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Homolytic and heterolytic cleavage of β -ether linkages in hardwood lignin by Steam Explosion

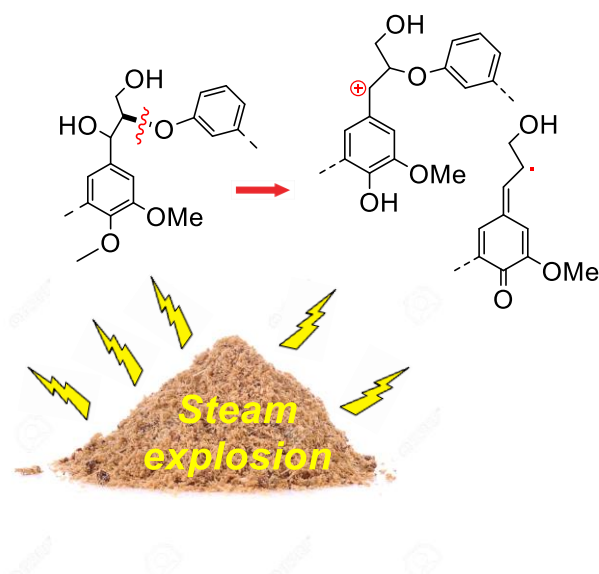
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Abstract

Steam explosion lignin (SEL) was extracted with ethanol from steam exploded hardwood (okoumé, *Aucoumea klaineana* Pierre) pretreated at various severities after neutral or acidic impregnation. The SELs were subjected to a structural characterization by 2D HSQC NMR, ^{31}P NMR, SEC and compared to Milled Wood Lignin (MWL). A strong decrease in the β -O-4 content is observed with increasing steam explosion severity accompanied with a gradual increase in molecular mass. α -oxydized S units (S', Hibbert's ketones) were quantified by NMR and used as a marker of the hydrolytic mechanism as well as naphthol used as a carbonium ion scavenger. It has been observed that mixed reactions of hydrolysis and homolysis are involved but the SEL is mainly cleaved homolytically, favouring recondensation through radical coupling even at low reaction severity. However, acidic pre-impregnation of wood prior to steam explosion enhanced the carbonium ion pathway.

Key words : Okoume; lignin; steam explosion

1. Introduction

Huge amounts of lignin are currently available as co-products of the pulp industry and it can be expected that even more important quantities will be produced in the next future by the emerging biorefineries. Lignin is of particular interest for new applications such as green adhesives, phenols, aromatics and carbon fibers [1]. However, owing to its complex structure, fractionation and valorization of lignin is still very challenging. It is formed by the in vivo polymerization of three monolignols namely p-coumaryl, coniferyl and syringyl alcohols, leading to p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units in the lignin polymer respectively. As a result of a random radical polymerization, H, G and S units are linked through various kinds of C-C and ether bonds. The most common linkages are β -O-4 followed by β - β and β -5. In addition to a large natural variability, the delignification or pulping processes used for lignin extraction significantly modified the polymer [2,3]. As a result, it is critical to figure out to how lignin processing can modify the reactivity and the structure of the resulting material.

The steam explosion pretreatment (SE) is one of the most promising process to open up the biomass fibers and to make the cellulose more accessible for subsequent processes such as enzymatic hydrolysis, fermentation or densification [4]. Since few years, industrial steam explosion facilities have been developed for bioethanol production from lignocellulosic agricultural residues at a commercial scale. SE is a thermo-mechanico-chemical treatment inducing a breakdown of lignocellulosic matrix due to an explosive decompression of biomass. The process results in an efficient depolymerization and extraction of hemicelluloses in the water stream and leaves most of the cellulose and lignin as a solid residue [5]. During the SE treatment, the steam explosion lignin (SEL) is not removed from

the pulp but is highly impacted [6]. In the current industrial pathways, the SE process does not specifically target lignin. Most of the SEL is left in the pulp and is recovered at the end of the biomass-to-ethanol process as an impure and degraded residue used for energy production. However, it has been described that a washing step of exploded fibers using an organic solvent [7] or an alkali [8,9] allows the recovery of low molecular mass lignin. However, compared to organosolv lignin which has been extensively studied [2,3,10], the literature is less abundant regarding the structure and composition of SEL. A better knowledge of the mechanisms involved during the process as well as the resulting lignin structure could then stimulate the developments of its further utilization.

From previous works regarding lignin modification after SE, a strong decrease of β -O-4 structures and a concomitant increase in C-C bonds due to SEL repolymerization have been observed [8,11,12]. A review of literature showed that two different mechanisms have been proposed for the SEL deconstruction / repolymerization. (1) An acid-catalyzed hydrolytic route involving a carbonium ion on the α position of the lateral chain was reported [8]. This ionic mechanism has been also extensively described for autohydrolysis and organosolv processes. This mechanism can be supported by an improvement in lignin extraction in presence of 2-naphthol. Naphthol acts as a carbonium ion scavenger limiting the lignin recondensation through electrophilic substitution reactions with aromatic moieties of lignin [12,13]. (2) An homolytic mechanism with a β -O-4 radical cleavage and subsequent coupling reactions has been also proposed [14]. However, taking into account the complexity of the lignin polymer and of its chemistry, the distinction between these two degradation pathways is tricky and the results or discussion from literature are sometime confusing.

Among the $400 \cdot 10^6 \text{ m}^3$ of potential exploitable volume for standing trees in Gabon, *Aucoumea klaineana* Pierre (Okoumé) represents $130 \cdot 10^6 \text{ m}^3$ accounting for 31 % of the total reserve of wood in Gabon forest [15] (Yoan et al., 2018). According to Nguema [16], approximately $1.5 \cdot 10^6 \text{ m}^3$ of transformed wood are produced in Gabon each year (veneers, plywood, trenching) producing approximately $750\,000 \text{ m}^3/\text{year}$ of waste. Currently more than 60% of these wood wastes are open-air burned or used as energy source. Recently studies have been undertaken for a better use of Okoumé co-products through organosolv pulping [17] and lignin characterization [18].

In the present study, we examined the steam explosion of Okoumé wood co-product. SEL was extracted from exploded wood in mild conditions using ethanol at low temperature and characterized. The impact of the severity of the process on the lignin yields and on its structure was examined through 2D HSQC NMR, ^{31}P NMR and Size Exclusion Chromatography (SEC). For comparison with SELs, Okoumé Milled Wood Lignin (MWL) was also extracted and characterized for the first time. The final goal of this work is to understand more detail hardwood lignin deconstruction during the steam explosion process.

2. Experimental

Experimental procedure is resumed in Fig. 1.

2.1. Raw materials

Aucoumea klaineana Pierre (Okoumé) sapwood used in this study ($\rho=0.484 \text{ g.cm}^{-3}$) is from a tree harvested in 2017 by the SNBG (Société Nationale des Bois du Gabon) at Mitzic natural forest located in the north of Gabon. The raw material had a dry moisture content of 4.1% and its chemical composition was: extractive = $2,96 \pm 0,15 \%$ (toluene : methanol : acetone 4 : 1 : 1); Klason lignin = $27.40 \pm 0.13 \%$; acid soluble lignin = $0.14 \pm 0.01 \%$; glucan = $59.57 \pm 2.64 \%$; xylan = $12.21 \pm 0,33 \%$; mannan = $2.34 \pm 0.10 \%$. Throughout the study, the samples were dried and then sheltered from moisture and fungal attacks in sealed plastic vials. Okoume slices were cut with a saw and then ground with a knife mill equipped with a 3 mm sieve and trilled with a 2 mm sieve.

2.2. Wood impregnation

Biomass was first impregnated during 15 h at room temperature in pure water or 0.5 % (w/w) dilute sulfuric acid, with a water/biomass ratio of 6/1. Regarding naphthol impregnation, 100 g (dry biomass) of Okoumé was impregnated in 1 L of ethanol containing 4 g of 2-naphthol (Sigma Aldrich $\geq 98\%$) [8]. After homogenization under stirring during 1 hour, the mixture was evaporated for 24 h at room temperature under a ventilated hood.

2.3 Steam explosion pre-treatment

The impregnated sawdust was transferred into a 2 L pressure-resistant steam gun where about 50 g (dry basis) of biomass were exposed to steam at varying temperatures and residence times. After the residence time, a pneumatic valve was opened leading to the

vapor phase exit from the reactor through a nozzle entraining the biomass. Pre-treatment parameters (using JMP software) were set at temperatures ranging between 170 and 210°C and a residence time of 2.5 to 7.5 mn. The operational conditions are summarized in Table 1. The severity factor of each treatment was determined using the following equation [19].

$$\text{LogR}_0 = \text{Log}(t \times e^{\left[\frac{T-100}{14.75}\right]}). \text{ (Eqn 1)}$$

Where t is the residence time (in mn) and T is the temperature (in °C).

After explosion, the solid was filtrated, washed with pure water and dried first, at room temperature for 48 hours and then in an oven at 105 °C for 24 h. SEL was then extracted with a soxhlet apparatus with the ethanol: water mixture (v/v 9: 1) during 24 h leading to a colorless solvent. The mixture was concentrated using a rotary evaporator to about 50 mL and the lignin was then precipitated in acidic medium (HCl, 6M) at pH 2, then centrifuged at 4000 rpm for 20 mn and dried at 40 °C for 48 h. This lignin was then quantified and characterized by NMR and SEC.

2.4. Milled Wood Lignin recovery

Milled wood lignin (MWL) was isolated after extensive milling of biomass in a ball mill prior to extraction with dioxane-water [2]. The wood chips were milled to pass through 0.05 mm pore-size screen, then the milled wood was extracted with 1: 2 (v / v) ethanol: benzene for 24 h in a Soxhlet apparatus. The extracted wood was left to dry in a hood. About 100 g of extracted wood was milled in a rotary ball mill at room temperature. The ball milled samples were mixed twice with a solution of water/dioxane (96% v/v) in a shaker for 24 h in the dark with a ratio liquid: solid of 10/1 (w/w). The dioxane-water extracts were combined, filtered and reduced to 100 mL of solvent at 35°C under reduced pressure. Lignin was then

precipitated in pure water and lyophilized. The lyophilized sample was then dissolved in 90% acetic acid and precipitated in deionized water (1 g/220 mL of water). After centrifugation, the solids were dissolved in 10 mL of 1,2-dichloroethane/ethanol mixture (2: 1 v/v) and precipitated in 200 mL of diethyl ether. This mixture was then centrifuged and washed with petroleum ether and finally air-dried at 40°C just before NMR analyses.

2.5 Insoluble lignin content

0.175 g of extractive-free wood meal, previously ground (0.160 mm of particle size), was put inside a plastic tube. 1.5 mL of sulfuric acid (72% w/w) were mixed with the sample and then incubated in a rotary water bath at 30°C for 1 h. The sample was taken out and 42 mL of pure water were added. The tube was then closed and autoclaved for 1 h. The solid, composed of insoluble lignin – also known as Klason lignin, was filtered, washed, dried at 105°C for 24 h and finally weighed. The liquid phase, containing monomeric sugars from cellulose and hemicelluloses, was completed with pure water to 100 mL and frozen for further analyses. Lignin content was calculated by using the mass variation, whereas monomeric sugars were quantified by High-Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection (HPAE-PAD).

2.6. Analyses

HSQC-NMR

For ^{13}C - ^1H Heteronuclear Single Quantum Coherence (HSQC) NMR analysis, the samples were prepared by dissolving 200 mg of purified (n-hexane) and non-acetylated lignin with 0.4 mL of dimethylsulfoxide- d_6 (99.8%). The mixture was homogenized with ultrasound for 30 min for complete dissolution. The spectra were recorded on Bruker Avance 400 MHz

instrument using standard Bruker program (reference). The analysis was made at 50°C to reduce the viscosity.

³¹P NMR

The two lignin samples were phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane in presence of cyclohexanol as an internal standard according to a method described by Granata and Argyropoulos [20]. The concentration of each hydroxyl functional group (in mmol.g⁻¹) was calculated on the basis of the hydroxyl content of the internal standard and its integrated peak area.

SEC

Size Exclusion Chromatography was carried out as follows: in a 2 mL vial, about 1.5 mg of dry non-acetylated lignin was accurately weighed and 1.5 mL of unstabilized tetrahydrofuran (THF) were added. The sample was then stirred magnetically for 24 h for complete solubilization. The samples were then filtered with a 0.45 µm PTFE membrane ensuring that no particles were visible in the filter thus confirming complete solubilization. Finally, the lignin analysis was carried out using a Shimadzu Prominence HPLC chromatograph equipped with a UV detector and 2 columns connected in series, Shodex GPC KF-806L 10 µm (8.0 x 300 mm) and Phenomenex Phenogel 5 µm 100 Å (7.8 x 300 mm). The mobile phase (~~eluent~~) was unstabilized THF with a flow rate of 0.7 mL.mn⁻¹. 20 µL of filtered solution were injected and separated at 35°C. The detection is carried out at a wavelengths of 280 and 254 nm (Shimadzu SPD-20A) Standard polystyrene samples were used for calibration.

HPAE-PAD

Monosaccharide contents of soluble fractions from lignin Klason hydrolysis were analyzed by HPAE-PAD (ICS-3000 Dionex) equipped with a Dionex CarboPac™ PA-20 (3x150 mm) analytical column. Filtered samples (20 µL) were eluted at 35°C and at 0.4 mL/mn with the following composition: pure water 99,2% /250 mM NaOH 0,8% : 0→20 mn ; pure water 75%/250 mM NaOH 20% /CH₃COONa (1 M)- NaOH (20 mM) 5% 20→37 mn ; pure water 40% /250 mM NaOH 20%/ CH₃COONa (1 M)-NaOH (20 mM) 40% 37→41 mn. Each elution was followed by a wash and subsequent equilibration time. External sugar and uronic acids standards were used for calibration with 7 points per curve. Fucose, glucose, xylose, galactose, mannose, rhamnose, arabinose and galacturonic acid, glucuronic acid were all provided by Sigma-Aldrich.

3. Results

Okoumé wood was steam exploded and the SELs were recovered according to scheme presented in Fig. 1. The lignin was then extracted from the water-washed exploded pulp in mild conditions with ethanol using a soxhlet apparatus. The experimental conditions of the SE reactions are compiled in Table 1 The severity factors (LogR₀) have been used for comparison of the process at different temperatures and residence times. Regarding the water impregnation, a hydrolysis of the acetyl groups present in the xylan backbone occurs at an early stage of the steaming step and gradually with the severity of the treatment, resulting in a decrease in pH.

3.1 Lignin yields

The variation of the SEL yields relative to the severity of the treatment and to the impregnation step conditions are given in the Table 1 and the Fig. 2. It appears that the lignin extractability is higher for dilute acid pre-soaking and increased with the severity of the SE

treatment. Based on Klason lignin content in the raw material and for the highest severity experimented ($\text{Log } R_0 = 4.1$), the lignin recovered after ethanol extraction was 4.5 % after a water pre-impregnation and 14.3 % after an acidic pre-soaking. Based on previous data, the main explanation of this observation is the acid-catalyzed depolymerization of lignin through the cleavage of aryl-ether linkages, producing small lignin fragments with an enhanced ethanol solubility [3, 14]. As seen in Table 1 and Fig. 2 and in accordance with previous reports [3, 8, 13], the presence of naphthol significantly increased the delignification yields. For a water impregnation and without naphthol, the SEL yields were 1.7 % and 2.6 % for $\text{Log } R_0 = 3.4$ and 3.6 respectively. In the same conditions but in presence of naphthol the yields were 3.8 % and 9.3 % respectively. It was previously reported that the presence of 2-naphthol resulted in a better yield of delignification during the organosolv [3] or steam explosion [8, 13] processes leaving a pulp with a relatively low lignin content.

3.2 Okoume MWL 2D HSQC Analysis

HSQC is a powerful technology for obtaining detailed structural characteristics of lignin inter-unit linkages. In this study, Milled Wood Lignin (MWL) was extracted from untreated Okoumé wood. It is well known that MWL is less changed than lignins isolated by physicochemical methods and can be considered as a representative of native lignin. The aromatic and the side chain regions of 2D HSQC spectrum of MWL are given in Fig. 3 (A and B respectively). Based on previous papers [21, 22] (Guo et al., 2017, Wang et al, 2017), the main characteristic hardwood lignin signals were detected and assigned (see Table 2), and the inter-units are depicted in Fig. 4. The presence of syringyl and guaiacyl units was confirmed by the cross peak at $\delta\text{H}/\delta\text{C}$ -7.45-7.14 ppm/102.61-108.31 ppm and $\delta\text{H}/\delta\text{C}$ -6.49-

7.32 ppm/110.05-122.64 ppm respectively. β -O-4 linkages were clearly detected at $\delta\text{H}/\delta\text{C}$ -3.97-4.32ppm/85.6-88.41ppm and $\delta\text{H}/\delta\text{C}$ -4.30-4.55/82.62-85.54 ppm for S and G respectively. The cross peaks $\delta\text{H}/\delta\text{C}$ -2.96-3.22 ppm/53.37-55.29 ppm are assigned to resinol units. Finally, phenylcoumaran were detected at $\delta\text{H}/\delta\text{C}$ -3.41-3.68 ppm/52.60-54.63 ppm. In the aromatic region in addition to the signals assigned to S and G units, a cross-signal at $\delta\text{H}/\delta\text{C}$ 7.01-7.48/105.82-108.67 was detected and assigned to $\text{C}\alpha$ -oxydized S units (S' unit). This oxidized function was confirmed by the presence of a cross-peak at $\delta\text{H}/\delta\text{C}$ 5.23-5.30-83.58-84.54 ppm assigned to $\text{C}\beta$ in $\text{C}\alpha$ -oxidized S units

3.3 Structural changes in lignin during SE

3.3.1 HSQC NMR

The HSQC spectra of SEL recovered under different conditions have been performed and compared to that of MWL. As an example, the aromatic and the side chain regions of SEL4 (Log $R_0=3,6$ without acid presoaking) and SEL8 (Log $R_0=3,6$ with acid presoaking) HSQC spectra are given in Fig. 3. In the oxygenated aliphatic region area, the absence of signals corresponding to polysaccharides ($\delta\text{C}/\delta\text{H}$ 63-65/3.2-3.8) indicate a very low concentration of residual sugars. The signal corresponding to the major structures of MWL such as β -O-4, resinol and phenylcoumarane were observed although the amounts of β -O-4 decreased after SE.

A semi-quantative HSQC analysis was performed in order to quantify the impact of the SE process on the SEL structure using C2 and/or C6 position in G and S units as internal standard. It is known that the cleavage of β -O-4 linkages is one of the most important process governing the lignin depolymerization. The content of β -O-4 structures was

estimated from the spectra by the intensity of C β H signal (which is well separated from other signals) related to the intensity of the aromatic C2H present in guaiacyl and syringyl units according to a previously described method [8, 23]. The variation of the β -O-4 content with the severity of the SE treatment as a function of the impregnation step (water or acid presoaking) is given in Table 1 and Fig. 5. As previously reported by other authors, a strong decrease in the β -O-4 content is observed with increasing in SE severity [8]. A quasi total degradation of the β -O-4 units was observed in acidic conditions for $\text{Log } R_0 > 3,6$. On the other hand and in accordance with Auxenfans et al. [23] β - β' and β -5' linkages are mostly preserved in SEL.

3.3.2 ^{31}P NMR

The lignin fractions have been analyzed after derivatization with 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane in presence of cyclohexanol as an internal standard [20]. The concentrations of aliphatic and phenolic OH groups in mmol.g^{-1} were calculated from the integrated peaks area and are given in Table 1. After a water impregnation (SEL1-5), the increase in severity of the SE treatment is associated with an increase in the aliphatic and phenolic OH content. This observation is rationalized by the hydrolysis of the β -O-4 units previously mentioned. In acidic conditions (SEL6-9), the high phenolic OH concentration at high severity (SE15) is in accordance with the strong decrease of the β -O-4 content observed by HSQC. As previously described for organosolv lignins, in acidic conditions a decrease in aliphatic OH due to dehydration reaction is also observed [2].

3.3.3 Molecular weight distributions

The MWL and SEL size exclusion chromatography (SEC) analysis was performed to determine in which extent the SE conditions affected the depolymerisation/repolymerization of lignin.

For water and dilute acid impregnation, it can be observed from the fig. 6A and 6B respectively a gradual increase in molecular weight with the increase of SE reaction severity. This observation may appear to be in contradiction with the previous conclusions regarding the cleavage of aryl-ether linkages. However, it can be explained by competing lignin recondensation reactions leading to new C-C linkages. These repolymerization reactions have been observed by several authors for different hydrolytic pretreatment processes including autohydrolysis, organosolv and SE. In our study and compared with previous papers, these repolymerization reactions appeared to be effective and relatively important even at low SE severity. Our results are in accordance with those of Li et al. [8] who have studied the autohydrolysis of aspen MWL in order to mimic the effect of SE process. These authors observed a decrease of the lignin molecular weight after an autohydrolysis performed in very mild conditions but for $\text{Log } R_0 > 1.3$, the repolymerization of lignin started to become prominent. On the other hand, Martin-Sampero et al [11] did not observed significative lignin recondensation during SE of Eucalyptus wood chips performed at $\text{Log } R_0 \sim 3.1-3.6$.

4. Discussion

The acidolytic breakdown of lignin has been extensively described in the literature for autohydrolysis, organosolv and steam explosion pretreatments (path a, Fig 7). During the hydrolysis of lignin, a carbonium ion at the benzylic position can be formed by an acid-catalyzed reaction, resulting to the cleavage of β -O-4 linkages and the formation of ketone groups arising from dehydration reactions (Hibbert's ketones). When neutral water or steam is used, hydrolysis of the acetyl groups from the hemicellulose lead to a pH drop initiating

the aforementioned acid-catalyzed reactions. Even if many studies from the literature regarding lignin hydrolysis refer to an heterolytic fragmentation of aryl-ether linkages, it however does not constitute the single mode of lignin degradation. Westermarck et al. [24] studied the hydrolysis of lignin dimers and concluded that even in mild conditions (160°C, pH = 2.7) the description of the hydrolytic mechanism could lead to erroneous and confusing results if homolytic cleavage is neglected. The homolytical β -O-4 fragmentation route (path b, Fig 7) may also occur and should be enhanced in the case of the SE process because of the high temperatures involved. Tanahashi [25] studied the mechanism of steam explosion depolymerization of lignin using model molecules (guaiacyl-glycerol- and syringyl-glycerol-p-guaiacyl ethers) subjected to SE at 230°C during 4 mn. Hibbert's ketones formed by acidolysis were scarcely detected while products formed by the coupling of cinnamyl alcohol radical were the main products isolated. Based on these observations, the author proposed that lignin is cleaved by steam explosion mainly homolytically. Starting from real lignin the distinction between the two degradation pathways (paths a & b, Fig. 7) is more difficult to determine because of the complexity of the structures formed. More recently Heikkinen et al [14] studied the chemical changes in lignin wheat straw during SE and found by experimental and computational approaches that the homolytical cleavage of β -O-4 bonds is the preferred route. The dissociation energy was calculated and the authors showed that G-O-G showed a higher dissociation energy than G-O-S (291 kJ.mol⁻¹ and 286 kJ.mol⁻¹ respectively).

From our results, it appeared that SEL1 recovered at low severity after a water impregnation exhibited structural features very closed to those of MWL. This result demonstrated that in the SE conditions used (170°C, 2.5 mn), fragments of lignin are recovered in unaltered form with high β -aryl ether and low Hibbert's ketones contents (S'). In the present work, the

saturated steam is directly injected in the reactor and the wood sample reached the set temperatures in less than 1 mn. As a result, the contact of wood with steam and water in the reactor being short, the hydrolytic pathway (path a, Fig. 7) is disadvantaged and the homolytic depolymerization could be the major degradation mechanism.

A progressive increase ~~of~~ in the SE process severity and/or the utilization of acidic conditions led to a substantial decrease of the aryl-ether bonds accompanied with the formation Hibbert's ketones and a decrease of S/S' values. Hibbert's ketones bearing α -carbonyl groups on the lateral chain were quantified through S' units detected by HSQC NMR. As previously mentioned, Hibbert's ketones are possibly formed through an heterolytic cleavage of β -O-4 ether linkages of lignin according to path a on Fig. 7. In accordance with previous works [8, 13] it is observed that the addition of a carbonium scavenger such as 2-naphthol (1) allowed a better lignin extractability during the washing step with ethanol, (2) strongly decreased the formation of Hibbert's ketones in SEL3N and SEL4N ($S/S' = 13.4$ and 16.6 respectively, Table 1). In acid conditions (SEL6-9) the lower S/S' values observed ($S/S' = 6.2$ - 2.7) are in accordance with an acid-catalyzed hydrolytic route producing Hibbert's ketones.

For SEL1-5 (water impregnation) a dispersion of the S/S' ratios as a function of the severity of the SE treatment (duration and temperature of the steaming step) was observed. This result can be rationalized by the two concomitant deconstruction mechanisms involved (path a and b, Figure 7) when increasing temperature and residence time: higher temperature promoted homolytic fragmentation but in the same time necessitated longer duration to heat the reactor and the biomass in order to reach the set temperature (>2 mn for 190°C), the steaming stage promoting the hydrolytic pathway.

These observations are in accordance with Tanahashi [25] who suggested that the mechanism of a SE lignin depolymerization could be function of the technology of the SE facility used. (1) If the steam treatment is carried out by a slow heating of the reactor, a mixed reactions of hydrolysis and homolysis can be involved; (2) if the steam is directly introduced at high pressure in the reactor, the lignin is degraded at high temperature mainly preferentially through an homolytic process.

In addition to lignin depolymerization, a lignin repolymerization occurs in particular through the reaction of the lignin intermediate with electron-rich carbon atoms such as the C-2 and C-6 of guaiacyl and syringyl rings, promoting the formation of new C-C linkages (path b, Fig. 7). This can explain the Mw increase with the increase severity of the treatment (see Fig 6). Guaiacyl units compared to syringyl display a higher nucleophilic reactivity and undergo repolymerisation reactions more easily. Thus the repolymerization reactions preferably take place among the guaiacyl units. As seen in Fig. 5, a higher S/G ratio ($S/G \sim 1.7 - 2.0$) was observed for a dilute acid impregnation attesting a higher recondensation rate for SEL at lower pH. An increase of the S/G ratio was clearly observed with the increase of severity of the SE treatment (from $S/G \sim 0.8$ for $S_0 = 2.5$ to $S/G \sim 1.7$ for $S_0 = 4.1$). In a previous paper, we studied the impact of the severity of the ethanol organosolv pulping ($T = 170^\circ\text{--}190^\circ\text{C}$, 60 mn, in presence of sulfuric acid as catalyst) on the recovered lignin molecular weight [3]. We observed a clear decrease of Mw with the severity of the treatment. In the present study, a reverse trend was observed with an increase in Mw from the lowest severity values. Taking into account the above-mentioned observations, we can conclude that this resulted essentially from recondensation reactions due to radicalar coupling processes (path b, Fig. 7).

To conclude, the results obtained in our study confirmed that the lignin is cleaved during SE through two concomitant pathways via homolytical and heterolytic mechanisms. The homolytic fragmentation is the major route and promotes recondensation through radical coupling even at low reaction severity. On the same time, the heating phase of the biomass and of the reactor, the steaming stage of the SE treatment and/or dilute acid impregnation promote the hydrolytic pathway. We demonstrated in this work that the S/S' ratio could be considered as a marker of the acidolysis mechanism.

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References

- [1] Ragauskas, A.J.; Williams, C.K.; Davison, B.H.; Britovsek, G.; Cairney, J.; Eckert, C.A.; Frederick, W.J.; Hallett, J.P.; Leak D.J.; Liotta, C.L.; Mielenz, J.R.; Murphy, R.; Templer, R.; Tschaplinski, T. The Path Forward for Biofuels and Biomaterials. *Science* **2006**, *311*(5760), 484–489.
- [2] El Hage, R.; Brosse, N.; Chrusciel, L.; Sanchez, C.; Sannigrahi, P.; Ragauskas, A. Characterization of milled wood lignin and ethanol organosolv lignin from miscanthus. *Polym. Degrad. Stab.* **2009**, *94*, 1632–1638.

- [3] El Hage, R.; Chrusciel, L.; Desharnais, L.; Brosse, N. Effect of Autohydrolysis of *Miscanthus x giganteus* on Lignin Structure and Organosolv Delignification. *Bioresour. Technol.* **2010**, *101*, 9321-9329.
- [4] Jacquet, N.; Maniet, G.; Vanderghem, C.; Delvigne, F.; Richel, A. Application of Steam Explosion as Pretreatment on Lignocellulosic Material: A Review, *Ind. Eng. Chem. Res.* **2015**, *54*, 2593–2598.
- [5] Heitz, M.; Capek-Menard, E.; Koeberle, P.G.; Gagne, J.; Chornet, E.; Overend, R.P.; Taylor, J.D.; Yu, E. Fractionation of *Populus tremuloides* at the pilot plant scale: optimization of steam pretreatment conditions using the STAKE II technology. *Bioresour. Technol.* **1991**, *35*(1), 23-32.
- [6] Donaldson, L.A.; Wong, K.K.Y.; Mackie, K.L. Ultrastructure of steam-exploded wood, *Wood Sci. Technol.* **1988**, *22*, 103-114.
- [7] Chen, H.; Liu, L. Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction. *Bioresour. Thechnol.* **2007**, *98*, 666-676.
- [8] Li, J.; Gellerstedt, G.; Toven, K. Steam explosion lignins; their extraction, structure and potential as feedstock for biodiesel and chemicals. *Bioresour. Technol.* **2009**, *100*, 2556–2561.
- [9] Sun, S.N.; Cao, X.F.; Xu, F.; Sun, R.C.; Jones, G.L. Structural Features and Antioxidant Activities of Lignins from Steam- Exploded Bamboo (*Phyllostachys pubescens*). *J. Agric. Food Chem.* **2014**, *62*, 5939–5947.
- [10] Jarrell, T.M.; Marcum, C.L.; Sheng, H.; Owen, B.C.; O'Lenick, C.J.; Maraun, H.; Bozell, J.J.; Kenttaemaa, H.I. Characterization of organosolv switchgrass lignin by using high

performance liquid chromatography/high resolution tandem mass spectrometry using hydroxide-doped negative-ion mode electrospray ionization. *Green Chem.* **2014**, *16*(5), 2713-2727.

[11] Martin-Sampedro, R.; Capanema, E.A.; Hoeger, I.; Villar, J.C.; Rojas, O.J. Lignin Changes after Steam Explosion and Laccase-Mediator Treatment of Eucalyptus Wood Chips. *J. Agric. Food Chem.* **2011**, *59*, 8761–8769.

[12] Li, J.; Henriksson, G.; Gellerstedt, G. Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion, *Bioresource Technology* **2007**, *98*, 3061–3068.

[13] Piellhop, T.; Amgarten, J.; Studer, MH.; Rudolf von Rohr P. Pilot-scale steam explosion pretreatment with 2-naphthol to overcome high softwood recalcitrance *Biotechnology for Biofuels* **2017**, *10*, 130.

[14] Heikkinen, H.; Elder, T.; Maaheimo, H.; Rovio, S.; Rahikainen, J.; Kruus, K.; Tamminen, T. Impact of Steam Explosion on the Wheat Straw Lignin Structure Studied by Solution-State Nuclear Magnetic Resonance and Density Functional Methods. *J. Agric. Food Chem.* **2014**, *62*, 10437–10444.

[15] Yoan, A.O.; Xue, Y.; Kiki, M.J.M. Gabon Wood Industry and Chinese Companies Activities. *OALib* **2018**, *05*, 1–15.

[16] Nguema, N.S. Présentation du Secteur Forestier au Gabon: Rapport sur l'évolution de la mise en oeuvre de la politique du Gouvernement dans les secteurs Forêts, Pêches et Aquaculture, Aires protégées et Formation [Internet]. Rapport sur l'évolution de la mise en oeuvre de la politique du Gouvernement dans les secteurs Forêts, Pêches et

- Aquatculture, Aires protégées et Formation. **2009**. [cited 2017 Nov 30]. Available from:<http://www.euflegt.efi.int/documents/10180/23275/Pr%C3%A9sentation+du+Secteur+Forestier+au+Gabon/eb4427e5-f61c-4b09-83c3-6ab5bcfa14b4?version=1.0>
- [17] Safou-Tchiama, R.; Obame, S.N.; Brosse, N.; Soulounganga, P.; Barhe, TA. Investigating the potential of *Aucoumea klaineana* Pierre sapwood and heartwood wastes to produce cellulosic ethanol. *African Journal of Biotechnology* **2016**, *15*(46), 2587-2595.
- [18] Safou Tchiama, R.; Soulounganga, P.; Engozogho Anris, PS.; Bikoro Bi Athomo, A.; Andzi Barhé, T.; De Jeso, B.; Charrier B.; Akagah, A.G. Understanding the natural durability of some African tropical heartwoods toward *Pycnoporus sanguineus* and *Antrodia* sp.: lignin structure and cellulose morphology control. *Journal of the Indian Academy of Wood Sciences*. **2018**, *15*(2), 162-171.
- [19] Overend, R.P.; Chornet, E. Fractionation of lignocellulosics by steam-aqueous pretreatments. *Phil Trans R Soc Lond A* **1987**, *321*, 523–536.
- [20] Granata, A.; Argyropoulos, DS. 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, a reagent for the accurate determination of the uncondensed and condensed phenolic moieties in lignins. *J Agric Food Chem*. **1995**, *43*, 1538–1544.
- [21] Guo, H.; Zhang, B.; Qi, Z.; Li, C.; Ji, J.; Dai, T.; Wang Aiqin.; Zhang, T. Valorisation of lignin to simple phenolic compounds over tungsten carbide: impact of lignin structure. *ChemSusChem*. **2017**, *10*, 523-532.
- [22] Wang, X.; Guo, Y.; Zhou, J.; Sun, G. Structural changes of poplar wood lignin after supercritical pretreatment using carbon dioxide and ethanol –water as co-solvents. *RCS Adv*, **2017**, *7*, 8314-8322.

- [23] Auxenfans, T.; Cronier, D.; Chabbert, B.; Paes, G. Understanding the structural and chemical changes of plant biomass following steam explosion pretreatment. *Biotechnology for Biofuels* **2017**, *10*, 36/1-36/16.
- [24] Westermarck, U.; Samuelsson, B.; Lundquist, K. Homolytic cleavage of β -ether-bond in phenolic beta-O-4 structures in wood lignin and in guaiacylglycerol- β -guaiacyl ether. *Res. Chem. Intermed.* **1995**, *21*(3-5), 343-352.
- [25] Tanahashi, M. Characterization and degradation mechanisms of wood components by steam explosion and utilization of exploded wood. *Wood research : bulletin of the wood research institute Kyoto University* **1990**, *77*, 49-117.

Fig 1. Experimental set-up

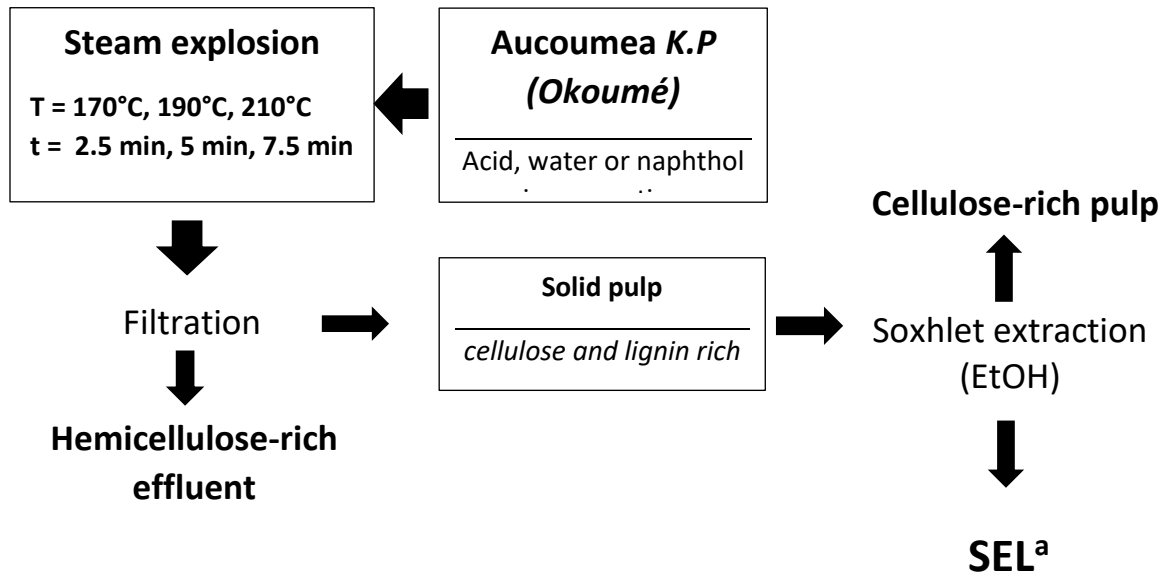


Fig. 2. SEL yields after dilute acid and water impregnation using various severity factors.

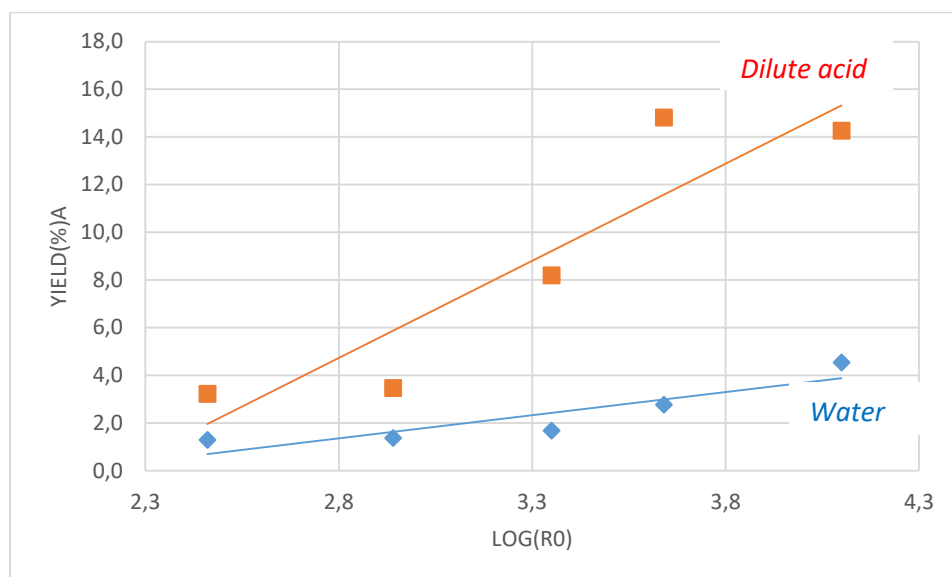


Fig. 3. MWL, SEL4 and SEL8 HSQC NMR spectra. A Lateral chains, B Aromatic region.

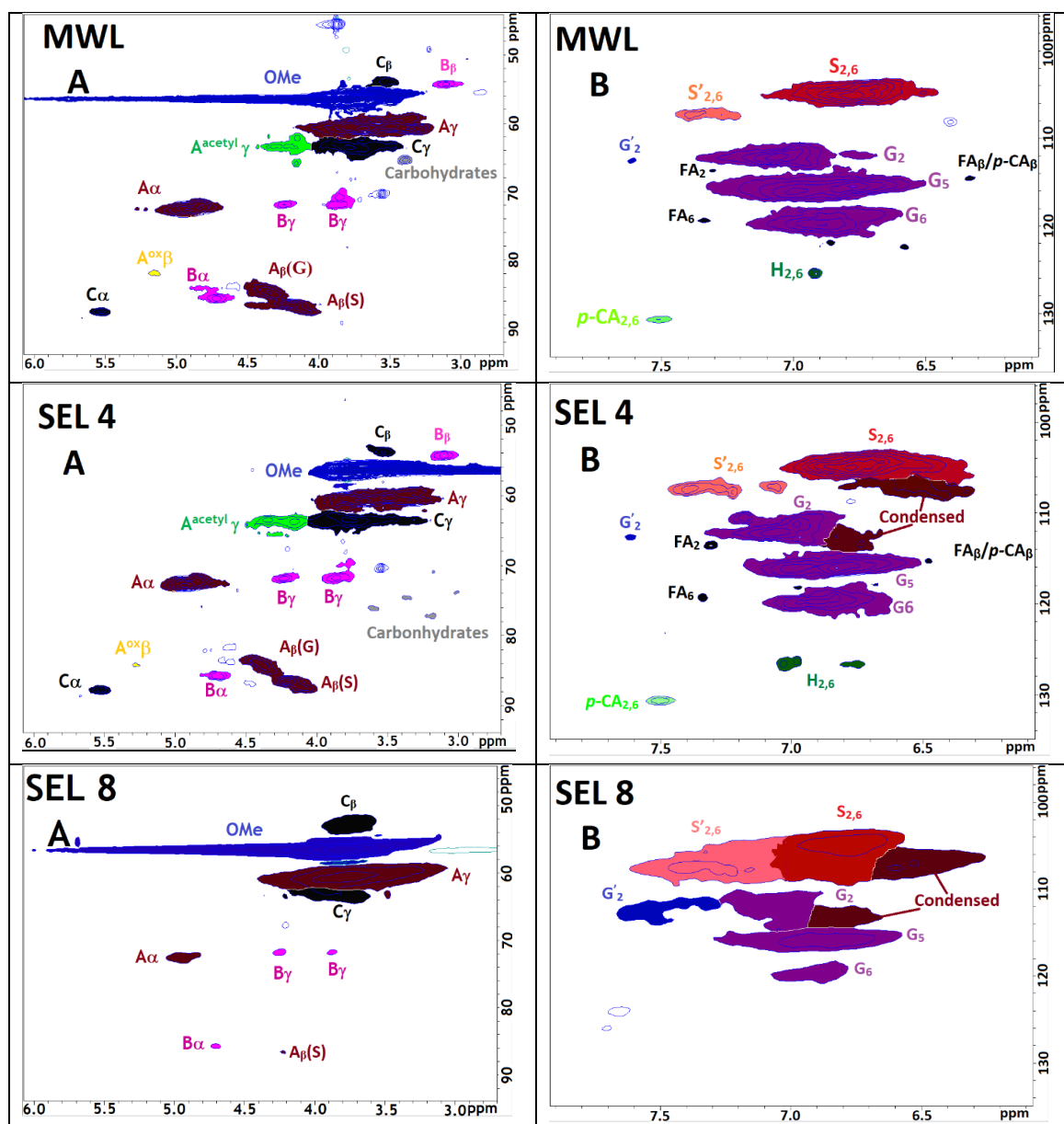


Fig. 4. Structure of identified lignin units.

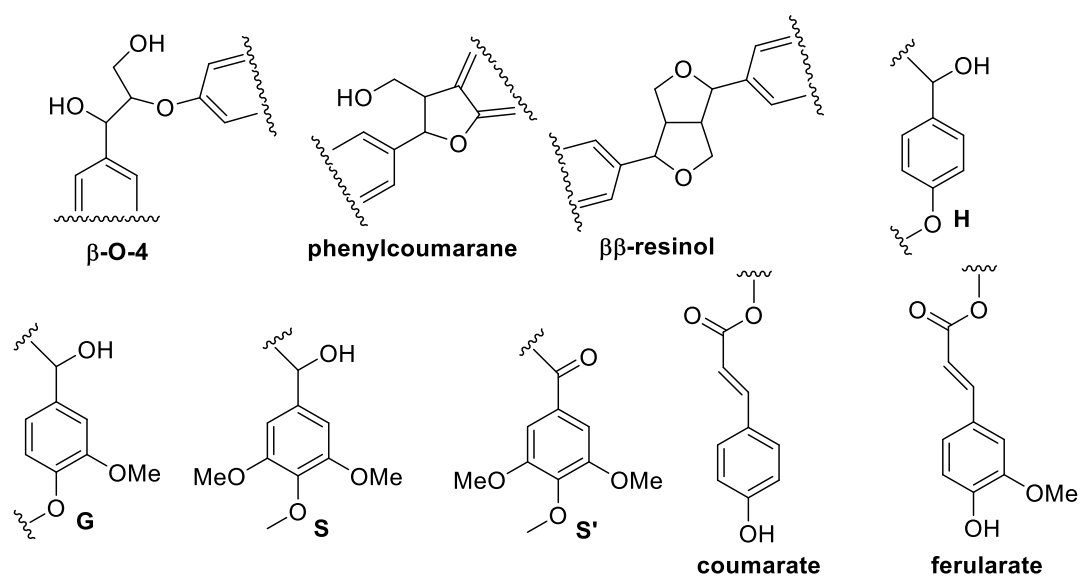
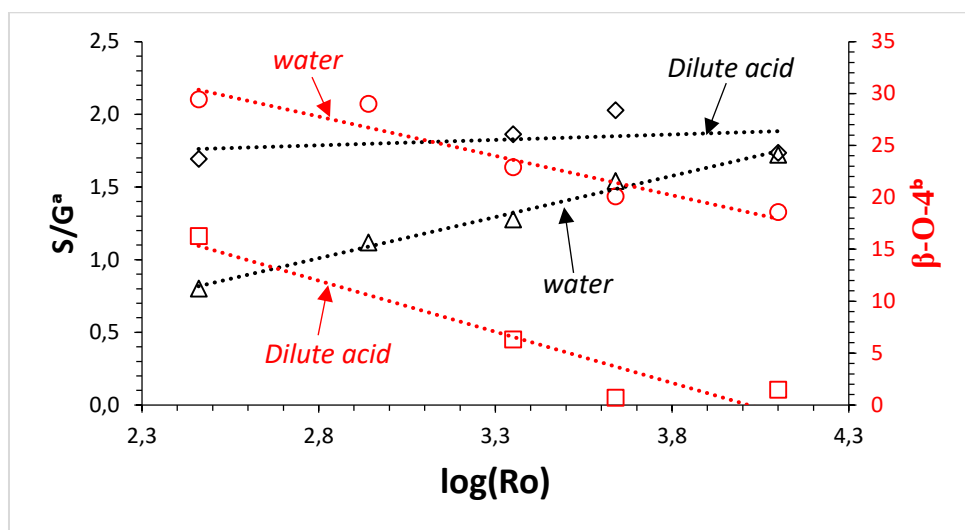


Fig. 5. Evolution of S/G ratios (in black) and β -O-4 linkages (in red) with the severity ($\log R_0$) of SE treatments.



^a S/G: determined by HSQC NMR. ^b β -O-4 content per 100 phenylpropane units.

Fig 6 Size exclusion chromatography (SEC) of SE lignin extracted: **A** water impregnation; **B** dilute acid impregnation

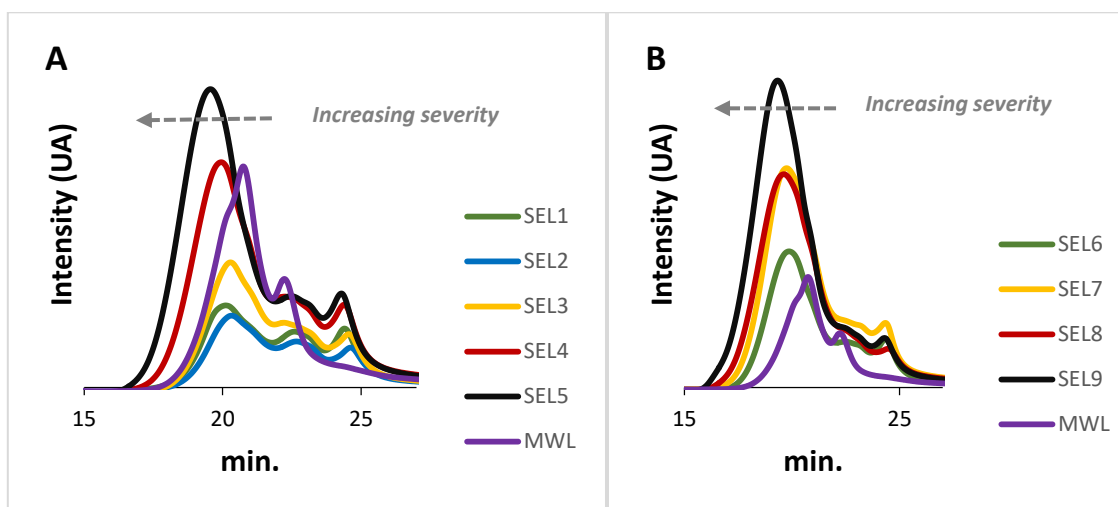


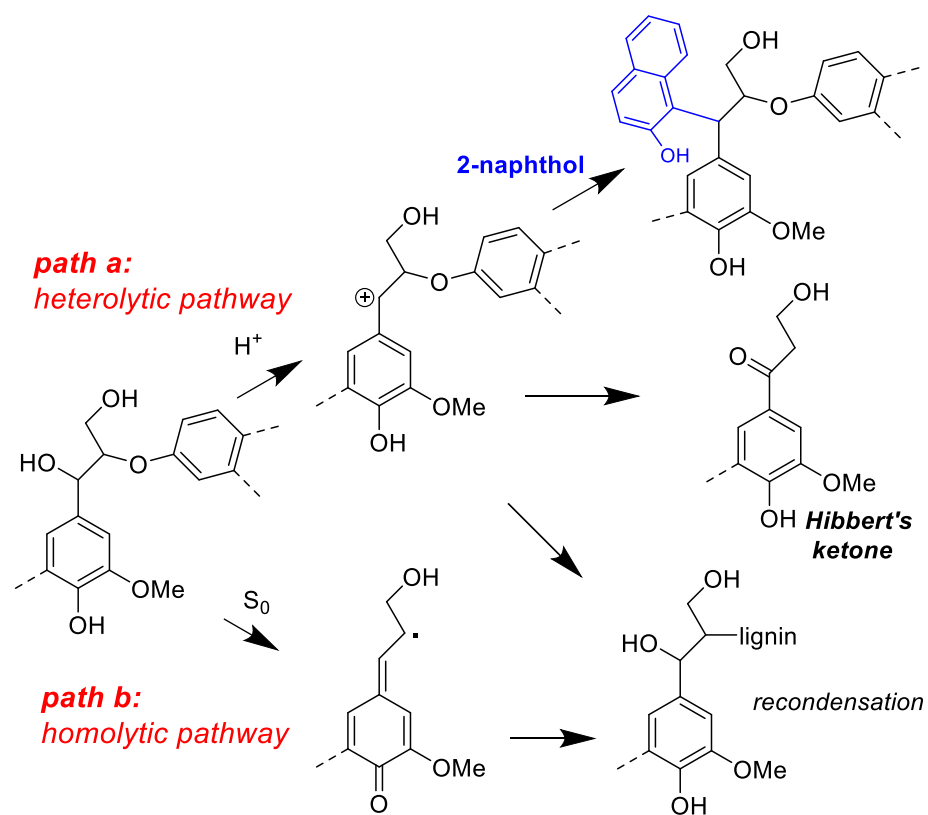
Fig. 7. Mechanisms of the cleavage of β -aryl ether linkages

Table 1: Pre-treatment conditions, MWL and SELs characterization.

assay	t (min)	T°C	H ₂ SO ₄ ^a %	Napht	LogR ₀ ^b	Lignin (%) ^a	β-O-4 (%) ^c	S/G	S/S' ^d	OH ^e aliph	OH ^e Phenol	Mw ^f	Mn ^f
MWL							30.2	0.9	13.3	3.0	2.1	498	356
SEL1	2.5	170	0	no	2.5	1.3	30.7	0.8	11.7	2.1	0.2	531	332
SEL2	7.5	170	0	no	2.9	1.4	29.0	1.1	8.5	2.9	0.9	434	291
SEL3	5.0	190	0	no	3.4	1.7	22.9	1.3	5.9	4.1	2.0	429	306
SEL3N	5.0	190	0	yes	3.4	3.8	19.8	1.3	13.4	3.2	2.4	1970	1287
SEL4	2.5	210	0	no	3.6	2.8	20.1	1.5	11.1	3.4	1.8	612	382
SEL4N	2.5	210	0	yes	3.6	9.3	16.8	1.5	16.6	3.0	2.3	514	361
SEL5	7.5	210	0	no	4.1	4.5	18.6	1.7	11.9	3.6	2.5	819	450
SEL6	2.5	170	0.5	no	2.5	3.2	16.3	1.7	6.2	3.2	2.1	615	367
SEL7	5.0	190	0.5	no	3.4	8.2	6.3	1.9	4.7	1.9	2.8	666	394
SEL8	2.5	210	0.5	no	3.6	14.8	0.7	2.0	2.7	1.1	2.6	807	418
SEL9	7.5	210	0.5	no	4.1	14.3	1.5	1.7	5.3	1.8	4.4	1029	497

^aWt%, based on weight of dry wood. ^bLogR₀= $\log(t \times e^{\frac{T-100}{14.75}})$. ^cQuantity of β-O-4 structures per 100 phenylpropane units.

^dG: guaiacyl and S: Syringyl units, S': α-oxydized S, determined by HSQC NMR, ^e in mmol.g⁻¹, determined from ³¹P NMR, ^fin Da, determined by SEC.

Table 2 Assignments of $^{13}\text{C}/^1\text{H}$ cross signals in HSQC NMR spectra

$\delta_{\text{C}}/\delta_{\text{H}}(\text{ppm})$	assignment
54.3/3.1	$\text{C}_{\beta}/\text{H}_{\beta}$ in resinol substructure (B)
53.8/3.5	$\text{C}_{\beta}/\text{H}_{\beta}$ in phenyl coumaran substructure (C)
56.4/3.8	CH in methoxyl group
60.4/3.6	$\text{C}_{\gamma}/\text{H}_{\gamma}$ in β -O-4 linkage (A)
63.5/3.8	$\text{C}_{\gamma}/\text{H}_{\gamma}$ in phenyl coumaran substructure (C)
63.6/4.3	Acetyl γ in β -O-4 linkage
67.7-77.7/3.0-3.62	Carbohydrates
68.9-72.7/3.7-4.3	$\text{C}_{\gamma}/\text{H}_{\gamma}$ in resinol substructure (B)
72.7/4.9	$\text{C}_{\alpha}/\text{H}_{\alpha}$ in β -O-4 linkage (A)
84.1/4.4	$\text{C}_{\beta}/\text{H}_{\beta}$ in β -O-4 linkage from guaiacyl units (A)
86.5/4.2	$\text{C}_{\beta}/\text{H}_{\beta}$ in β -O-4 linkage from syringyl units (A)
85.7/4.7	$\text{C}_{\alpha}/\text{H}_{\alpha}$ in resinol substructure (B)
84.2/5.3	$\text{A}^{\text{ox}}\beta$
87.7/5.5	$\text{C}_{\alpha}/\text{H}_{\alpha}$ in phenyl coumaran substructure (C)
104.9/6.8	$\text{C}_{2,6}/\text{H}_{2,6}$ in syringyle units (S)
107.6/6.5	$\text{C}_{2,6}/\text{H}_{2,6}$ condensed in syringyl units (S)
106.0-108.5/7.0-7.5	$\text{C}_{2,6}/\text{H}_{2,6}$ in oxydised syringyl units (S')
111.6/7.0	C_2/H_2 in guaiacyl units (G)
112.9/6.8	C_2/H_2 condensed in guaiacyl units
112.6/7.6	C_2/H_2 in oxydised guaiacyl units (G')
115.8/6.9	C_5/H_5 in guaiacyl units (G)
119.5/6.8	C_6/H_6 in guaiacyl units (G)
113.5/7.3	C_2/H_2 in Ferulate (FA)
119.3/7.3	C_6/H_6 in ferulate (FA)
115.2/6.5	$\text{C}_{\beta}/\text{H}_{\beta}$ in ferulate (FA)
125.5-127.5/6.7-7.1	$\text{C}_{2,6}/\text{H}_{2,6}$ in p-hydroxyphenyl (H)
130.6/7.5	$\text{C}_{2,6}/\text{H}_{2,6}$ in p-hydroxybenzoate units (p-CA _{2,6})