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Investigating the potential of *Aucoumea klaineana Pierre* sapwood and heartwood wastes to produce cellulosic ethanol

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This work investigates for the first time the different reactivity exhibited by sapwood and heartwood wastes under two conditions: They were pretreated with catalyzed ethanol organosolv in the presence of sulfuric acid and their pulps underwent enzymatic hydrolysis by *Trichoderma reesei* which causes them to release neutral sugars for the production of cellulosic ethanol. *Aucoumea klaineana Pierre* (Okoumé) wood wastes were used for the experiment. Organosolv pretreatment was performed to investigate how to harness the benefits of fermentable sugars and lignin, which were reacted at varying defined severity levels as follows: 160°C≤T≤210°C and 0%≤[H_2SO_4]≤2% (w/w). The highest ethanol organosolv lignin content was obtained at T=160°C and [H_2SO_4]=1% for sapwood (18.10%) and heartwood (19.46%) (w/w). Enzymatic hydrolysis of the pretreated wood samples displayed that sapwood and heartwood pulps released their highest sugars content under free acid pretreatment conditions. The maximum neutral sugar released by heartwood pulps pretreated at T=160°C and [H_2SO_4]=0% was 0.126 g/L while that from sapwood pretreated at T=185°C and [H_2SO_4]=0% was 0.125 g/L. It is noteworthy that, the neutral sugars from sapwood and heartwood do not have the same sensitivity to temperature increase. When pretreated at T=160°C and [H_2SO_4]=0%, sapwood pulps yielded the following neutral sugars: Xyl< Ara≈Gal < Man < Glu, while heartwood was: Ara < Gal < Xyl < Man < Glu. However, with increased temperature (T=185°C and [H_2SO_4]=0%), sapwood yielded the following neutral sugars: Ara < Gal < Xyl < Man < Glu, while heartwood yielded the following: Xyl < Ara≈Gal < Man < Glu. Similar trend was observed in both sapwood and heartwood pulps when sulfuric acid concentration was increased.

Key words: *Aucoumea klaineana Pierre,* wood wastes, ethanol organosolv lignin, enzymatic hydrolysis, bioethanol.

INTRODUCTION

Biofuels help to overcome the global warming concern because they use carbon from the atmosphere and give it back to it, while fossil energies increase the carbon content in the atmosphere. Biofuel was first mentioned in 1903 and 1926, which corresponds to the beginning of the automotive era (Payen, 1982). Nowadays, first generation bioethanol from sugarcane in Brazil or corn in USA (Wheals et al., 1999) is blended with gasoline and used in all petrol engines without modifications (Miller, 2003; Steven and Verhe, 2004).

Considering that the fossil fuels reservoirs will serve man’s needs for a very limited time period, scientific search for alternatives has already been initiated. Amongst the renewable resources for emerging biotechnological strategies used to produce high energy-less volume fuels, lignocellulose like cellulose which is the most abundantly synthesized and stable carbohydrate of the biosphere has been identified. However, the stable nature of the substrate and some fermentation difficulties of its monomeric products have been the major hurdles in using cellulose. But due to its ubiquitous nature and abundant availability as renewable resource, research on the utilization of cellulose for obtaining bioethanol continues in diverse fields.

There are still many issues to be addressed in considering the future of ethanol fuels. Many critics have charged that the energy balance of ethanol fuels is flawed, arguing that the energy inputs exceed the energy content of the final product. However, a study released by the United States Department of Agriculture in July of 2002 refutes these claims and finds that ethanol has an output: input energy ratio of 1.34:1 (Shapouri et al., 2002). Furthermore, another study found that the energy balance of gasoline is actually negative, giving ethanol a 1.42:1 output energy ratio compared to gasoline (Alternative Fuels Data Center, 2003). On the other hand, converting the entire feedstock plant to useful end products allows ethanol costs to decrease as manufacturing becomes more profitable. Besides being a renewable fuel made from plants, with high octane at low cost, ethanol is a much cleaner fuel than petrol. Ethanol blends dramatically reduce emissions of hydrocarbons, (major sources of ground level ozone formation), cancer-causing benzene and butadiene, sulphur dioxide and particulate matter.

However, the use of ethanol for fuel will almost certainly require cellulose technology. Likewise, Taherzadeh and Kairimi (2007) have recently indicated that lignocelluloses can be expected to be major feedstocks for ethanol production in the future. Evans (2005) has explained that, being 50% of the total dry matter of plant, cellulose is potentially a huge renewable energy store and vast amounts of this material are routinely thrown away. Thus, ethanol cellulose from the wood waste timber industries is an opportunity for equatorial oil-producing developing countries of Congo basin like Gabon to receive transfer of technologies. This will allow those countries to produce much ethanol at cheaper rates to obtain fuels at a reduced cost. A prospective study has stated that ethanol (from sugar cane, maize, sorghum and manioc fibers) or biodiesel (from palm oil and Jatropha) demand in Gabon will be 12 and 46 millions of liters respectively, in the year 2020 (Sielhorst et al., 2008). However, the first boards of trunk wastes from sawmill and the inner heartwoods from the rotary cutting logs of Okoumé’s plywood industry which remain undervalorized can be suitable source for ethanol cellulose production. Previous studies have shown that ethanol can be obtained from wood specie like Eucalyptus (Ferrari et al., 1992, Romani et al., 2012, Yanez-S et al., 2014).

Despite that hydrolysis of lignocelluloses by sulfuric acids is relatively old, it remains the most investigated process (Harris et al., 1945). Advantages and disadvantages of lignocellulose pretreatment process for ethanol cellulose production with sulfuric acid have been extensively discussed by Taherzadeh and Karimi (2007). Nevertheless, Organosolv pretreatment released a cellulosic residue which can be used for ethanol production, high neutral sugars yield in aqueous phase, better delignified fibers and relatively pure lignin for which interest grows in various industrials (Brosse et al., 2010; El Hage, 2010).

However, the differences in the reactivity of sapwood and heartwood pretreated with sulfuric acid and the behavior of their partially delignified fibers through enzymatic hydrolysis have not been investigated. The aim of this study is to investigate the potential of sapwood and inner heartwood of Okoumé as biomass candidates treated with ethanol organosolv lignin in an experimental design to find better conditions for lignin extraction. The neutral sugar contents obtained from enzymatic hydrolysis of the partially delignified pulps shall be used to discriminate between the two wood wastes. The relative sensitivity of the cellulose-hemicellulose matrix sugars from sapwood and heartwood to temperature and sulfuric acid concentration increase will be investigated.

MATERIALS AND METHODS

The raw material of Okoumé was collected in February 2016 at SNBG (Société Nationale des Bois du Gabon). The air-dry material was ground and milled to a particle size of 0.25 mm (for Klason lignin) and 0.6 mm (for Ethanol Organosolv Lignin) using a Willey

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mill. It was stored at room temperature during the course of this study. All chemical reagents used in this study were purchased from Aldrich and VWR (France) and used as received. *Trichoderma reesei* (product number C2730) was supplied by Sigma Aldrich. Other chemicals used include absolute ethanol, sulfuric acid, sodium hydroxide, potassium sodium tartrate, acetic acid glacial.

Extractives and ash content

The extractive contents were obtained as follows: Triplicates of 1 g of unextracted heartwood or sapwood sawdust were subjected to soxhlet extraction with acetone of 70% (v/v) for 24 h to remove the extracts. The solid to liquid ratio was 2% (w/v). Then, the wood was oven-dried at 105°C for 24 h and cooled to ambient temperature in a desiccator containing phosphorus pentoxide (P_2O_5).

The ash content of the wood samples was obtained at Tappi T 211 cm-86 (2003) adapted as follows: 1 g of unextracted and dried heartwood or sapwood sawdust was weighted with an analytical and accurate balance of 0.1 mg. The samples were deposited in a ceramic ashing crucible, and introduced in a muffle furnace equipped with a thermostat set at 575±25°C equipped with optional ramping program. The wood biomass was ignited in a first step at 250°C for 2 h, and then a second step was carried out at 500°C for 24 h. At the termination of the pyrolysis, the ceramic ashing crucible was removed to the furnace directly into a desiccator containing P_2O_5 for cooling at room temperature until constant weight was obtained. The ash content was calculated as follows:

$$Ash(\%) = \frac{M_{ash}}{M_0} \times 100$$

M_{ash} is the weight of ash obtained after pyrolysis and M_0 is the unextracted moisten-free dry wood samples weight.

Klason lignin measurement

175 mg extractive-free and dried wood materials were hydrolyzed with 72% (w/v) sulfuric acid and stirred for 1 h before autoclaving at Tappi T 222 om-11 (2006). The autoclaved samples were filtered with Buchner and the dried residue was weighed to give the Klason lignin (KL) content; while the liquid fraction was keep at -5°C for the neutral sugar content determination.

Ethanol organosolv lignin extraction

15 g of unextracted and dried material samples were treated with 65% (v/v) ethanol in the presence of sulfuric acid as catalyst and different levels of temperature (Table 1), using a published protocol (Pan et al., 2006, 2007; El Hage, 2010). The solid to liquid ratio was 1/7 (w/v). The process was optimized by an experiment design run with the statistical *JMP*® 11 Découvrir *JMP.* Cary, North Carolina: SAS Institute Inc.2013 software. The pre-treatments were carried out in a 1.0 L glasslined pressure Parr reactor equipped with a 4842 temperature controller (Parr Instrument Company. Mole, IL) for 1 h (manually controlled). The reactor was cooled until 30°C, and the pre-treated wood pulps were washed with warm (60°C) ethanol/water (2:1, 3x50.00mL). The washes were combined and 3 volumes of water were added to precipitate the Ethanol Organosolv Lignin (EOL), which was collected by centrifugation (3000 rpm, 15 min and 10°C) and dried. The EOL content is calculated as follows:

$$EOL(\%) = \frac{M_{EOL}}{M_0} \times 100$$

Table 1. Experimental matrix of the face centered composite design conditions used for the pretreatments of Okoumé's heartwood and sapwood.

Number of reactions	Temperature (°C)	$[H_2SO_4]$ (%)
1	0	0
2	-1	0
3	0	1
4	1	1
5	0	0
6	-1	1
7	0	0
8	1	0
9	0	-1
10	1	-1
11	0	0
12	-1	-1

Experimental conditions: 160°C≤T≤210°C such as -1=160°C; 0=185°C and +1=210°C. $1\% \leq [H_2SO_4] \leq 2\%$ (w/w) : -1=0% ; 0=1% and 1=2%. The reaction time is 1hour. Ethanol (65%)/H_2O=1v/2v and the solid to liquid ratio =1/7. The central point was R1; R5; R7 and R R11.

M_{EOL} is the weight of dried EOL and M_0 is the moisten-free dry wood samples weight. For each residual heartwood or sapwood pulp obtained, the KL content was determined as described previously.

Enzymatic hydrolysis of the pretreated wood samples

Enzymatic hydrolysis of the pulps obtained after EOL extraction was carried out using a published procedure with slight modifications (El Hage, 2010; Brosse et al., 2010). The sapwood and heartwood pulps obtained after EOL treatment were grinded to pass 0.5 mm, oven-dried at 103°C for 24 h and cooled at room temperature in a desiccator which contained P_2O_5. 2.0 g of the pretreated and dried pulps was poured into an Erlenmeyer flask with 98 mL of sodium acetate buffer (0.05 M; pH=4.8). Then, 0.38 mL of the enzymatic solution of 15 FPU per gram of substrate (2% w/v) was added to the mixture and stirred. It was incubated at 50°C for 48 h in an air-bath shaking incubator at 150 rpm. About 0.2 mL samples were drawn periodically, diluted in 1 mL distillated water, and placed in 1.5 mL Eppendorf safe-lock tube. In order to arrest enzymatic activity, the samples in the Eppendorf tube were placed in boiling water at 97°C for 5 min, cooled and kept in a refrigerator at -5°C until further analysis.

Monosaccharides and uronic acids reducing measurement

An aliquot (2.5 mL) from the filtrates obtained after enzymatic hydrolysis was freeze-dried and diluted to 10%, then the monosaccharide and uronic acids content in the filtrate were quantified by the High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) procedure. A Dionex ICS-3000 HPLC system was used to separate and quantify neutral sugar and uronic acids. The system consisted of a SP gradient pump, an AS auto-sampler, an ED electrochemical detector with gold working electrode, an Ag/AgCl reference electrode,

Table 2. Dry density, ash, extractives and Klason lignin content of Okoumé's sapwood and heartwood.

Samples	Dry density (g/cm^3)^a	Ash (%)^a	Extractives (%)^a	Klason lignin (%)^a
Sapwood	0.484±0.034^b	0.232^d	0.530±0.100^e	29.43±1.00^d
Heartwood	0.429±0.046^c	0.224^d	1.912±0.090^f	29.91±1.19^d

Ash, extractives (n=3) and Klason lignin (n=3) content is based on dried wood samples. Density (n=6). Means ith the same letters are not statistically different at the 0.05 level of significance. [a] Mean±S.D

Table 3. Chemical composition of Okoumé as published by various authors.

Components	Savard (1959)	Lal et al. (1977)	Brunck et al. (1990)	Minkuè M'Eny (2000)	Safou-Tchiama, 2005*; Safou-Tchiama et al., 2007)	Medzegue (2007)
Ash (%)	0.4	1.8	0.33	0.43	0.55	-
Holocellulose (%)	-	77.20	-	75.60	80.0±0.30	78.2±0.20
Hemicelluloses (%)	-	-	-	-	32.3±0.50*	-
Cellulose (%)	-	-	-	-	47.9±1.00*	-
Glucose (%)	-	-	-	-	55.70±3.70	-
Xylose (%)	-	-	-	-	10.30±0.70	-
Arabinose (%)	-	-	-	-	0.30±0.05	-
Galactose (%)	-	-	-	-	0.30±0.04	-
Mannose (%)	-	-	-	-	4.10±0.30	-

The ash content was based on percentage of dried wood sawdust. The sugar content was based on percentage of dried hollocellulose.

and Chromeleon software version 6.8 (Dionex Corp, USA). The stationary phase used was a Carbopac PA20 (3 × 150 mm), Dionex column with a guard column (3 × 50 mm, Dionex), while mobile phase was water, 250 mM NaOAc, and 1 M NaOH/20 mM NaOH. Monomers and uronic acids were separated using isocratic conditions and linear gradient elution. All eluents were degassed before use by flushing with helium for 20 min with 250 mM NaOH solution and re-equilibrated for 10 min in the starting conditions. Samples were injected through 25L full loop at a discharge rate of 0.4 mL/min and separations effected at 35°C column temperature. The pulse sequence for pulsed amperometric detection consisted of potential +100 mV (0 to 200 ms), +100 mV integration (200 to 400 ms), -2000 mV (410 to 420 ms) +600 mV (430 ms), and -100 mV (440 to 500 ms).

Statistical analysis

The data were analyzed by a one-way analysis of variance (ANOVA) followed by the Fischer's LSD (last significant difference) test at α=0.05 level of significance with Rr643.0.2 software.

RESULTS AND DISCUSSION

Dry density, ash, extractives and Klason lignin content

The analysis of the biomass (Table 2) pointed out a significant difference (P<0.05) between the sapwood and heartwood dry density. The former displayed the highest dry density. However, the heartwood was more abundant in the extracts than the sapwood. Similar trend was previously observed for lodgepole pine (Campell et al., 1990) and loblolly pine (Thompson et al., 2006). Furthermore, the extractive content released by the heartwood is close to those previously reported by Safou-Tchiama (2005) and Medzegue (2007) who obtained 2.1±0.1 and 2.0±0.4% respectively. No significant difference was found (P>0.05) between the ash content of sapwood and heartwood (Table 2) which is lower than those reported by previous works (Table 3). In addition, no statistical difference (P>0.05) was found in the KL content of the selected samples. The lack of variability in the lignin content released by the sapwood and heartwood was observed for other wood species (Thompson et al., 2006). However, the KL content obtained was in the same range with those published by Brunck et al. (1990) and Minkuè 'Eny (2000) who found 30 and 30.9% (g/dry weight), respectively.

The hollocellulose deducted from the KL pointed out that the sapwood should be richer (70.04%) in cellulose-hemicelluloses complex than the heartwood (68.18%) as observed in lodgepole pine (Campbell et al., 1990). Nevertheless, the holocellulose content obtained from the inner heartwood is accordingly lower than that published previously by Lal et al. (1977), Minkué M'Eny (2000), Medzegue, (2007); Safou-Tchiama, 2005 and Safou-Tchiama et al. 2007) who investigated the whole heartwood including mature wood assumed to be richer in fibers than the inner heartwood which is mainly composed of juvenile wood. The latter is poor in pulps (Campbell et al., 1990). The holocellulose content

Table 4. Pretreatment conditions, ethanol organosolv lignin (EOL) and Klason lignin recovery from Okoumé's sapwood (SW) and heartwood (HW).

Reactions	Temperature (°C)		Sulfuric acid (%)		EOL (%)		Klason lignin (%)	
	SW	HW	SW	HW	SW	HW	SW	HW
R1	185	185	1	1	16.77	15.43	73.44	81.42
R2	160	160	1	1	19.46	18.10	22.74	28.65
R3	185	185	2	2	17.24	15.02	73.75	76.72
R4	210	210	2	2	06.70	8.25	98.23	93.29
R5	185	185	1	1	11.15	13.98	91.37	83.60
R6	160	160	2	2	17.55	16.93	74.22	72.44
R7	185	185	1	1	11.46	14.75	90.43	83.87
R8	210	210	1	1	07.20	10.06	94.56	88.71
R9	185	185	0	0	09.83	4.02	22.26	23.80
R10	210	210	0	0	15.85	11.77	13.70	16.84
R11	185	185	1	1	14.44	12.36	97.57	90.71
R12	160	160	0	0	0.00	0.00	29.86	26.90

displayed by the sapwood and the inner heartwood of Okoumé is very close to that found for promising lignocellulose biomass like *Miscanthus x Giganteus* which displayed sugar content of 70% (El Hage, 2010).

Ethanol organosolv lignin

The EOL extracted from the wood samples is presented in Table 4. Reactions R9 and R10 suggest that EOL could be obtained from the sapwood and heartwood of Okoumé by heating free acid at T=185°C and T=210°C, respectively, and the highest acid free EOL content was yielded by sapwood heated at 210°C. Nevertheless, the KL released by R10 is very weak compared to the untreated wood samples (Table 2). This suggests that a mass was lost when the sapwood and heartwood were subjected to high temperatures. The polysaccharide matrix of the sapwood was more sensitive to temperature increase than the heartwood at $[H_2SO_4]=0\%$ (Table 4).

Increasing acid concentration shows that both sapwood and heartwood released their highest EOL in R2 pretreatment (Table 4). Their respective content is very close to that obtained for a cellulose ethanol candidate like *Pinus taeda* which yielded 19% of EOL (Sannigrahi et al., 2010). Nevertheless, the pretreatment conditions used for that wood specie were energy and acid consuming (T=170°C; $[H_2SO_4]=1.1\%$). On the other hand, both the wood wastes from Okoumé exhibited higher EOL than that extracted from *Miscanthus* (16%) (El Hage, 2010).

When both temperature (T>160°C) and sulfuric acid concentration ($[H_2SO_4]>1\%$) increased, the residual KL content of the sapwood and heartwood pulps increased as well (Table 4). Both the wood samples exhibited similar trend and their highest KL content was displayed in R4 conditions which corresponds to the maximum process severity. That result suggests a strong susceptibility of the sapwood and heartwood sugars to degradation, both at high temperature and acid concentration as observed for other lignocelluloses biomass (El Hage, 2010; Audu et al., 2012). This results in a pseudo-lignin formation which would increase strongly the KL content above the average value of untreated sapwood and heartwood as observed for other lignocellulosic materials (Hu et al., 2012; Li et al., 2007).

Enzymatic hydrolysis and sugar content

The pretreated sapwood and heartwood pulps were subjected to enzymatic hydrolysis and the total sugar based on dried weigh per L (g/L) is presented in Figure 1. Reactions R2 (T=160°C; $[H_2SO_4]=1\%$), R6 (T=160°C; $[H_2SO_4]=2\%$) and R9 (T=185°C; $[H_2SO_4]=0\%$) were the most promising for the sapwood while the heartwood exhibited its highest neutral sugars content under R2, R10 (T=210°C; $[H_2SO_4]=0\%$) and R12 (T=160°C; $[H_2SO_4]=0\%$) pretreatment conditions. Those results have pointed out the variability between the sapwood and heartwood of Okoumé under different temperature and acid concentrations.

Effect of acid concentration on neutral sugar content

The total sugar content released through enzymatic hydrolysis of the pulps is presented in Figure 1. The impact of acid concentration on enzymatic hydrolysis of the pulps was studied in comparison with the acid free reaction R12 (T=160°C; $[H_2SO_4]=0\%$). It is obvious that all the neutral sugars content released by the sapwood are lower than those from heartwood. On the other hand, the sugar yield order from the sapwood pretreated in R12

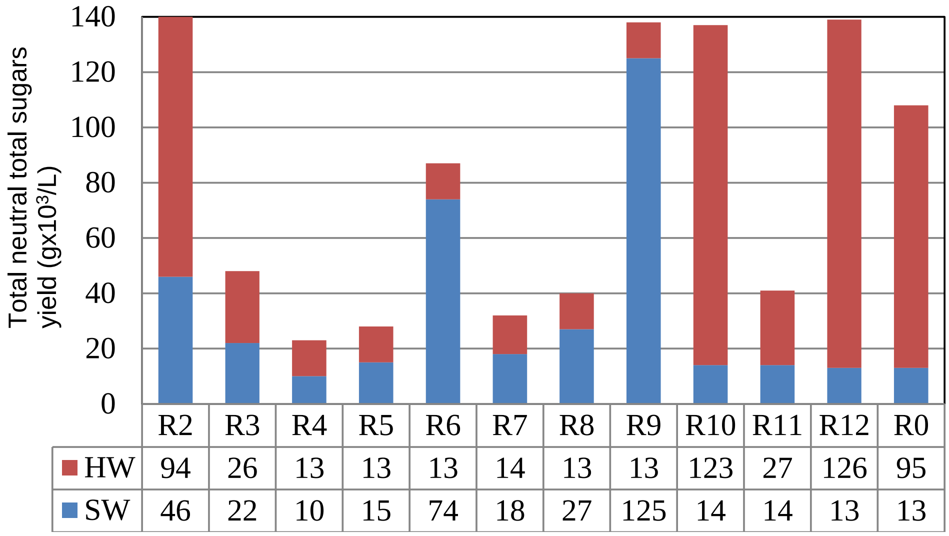

Figure 1. Total neutral total sugars yield obtained after enzymatic hydrolysis of pretreated heartwood (HW) and sapwood (SW) pulps at 160°C≤T≤210°C and 1%≤[H_2SO_4]≤2%. R0 (T=25°C; [H_2SO_4]=0%) is the reaction from which enzymatic hydrolysis was performed with unpretreated heartwood and sapwood.

Table 5. Monosacharaides and uronic acids reducing content (mg/L) of the liquid fraction of Ethanol Organosolv Lignin pulps from Okoumé sapwood (SW) and heartwood (HW) enzymatically hydrolyzed according to the pretreatment conditions.

Reaction	Rha		Ara		Gal		Glu		Xyl		Man		GalAc		GluAc	
	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW
R2	0.00	0.00	1.90	1.33	0.44	0.34	39.28	85.97	2.94	0.61	0.91	5.37	0.26	0.00	0.12	0.22
R3	0.00	0.00	3.80	2.49	0.29	0.33	13.14	18.34	4.43	0.93	0.47	3.56	0.05	0.00	0.00	0.04
R4	0.00	0.00	0.90	0.37	0.30	0.40	7.62	8.12	1.19	0.16	0.52	4.13	0.07	0.00	0.12	0.05
R5	0.00	0.00	1.54	0.78	0.35	0.24	9.94	9.53	2.19	0.41	0.63	2.42	0.07	0.00	0.11	0.04
R6	0.00	0.00	0.93	0.38	0.32	0.39	66.93	8.24	0.40	0.15	4.42	4.24	0.00	0.00	0.16	0.05
R7	0.00	0.00	1.25	0.38	0.36	0.40	11.71	8.32	0.57	0.15	3.76	4.31	0.00	0.00	0.06	0.05
R8	0.00	0.00	1.80	0.37	0.49	0.39	17.66	8.08	0.68	0.15	5.89	4.13	0.00	0.00	0.08	0.05
R9	0.17	0.00	0.42	0.37	1.17	0.38	94.97	8.13	8.22	0.15	18.58	4.16	0.00	0.00	0.16	0.04
R10	0.00	0.00	0.38	0.12	0.41	0.46	8.32	99.97	0.15	4.82	4.32	13.99	0.00	0.00	0.05	0.07
R11	0.00	0.00	0.38	1.00	0.41	0.37	8.30	18.92	0.15	0.41	4.34	5.76	0.00	0.00	0.04	0.07
R12	0.00	0.22	0.37	0.73	0.37	0.93	8.15	96.63	0.15	7.63	4.18	17.68	0.00	0.00	0.04	0.36
R0	0.00	0.06	0.37	0.38	0.40	0.56	8.18	80.38	0.15	2.74	4.20	10.48	0.00	0.05	0.05	0.10

R0 (T=25°C; [H_2SO_4]=0%). Sulfuric acid concentration is based on (w/w). Rha, rhamnose; Ara, arabinose; Gal, galactose; Glu, glucose; Xyl, xylose; Man: mannose; GalAc, galacto-uronic acid; GluAc, glucuronic acid.

is as follows: Xyl< Ara≈Gal < Man < Glu while that from the heartwood pulps is: Ara < Gal < Xyl < Man < Glu (Table 5). That result has pointed out that there is strong variability between the sapwood and heartwood pulps in enzymatic hydrolysis. The enzymatic hydrolysis of the heartwood led to a release of a high mannose content, confirming the abundance of mannose within Okoumé cell wall fibers (Safou-Tchiama et al., 2007).

Within the sapwood, Figure 1 shows that increasing acid concentration of the pretreatment leads to significant increase of the neutral sugar released by enzymatic hydrolysis of the pulps in R2 (T=160°C; [H_2SO_4]=1%) and R6 (T=160°C; [H_2SO_4]=2%) compared to R12 (T=160°C; [H_2SO_4]=0%). The neutral sugars yielded from R2 were: Gal < Man < Ara < Xyl < Glu (Table 5). The relative neutral sugars increase (Figure 2) shows clearly that increasing acid concentration under the pretreatment conditions of R2 hydrolyzed strongly mannose of the sapwood pulps, while the enzymatic hydrolysis of all the other neutral sugars was facilitated. The highest increase

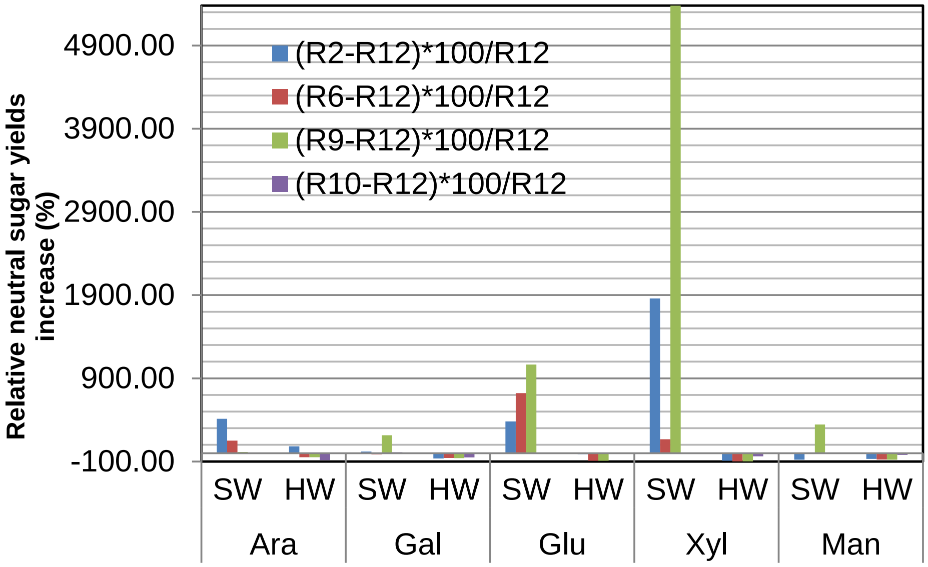

Figure 2. Relative neutral total sugar yield increase after enzymatic hydrolysis of sapwood (SW) and heartwood (HW) pulps from the promising pretreatments R2 (T=160°C, $[H_2SO_4]$=1%); R6 (T=160°C, $[H_2SO_4]$=2%), R9 (T=185°C; $[H_2SO_4]$=0%) and R10 (T=210°C; $[H_2SO_4]$=0%) in comparison with the free acid pretreated heartwood and sapwood pulps from reaction R12 (T=160°C; $[H_2SO_4]$=0%).

was observed for arabinose, glucose and xylose. Furthermore, increasing acid concentration for +2% in R6 leads to an increase in the neutral sugars yielded by the enzymatic hydrolysis of sapwood pulps (Figure 1). The corresponding sugars were released as follows: Gal < Xyl< Ara < Man < Glu (Table 5). Increasing acid concentration under R6 conditions was favourable to the enzymatic hydrolysis of glucose. Arabinose and xylose yields remained higher in R12 (Figure 2) whereas they were lower in R2 (Table 5). This suggests the inhibiting effect of sulfuric acid concentration on enzymatic hydrolysis of arabinose and xylose within the sapwood pulps pretreated in R6 (T=160°C; $[H_2SO_4]$=+2%). That inhibiting effect was more marked for galactose (Figure 2).

Raising acid concentration either in R2 or R6 reduced the total sugar yielded from the heartwood fibers in R12 (Figure 1). Nevertheless, in R2 reaction, the total sugar yielded from enzymatic hydrolysis of heartwood pulps is higher than that of sapwood (Figure 1); glucose and mannose remained the major neutral sugars within the heartwood pulps (Table 5). With the exception of arabinose for which raising acid concentration according to R2 increased its yield after enzymatic hydrolysis of the heartwood pulps, the other neutral sugars content decreased dramatically (Figure 2). That decrease was observed for all the neutral sugars released by the enzymatic hydrolysis of the heartwood pulps from R6 pretreatment; and the following sugar yields order was obtained: Xyl < Ara < Gal < Man < Glu, while that from R2 was: Gal < Xyl < Ara < Man < Glu (Table 5). It is noteworthy that xylose remained strongly sensitive to acid concentration at high temperature as previously found in other biomass (Xiang et al., 2004). In addition, glucose and mannose have pointed out a strong sensitivity towards increased acid concentration within the heartwood pretreated either in R2 (T=160°C; $[H_2SO_4]$=1%) or R6 (T=160°C; $[H_2SO_4]$=2%) as shown in Figure2. Those results have pointed out that heartwood exhibited an opposite response towards acid sulfuric increase compared to sapwood. That should be explained by the difference in chemical composition between *Aucoumea klaineana* heartwood and sapwood. The latter was found to be rich in terpens, furans or phenol compounds bearing esterified 4-hydroxybenzoic acid (unpublished data) described as inhibitors for enzymatic hydrolysis of wood pulps (Taherzadeh and Karimi, 2007).

Effect of temperature on sugar content

The total neutral sugars yield (Table 5) shows that sapwood and heartwood pulps do not have the same sensitivity towards temperature increase. Enzymatic hydrolysis of the sapwood pulps pretreated under R9 (T=185°C; $[H_2SO_4]$=0%) conditions released the highest total sugars content (Figure 1), and the following sugar yield order was obtained: Ara < Gal < Xyl < Man < Glu (Table 5). Raising temperature in R9 reaction increased strongly the galactose, glucose, xylose and mannose content released from the enzymatic hydrolysis of sapwood pulps when compared to heartwood which released one of the lowest neutral sugars yielded as follows: Xyl < Ara≈Gal < Man < Glu (Table 5). The results above have pointed out a strong sensitivity of all the heartwood neutral sugars to temperature increase. They decreased dramatically under the pretreatment conditions of R12 (T=160°C; [H_2SO_4]=0%) (Figure 2).

Nevertheless, enzymatic hydrolysis of the wood pulps pretreated under R10 (T=210°C; [H_2SO_4]=0%) conditions was favourable to heartwood. The latter exhibited one of the highest total sugar content (Figure 1) and the same sugars yield order like R12 pretreatment (Table 5). But raising temperature from 160 to 210°C hydrolyzed dramatically the arabinose, galactose, xylose and mannose of the heartwood pulps while enzymatic hydrolysis of glucose was favored (Figure 2); confirming that pretreating the heartwood at T=185 or 210°C with [H_2SO_4]=0% hydrolyzed the neutral sugars in the same extent. Major neutral sugars like glucose and xylose were more sensitive to temperature hydrolysis when the heartwood was pretreated at T=185°C.

Finally, comparing the sensitivity of the wood samples to temperature or sulfuric acid concentration increase has pointed out that sapwood and heartwood do not display the same behavior (Figure 2). The heartwood's galactose, xylose and mannose yielded from the enzymatic hydrolysis decreased systematically when temperature and sulfuric acid concentration increased in R12. The same trend was observed not only for arabinose from R6, R9 and R10 pretreatment conditions; but also for the heartwood's glucose from R2, R6 and R9 (Figure 2). However in Figure 2, it is noteworthy that increasing temperature and acid concentration point out an inverse trend for the sapwood. Arabinose, glucose and xylose yielded by enzymatic hydrolysis of the sapwood pulps from R2, R6, R9 and R10 increased indeed, and the galactose yielded from R6, R9 and R10 increased in R12 as well; whereas mannose content from the R6, R9 and R10 pretreatment conditions increased compared to the heartwood.

Conclusion

Aucoumea klaineana Pierre sapwood and heartwood have pointed out a strong potential for EOL extracted under middle conditions. Assuming that lignin is the second most abundant polymeric material on earth, and it is still underutilized, the high content of EOL displayed by *A. klaineana Pierre* can offer opportunities for producing sustainable alternatives to non-renewable products such as polyurethanes, thermoplastic polymers, epoxy and phenolic resins, as well as corrosion inhibitors. On the other hand, enzymatic hydrolysis by *Trichoderma reesei* has pointed out that the highest sugar contents are obtained from the acid free pretreated sapwood (T=185°C) and heartwood (T=160°C) pulps. However, increasing temperature and sulfuric acid concentration in the pretreatment process of the sapwood pulps leads to an increase in the total neutral sugars content released by the enzymatic hydrolysis. Nevertheless, the heartwood exhibited a strong sensitivity to temperature and sulfuric acid concentration increase; most of all the neutral sugars tend to be hydrolyzed during the heartwood pretreatment process. But further investigations are necessary for a better understanding of the molecular composition and supramolecular organization of the two EOL. The lignin-hemicellulose linkages as well as cellulose-hemicellulose interactions which control the access of chemicals and enzymatic hydrolysis shall be performed. The yield of the fermentation process of the neutral sugars from the promising reactions will discriminate between the potential of sapwood and heartwood of Okoumé to be used as source for cellulosic ethanol production.

Conflicts of Interests

The authors have not declared any conflict of interests.

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