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► **To cite this version:**

Arnaud Besserer, Sebastien Ngwa Obame, Rodrigue Safou-Tchima, Safwan Saker, Isabelle Ziegler-Devin, et al.. Biorefining of *Aucoumea klaineana* wood: Impact of Steam explosion on the composition and Ultrastructure the Cell Wall. *Industrial Crops and Products*, 2022, 177, pp.114432. 10.1016/j.indcrop.2021.114432 . hal-03502862

HAL Id: hal-03502862

<https://hal.univ-lorraine.fr/hal-03502862>

Submitted on 26 Dec 2021

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Biorefining of *Aucoumea klaineana* wood : Impact of Steam explosion on the composition and Ultrastructure the Cell Wall

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Abstract

Aucoumea klaineana Pierre (okoumé) is a tropical hardwood used in the timber industry in the Congo area to produce veneers and plywood. This paper described the utilization of sapwood co-products from this industry according to a biorefinery approach to generate both bioethanol and lignin. A batch Steam Explosion (SE) was used as a pretreatment after acid impregnation (H_2SO_4 0.25 % or 0.50 % w/w) at various pressure (8-19 MPa) and residence times (2.5-7.5 min). The impact of the process was examined at the molecular and parietal scales. The pulp composition was examined in terms of lignin, glucose and xylose content, cellulose crystallinity (CrI %) and degree of polymerization (DP). The conditions of

high pretreatment severity led to an almost total xylans removal (residual xylans < 2 %), to a strong decrease of cellulose DP and to a lignin fragmentation and relocation increasing its extractability with ethanol as solvent. At the parietal scale, the effect of the pretreatment on wood cell wall was analyzed by optical microscopy after dyeing, fluorescence microscopy and scanning electron microscopy. Enzymatic hydrolysis of the cellulosic pulps was experimented using commercial cellulase from *T. Reesei*. A SE pretreated wood produced at 210°C during 5 min with an impregnation with 0.25 w/w% acid resulted in a glucose yield > 80 % with an excellent fermentability into ethanol by *S. Cerevisiae* . Using these operating conditions, one ton of okoumé sapwood could produce 112 kg of ethanol and 82 kg of high purity lignin.

Key words : *Aucoumea klaineana* Pierre; steam explosion; pretreatment; bioethanol; lignin

1. Introduction

One of the consequences of the Asian financial crisis (1998 – 2001) was the reorientation of Gabon's industrial policy including a local development of okoumé industry (peeling and slicing units). This led to the local production of huge quantities of wood co-products (abandoned tree crown in the logging area, peeling core, shavings, sawing slabs, etc.), currently of low value and not properly valorized (Mougnala Moukagni et al., 2021). The accumulation of these co-products currently raises environmental concerns but they also constitute a development opportunity for the Gabonese economy. In the past decades, there has been great interest in transforming wood into commodity molecules and fuels through biotechnological pathways (Shen and Sun, 2021). However, this effort has been focused on temperate wood species such as poplar and few studies have been devoted to tropical species.

Wood and more generally lignocellulosic biomass (LB) are composed of 3 main polymers cellulose, hemicellulose and lignin, closely associated through covalent and non-covalent bonding. This organization makes LB strongly resistant to enzymatic attack, lignin being one of the most important factors of recalcitrance (Yoo et al., 2020). In this context, overcoming this natural recalcitrance towards biocatalyst is a key step. A pre-treatment is generally required in order to alter the LB structure and to increase the polysaccharides digestibility for the production of fermentable sugars.

The main goals of the pretreatment are (1) the destruction of the lignin seal and the reduction of lignin / hemicellulose contents, (2) the increase of cellulose accessibility and (3) the increase of the cellulose surface area and porosity (Silveira et al., 2015). Among all the pretreatment technologies described in the literature, Steam Explosion (SE) has been

considered as one of the more promising especially for large scale developments because of its low environmental impact and energy consumption. In a SE pretreatment, LB is first impregnated generally in water or in dilute acid and then treated with saturated steam at high pressure (15-25 bars) for a short time (typically few minutes). This step promotes the hydrolysis of polysaccharides and lignocellulosic complex. The second stage of the process consists of a rapid depressurization causing water expansion and physical degradation of the LB (Jacquet et al., 2015; Ziegler-Devin et al., 2021). The combination of chemical and mechanical effects in SE treatment leads to a significant degradation and modification of the cell wall components, producing a cellulose-rich residue bearing a higher enzyme accessibility (Auxenfans et al., 2017; Sun et al., 2020). As a result, SE has been widely described and developed to convert biomass into ethanol and also for hemicellulose extraction and lignin removal (Jin et al., 2016; Sun et al., 2020). It has been recently shown that combined SE and green liquor treatment can reduce biomass recalcitrance to maximize bioethanol production (Gao et al., 2021).

In the current SE processes developed at the industrial scale, the lignin is not recovered after the pretreatment step, but at the end of the whole process, after the biotechnological steps as an impure co-product, used for energy production. The isolation of lignin from exploded LB has been reported by several authors using an organic solvent or alkali solutions (He et al., 2020; Matsakas et al., 2018).

In previous published papers, we studied the pretreatment of *Aucoumea klaineana* Pierre (okoumé) wood by SE, the subsequent polymeric hemicelluloses (Mougnala Moukagni et al., 2021) and lignin (Obame et al., 2019) extraction and characterization. In the present work, the impact of the SE treatment severity on the parietal and molecular scale has been fully

examined and the pretreatment conditions were optimized (pH of impregnation, T, t) for both fermentable sugars and lignin production. The novelty lies in the study of the chemical and structural characteristics having an impact on the recalcitrance of a tropical wood species and its complete valorization according to the concepts of the biorefinery.

2. Material and methods

2.1. Okoumé sapwood

The Okoumé (*Aucoumea klaineana* Pierre) wood used in this study was harvested in 2017 in N'Zamaligué natural forest located in the north of Gabon. The raw material consisted of sapwood with a low density ($\rho = 0.484 \text{ g}\cdot\text{cm}^{-3}$) and an initial dry moisture content of 4.1%. The Okoumé chemical composition was as follows: extractives (toluene/methanol/acetone, 4v:1v:1v for 24h followed by water extraction for 24 h) $3.0 \pm 0.3\%$; Acid insoluble lignin (Klason lignin) 26.6 ± 0.1 . Acid soluble lignin 2.0 ± 0.0 . Ashes (500 °C for 24 h) $0.4 \pm 0.1 \%$. Polysaccharides $58.0 \pm 0.9\%$ including $39.8 \pm 0.3\%$ glucose. Okoumé slices were cut with a saw and then milled with a knife mill to a sawdust size of 2 mm. Before use, wood samples were oven-dried then stored in sealed plastic bags à RH below 4 % to prevent fungal attack.

2.2. Steam explosion pretreatment

The sapwood samples were first impregnated in ultrapure water, or in 0.25 % (w/w) or 0.5 % (w/w) dilute H_2SO_4 for 24 hours at room temperature; with a ratio (liquid/solid) of 6:1 (v/w). Presoaked samples were then filtered. Concerning steam explosion process, the apparatus consists of three main parts: a vapor generator, a pressurized reactor (2 L) and a discharge tank. About 50g (dry basis) of the presoaked sawdust were loaded into the reactor and exposed to several high-pressure steams [6.9 bar – 18.4 bar] corresponding to temperatures ranging between [170 °C-210 °C] and for several residence times [2.5 min-7.5 min]. The

wood sawdust was then exploded by sudden dropped in pressure and released in the discharge tank. The exploded samples were separated from the liquid fraction using vacuum filtration and were dried at room temperature for 48 hours. The severity factor (S) of each treatment was determined by combining both temperature and residence time using the following equation:

$$S = \log [t_s \cdot \exp\left(\frac{T - 100}{14.75}\right)]$$

where t_s is the residence time (min) and T is the temperature ($^{\circ}\text{C}$).

2.3. Cellulose recovery

Lignin SE was then extracted from SE sawdust using a Soxhlet apparatus with mixture of ethanol/water (9:1, v/v) over 24 hours, resulting in a colorless solvent and a cellulose-rich pulp. The SE cellulose fraction was dried and then quantified and characterized by size exclusion chromatography (SEC) and X-ray diffraction (XRD).

2.4. Size exclusion chromatography (SEC)

Before SEC analyses, the cellulose was chemically modified with phenyl isocyanate into its tricarbanilate derivative (CTC). The procedure was as follows: in a 50 mL flask, 30 mg of cellulose were mixed with 8 mL of pyridine and 1 mL of phenyl isocyanate. The flask was carefully sealed with a Teflon cap and then placed in an oil bath set at a temperature of 65 $^{\circ}\text{C}$. The mixture was kept at this temperature under stirring for 3 days until complete dissolution of the cellulose, then the mixture was cooled, and 2 mL of methanol were added to remove excess of phenyl isocyanate. Subsequently, the cellulose solution was poured into a beaker containing 200 mL of a water/methanol mixture (3/7 v/v) to precipitate the CTC. The CTC was washed three times with the water/methanol mixture, and twice with ultrapure

water, then it was freeze-dried. For analysis, 15 mg of CTC was solubilized in 3 mL THF until being completely dissolved.

Size-exclusion chromatography analyses were performed using a Shimadzu Prominence HPLC chromatograph equipped with a UV detector and two columns linked in series [Shodex GPC KF-806L 10 μm (8.0 \times 300 mm) column and Phenomenex Phenogel 5 μm 100 \AA (7.8 \times 300 mm) column]. The mobile phase was non-stabilized THF with a flow rate of 0.7 mL \cdot min $^{-1}$. Filtered solutions of CTC (20 μL) were injected and separated at 35 $^{\circ}\text{C}$. The detection was carried out at wavelengths of 280 and 254 nm. Five standard polystyrene samples were used for calibration.

2.5. Cellulose X-ray diffraction analyses

The diffractometer used (Oxford Diffraction (Agilent) Supernovae diffraction model) was equipped with a copper anode (λ K α 1 = 1.54056 nm, λ = K α 2 = 1.544339 nm) and a PW3050/60 goniometer. The applied voltage was 40 V. The SE cellulose samples were uniformly spread on a quartz glass and carried on the acquisition support. The diffractograms were obtained by accumulation of the intensities I (2θ) from $2\theta = 5^{\circ}$ to $2\theta = 40^{\circ}$ at room temperature. For each sample, the resulting intensity ranging between $22.4^{\circ} \leq 2\theta \leq 22.6^{\circ}$ corresponded to the intensity of the diffraction ray crystallographic plane 002 (I_{002}) of the cellulose I_{β} . Whereas the intensity ranging between $18^{\circ} \leq 2\theta \leq 19^{\circ}$ corresponded to that of the amorphous phase (I_{am}). The calculation of the crystallinity index used both these intensities.

2.6. Enzymatic hydrolysis of SE cellulose

In a screw-capped Erlenmeyer flask, 2 g of milled SE cellulose pulp (0.16 mm) were added to 100 mL of 50 mM sodium acetate buffer solution (pH 4.8). The mixture was incubated at 50

°C by shaking at 170 rpm for 30 min. Enzymatic hydrolysis was initiated by the addition of 1.14 mL of cellulase from *Trichoderma reesei* (≥ 700 units/g - reference C2730 Sigma Aldrich) and prolonged by shaking (170 rpm) at 50 °C for 72 h. Hydrolysate samples were periodically collected over 72 h to monitor the conversion kinetics of cellulose to glucose. During the first 12 h a sample was taken after 3h and then every 12 h. Enzymatic hydrolysis was stopped using boiling water for 10 min, then samples were centrifuged for 15 min (4000 rpm). The recovered liquid phases were subsequently diluted and analyzed by HPAEC-PAD for quantification of glucose released by the enzymes.

2.7. Glucose quantification – HPAEC-PAD

Glucose content of the hydrolysate fraction were analyzed by HPAEC-PAD (ICS-3000 Dionex) equipped with a Dionex CarboPacTM PA-20 (3 × 150 mm) analytical column. Diluted and filtered samples (20 μ L) were eluted at 35 °C, at 0.4 mL.min⁻¹, with the following composition: 99.2 % ultrapure water / 0.8 % of 250 mM NaOH (0–20 min); 75 % ultrapure water / 20 % of 250 mM NaOH / 5 % 1 M of CH₃COONa - 20 mM NaOH (20–37 min) and 40 % of ultrapure water / 20% 250 mM of NaOH / 40% of 1 M CH₃COONa - 20 mM of NaOH (37–41 min). Each elution was followed by a wash and subsequent equilibration time of both the column and the PAD. Glucose standards provided by Sigma- Aldrich were used for external calibration.

2.8. Microscopy

For bright field transmission observations, thin sections (25 μ m) were prepared in the transversal direction with a sliding microtome (SM200 R, Leica, Germany). Sections were stained with 1% astra blue solution and counterstained with 0.1% safranin solution according the protocol described in (Srebotnik and Messner, 1994). Acquisitions were

performed with an Olympus CX31 microscope equipped with an Olympus Color view III CCD camera. Objective used were 10X and 40X. Image computation were carried out with FIJI software with a lab written script (Schindelin et al., 2012).

Fluorescence observations were carried out on thin section stained with 0.1% aqueous safranin for 2 min and rinsed 3 times with water (Bond et al., 2008). Observations were done on a Leica DM 2700M microscope equipped with a DFC7000T CCD camera and a XYZ scanning stage. The filter cubes L5 (EX=480/40, LP505 dichroic, BP527/30), N2.1 (EX=515-560, LP580 dichroic, LP590) were used for visualization of the fluorescence of the cellulose + lignin (green channel) and the lignin (red channel). Extended depth of field computation of Z-stack (37 stacks over 21.33 μm) was used to generate the final picture. N PLAN 40x/0.65 DRY objective was used.

Scanning electron microscopy acquisition were done with a Tabletop Microscope TM3000 (Hitachi) equipped with secondary electron detector. Sample were coated with Gold/palladium using SC7620 Mini Sputter Coater (Quorum Technologies).

2.9. Fermentation of the hydrolysate

The fermentations were run in 3 L bioreactor (Pro-Lab, CBIO2, Global process Concept SAS, France). The wood hydrolysates (900 mL) were filtered (0.2 μm) and added the bioreactor and autoclaved at 110 °C for 30 min. Fermentations were carried out with a commercial strain of *Saccharomyces cerevisiae* yeast (CHY1011), under anaerobic conditions at 30 °C and at 400 rpm. The pH was controlled at 5.0 by automatic addition of 1 M NaOH or HCl, each experiment was performed in duplicate. Yeast biomass was grown overnight in YPD medium (YPD Broth, Sigma Aldrich), then harvested by centrifugation (10000 x g, 4 °C). Yeast biomass was diluted using the hydrolysate to get $\text{OD}_{600\text{nm}} = 4$ in the bioreactor. Glucose consumption

and ethanol production were controlled each 30 min using HPLC/RID. The fermentation was stopped after about 2 h when there was no more ethanol formation.

3. Results

The SE conditions used in this study (170-210 °C, 2.5 – 7.5 min, water or sulfuric acid impregnation 0.25 and 0.5 w%) were selected considering previous studies dealing with hardwood pretreatment. The severity factors ($S = \text{Log } R^\circ$) have been used to evaluate the process using different conditions. After pretreatment, the solid pulps isolated were extracted with ethanol at atmospheric pressure to recover SE lignin (SEL) and the cellulosic pulp was converted into fermentable sugars with enzymes. The whole process is depicted in Fig. 1 and Table 1 gathers all the conditions and results including the composition of the solid pulp, liquid effluents and the amount of SEL. The composition of the raw material is typical of hardwood with a high glucans content which makes it a good candidate for bioethanol production.

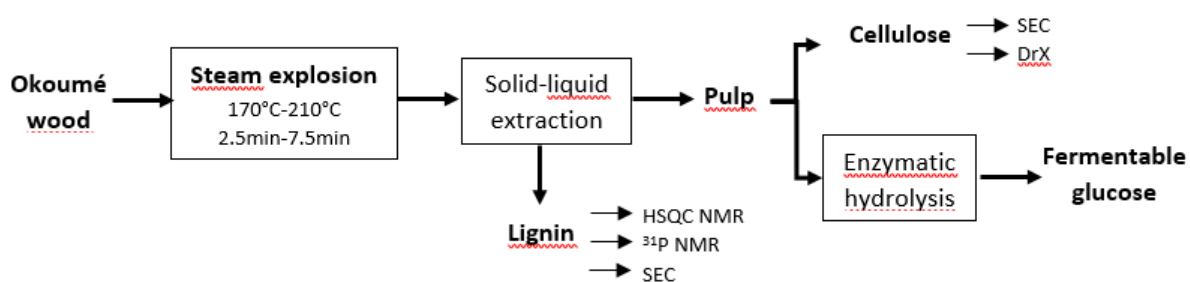


Fig. 1. Schematic of the process

Table 1. Experimental conditions, compositions of solid residues and liquid fractions and glucose produced after enzymatic hydrolysis^a

Conditions					Solid			Liquid			SE lignin		Enzym. hydrolysis
Essays	T°C	Tmin	H ₂ SO ₄ %	S ^b	KL	Glc.	Xyl.	Glc.	Xyl.	Furf	%	β-O-4 ^c	Glc
Raw material					27.4	45.8	12.2				30.2 ^d		
1	170	2.5	0.5	2.5	22.2	37.5	2.5	0.1	0.8	0	2.6	16.3	13.7
2	170	5	0.25	2.8	23.7	42.1	3.8	0.1	0.6	0	2.3		14.5
3	170	7.5	0.5	2.9	19.3	39.6	2.4	0.2	0.9	0.1	2.8		5.5
4	190	2.5	0.25	3.1	20.5	40.0	1.7	0.4	1.8	0.1	4.8		11.8
5	190	5	0.25	3.4	20.3	37.9	2.0	0.2	0.8	0.2	5.0		19.2
6	190	5	0.5	3.4	18.2	42.0	0.8	0.3	0.6	0.2	5.8	6.3	9.6
7	190	7.5	0.25	3.5	19.0	40.2	1.9	0.3	0.9	0.1	5.1		22.5
8	210	2.5	0.5	3.6	12.9	32.1	0.6	1.2	0.5	0.5	8.9	0.7	17.5
9	210	5	0.25	3.9	21.2	28.4	0.6	1.0	0.9	0.5	8.2		23.4
10	210	7.5	0.5	4.1	20.0	27.0	0.9	1.7	0.8	0.9	8.8	1.5	17.8

^aall data are yields of components (g) per 100 g dry weight of wood, ^b Severity factor ^c see (Obame et al., 2019),

^ddetermined from Milled Wood Lignin (Obame et al., 2019)

3.1. Chemical characterization of pretreated Okoumé wood

The variation of the mass loss, glucans and xylans content in the solid pulp respectively as a function of the severity of the SE treatment are presented in Fig. 2 A, B and C. The mass loss increased with the treatment severity. For S = 4.1, about 40 % of the wood was solubilized.

A similar trend has recently been reported for SE of beechwood (Simangunsong et al., 2020).

This is consistent with the well-known hydrolysis of hemicelluloses during the steaming step of SE. This result can also be correlated with the decrease of the xylans content in the pulp with the SE severity (Fig. 2B), xylose being the main hemicellulosic monosaccharide in hardwood hemicelluloses. Indeed, significant decrease in xylans content in SE pulp was

observed, particularly at high severity and at $S > 3,4$, xylans were almost totally removed from the solid pulp. In contrast, glucans content of the pulp is slightly affected due to the lower hydrolysability of cellulose in the SE conditions.

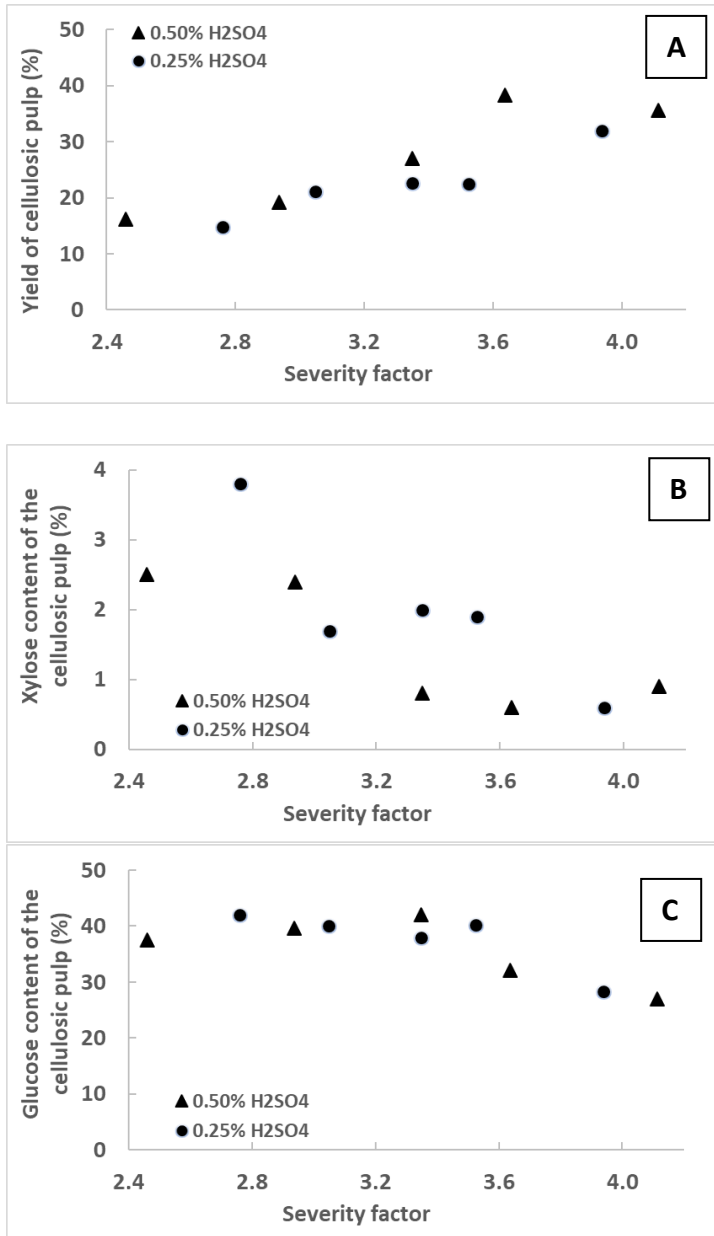


Figure 2. Pulp yield, composition of pulps in xylose and glucose as a function of the SE severity. The amounts of glucose and xylose were determined after a post hydrolysis step (see section 2).

The SE conditions generated furan-derived sugars (furfural, see Table 1), and up to 1% furfural (calculated on the basis of the initial wood mass) was detected in the liquid treatment effluent.

In order to study the impact of SE treatment on the properties and hydrolysability of cellulose, its degree of polymerization (DP) and crystallinity (CrI) as a function of the experimental conditions were examined. DP and CrI have been described to be an important factors affecting the cellulose digestibility. Huang et al. (2015) described a reduction of cotton stalk cellulose DP by \approx 50-65 % after SE ($S = 4.22$), they showed that reduction of DP was the main factor promoting enzymatic hydrolysis. In our study, the evolution of cellulose DPs as a function of the severity of the SE treatment was estimated by SEC analyses after derivatization and using polystyrene as standard. This method allows comparison between samples produced under different conditions but is not accurate for PD quantification. The evolution of the DPs as a function of S is presented in Fig. 3A. As expected and in accordance with previous papers, the acidic medium of SE strongly decreased the DP, because of the well-known hydrolysis of glycosidic linkages. This degradation of the cellulose chains by acid hydrolysis of the glycosidic bonds by SE has been described by different authors (Diop et al., 2015; Huang et al., 2015). An increasing drop in cellulose DP with residence time and temperature was also observed for dilute acid pre-treatment (Foston and Ragauskas, 2010) on poplar and Switchgrass. The effect of SE conditions on cellulose crystallinity has been examined by X-Ray diffraction. In accordance with several authors, an increase in crystallinity has been observed, CrI % steadily increasing with the severity of the treatment (Fig. 3B). However, based on literature, the reasons of this trend are still unclear. It could be due to a partial hydrolysis of the amorphous regions and/or to a reorganization of amorphous /

paracrystalline cellulose due to the high pressure and temperatures involved (Beyene et al., 2020).

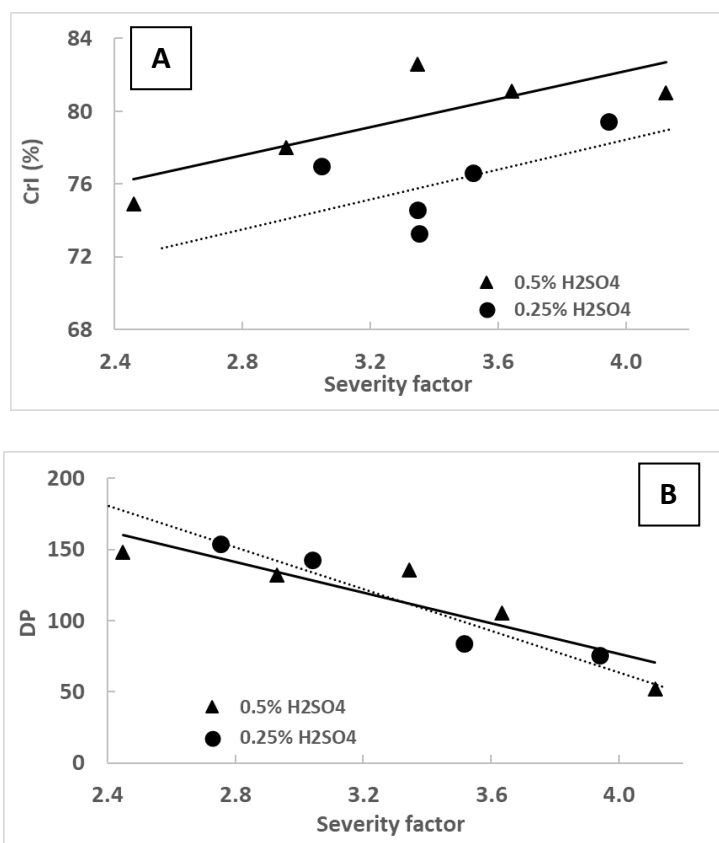


Figure 3. Evolution of cellulose crystallinity and degree of polymerization with the severity of the SE pretreatment. CrI% (A) and DP (B) were determined by DRX and SEC respectively.

The influence of the SE treatment severity of okoumé wood on the lignin structure and yields has been previously fully described in a previous paper (Obame et al., 2019). HSQC spectrum of SE-lignin essay 9 is given in Supplementary Material section. The lignin purity and the very low concentration of sugars was confirmed by the absence of signals in the range $\delta\text{C}/\delta\text{H}$ 63–65/3.2–3.8. Relatively weak signals were detected in the area corresponding to

the inter-unit linkages showing the strong deconstruction of the lignin: residual β -O-4 were detected at $\delta\text{H}/\delta\text{C}$ 4.1-4.2/86.8-87.7 ppm ($\text{C}\beta/\text{H}\beta$), resinol units at $\delta\text{H}/\delta\text{C}$ 4.7/85.6 ppm ($\text{C}\alpha/\text{H}\alpha$) and $\delta\text{H}/\delta\text{C}$ 3.9 and 3.2 /71.8 ppm ($\text{C}\gamma/\text{H}\gamma$) and phenylcoumaran at $\delta\text{H}/\delta\text{C}$ 5.5/87.7 ppm ($\text{C}\alpha/\text{H}\alpha$). In the aromatic region, in addition to the correlations assigned to the S and G moieties (at $\delta\text{H}/\delta\text{C}$ 104.4-6.8 ppm ($\text{C}_{2,6}/\text{H}_{2,6}$) and $\delta\text{H}/\delta\text{C}$ 111.6/7.0 (C_2/H_2); 115.8/6.9 (C_5/H_5); 119.5/6.8 ppm (C_6/H_6) respectively), additional cross-signals attested the recondensation reactions (recondensed S at $\delta\text{H}/\delta\text{C}$ 107.7/6.5 ppm; recondensed G at $\delta\text{H}/\delta\text{C}$ 112.1/7.6 and 113.3/6.8 ppm). The β -O-4 contents in lignins determined by a semi-quantitative HSQC NMR method are given in Table 1 for raw material and essays 1, 6, 8 and 10 (Obame et al., 2019). A strong decrease of the β -O-4 contents was observed with the treatment severity accompanied by an increase in SE lignin yield.

3.2. Parietal characterization of pretreated Okoumé wood

The changes occurred during steam explosion treatment of okoumé wood at the cell wall level were analyzed. Effects of steam explosion were well described in the literature for temperate wood species and lignocellulosic biomass (Auxenfans et al., 2017; Donaldson and Vaidya, 2017; Donaldson et al., 1988). However, characterization of steam explosion effect on tropical wood species at ultrastructural level remains poorly documented. Indeed, compared with temperate wood species, tropical wood such as *A. klaineana* showed increased extractive and lignin contents and relatively low amounts of pentosans (Table I and Rowell, 2012). A study at the cellular level can thus bring complementary information to the chemical analyses. Pulps isolated after steam explosion from essays 9 and 10 were stained with Astra blue and Safranine (Fig. 4) (Mihiretu et al., 2019; Silva et al., 2018; Wang et al., 2015). The pretreated samples (Fig.4 C-F) exhibited altered cell structure and patchy

delignification characterized by bluish areas. This high variability is illustrated on the high magnification panels of the figure 4 (B, D and F). The most visible effect of the treatment was the deformation of the fiber cell wall particularly marked in blue dignified area. This suggested significant alterations in the cell wall chemical composition with the formation of numerous cracks. To evaluate the effect of several pretreatment condition on the lignin removal, we developed a semi-quantitative evaluation of the delignification process occurring in wood after steam explosion pretreatment based on image computation. For both samples, depending of the image location, delignification varied greatly with values from $\approx 3\%$ to $\approx 75\%$ with an average of $\approx 25\%$. This delignification % computed from the microscopical data was in a good agreement with the delignification % obtained from the chemical analysis (Table 1 for raw and pretreated material (delignification of 23 % and 26 % for essay 9 and 10 respectively).

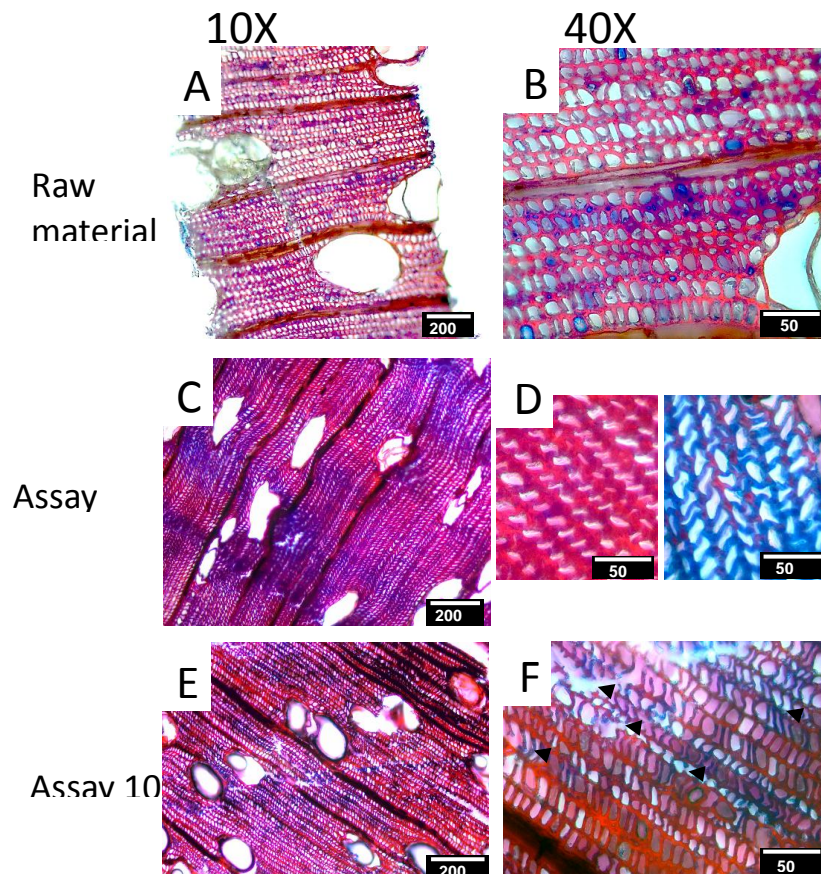


Figure 4. Effect of the steam explosion pretreatment on wood cell wall observed by optical microscopy after dyeing. Thin section of sample A, B: Raw material, C,D: assay 9 (210°C, 5 min, Sulf. Acid 0.25%); E, F : assay 10 (210°C, 7.5 min, Sulf. Acid 0.5%). Arrowheads indicate cracks in the wood structure.

Changes in safranin fluorescence emission was used to investigate lignin distribution in wood (Bond et al., 2008). The relative cellulose/lignin distribution was examined at the cell wall scale by fluorescence microscopy. Representative images are shown of figure 5. In untreated sample (figure 5A), cells have of mixture of lignin and cellulose in their cell walls and appeared in shades of yellow. The fluorescence profile (figure 5B) shows that lignin mainly occurred only in middle lamella whereas it occurred in mixture in S1-S3 layers as previously reported (Bond et al., 2008; Brändström, 2001; Harris, 2006; Lancha et al., 2021).

For the pretreated samples, a gradient of cellulose and lignin distribution was observed. In accordance with the chemical characterization, not only lignin but also cellulose and thus likely hemicellulose content were altered. However, neither complete delignification nor total carbohydrate removal were observed. Interestingly, cell shape and ultrastructure of the cell wall appeared to be altered in correlation with treatment severity that might be due to a decrease of lignin content in the middle lamella as previously reported (Lancha et al., 2021). A high variability in polymer content distribution was observed. For essay 9, the lignin relocation at the surface of S3 layer was coupled with a decrease in carbohydrate content (figures 4C and 4D). Lignin relocation at the surface of the S3 layer was also detected on these graphs. As illustrated on fig. 4, the assay 9 lead to a patch pattern of cell wall modification when compared with assay 10. Conditions of assay 10 seemed to promote

lignin removal from cell corner and its relocation at the surface of S3 layer as well as global carbohydrate removal with a homogeny pattern at the tissue level.

The effect of the steam explosion treatment was also examined by scanning electron microscopy. Results illustrated on the figure 6 clearly showed that steam explosion triggered structural alteration of middle lamella and reduction of cell wall thickness. In the harsher condition tested (essay 10), severe changes in cell wall porosity and structure were observed. In addition, numerous lignin aggregates were observed at the cell surface exclusively in steam explosion pre-treated samples. The size of these particles ranged from 0.18 μm to 0.61 μm .

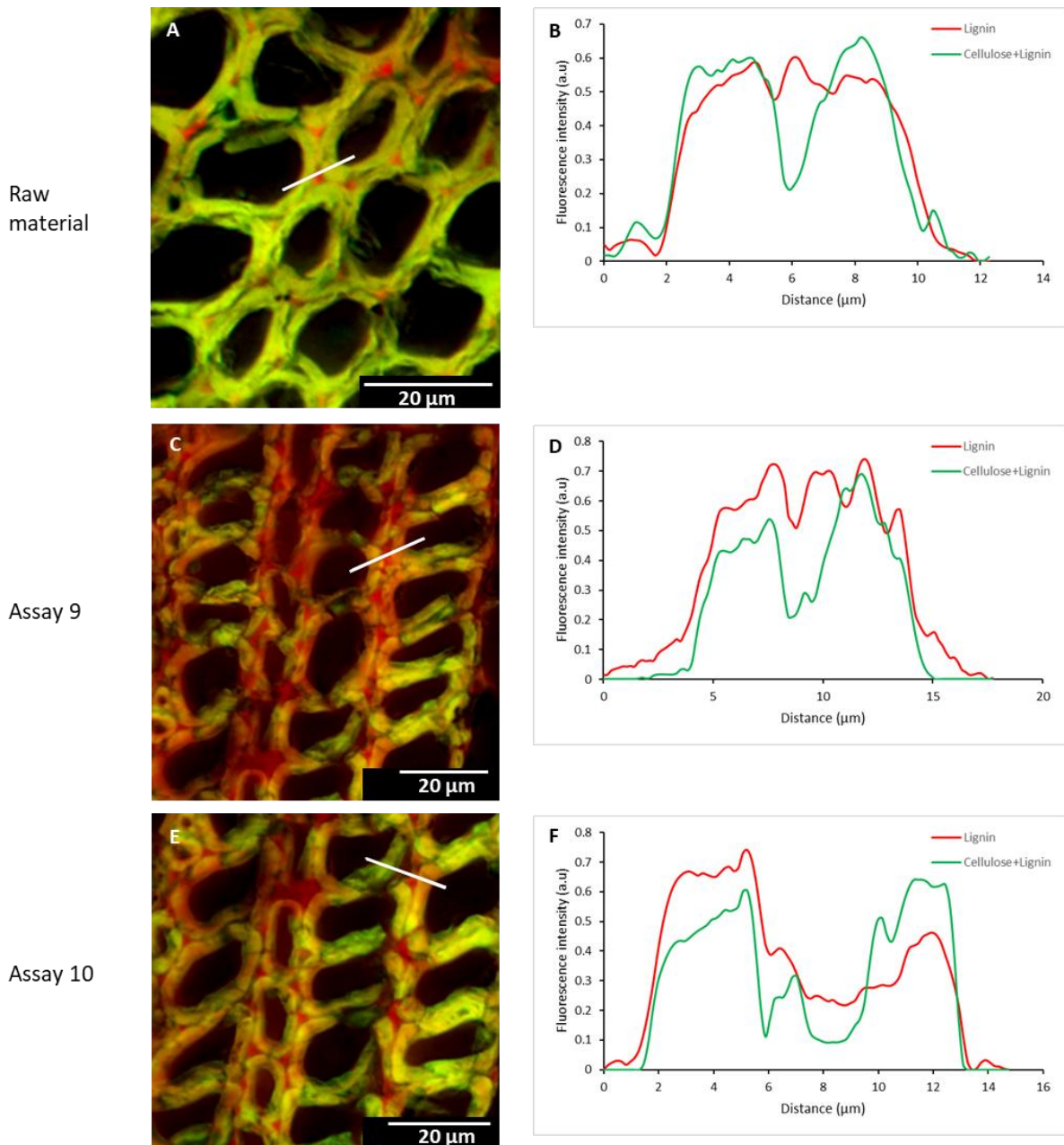


Figure 5. Cell morphology and cellulose/lignin distribution in control (A-B); assay 9 (210°C, 5 min, Sulf. Acid 0.25%) (C-D) and assay 10 (210°C, 7.5 min, Sulf. Acid 0.5%) (E-F)

These results are consistent with other reports showing that after hydrothermal pre-treatment, lignin in the cell corner formed dense lignin droplets, while lignin in the secondary cell wall was released with hemicelluloses forming loosely packed lignin droplets (Auxenfans et al., 2017; Donaldson et al., 1988; Lancha et al., 2021; Zeng et al., 2015).

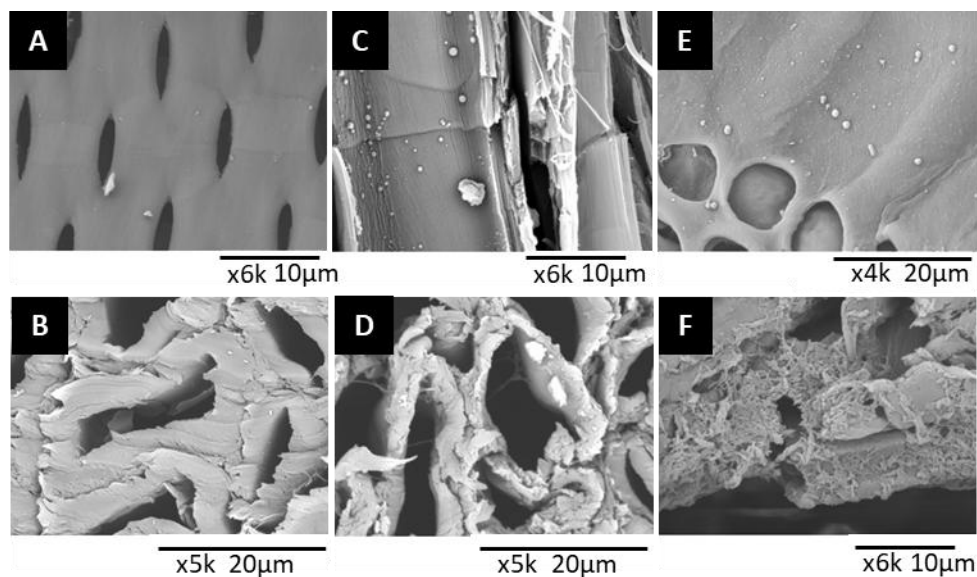


Figure 6. SEM of raw and treated materials. A-B Raw sample; C-D essay 9 (210°C, 5 min, Sulf. Acid 0.25%); E, F Essay 10 (210°C, 7.5 min, Sulf. Acid 0.5%).

3.3. Enzymatic hydrolysis and ethanol production

Enzymatic hydrolysis of the cellulosic pulps produced after steam explosion and ethanol extraction were performed using commercial cellulase cocktail isolated from *T. Reesei*. The glucose yields (based on initial dry wood) after 40 h of incubation are given in Table 1. The Cellulose-to-Glucose conversion (%) at different points during the enzymatic hydrolysis experiments are also given in Supplementary Material section. Regardless of impregnation conditions (sulfuric acid 0.25% or 0.5%), low glucose yields were obtained for the SE performed at low severity. On the other hand, it can be observed that an increase in severity leads to a gradual improvement in glucose production. For essay 9, glucose yield of 23.4% w/w (based on initial dry weight of wood) was obtained, corresponding to a cellulose-to-glucose conversion \approx 82%. The fermentability of the sugars solutions resulting from enzymatic hydrolysis of experiments 8-10 has been checked using *S. cerevisiae*. A

quantitative conversion of glucose into ethanol was observed for the three samples attesting the very good fermentability of the liquors. Ethanol yields of 8.9 %, 11.9 % and 9.1 % (w/w based on initial dry weight of wood) have been obtained for essays 8, 9 and 10 respectively.

4. Discussion

The cell wall of okoumé wood was strongly altered with the acid-catalyzed SE treatment: it was thinner, deformed with cracks. Beside specific anatomical and chemical properties, wood of *Aucoumea klaineana* is well-known to exhibit great variability in tree. Interestingly, anatomical characterization of the effect of SE (assay 9) reflect this high variability. The correlation between the intrinsic variability of wood biomass and the effect of SE pretreatment is new. The conditions of high severity led to an almost total hydrolysis of xylans. A relatively slight lignin removal was observed, however this polymer was strongly impacted and depolymerized especially when the severity was high, leading to the formation of fragments of low β -O-4 contents and molar masses redeposited on the surface of the fibers, easily extractable during the ethanol washing stage. A decrease of cellulose DP was observed with an increase in SE severity making it more accessible to enzymes. As a result, cellulose-to-glucose conversion yields up to 82% were obtained with an excellent fermentability into ethanol. The good fermentability of glucans can be explained by the process used in our approach, which involves extraction and washing of the pulp with ethanol and then water. These steps thus have the dual advantage of washing the cellulosic residue (in order to remove both some of the lignin redeposited on the fibers and the inhibitors) to make it more digestible for biotechnological processes and solubilizing lignin that can be recovered prior to biotechnological steps. Then this process also produced a large amount SE lignin displaying interesting features including solubility, low molecular

mass, high phenolic OH contents which could find valuable applications in various fields (Obame et al., 2019). In addition to cellulose and lignin, we showed in a previous paper (Mougnala Moukagni et al., 2021) that okoumé hemicelluloses primarily glucuronoxylans can be recovered from the liquid effluent of SE in polymeric forms.

5. Conclusion

Steam explosion was evaluated and optimized as a pretreatment of *Aucoumea klaineana* Pierre sapwood, an abundant tropical feedstock for the production of bioethanol and lignin. This study highlights the chemical and structural features impacting the full utilization of the biomass and brings new data on the effect of SE at the ultrastructural level on tropical wood species. It was anticipated that from 1 ton of okoumé sapwood and using the optimum conditions (210°C, 5 min, 0.25 w/w% sulf. acid), \approx 112 Kg of ethanol and \approx 82 Kg of lignin of high purity can be recovered. A strategy including the fermentation of pentoses (xylose in particular) using appropriate microorganisms could significantly increase the quantities of ethanol produced. We hope that this work opens up prospects for the full valorization of Gabon's forest industry co-products for the production of biofuel and biosourced molecules through a "biorefinery" approach.

Acknowledgements:

EA 4370 LERMAB is supported by the French National Research Agency through the ARBRE Laboratory of Excellence (ANR-12-LABXARBRE-01). The authors thank the Agence Nationale des Bourses du Gabon (ANBG) and Campus France for the financial support. The authors also thank the RMN platform, Institut Jean Barriol, CRM2, Université de Lorraine - CNRS.

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