removal reached 89.2% and 88.9% respectively. On the other hand, the structure of the acid precipitate, CL, was studied. CL has lower molecular weight and about a 10% higher ratio of β-O-4 than MWL, was regarded as a better feedstock for further lignin valorization. Thus, this precipitation method has remarkable application potential and is possibly an alternative technology for the treatment of effluent for biomass pretreatment.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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