Efficient pretreatment using dimethyl isosorbide as a biobased solvent for potential complete biomass valorization

Shuang Yang,[a,b,c] Xianpeng Yang,[b,c] Xianzhi Meng[d] and Lei Wang*[b,c]

[a] College of Environmental and Resource Sciences, Zhejiang University, Hangzhou, 310058, Zhejiang Province, China

[b] Key Laboratory of Coastal Environment and Resources of Zhejiang Province, School of Engineering, Westlake University, 18 Shilongshan Road, Hangzhou, 310024, Zhejiang Province, China

[c] Institute of Advanced Technology, Westlake Institute for Advanced Study, 18 Shilongshan Road, Hangzhou, 310024, Zhejiang Province, China

[d] Department of Chemical & Biomolecular Engineering, University of Tennessee Knoxville, Knoxville, TN 37996, USA

*Corresponding author. E-mail: wang_lei@westlake.edu.cn

Abstract

An efficient and sustainable pretreatment, such as organosolv pretreatment that produces high-quality lignin and highly digestible carbohydrates, could enable the potential complete utilization of lignocellulosic biomass. Demand for bio-based solvents with a high boiling point, low viscosity, and negligible toxicity is increasing. Herein, we report the use of dimethyl isosorbide (DMI) as a solvent to fractionate lignocellulosic biomass into its main components for the first time. High lignin removal efficiency (91.2%) with good cellulose retention (around 80%) could be achieved during the pretreatment of *Eucalyptus* by DMI/H_2O co-solvents under a mild condition. A near-complete cellulose conversion to its monosaccharide could be realized at a relatively low enzyme loading of 20 FPU g^{-1} glucan. The addition of water could suppress the condensation of lignin, yielding high-quality lignin with a good fraction of β-O-4 linkages reserved (24.8%) and homogeneous molecular weight (Đ<2) suitable for depolymerization to mono-aromatic chemicals. Besides its highly digestible nature, the high quality of the cellulose-rich residue is also demonstrated from a material perspective. A more efficient fibrillation of obtained pulp to nanocellulose was developed, leading to a promising potential of energy saving compared to the traditional bleaching pathway. Overall, this work developed a mild pretreatment technology as a potential basis for a green and closed-loop biorefinery concept for converting lignocellulosic biomass to multiple products (high-quality lignin, fermentable sugars, or functional materials).

Keywords

Biomass pretreatment, Biobased solvents, Dimethyl isosorbide, Closed-loop biorefinery, Cellulose, Lignin.

1. Introduction

Conversion of lignocellulosic biomass is one of the most promising pathways to tackle the emerging shortage in fossil energy resources and environmental issues caused by their combustion.[1] Through the biorefining process, bioethanol, butanol, and other liquid fuels, or nanocellulose, lignin, and other high-value added materials or chemicals could be produced from agricultural and forestry wastes such as corn cob, bagasse, straw, waste papers, and wood chips.[2-4] Along the bio-refining supply chain, pretreatment is a critical step to overcome biomass recalcitrance to fractionate cellulose, hemicellulose, and lignin for downstream utilizations. The pretreatment step accounts typically for 20–30% of the total cost of biofuel production due to the large amount of chemicals and energy used in traditional pretreatment technologies such as steam explosion, acidic and alkaline-based methods.[5] Combining the interests in cost reduction and approaching more environmentally friendly pretreatment method, advanced organosolv pretreatment using non-toxic/low-toxic green solvents have attracted increasing attentions.[6, 7] In addition, compared to extensive acid or alkaline pretreatments, organosolv pretreatment could well preserve lignin structure, enabling the complete utilization of biomass by sequentially converting lignin to valuable chemicals or materials.[8]

Traditional organosolv pretreatment technologies usually use alcohols, polyols, organic acids, or ketones due to their low-cost and wide availability, for example, the well-known ALCELL process.[9] However, these processes generally suffer from volatility, flammability, and energy consumption, for example, maintaining the negative pressure environment of operation. Towards a sustainable green chemistry future, a green solvent should follow 12 principles according to a review entitled 'Designing for a green chemistry future' by Zimmerman et al.[10] It shall be renewable, non-toxic/low-toxic, recyclable, thermal stabile, non-flammable, degradable, efficient, economically feasible, easy to store and transport, with low volatility and low vapor pressure.[10] In the recent decade, an emerging concept for converting biomass to fuels and chemicals using biobased and low toxic organic solvents, such as 2-methyltetrahydrofuran (2-MeTHF),[11] γ-valerolactone (GVL),[12] and

dihydrolevoglucosenone (Cyrene™)[13] have been investigated to achieve a "closed-loop" biorefinery. These novel solvents present excellent performances in the organosolv pretreatment of biomass and show potential for industrial production. However, GVL and Cyrene™ have a common disadvantage, that is both lead to the grafting of functional groups on the cellulose surface, ultimately blocking the access of enzymes to cellulose.[12, 13] Meanwhile, the high viscosity of Cyrene™ (about 7 times that of DMSO and 15 times that of DMF) could make the filtration or centrifugation processes difficult.[14] Moreover, unlike the fact that various kinds of traditional organic solvents are available, the variety of biobased green solvents is still very limited. There is an urgent need to explore and evaluate new solvents for biomass pretreatment.

Dimethyl isosorbide (DMI) is a renewable solvent as it can be synthesized from d-glucose via a catalytic process (SI Fig. S1).[15] In addition to high boiling point (235 °C), low level of volatility, good stability, and low viscosity, DMI has negligible bioaccumulation, therefore a very low acute toxicity due to its greater affinity for water than by organic compounds.[16, 17] It has been used as a green solvent to replace toxic solvents such as DMF, NMP, and THF in membrane production, [18] pharmaceutical additives, [19] and cosmetics.[20] The estimation of solvents' solubility based on Hansen Solubility Parameter (HSP) theory suggest that DMI should have a good capacity for dissolving lignin, as the estimated Hansen relative energy difference (RED) between lignin and DMI is only 0.89. In addition, DMI is miscible with water which could be used to further tune the solubility of lignin in DMI-water mixture accordingly. As a result, we propose that DMI might be a promising solvent candidate for the organosolv pretreatment of biomass, which has not been reported to our best knowledge. Therefore, the objective of this study is to evaluate the potential of DMI, with or without water, as a green solvent in pretreatment of biomass (e.g., *Eucalyptus*) from several perspectives (e.g., fractionation efficiency, lignin quality, enzymatic digestibility, and fibrillation feasibility of obtained pulp) to produce multiple products towards a potential close-loop biorefinery.

2. Experimental

2.1 Materials and chemicals

Eucalyptus (E. urophylla×E. grandis), obtained from a forest farm in Guangxi, China, was milled with a cutting mill (SM 300, RETSCH, Hana, Germany) after air-drying and debarking. The

Eucalyptus particles with the size of 60–100 mesh were screened, followed by removal of extractives by refluxing with toluene/ethanol (2:1, v/v) for 8 hours. Compositional analysis was performed based on NREL protocols.[21] The cellulose, hemicellulose, lignin and extractive contents were 50.3%, 16.0%, 31.1% and 2.6%, respectively. DMI (99%) was purchased from Wuhan Carnoss Technology Co., Ltd (Wuhan, China). Other chemicals were provided by Sinopharm Chemical Reagent Co., Ltd, (Shanghai, China). All the chemicals were used as received unless mentioned specifically.

2.2 Biomass pretreatment and lignin recovery

Pretreatments were performed using DMI/H_2O co-solvents with different ratios (10:0, 9:1, and 8:1) at 120 ℃ for 20 min and 60 min, respectively. 1.5 g of the extractive-free *Eucalyptus* samples and 15 mL of DMI with 75 mM H_2SO_4 were transferred to a Synthware™ cylindrical pressure vessel. The reaction was performed in a Synthware™ heating aluminum block bath at 120 ℃ with a thermometer monitoring for 20 and 60 min. After pretreatment, the pressure vessel was quenched in a water bath at room temperature. Then, the solid was separated from the liquid by vacuum filtration and washed with about 15 mL of the same DMI/H_2O mixture, followed by a large amount of Milli-Q water. The wet pulp was stored at 4 ℃ until further use. The DMI/ H_2O filtrate was dropped into 200 mL of 10 wt% NaCl solution under stirring to precipitate lignin which was then isolated by filtration and washed with acidified water (pH 2.0). The obtained lignin was dried at 45 ℃ overnight. All pretreatment experiments were conducted three times, and the obtained solid residue and lignin substrates were combined accordingly. Pulp yield, lignin yield, lignin removal efficiency, and cellulose conversion were calculated using the following equations:

$$\text{Pulp yield}(\%) = \frac{m_p}{m_r} \times 100 \quad (1)$$

$$\text{Lignin yield}\ (\%) = \frac{m_{rl}}{m_{lr}} \times 100 \quad (2)$$

$$\text{Lignin removal efficiency}\ (\%) = \left(1 - \frac{m_{lp}}{m_{lr}}\right) \times 100 \quad (3)$$

Where m_p is the weight of dried pretreated pulp, m_r is the weight of raw *Eucalyptus*, m_{rl} is the weight of recovered lignin, m_{lr} is the weight of lignin in raw *Eucalyptus*, and m_{lp} is the weight of lignin remaining in the pretreated pulp.

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis of raw *Eucalyptus* and pretreated samples was performed in 50.0 mM citrate buffer at pH 4.8 with Cellic® CTec 2 (Sigma, SAE0020). The cellulase loadings of each sample mentioned above were 20 FPU g^{-1} and 60 FPU g^{-1} glucan, respectively. The antibiotic antimycotic solution (Thermo Fisher Scientific, 15240062) with 1% (v/v) was diluted 100 fold and added to the buffer mixture to avoid microbiological contamination.[13] The mixture (1% substrate concentration) was incubated at the conditions of 50 °C, 150 rpm for 72 h in a shaking incubator (ZWYRb2102, ZHICHENG, China). After enzymatic hydrolysis, hydrolysis liquid was quenched in a boiling water bath for 10 min followed by centrifugation at 5000 rpm for 10 min, and then immediately kept at -20 °C prior to further analysis.

2.4 Sugar analysis

The sugar composition of carbohydrate-containing samples was determined by an ion chromatograph (Dionex™ ICS-6000, Thermo Fisher Scientific, Waltham, MA, USA) equipped with an electrochemical detector Dionex ED. A Dionex CarboPac™ PA20 guard column (3 × 30 mm) and an analytical column (3 × 150 mm) were used, with the mobile phase composed of (A) Milli-Q water, (B) 100 mM NaOH, and (C) 500 mM NaOH. Elution was performed with 98% A and 2% B for 20 min, followed by 60% A and 40% C for 5 min, and finally equilibration to starting conditions (98% A and 2% B) for 5 min. Analysis was conducted at 30 ℃, with an injecting volume of 10 μL and a flow rate at 0.5 mL/min.

2.5 Milled wood lignin and residual lignin isolation

Another two kinds of lignin, namely milled wood lignin (MWL) from the untreated biomass and residual lignin that remained in the pretreated pulp, were analyzed as references. MWL was isolated by ball milling and dioxane extraction, according to a previously reported method.[22] While the residual lignin was extracted from the pretreated biomass following a modified cellulolytic enzyme lignin (CEL) isolation procedure.[23]

2.6 Pulp fibrillation

The pretreat pulp was fibrillated by an automatic sample grinding machine (JXFSTPRP-24, Shanghai Jingxin, China). 1 mL of pulp suspension with a concentration of 0.5 wt% and a 5-mm ZrO_2 bead were added into a 2-mL centrifuge tube. The grinding was performed at 50 Hz for 1 hour. The sodium chlorite-bleached pulp was also fibrillated as a reference at the same condition.

2.7 Characterizations

2.7.1 Lignin characterization

The chemical structure of lignin was analyzed by 2D ^{1}H–^{13}C heteronuclear singular quantum correlation (HSQC) and ^{31}P spectra on a 600 MHz NMR spectrometer (AVANCE NEO, Bruker, Germany) equipped with a QCI-F Cryoprobe. For HSQC analysis, 30 mg of lignin was dissolved in 500 µL of deuterated dimethyl sulfoxide, and the standard pulse sequence "hsqcetgpsisp.2" was used.[24] For ^{31}P NMR analysis, 25 mg of lignin was dissolved in a pyridine/$CDCl_3$ (1.5/1.0, v/v, 500 µL) solution and derivatized with 75 µL 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane. Chromium acetylacetonate and endo-N-hydroxy-5-norbornene-2,3-dicarboximide were added into the solution as relaxation agent and internal standard, respectively.[13, 25] The molecular weight of lignin were measured by advanced polymer chromatography (APC, Waters, USA) with a refractive index detector after acetylation[13]. ACQUITY APC™ XT 200 column (4.6 × 150 mm, 2.5 µm) and XT 45 column (4.6 × 150 mm, 1.7 µm) were used for separation. Acetylated lignin was dissolved in tetrahydrofuran (THF) with a concentration of 1.5 mg/mL. THF was used as the mobile phase and the flow rate was 1 mL/min. Polystyrene standards with different molecular weights were used to prepare the standard calibration curve.

2.7.2 Pulp and cellulose characterization

The morphology and size of samples were investigated on a field emission scanning electron microscope (FE-SEM, Regulus 8230; Hitachi Co, Tokyo, Japan) with an acceleration voltage of 5 kV. All samples were sprayed with a thin platinum layer. Cellulose crystallinity index (CrI) of bleached raw eucalyptus and pretreated biomass were measured by powder X-ray diffraction (D8 Advance, Bruker, Germany) using Cu Kα radiation at 40 kV and 40 mA. The samples were tested with 2θ ranging from 5° to 40°. The CrI of all dried neat films were determined using the equation (4),[26] and the Scherrer equation (5) was used to calculate the crystallite size, D (nm).[27]

$$\mathrm{CrI}(\%) = \frac{\mathrm{I}_{200} - \mathrm{I}_{\mathrm{am}}}{\mathrm{I}_{200}} \times 100 \quad (4)$$

$$D = \frac{k \times \lambda}{\beta \times \cos\theta} \quad (5)$$

Where I_{200} and I_{am} were the value of maximum intensity at 2θ between 22° and 23° and minimum intensity at 2θ between 18° and 19°, respectively (Fig. S4). k is Scherrer constant (0.94),

λ is the X-ray wavelength (0.154 nm for Cu), θ is the half of the (200) Bragg diffraction peak position (2θ max position) in radians, β is the width of the peak at half maximum height. Weight-average degree of polymerization (DPw) of cellulose was measured on an Ubbelohde viscometer (IVS 300, ZONWON, China). For the sample with a lignin content higher than 3.0%, a further delignification step was performed by sodium chlorite bleaching.[28] This method can delignify selectively and does not degrade cellulose.[29] Then, with continuous nitrogen flushing, 0.1 g dried bleached raw *Eucalyptus* and cellulose-rich pulp were dissolved in 0.5 M copper ethylenediamine (20 mL) for 30 min.[29] The DP of cellulose was calculated based on the intrinsic viscosity taking the effect of hemicellulose on viscosity into account:[30]

$$\mathrm{DP} = ((1.65 * \eta - 116 * \mathrm{H})/\mathrm{G})^{1.111} \quad (6)$$

Where η is the intrinsic viscosity, and H and G are the mass fractions of hemicellulose and glucan, respectively, obtained from the sugar analysis.[31]

3. Results and discussion

3.1 Fractionation of *Eucalyptus* with DMI/H_2O co-solvents

Hansen and Hidebrand approach has been successfully used to pre-screen solvents for extraction purposes.[32] RED (relative energy difference), as a qualitative criterion that estimates the polymer miscibility into a defined solvent, was calculated for DMI/H_2O co-solvents with various ratios (SI for calculation method). According to the results (SI Table S1), solvents (at DMI/H_2O ratios =10:0, 9:1 and 8:2) with the presence of sulfuric acid (75 mM) as catalyst were used in the pretreatment. Pre-screening experiment results indicate that reactions lower than 120 ℃ were not effective for lignin removal (SI Table S3). Therefore 120 ℃ was selected which is much lower than traditional organosolv pretreatment using ethanol and acetic acid.[33, 34] Owing to the low viscosity of the co-solvent, cellulose-rich pulp could be easily obtained via filtration from the solvent where dissolved lignin was precipitated and recovered (Fig.1).

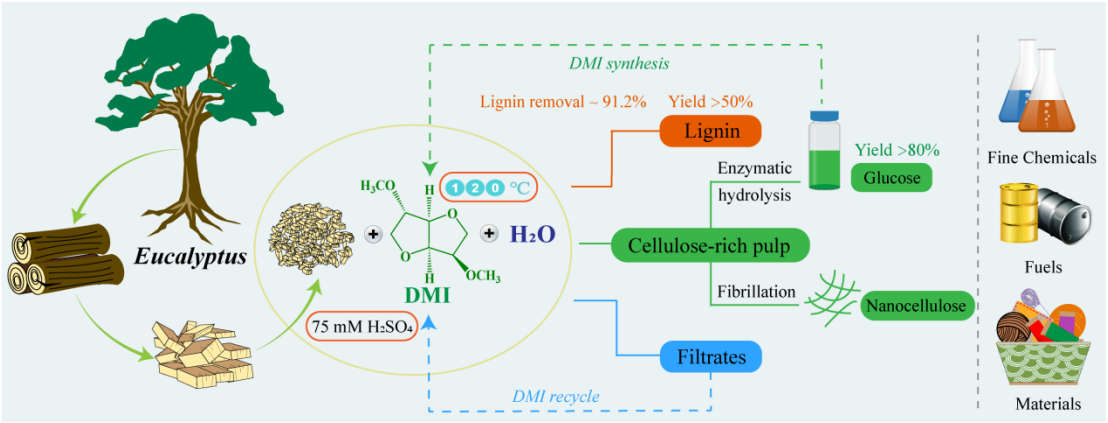

Fig 1. Schematic diagram of DMI/H_2O co-solvent pretreatment

As shown in Fig. 2, pulp yield, lignin yield, lignin removal efficiency, and pulp compositions were measured. It is found that lignin removal efficiency and cellulose contents in the pretreated residue increase with prolonged pretreatment time while cellulose-rich pulp yield, lignin and hemicellulose contents decrease. Take 60 min as an example, the lignin removal efficiency and lignin yield using co-solvent (60 min_9:1) achieved over 90% and 50%, respectively. As a result, the pulp containing 92.7% cellulose, 0.6% hemicellulose and 6.7% lignin was obtained. Around 80% of cellulose in original biomass was maintained, while over 98% of hemicellulose was removed (SI Table S4), demonstrating a low degradation of cellulose and a high fractionation selectivity during the pretreatment.[35] For 60 min pretreatment using pure DMI, the lignin removal efficiency was lower than that using DMI/H_2O co-solvent (60 min_9:1). This is probably due to the increased mass transfer and interactions between solvent/acidic catalyst and lignin induced by the addition of water. Similar phenomena were reported for deep eutectic solvent (DES) and THF.[36, 37] H_2O in these co-solvents (e.g., THF/H_2O) is considered as an efficient plasticizer favoring organic solvent diffusion into the compact lignin complexes, leading to the solubilization of lignin in solvent-water mixture. In addition, H_2O was revealed to be a nucleophile agent to break the linkages between lignin and hemicellulose (e.g., hydrogen bonds, ether and ester bonds) owing to its higher hydrogen bond acceptor ability compared to THF.[37] For pretreatment using DMI/H_2O at lower ratio e.g., 4:1, decreased lignin removal efficiency was observed which was echoed by findings for THF/H_2O[37] and GVL/H_2O[38] co-solvent systems. This is probably due to the reduced cleavage activity of sulfuric acid and the decreased dissolving ability of solvent due to the presence of water at a higher percentage. In summary, DMI/H_2O is found to be a promising co-solvent system to fractionate

lignocellulosic biomass at a mild condition. Similar to other co-solvent systems, H_2O was observed to play an important role in altering lignin and hemicellulose removal efficiency.

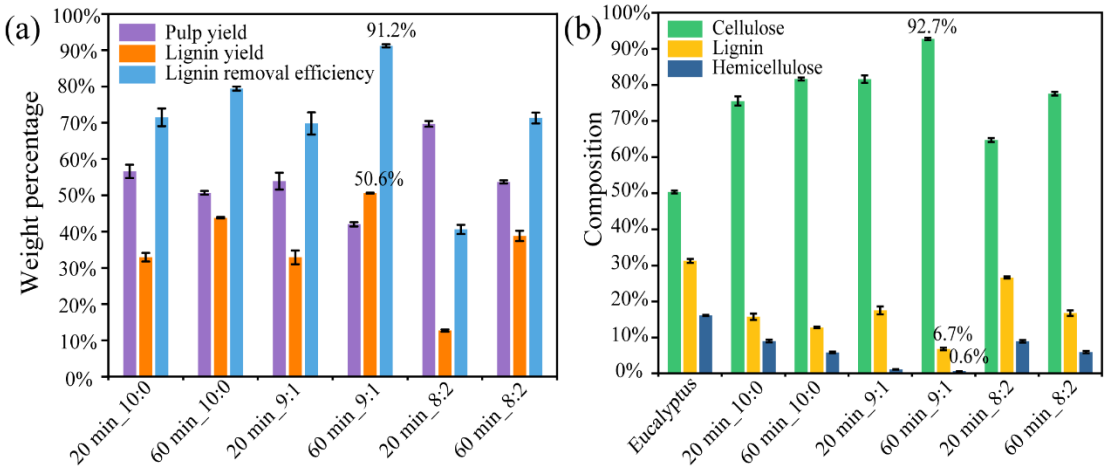

Fig 2. Pretreatment efficiency and composition analysis. (a) pulp yield, lignin yield and lignin removal efficiencies, (b) composition of cellulose-rich pulp under different pretreatment conditions.

3.2 Lignin characterization

Lignin, a 3D heterogeneous phenolic polymer in the plant cell walls, primarily comprises of guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) subunits. In general, softwood lignin is mainly composed of G units with a small amount of H unit lignin, while hardwood lignin consists of G and S units.[39] After above pretreatments, lignin fractions were precipitated and recovered. The structures and functional groups of the obtained lignin were investigated via 2D HSQC NMR, ^{31}P NMR, and GPC analysis. Milled wood lignin (MWL) from the same *Eucalyptus* was generated and used as 'model native lignin' for comparison purposes.

The 2D HSQC NMR spectra of MWL and the recovered lignin from the 60 min pretreatments were shown in Fig. 3 (a–b), while the corresponding subunits assignments and a semi-quantitative analysis were illustrated in Fig. 3 (c) and Table 1, respectively. Due to the cleavage of the reactive ether bonds during acid-catalyzed pretreatment, contents of β-O-4 aryl ether linkages decreased to various extent in all lignin samples depends on the pretreatment severity as well as the DMI/H_2O ratio applied. For example, the aryl ether linkages were completely removed during the pure DMI pretreatment, while nearly 37.6% amount of ether linkages were preserved after the 20 min_8:2 DMI/H_2O co-solvent pretreatment. A slight decrease in the content of ether linkages was also observed as the pretreatment time extended from 20 to 60 min. In the meantime, the cleavage of

ether linkages (e.g., β-O-4) were also accompanied by the formation of new carbon-carbon linkages (e.g., $S_{2,6\ condensed}$ and $G_{2\ condensed}$) as observed in Fig. 3 (b). It was observed that adding a small amount of water could reduce the breakage rate of β-O-4 linkages significantly (60 min_10:0 vs. 60 min_9:1), which eases the condensation dramatically (Fig.3b) and synergistically enhance lignin removal efficiency (Fig.2a). Consequently, both lignin S and G subunits were largely condensed to some extent in the pure DMI pretreatment (See Fig. 3b), while only a small amount of the S subunits were condensed in the DMI/H_2O co-solvent pretreatment. These findings were echoed by a recent study on GVL/H_2O co-solvent.[38] It was shown that the addition of water into the GVL system could slow the lignin depolymerization over the ether bond cleavage and ease the lignin condensation reactions. The model compound study revealed that the inhibition of dimeric or trimeric structures is due to the fact that water as an external nucleophile could stabilize the benzyl carbocation-type intermediates, introducing reactive OH groups[38]. Besides β-O-4 aryl ether, β-β, and β-5 linkages, changes in the S/G ratio were also investigated during the DMI/H_2O co-solvent pretreatment. The S/G ratio of MWL was 0.92, which was much lower than that of the pretreatment samples. Meanwhile, the S/G ratios were positively correlated with the DMI ratio in the co-solvent system. The increased S/G ratio after pretreatment may be due to the low molecular weight of G-type lignin is preferentially degraded than S-type lignin at the mild temperature (120 ℃).[40] These findings were also echoed by previous studies showing that organic solvents with different structures and properties influenced the solubilization of lignin with various structure units. For example, MeTHF, EAC and GVL dissolved S- and G-type lignin, while THF solvent showed high selectivity to fractionate and solubilize lignin with G and H units.[37]

Table 1. Semi-quantitative information for subunits and interunit linkage in lignin before and after DMI/H_2O co-solvent pretreatment.

	β-O-4[a]	β-β[a]	β-5[a]	S/G
MWL	57.6%	7.3%	3.1%	0.92
20 min_10:0	N/A[b]	6.4%	N/A	3.84
60 min_10:0	N/A	5.0%	N/A	4.53
20 min_9:1	33.0%	5.7%	1.0%	2.72
60 min_9:1	24.8%	6.5%	0.4%	3.55
20 min_8:2	37.6%	6.1%	1.1%	2.06
60 min_8:2	30.2%	5.6%	0.6%	2.87

[a] Content (%) expressed as a fraction of S + G.

[b] N/A: below the NMR detection limit.

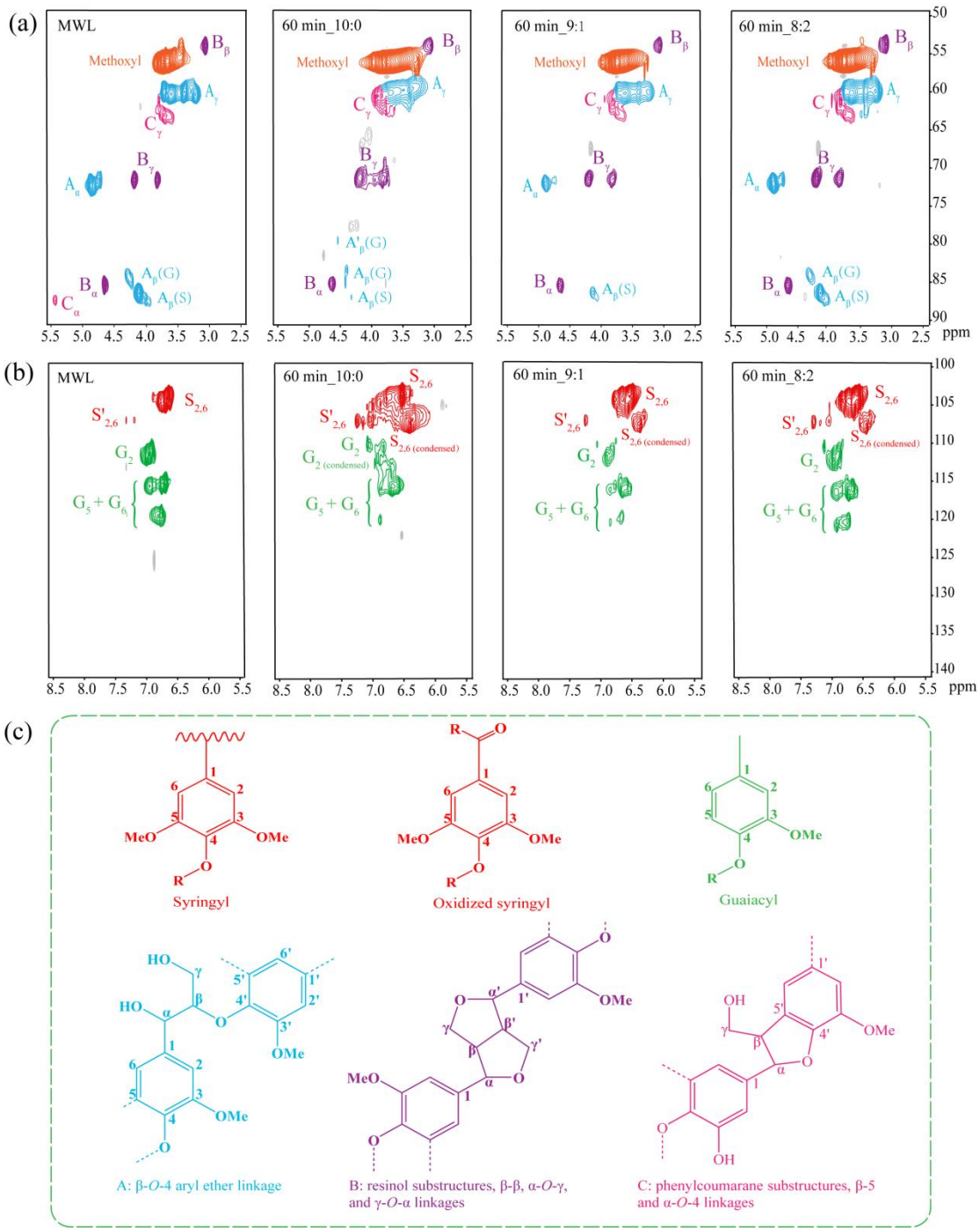

Fig. 3 (a) Side-chain regions and (b) aromatic region in the 2D HSQC NMR spectra of MWL and DMI lignin; (c) main substructures of lignin identified by 2D HSQC NMR, involving aromatic units and side-chain linkages.

Considering the valorization potential of DMI lignin, the types and contents of hydroxyl groups are also crucial parameters, as they strongly determine the physicochemical properties of lignin.[41] In the quantitative ^{31}P NMR spectra, four different types of hydroxyl groups were observed in MWL

(Fig. 4a), including aliphatic hydroxyl, C_5 substituted hydroxyl, guaiacyl hydroxyl, and carboxylic hydroxyl (Fig. 4b). The aliphatic hydroxyl group was the dominant hydroxyl group in MWL (4.58 mmol g^{-1}), and its content decreased to 1.54 mmol g^{-1} and 1.31mmol g^{-1} after pure DMI (10:0) pretreatment 20 min and 60 min, respectively (Fig. 4c). These reductions in aliphatic hydroxyl groups mainly due to the loss of γ-methylol group as formaldehyde and OH groups on the $C_{\alpha,\beta}$ or the whole side chain to form β-1′ linkages which are then converted to stilbene type of structures.[42] It is observed that the declining trend of aliphatic hydroxyl group content was hindered when water was added in the pretreatment system, probably due to the introduction of active OH groups instead of forming larger structures as revealed in GVL/H_2O co-solvent system.[38] According to related reports, syringyl and condensed guaiacyl were combined into C_5 substituted phenolic units to avoid a possible overestimation of syringyl units or underestimation of condensed guaiacyl units.[13, 43] The C5 substituted hydroxyl group content increased from 0.44 mmol g^{-1} in MWL to 1.16 mmol g^{-1}, 1.62 mmol g^{-1} and 2.06 mmol g^{-1} after 60 min_8:2, 60 min_9:1 and 60 min_10:0, respectively. Guaiacyl hydroxyl content was decreased to a similar level of around 0.70 mmol/g, compared with 1.27 mmol/g in MWL. The increased C_5-substituted hydroxyl and decreased of guaiacyl hydroxyl content are mainly due to the lignin condensation ($S_{2,6\ condensed}$), consistent with findings in changes of S/G ratio (Table 1). The contents of carboxylic hydroxyl were not significantly affected by the pretreatments, echoed by findings from other studies.[44]

Molecular weight is another index to evaluate the physicochemical properties of lignin. The weight-average molecular weight (M_w), number-average molecular weights (M_n) and dispersity (Đ, Mw/Mn) of the seven-lignin preparations were determined by APC analysis. As shown in Fig. 4d, M_w and M_n were all significantly reduced after pretreatment. The M_w and M_n of MWL are 6708 g $mol^{-1}$and 4500 g mol^{-1}, while the that of 60 min_10:0 lignin are 2804 g mol^{-1} and 1953 g mol^{-1}, respectively. This is because the co-solvent pretreatment was accompanied by lignin depolymerization reactions. In other words, the cleavage of inter-units linkages results in a smaller molecular weight of fractionated lignin after pretreatment as shown in 2D HSQC NMR results (Table 1). The molecular weights of 60 min_9:1 lignin were slightly smaller than that of 60 min_8:2 and MWL obtained from raw *Eucalyptus*, however, the difference between them was not significant. The Đ of each sample was calculated to be small (<2), indicating the lignin molecular weight distribution was relatively uniform and offering a have a great potential in high-value applications

of lignin in chemical industry.[40]

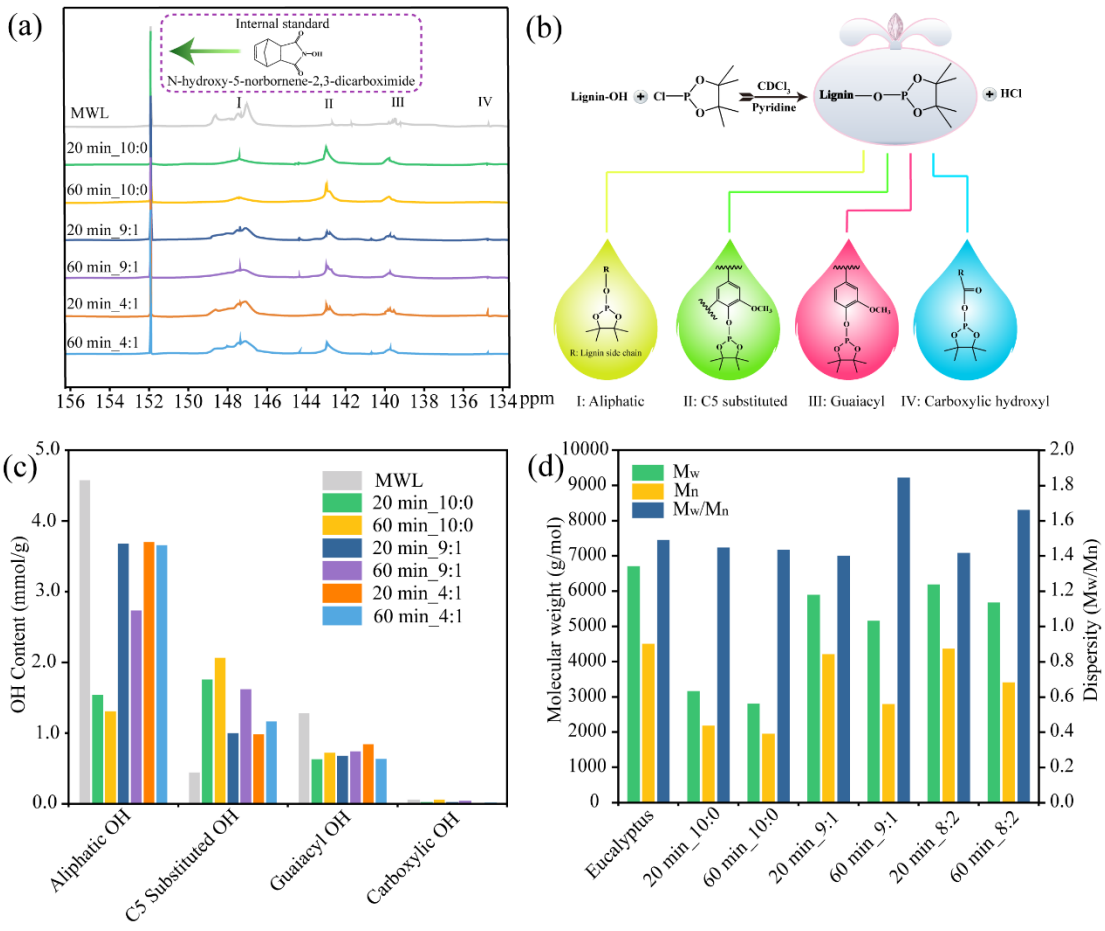

Fig.4 ^{31}P NMR and APC analysis of MWL and DMI lignin. (a) quantitative ^{31}P NMR spectra, (b) the phosphorylated products of the reaction between hydroxyl groups in lignin and TMDP, (c) contents of various aliphatic, phenolic, and carboxylic hydroxyl groups, (d) weight-average and number-average molecular weight of lignin.

3.3 Pulp and cellulose characterization

Cellulose-rich pulp was another product from the DMI/H_2O co-solvent pretreatment, while the features of the pulp will affect its enzymatic hydrolysis process. The CrI, crystallite size, DPw, and morphologies of cellulose or pulp were determined to reveal the changes of cellulose duirng biomass pretreatment. As shown in Fig.5a, the CrI of the raw material was determined to be 72.6% while the CrI increases slightly to 73.8%, 79.5% and 78.3% for the 60 min_10:0, 60 min_9:1 and 60 min_4:1 pretreatment (SI Table S6). These increases are due to the removal of amorphous hemicellulose and lignin during pretreatment, which was confirmed by composition analysis (SI Table S4). Crystallite

size was also calculated from XRD patterns (SI Fig.S3), which had the same trend with CrI, a slight increase after pretreatment. These changes are probably due to the swelling of cellulose after penetration of solvent into the crystalline region.[27] The DPw of cellulose, as the average number of cellulose monomer units, is another important property of cellulose that may affect the mechanical properties of cellulose composite materials.[45] The DPw of cellulose in the pretreated pulp was found to dramatically decrease from 2310 in raw material to 1144, 1478 and 823 after for the 60 min_10:0, 60 min_9:1 and 60 min_8:2 pretreatment, which is due to the extensive scission of cellulose glycosidic bonds under acidic conditions.[46] It has been reported that shorter cellulose chains have more reducing ends and are beneficial to enzyme hydrolysis of cellulose.[13]

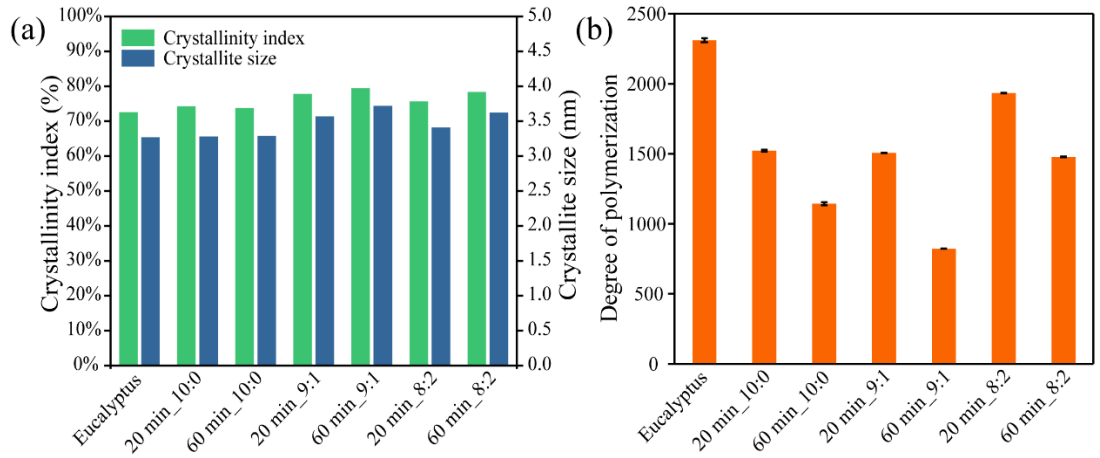

Fig 5. Characterization of cellulose obtained from original and the pretreated *Eucalyptus* under different conditions. (a) cellulose crystallinity and crystallite size, (b) weight-average degree of polymerization.

The morphological changes of *Eucalyptus* after pretreatment were revealed by SEM (Fig. 6). The width of the raw particles was around 170 μm, which is consistent with the size of 60–100 mesh (Fig. 6a). At higher magnification, the lumen was clearly observed (Fig. 6b), and the cell wall was kept compact (Fig. 6c). After 60 min_10:0 and 60 min_9:1 pretreatment, the raw materials fractured into much smaller fibrillar particles with a diameter of around 20-50 μm (Fig. 6d,g), owing to high lignin removal efficiency (Fig. 2a). Meanwhile, the lumen was not observed anymore and nanofibrils were partially exposed (Fig. 6e,f,h,i). In contrast, the shape and size of majority materials after 60 min_4:1 pretreatment remained unchanged, probably due to a lower lignin removal efficiency (Fig. 6j).

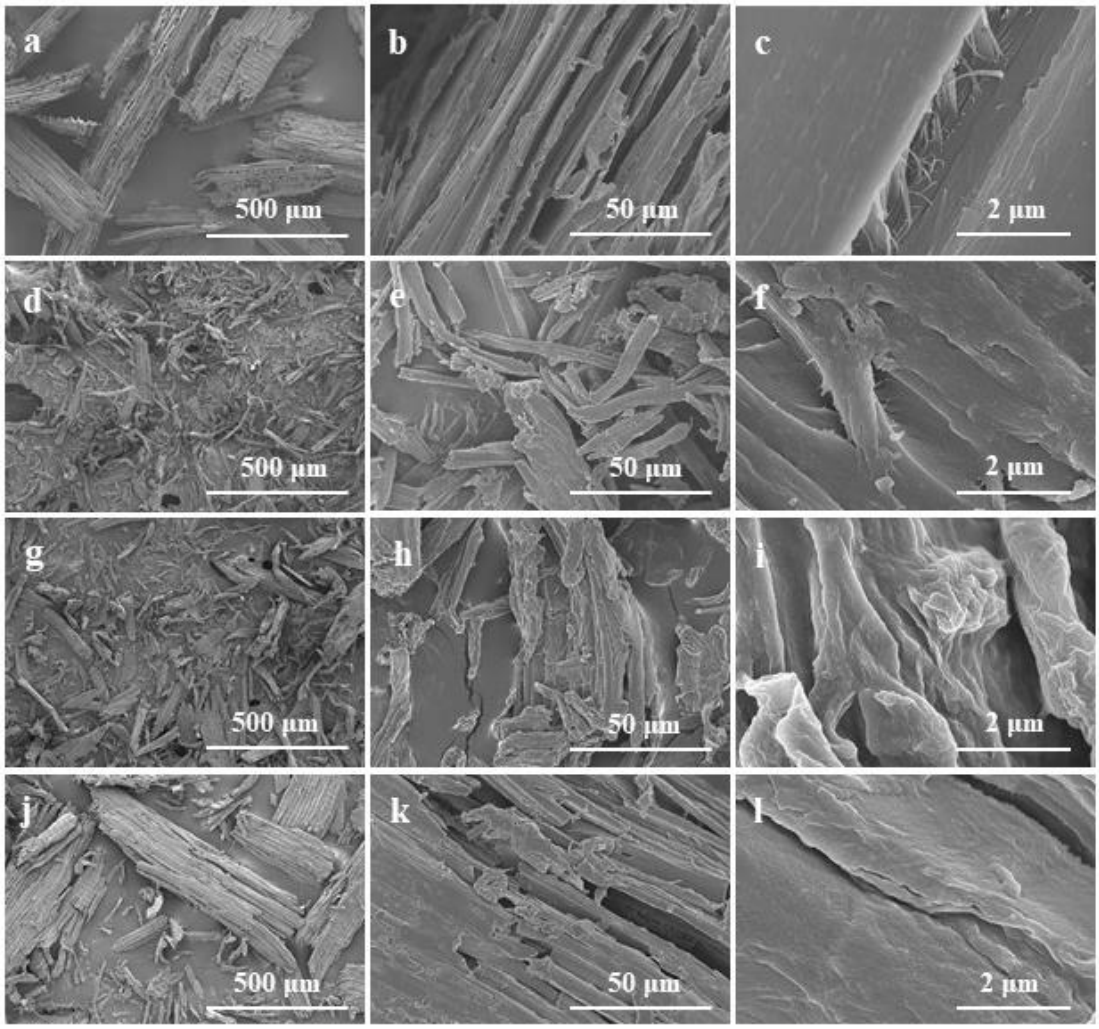

Fig. 6 SEM images of the raw and pretreated substrates under different conditions. (a) Raw *Eucalyptus*_100x, (b) Raw *Eucalyptus* _1000x, (c) Raw *Eucalyptus*_20000x, (d) 60 min_10:0_100x, (e) 60 min_10:0_1000x, (f) 60 min_10:0_20000x, (g) 60 min_9:1_100x, (h) 60 min_9:1_1000x, (i) 60 min_9:1_20000x, (j) 60 min_8:2_100x, (k) 60 min_8:2_1000x, (l) 60 min_8:2_20000x.

3.4 Enzymatic hydrolysis

The enzymatic hydrolysis of the pretreated pulp was carried out with Cellic® CTec 2 at two loadings (20 FPU and 60 FPU g^{-1} of glucan), to produce fermentable sugar. After 72 hours of enzymatic hydrolysis, the sugar yield of the 60 min_9:1 sample achieved over 80% and 97% under 20 FPU and 60 FPU g^{-1} glucan, respectively, obviously higher than that of raw material, 60 min_10:0 and 60 min_8:2 samples. Smaller particle size, lower lignin and hemicellulose contents, and lower DPw of cellulose together offer more accessible sites to enzymes in 60 min_9:1 sample (characterization and SEM in section 3.3).[47] This results are comparable to those using Cyrene™ with high enzyme loading (60 FPU g^{-1} of glucan), and better than those at low enzyme loading (20

FPU g^{-1} of glucan), and no post-NaOH washing is need to enhance the enzymatic hydrolysis performance.[13] However, the enzymatic hydrolysis of 60 min_8:2 pulp resulted in higher sugar yield than that of 60 min_10:0 pulp (Fig. 7a), which could not be explained according to the pulp size (Fig.6d,j), lignin and hemicellulose contents (Fig. 2b), and DPw of cellulose (Fig. 5b). Alternative possibilities could be the enzyme inhibition caused by (1) lignin deposited on pulp during lignin precipotation,[48] and (2) structural changes of the residual lignin remained in the pretreated pulp.[23] To verify former one, the pretreated pulp was extracted with 1,4-dioxane to remove possibly deposited lignin before the enzymatic hydrolysis experiment. However, there was no significant changes found in sugar yield after the 1,4-dioxane extraction (Fig. S4), revealing that DMI/H_2O could prevent lignin redepostion, similar to THF/H_2O co-solvent.[49] To verify the second possible reason, the remained cellulolytic enzyme lignin (CEL) in the pretreated pulp was harvested after over-dosed enzymatic hydrolysis. As shown in Fig. 7b, the remained CEL lignin in the 60 min_10:0 pulp was demonstrated to be more severely condensed, while the structure of the remained lignin on the 60 min_8:2 pulp was close to MWL (Fig. 3b). It has been reported the condensed structure of the remained lignin could increase the hydrophobicity of lignin,[23] which was recognized as one of the major inhibition factors to enzyme. In addition, Sun et al. also reported a strong association of condensed phenolic (S+G) moieties in isolated lignins with their inhibition of enzymatic hydrolysis.[50] This could support the findings that a lower sugar yield from 60 min_10:0 pulp though a relatively good level of lignin removal could be achieved under this condition. Nevertheless, pulp obtained from DMI/H_2O co-solvnet pretreatment (60 min_9:1) is a promising feedstock for fermentable sugar towards chemical and fuels production.

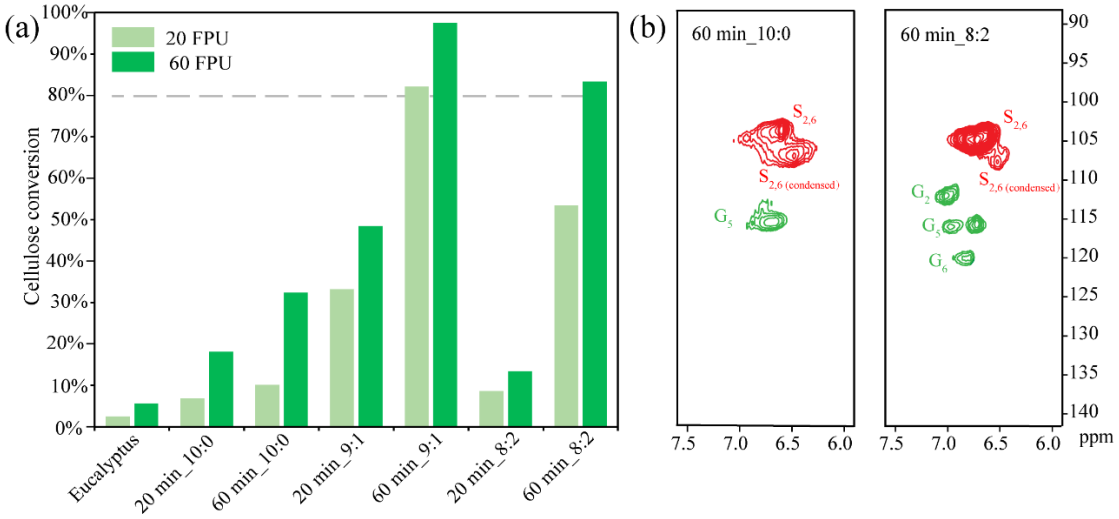

Fig.7 (a) Enzymatic hydrolysis results of the raw material and pretreated pulp under different conditions. The enzymatic hydrolysis was performed at enzyme loading of 20 FPU g^{-1} glucan and 60 FPU g^{-1} glucan for 72h, (b) 2D HSQC NMR spectra of the remained lignin on the pretreated pulp.

3.5 Pulp fibrillation

Nanocelluloses, which can be produced by the deconstruction of cellulosic fibers, have been considered as promising building blocks for multifunctional materials.[51] Another extensive utilization of organosolv products is to produce nanocellulose, which has been rarely reported.[52] The sodium chlorite-bleached *Eucalyptus* pulp was used as a comparison, since it can be easily fibrillated owing to the preserved hemicellulose.[53] The pulp suspensions were mildly milled in 1.5 mL centrifuge tubes. As shown in the SEM images (Fig.8), the 60 min_9:1 pulp (Fig. 8d) could be fibrillated surprisingly better than the bleached pulp (Fig. 8b). It was observed that the 60 min_9:1 pulp was delaminated into thin layers, while the bleached pulp kept the thickness of cell walls. At a higher magnification of the 60 min_9:1 sample, more nanofibrils were exposed. Unlike traditional organosolv or kraft pulping at high temperatures (for example 180 ℃), the current DMI/H_2O pretreatment was at a lower temperature (120 ℃), which might eliminate the fiber hornification. In addition, the high lignin removal efficiency of the 60 min_9:1 pretreatment loosened the cell walls and facilitated the fibrillation process. Considering that a large amount of energy consumption is one of the major challenges for nanocelluloses fibrillation, [54] the current DMI/H_2O pretreatment may provide a potential solution.

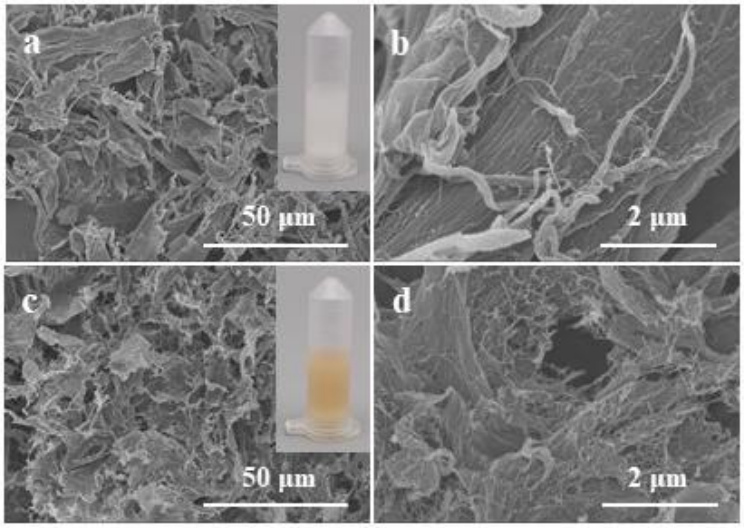

Fig.8 SEM images of cellulose nanofibers after defibrillation (a) Bleached *Eucalyptus*_1000x, (b)

Bleached *Eucalyptus* _20000x, (c) 60 min 9:1_1000x, (d) 60 min 9:1_20000x,

3.6 Comparison with other organosolv pretreatments

The advantage of DMI/H_2O co-solvent pretreatment in lignin removal was demonstrated by comparing with other advanced co-solvent (2-MeTHF, GVL and Cyrene™) pretreatments, as shown in Table 2. It has been widely recognized that the content of β-O-4 linkages was an important index to evaluate the quality of lignin obtained after pretreatment, especially for the purpose of production of aromatic lignin monomers.[55] To preserve the reactive β-O-4 linkages, biomass pretreatment should be either pretreated at a mild condition or a complicated lignin stabilization strategy should be used. However, the delignification efficiency is normally correlated with the lignin-target pretreatment severity, and it could suffer from a mild pretreatment condition thus subsequently affecting the carbohydrate conversion and lignin yield. Considering the difference among different tree species, we compared the β-O-4 content of *Eucalyptus* pretreated by different organic solvents (EtOH, DES, THF and GVL), demonstrating DMI/H_2O co-solvent pretreatment has great advantages in preserving lignin integrity while still maintaining high delignification efficiency (>90%). Enzymatic hydrolysis efficiency of obtained cellulose-rich pulp was at the higher bound of range at relatively low enzymatic loading. Furthermore, DMI solution is stable under this mild condition (120 ℃ and 60 min) revealed by ^{1}H NMR (SI Fig.S6), providing the possibility of recycling the solvent.

Table 2 Comparison with other organosolv pretreatments in terms of lignin removal, β-O-4 linkages and sugar yield.

Feedstock	Solvent	Conditions	Lignin removal	β-O-4	Sugar yield	Ref.
Bamboo	2-MeTHF: water (50:50, vol)	180°C, 1M oxalic acid, 20 min	41.9%	-	92.9% (27.8 FPU g^{-1} glucan, 96 h)	[56]
Beech	GVL: water (80: 20, vol)	120°C, 75 mM H_2SO_4, 60 min	77%	-	55% (15 FPU g^{-1} glucan, 48h)	[57]
Poplar	Cyrene™: water (80:20, vol)	120°C, 75 mM H_2SO_4, 60 min	68%	~30%	~70% (20 FPU g^{-1} glucan, 96 h)	[13]
Eucalyptus	EtOH: water (65: 35, vol)	170°C, 20 mM H_2SO_4, 60 min	>60%	17%	~90% (7.5 FPU g^{-1} glucan, 48 h)	[23]
Eucalyptus	DES (ChCl: lactic acid=1: 10, mol)	110°C, 240 min	>80%	11.84%	94.3% (22.5 FPU g^{-1} glucan, 72 h)	[58]
Eucalyptus	THFA: water (80:20, vol)	180°C, 1% HCl, 30 min	93.9%	-	82.5% (22.9 FPU g^{-1} glucan, 72 h)	[59]

Eucalyptus	GVL: water (80:20, vol)	120°C, 50 mM H_2SO_4, 60 min	90.7%	3.9%	94.3% (17.5 FPU g^{-1} glucan, 72 h)	[60, 61]
Eucalyptus	DMI: water (90:10, vol)	120°C, 75 mM H_2SO_4, 60 min	91.6%	24.8%	82.1% (20 FPU g^{-1} glucan, 72 h)	This study

4. Conclusions

Biobased solvent is an emerging alternative to traditional organic solvent used in the pretreatment of lignocellulosic biomass. In the current research, we demonstrated the potential capability of DMI/H_2O co-solvent for the fractionation of *Eucalyptus* towards yielding multiple products with a potential complete valorization. Under a mild condition (120 °C, 60 min), the removal efficiencies of lignin and hemicellulose were above 90% and 98% with over 80% of cellulose maintained. When 10% of water was incorporated in the co-solvent system, the structure of the recovered lignin was well maintained (around 25% β-O-4 links preserved), yielding a more 'native-like' lignins attractive or depolymerization to aromatic chemicals. The yield of fermentable sugars after enzymatic hydrolysis could achieve over 80% at a relatively low enzyme loading. H_2O is found to play an important role in tailoring the lignin removal efficiency and structure, affecting the enzymatic hydrolysis performance consequently. As an alternative application of pulp, nanocelluloses could be obtained via a relatively simple fibrillation. This demonstrates DMI/H_2O could be a promising treatment to produce these functional material precursors with a potential of saving energy consumption in the fibrillation step. Overall, the current research developed a novel DMI/H_2O co-solvent pretreatment technology that could contribute to a closed-loop biorefinery. For the future investigation, we will focus on realizing the recycling of DMI, demonstration on different feedstocks, screening for more selective catalysts to further improve the quality of lignin, and performing economic and environmental assessment aiming at scaled-up applications.[46]

Conflicts of interest

There are no conflicts to declare.

Acknowledgement

This project was supported by the foundation of Westlake University (Grant no. 103256021901). The authors would like to acknowledge the Instrumentation and Service Center for Molecular Sciences and Physical Sciences at Westlake University for most of the characterizations conducted in this study.

Reference

1. J. Zhang, H. Zhou, D. Liu and X. Zhao, *Lignocellulosic Biomass to Liquid Biofuels*, 2020, 17-65.
2. F. C. herubini, *Energy Conversion & Management*, 2010, **51**, 1412-1421.
3. W. Lei, J. Littlewood and R. J. Murphy, *Renewable & Sustainable Energy Reviews*, 2013, **28**, 715-725.
4. L. Wang, M. Sharifzadeh, R. Templer and R. J. Murphy, *Energy & Environmental Science*, 2012, **5**, 5717-5730.
5. B. Yang and C. E. Wyman, *Biofuels Bioproducts & Biorefining*, 2010, **2**, 26-40.
6. F. Liu, X. Dong, X. Zhao and L. Wang, *Energy Conversion and Management*, 2021, **246**, 114653.
7. X. Yin, L. Wei, X. Pan, C. Liu and K. Wang, *Frontiers in Plant Science*, 2021, **12**, 1-14.
8. C. Nitsos, U. Rova and P. Christakopoulos, *Energies*, 2018, **11**, 1-23.
9. Z. Zhou, F. Lei, P. Li and J. Jiang, *Biotechnology and Bioengineering*, 2018, **115**, 2683-2702.
10. J. B. Zimmerman, P. T. Anastas, H. C. Erythropel and W. Leitner, *Science*, 2020, **367**, 397-400.
11. S. X. Li, M. F. Li, J. Bian, S. N. Sun, F. Peng and Z. M. Xue, *Bioresource Technology*, 2017, **243**, 1105-1111.
12. L. Shuai, Y. M. Questell-Santiago and J. S. Luterbacher, *Green Chemistry*, 2016, **18**, 937-943.
13. X. Meng, Y. Pu, M. Li and A. J. Ragauskas, *Green Chemistry*, 2020, **22**, 2862-2872.
14. A. Duval and L. Avérous, *Green Chemistry*, 2022, **22,** 1671-1680.
15. P. Tundo, F. Arico, G. Gauthier, L. Rossi, A. E. Rosamilia, H. S. Bevinakatti, R. L. Sievert and C. P. Newman, *ChemSusChem*, 2010, **3**, 566-570.
16. M. Annatelli, D. D. Torre, M. Musolino and F. Aricò, *Catalysis Science & Technology*, 2021.
17. F. Aricò, A. S. Aldoshin and P. Tundo, *ChemSusChem*, 2017, **10**, 53-57.
18. F. Russo, F. Galiano, F. Pedace, F. Aricò and A. Figoli, *ACS Sustainable Chemistry & Engineering*, 2020, **8**, 659-668.
19. M. L. Weaver, A. B. Grossi, J. Schützsack, J. Parish, J. Løgsted, I. B. Bøgh, D. Cameron, W. Harvey, M. Festag, N. Downes, S. Venturella, J. Schlichtiger, S. Mhedhbi, V. Ross, T. Kissner, C. Stark, S. Milano, P. Heining and M. Sanchez-Felix, *Toxicologic Pathology*, 2016, **44**, 367-372.
20. P. Tundo, F. Aricò, G. Gauthier, L. Rossi, A. E. Rosamilia, H. S. Bevinakatti, R. L. Sievert and C. P. Newman, *ChemSusChem*, 2010, **3**, 566-570.
21. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templaton and D. Crocker, 2010.
22. D. S. Zijlstra, C. W. Lahive, C. A. Analbers, M. B. Figueirêdo and P. J. Deuss, *ACS Sustainable Chemistry & Engineering*, 2020, **8**, 5119-5131.
23. C. Dong, X. Meng, S. Y. Chi, H. Y. Tse, A. J. Ragauskas and S. Y. Leu, *Green Chemistry*, 2019, **21**, 2788-2800.
24. H. Chen, P. Gnanasekar, S. S. Nair, W. Xu, P. Chauhan and N. Yan, *ACS Sustainable Chemistry & Engineering*, 2020, **8**, 17165-17176.
25. X. Meng, C. Crestini, H. Ben, N. Hao, Y. Pu, A. J. Ragauskas and D. S. Argyropoulos, *Nature Protocols*, 2019, **14**, 2627-2647.
26. *Textile Research Journal*, 1959, **29**, 786-794.
27. B. Qi, Z. Wang, J. Luo and Y. Wan, *Cellulose*, 2021, **28**, 10127-10141.
28. R. Kumar, G. Mago, V. Balan and C. E. Wyman, *Bioresource Technology*, 2009, **100**, 3948-

3962.
29. Z. Fang, B. Li, Y. Liu, J. Zhu, G. Li, G. Hou, J. Zhou and X. Qiu, *Matter*, 2020, **2**, 1000-1014.
30. A. V. Heiningen, M. S. Tunc, Y. Gao and D. Perez, *Journal of Pulp & Paper Science*, 2004, **30**, 211-217.
31. R. Kumar, G. Mago, V. Balan and C. E. Wyman, *Bioresource Technology*, 2009, **99**, 3948-3962.
32. Q. Zhang, X. Tan, W. Wang, Q. Yu, Q. Wang, C. Miao, Y. Guo, X. Zhuang and Z. Yuan, *ACS Sustainable Chemistry & Engineering*, 2019, **7**, 8678-8686.
33. C. S. Lancefield, I. Panovic, P. J. Deuss, K. Barta and N. J. Westwood, *Green Chemistry*, 2017, **19**, 202-214.
34. A. P. Trzcinski and D. C. Stuckey, *Bioresource Technology*, 2015, **185**, 441-444.
35. D. Weidener, W. Leitner, P. Domínguez de María, H. Klose and P. M. Grande, *ChemSusChem*, 2021, **14**, 909-916.
36. M. Zhou, O. A. Fakayode, A. E. Ahmed Yagoub, Q. Ji and C. Zhou, *Renewable and Sustainable Energy Reviews*, 2022, **156**, 111986.
37. J. Li, W. Zhang, S. Xu and C. Hu, *Frontiers in Chemistry*, 2020, **8**, 1-17.
38. E. Jasiukaitytė-Grojzdek, M. Huš, M. Grilc and B. Likozar, *ACS Sustainable Chemistry & Engineering*, 2020, **8**, 17475-17486.
39. R. Katahira, G. T. Beckham and T. J. Elder, *Chapter 1: A Brief Introduction to Lignin Structure*, London, UK: The Royal Society of Chemisty (RSC), United States, 2018.
40. C. Wang, H. Li, M. Li, J. Bian and R. Sun, *Scientific Reports*, 2017, **7**, 593.
41. M. Barde, S. Adhikari, B. K. Via and M. L. Auad, *Green Materials*, 2018, **6**, 76-84.
42. X. Wang, Y. Guo, J. Zhou and G. Sun, *RSC Advances*, 2017, **7**, 8314-8322.
43. M. Balakshin and E. Capanema, *Journal of Wood Chemistry & Technology*, 2015, **35**, 220-237.
44. C. G. Yoo, M. Li, X. Meng, Y. Pu and A. J. Ragauskas, *Green Chemistry*, 2017, **19**, 2006-2016.
45. X. Meng, S. Bhagia, Y. Wang, Y. Zhou, Y. Pu, J. R. Dunlap, L. Shuai, A. J. Ragauskas and C. G. Yoo, *Industrial Crops and Products*, 2020, **146**, 112144.
46. M. Li, S. Cao, X. Meng, M. Studer, C. E. Wyman, A. J. Ragauskas and Y. Pu, *Biotechnology Biofuels*, 2017, **10**, 237.
47. Y. Kim, T. Kreke, J. K. Ko and M. R. Ladisch, *Biotechnology and Bioengineering*, 2015, **112**, 677-687.
48. C. Huang, X. Jiang, X. Shen, J. Hu, W. Tang, X. Wu, A. J. Ragauskas, H. Jameel, X. Meng and Q. Yong, *Renewable and Sustainable Energy Reviews*, 2022, **154**, 111822.
49. L. Yao, H. Yang, X. Meng and A. J. Ragauskas, *Frontiers in Energy Research*, 2022, **9**, 1-15.
50. S. Sun, Y. Huang, R. Sun and M. Tu, *Green Chemistry*, 2016, **18**, 4276-4286.
51. X. Yang, S. K. Biswas, J. Han, S. Tanpichai, M. C. Li, C. Chen, S. Zhu, A. K. Das and H. Yano, *Advanced Materials*, 2021, **33**, 2002264.
52. X. Wang, H. Chen, X. Feng, Q. Zhang, N. Labbé, K. Kim, J. Huang, A. J. Ragauskas, S. Wang and Y. Zhang, *Wood Science and Technology*, 2020, **54**, 503-517.
53. S. Iwamoto, K. Abe and H. Yano, *Biomacromolecules*, 2008, **9**, 1022-1026.
54. O. Nechyporchuk, M. N. Belgacem and J. Bras, *Industrial Crops and Products*, 2016, **93**, 2-25.
55. L. Shuai and B. J. G. C. Saha, *Green Chemistry*, 2017, **19**, 3752-3758.
56. S.-X. Li, M.-F. Li, J. Bian, S.-N. Sun, F. Peng and Z.-M. Xue, *Bioresource Technology*, 2017, **243**, 1105-1111.
57. L. Shuai, Y. M. Questell-Santiago and J. S. Luterbacher, *Green Chemistry*, 2016, **18**, 937-943.

58. X.-J. Shen, J.-L. Wen, Q.-Q. Mei, X. Chen, D. Sun, T.-Q. Yuan and R.-C. Sun, *Green Chemistry*, 2019, **21**, 275-283.
59. Y.-H. Xu, Q. Zhou, M.-F. Li, J. Bian and F. Peng, *Bioresource Technology*, 2019, **280**, 489-492.
60. Y.-J. Li, H.-Y. Li, X.-F. Cao, S.-N. Sun and R.-C. Sun, *ACS Sustainable Chemistry & Engineering*, 2018, **6**, 12124-12131.
61. Y.-J. Li, H.-Y. Li, S.-N. Sun and R.-C. Sun, *Renewable Energy*, 2019, **134**, 228-234.