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VESSEL PICKING AND INK REFUSAL: EFFECT OF ENZYME ACTIVITY

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SUMMARY

Vessel enzymatic passivation, altering its adhesion to the fiber network and reducing its hydrophobicity may address industrial offset printing problems, namely vessel picking and ink refusal. The primary object of this study was to study how enzymatic treatment by xylanase and by an enzymatic cocktail containing cellulases and laccases affect ECF bleached *Eucalyptus globulus* vessel and fiber porosities, bulk chemistry, and surface chemical composition, and how these parameters may correlate with vessel picking and ink refusal problematics. Differences in vessel and fiber porosity structures lead to differential enzymatic attacks, improving vessel bonding to the paper structure and decreasing vessel hydrophobicity, revealing the potential of combining mechanical and enzyme activities to address vessel picking and ink refusal, eventually causing vessel passivation.

KEYWORDS: vessel picking, ink refusal, Eucalyptus globulus, enzyme activity, vessel elements.

INTRODUCTION

Hardwood species have complex cellular structures consisting of fibers, vessel elements and parenchyma cells with different chemical compositions [1-3]. However, the presence of vessels with significant dimensions in their structure is a recurrent problem in the operation of industrial UWF paper printing [3, 4]. Vessel picking occurs due to picked vessel elements in the paper surface during printing. Ink refusal is caused by the hydrophobic character of vessel elements, resulting in further printing problems due lack of ink absorption [3,5]. Vessel picking and ink refusal are problems that paper professionals have tried to solve, but solutions for these have not yet been fully found [6, 7]. Vessel enzymatic passivation, altering its adhesion to the fiber network and reducing its hydrophobicity may address these phenomena [7]. If vessels are concentrated in a stream, they can be pre-treated (e.g., by mechanical refining) and reincorporated into the pulp [5]. Other strategies aim at vessel enzymatic and/or chemical passivation and sheet surface chemical treatment, altering the vessel adhesion to the fiber network. Thus, the importance of knowing the passivation effect on fibers and vessels surface composition, that can be significantly different from its bulk composition. Fiber and vessel bulk chemistry, carbohydrate composition, e.g., neutral sugars, must be known as a basis to understand chemical differences and the bulk effect of enzymes activities [5,7]. X-ray Photoelectron Spectroscopy (XPS), through samples exposure to x-radiation releases electrons, may reveal the surface chemical composition at a depth of 1–5 μm [7-9]. Pore structure of fibers and vessels may serve as a basis for understanding enzymes access and activity [7]. A variety of physical techniques are available for determining the geometry of the micropores of the cell wall, such as gas sorption isotherms, mercury intrusion porosimetry, solute exclusion, thermoporosimetry, nuclear magnetic resonance and microscopic techniques [10]. Thermoporosimetry uses differential scanning calorimetry to explore the fact that water has a depressed melting temperature in small pores. Tree fractions of the water inside the cell wall may be considered: freezing and nonfreezing water in relatively small pores (micropores) and bulk water in relatively large pores (macropores). To be able to make proper comparisons of bulk chemistry, surface chemistry and structural differences between fibers and vessels, methodologies were

implemented to obtain enriched fiber and enriched vessel suspensions [11-13]. The primary object of this study was to study how enzymatic treatment by xylanase and by an enzymatic cocktail containing cellulases and laccases affect ECF bleached *Eucalyptus globulus* vessel and fiber porosities, bulk chemistry, and surface chemical composition, and how these parameters may correlate with vessel picking and ink refusal problematics. Fiber rich and vessel rich suspensions were obtained with implemented laboratorial separation methods. Differences in fiber and vessel pore structures were expected to result in different enzymes accessibility, with preferential enzyme action in vessels. Enzymatic activity was expected to alter surface and bulk chemical composition, reducing vessel picking by increasing vessel to fiber bonding strength, and decreasing vessel hydrophobicity. To understand these phenomena, methodologies were implemented to evaluate vessel picking and ink refusal in untreated and treated samples.

EXPERIMENTAL PART

Raw materials

The pulp used in the production of fiber and vessel rich suspensions was ECF bleached *E. globulus* reference pulp provided by RAIZ – Forestry & Paper Research Institute (code Navigator E.B. Cacia 2019-FEU, of 3 April 2019). The Navigator Company E. globulus cultivated forests are certified by the Forest Stewardship Council (2007) and by the Program for the Endorsement of Forest Certification (2009).

Separation method

Enriched vessel and fiber rich samples were obtained via size exclusion methodologies [11-13]. Primary size exclusion separation was carried out using a three stages Bauer-McNett, with U.S. mesh # 30, # 50 and # 200 screens, corresponding to opening sizes respectively of 595 µm, 297 µm and 74 µm. The #30 screen retained long fibers, while #200 screen let fines pass through. A Britt Dynamic Drainage Jar was also set up according to the method earlier generically described, adapted with a recirculation system. A two-step system was developed, initially for vessels accumulation within the jar and afterwards for vessels extraction. Two extracts were obtained, the fiber rich one containing practically pure fibers, while the vessel rich fraction contained around 1:1 ratio of vessels and fibers.

Enzymatic hydrolysis

Parallel enzymatic hydrolysis treatments of the two samples (fiber rich samples and vessel rich samples) were performed with Novozymes endo-1,4-xylanase NS51121 and the Celodev commercial enzymatic cocktail Celodase-083S with cellulase and laccase activities (containing sorbitol, cellulase, laccase and 1,2-benzisothiazol-3-one). The xylanase treatment was carried out at 45ºC, with constant agitation at a sample consistency of 1%, and an enzymatic dosage of 0.01% (0.75 IU/g dry pulp, according to the safety datasheet). The treatment with the enzymatic cocktail was carried out at 40ºC, with constant agitation at a sample consistency of 4.5%, and an enzymatic dosage of 1% (10-100 IU/g dry pulp for cellulase and 0.5-5 IU/g dry pulp for laccase). Both sets of treatments were performed with no pH control requirement (samples pH of 7) for 60 minutes. To remove the enzymes, the samples were then subjected to three sequential washings in distilled water followed by centrifugations at 3000g to recover the fibrous material.

Thermoporosimetry

The thermoporosimetry measurements were performed on a Mettler Toledo DSC 3+ (Mettler-Toledo Intl. Inc. Instrument, USA) differential scanning calorimeter equipped with an intracooler. A 2-6 mg sample of pulp was hermitically sealed in 40 µL aluminum pans. The masses of the sealed crucibles were recorded before and after the measurements to ensure that all pans remained sealed during the measurement. After the measurements, the crucibles were punched with a needle and dried in an oven at 105 °C overnight to determine the moisture content. The temperature was first brought to –50 ºC at 20 K/min causing all the freezable water in the samples to crystalize. The temperature was then increased to –0.2ºC and held constant until the melting transition was completed i.e., until all the water in the small capillaries melt. This step is essential to prevent supercooling during the subsequent recrystallization

step. The temperature was then decreased at 2 K/min to –30ºC. In the next step, the temperature was increased at 20 K/min to +50ºC and the total freezable water in the sample was calculated by integrating the resulting peak. The nonfreezing water (NFW) was then determined from the difference between the moisture content and the total amount of freezable water.

Bulk carbohydrate composition

The two fractions (fiber rich and vessel rich) were hydrolyzed following the laboratory analytical procedure for the determination of structural carbohydrates and lignin. Concisely, 300 mg of sample was successively submitted to a 72% (m/m) and 3%(m/m) sulfuric acid hydrolysis during 1 hour at ambient temperature and 1 hour at 121ºC, respectively. Neutral sugars, organic acids, and carbohydrate byproducts (furfural and hydroxymethylfurfural, HMF) in the hydrolysates were analyzed by using the high-performance liquid chromatography (HPLC) system. In this case a Rezex ROA (Phenomenex®) organic acid column was used, using a 0.005N sulfuric acid ultra-pure water, as eluent. The column was maintained at 60ºC, and the flow was 400 µL/min. Cellobiose, glucose, xylose, mannose, galactose, and arabinose were used as standards. Although this column elutes xylose, mannose, and galactose at the same retention time, not enabling their separation, it has the advantage of analyzing the samples without post-treatment procedures.

X-ray Photoelectron Spectroscopy

The surface chemical composition of the samples was analyzed with an X-ray photoelectron spectroscopy, using an AXIS Ultra spectrometer with monochromatic Al Kα irradiation source at 100 W. Before the measurements, the samples were evacuated overnight. Survey scans as well as high-resolution regional carbon and oxygen were acquired from 2-3 locations.

Vessel picking

The IGT Reprotest (Amsterdam, The Netherlands) testing system was used to characterize vessel picking; the system consists of an ink distribution system (IGT Reprotest B.V. Inking unit AE) and a printing apparatus (IGT Reprotest B.V. AIC2-5 printability tester). The printing tests were carried out with inks of medium viscosity and increasing printing speed (from 0 up to 6 m/s). An optical/digital method was developed to quantify the picked-up vessels in the printing tests. First, samples with the same size were scanned with an HP ENVY 4520 scanner system, generating images with a resolution of 1200 dots per inch (dpi). The scanned images were then binarized to black and white images using a FOTOR software and were afterwards processed and analyzed with ImageJ© version 1.53j, after setting appropriate size and circularity parameters. The software counted the white spots on the image, which corresponded to the vessel picks.

Surface energy

Water contact angles measurements and surface energy studies were made on fibers and vessel pellets using an OCAH 200 apparatus.

RESULTS AND DISCUSSION

In thermoporosimetry, differential scanning calorimetry is used to measure the amount of hydration water in pulp fibers, measuring the energy absorbed when the water in a frozen sample of pulp fibers is melted. Assuming fiber pores to be cylindrical with radius r Gibbs-Thompson equation calculates the pore size using the melting point and melting point depression of water at normal pressure, the specific molar volume of ice, and water surface tension. Pores can be classified into micropores (<2 nm), mesopores (2–50 nm) and macropores which are above 50nm as it is done by the IUPAC system. According to its behavior during the differential scanning calorimetry process, water may be classified in unbound water whose transition temperature, enthalpy and peaks are equal to those of pure water, bound water (FBW) with a transition temperature lower than that of pure water, and non-freezing water (NFW) as a kind of bound water whose transition is not detected. The thermoporosimetry results in both fibers and vessels before and after enzymatic treatment are compiled in Table 1, namely pore total

volume (V_{total}), micropores volume (V_{micro}) and mesopores volume (V_{meso}) for untreated and enzymatically treated fibers and vessels. We can observe that vessels the total vessel porosity is 6.1% higher than that of fibers, vessels having a lower microporosity (-15.6%) and a higher content of mesopores (+16.5%). In terms of porosity structure, fibers decrease total porosity with xylanase (-6.3%) and increase marginally (+0.4%) with the cocktail; fibers microporosity decrease significantly with both enzymes (-20.3% with xylanase, -33.8% with the cocktail); while mesopores have a small increase with xylanase (+0.4%) and a greater increase with the cocktail (+15.6%). Vessels by its side increase total porosity both with xylanase (+11.7%) and with the enzymatic cocktail (+19.5%) with the cocktail; vessels microporosity decrease with both enzymes (-7.5% with xylanase, -34.0% with the cocktail); mesopores volume also increase with xylanase (+16.9%) and with the enzymatic cocktail (+36.6%). Both enzymes seem to have a more significant effect on vessels than on fibers in terms of porosity structure, more pronounced in the case of the enzymatic cocktail comparatively to xylanase. Fibers have no significant alteration of its total porosity, even though microporosity reduces significantly, especially with the cocktail. This may be explained by further fibers collapse. Vessels, on the other hand, have significant total porosity increase at the expenses of micropores decrease and mesopores increase, situation more visible with the enzymatic cocktail in comparison to xylanase.

Table 1. Thermoporosimetry (fibers and vessels, treated and untreated) [7].

Samples[(a)]	V_{total} (mL/g)	V_{micro} (mL/g)	V_{meso} (mL/g)
Fibers	0.768	0.237	0.514
Vessels	0.815	0.200	0.599
Fibers (Xylanase)	0.720	0.189	0.516
Vessels (Xylanase)	0.910	0.185	0.700
Fibers (Cocktail)	0.771	0.157	0.594
Vessels (Cocktail)	0.974	0.132	0.818

Table 2 compiles the values for the bulk hemicellulose content (percentage of xylose, mannose, and galactose, referred as XMG), XPS surface C_1 (C-C) content and O/C ratio, and the pulp suspension Zeta potential (mV) as measured in Mütek SZP-06 System Zeta Potential at a 0.2 % consistency. XMG content in vessels is 35% higher than in fibers, thus having a higher hemicellulose percentage. This result agrees with previous ones reported by other authors [7]. Enzyme activity, particularly of the cocktail, reduced vessels XMG content, approaching its values to those of fibers. Concerning the relative amount of carbon in the different forms measured by XPS, the C_1 peak (C-C or C-H) is due mainly to lignin and extractives. As can be seen in Table 2, vessels have shown to have higher C_1 content (+21.4%). Thus, vessels must have a higher surface content of extractives and/or lignin. Higher XPS surface coverage of extractives and lignin have been obtained elsewhere in previous works [7]. A higher surface O/C ratio was observed in fibers comparatively to vessels, confirming previous studies [11-13]. This difference may arise from lignin deposition as from extractives deposition. Xylanase reduces this ratio for both fibers and vessels, while the enzymatic cocktail provokes distinct effects on fibers (3% increase) and vessels (8% decrease). Zeta potential results agree with the previous results showing that fibers are more anionic than vessels.

The results shown in Table 3 clearly indicate that vessels are more hydrophobic than fibers [7, 5]. Water-contact angles and surface energy studies clearly showed vessels to be more hydrophobic than fibers. These trends are supported by the results obtained in the measurements of the fibers and vessels surface free energies. The enzymatic treatments made vessels more hydrophilic, while the dispersive/polar ratio showed a tendency of treated vessels to approach the value of fibers.

Table 2. Sugar XMG, XPS and Zeta potential (fibers and vessels, treated and untreated) [7].

Samples	XMG[(a)] (%)	C_1 (C–C) (%)	O/C ratio	Zeta potential 0(mV)
Fibers	18.8	17.5	0.63	-11.8
Vessels	25.4	21.3	0.61	-4.5
Fibers (Xylanase)	18.8	18.0	0.62	-16.6
Vessels (Xylanase)	23.9	21.0	0.60	-8.7
Fibers (Cocktail)	15.3	15.0	0.65	-3.7
Vessels (Cocktail)	17.7	22.7	0.70	-3.6

(a) XMG: xylose, mannose, and galactose.

Table 3. Surface properties (fibers and vessels, treated and untreated) [5].

Samples	Contact angle (º)	Surface Energy (mN/m)	Dispersive-polar ratio
Fibers	54.1	45.8	7.2
Vessels	63.7	41.7	18.3
Vessels (Xylanase)	62.1	45.9	6.0
Vessels (Cocktail)	58.4	48.4	11.3

Table 4. Picking count values for beaten McNett pulps with vessels addition, with and without enzymatic treatment [5].

Sample	ºSR	Final Printing Speed (m/s)	Pressure (N/m)	Picking Count (picks/dm^2)	Picking Reduction (%)
McNett pulp	14	2	125	109±5	Control *1*
McNett pulp → PFI beating	28	2	125	9±1	91
(McNett pulp → PFI beating) + Vessels	28	2	125	161±8	Control *2*
(McNett pulp → PFI beating) + Xylanase treated vessels (100 g/ton)	1.7	2	125	39±2	76
(McNett pulp → PFI beating) + Enzymatic cocktail treated vessels (10 kg/ton)	1.5	2	125	10±1	94

Beating has an important effect on vessel picking as the results from Table 4 show for the McNett pulp, reducing picking count over 90 % (from 109 picks/dm^2 to 9 picks/dm^2) after a PFI beating of 2000 revolutions. Pulp sheets apparent density [5] was seen to increase 53 % with PFI beating of the McNett

pulp, indicating a significative increase in inter-fiber bonding due to internal and external fibrillation, which was confirmed by the internal cohesion and tensile measurements [5]. Vessel elements were also subject to breakage and dimensions reduction, as observed by microscopy, minimizing the effect of vessel picking in the printing process (as seen in Figure 1). The vessel elements smaller dimension and aspect ratios promotes its retention in the structure, at the same time reducing the printing flaw area. The relative role of these two mechanisms, paper structure (including surface structure), and vessel morphological characteristics, requires further investigation.

To study the isolated effect of enzymatic treatments, "concentrated vessel" obtained from the vessel rich pulp was added to the "McNett pulp" previously beaten to 28ºSR, according to the delineated experimental design [5]. The same amount of "concentrated vessel" without and with enzymatic treatment was added to the pulp. The final vessel content of the pulp was about 3-5 %.

As observable in Table 4, it is evident the effect of vessel addition on the McNett pulp; the picking count increase from 9 picks/dm^2 to 161 picks/dm^2. Although the samples have different vessel contents, these results clearly indicate that vessels without any kind of treatment (mechanical or enzymatic) are extremely susceptible to picking. However, when the same amount of vessel pre-treated with xylanase or the enzymatic cocktail was added to the same beaten pulp, the vessel picking was substantially reduced. The picking count decreased from 161 picks/dm^2 to 39 picks/dm^2 and 10 picks/dm^2, respectively with the xylanase (100 g/ton) and the enzymatic cocktail (10 kg/ton) treatments. As the apparent density of the pulp sheets remained practically unchanged [5], all the effect can be attributed to the action of the enzymes on vessels, highlighting the positive role of the enzymatic treatment on the vessel adhesion/cohesion to the other stock elements (mainly fibers). Interestingly, the internal cohesion of the pulp structure decreases significantly (from 652 J/m2, Table 2, to around 520 J/m2 [5]) with vessels addition, either enzymatically treated or not, despite the apparent density remaining practically unchanged, suggesting that the insertion of the vessels, with low potential of adhesion with the fibers, as the main responsible for this loss of internal cohesion. The other mechanical properties also decrease accordingly [5]. Despite the internal cohesion and the other mechanical properties remaining low, vessel picking decreased between 76.1 % and 94 % with the enzymatic treatments (Table 4), suggesting that the conditions of the vessels are more important that the conditions of the fibrous structure.

Summing up, enzymes have shown to cause different effects on porosity, bulk and surface composition of fibers and vessels, affecting vessel adhesion and hydrophobicity. This differential action may be explained by differential accessibility of enzymes to fibers and vessels pore structure.

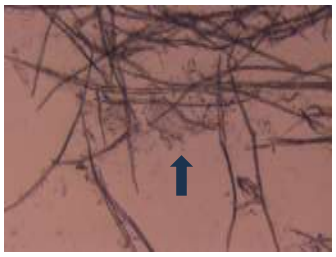

Figure 1. Vessels subject to ball mill beating (see arrow).

CONCLUSIONS

Vessels revealed to be more porous than fibers, with a higher content of mesopores and a lower content of micropores. Both enzymes seem to have a more significant effect on vessels than on fibers in terms of porosity structure, more pronounced in the case of the enzymatic cocktail comparatively to xylanase.

Vessels have shown to have higher hemicellulose content. Enzyme activity, particularly of the cocktail, reduced vessels XMG content, approaching its values to those of fibers. As revealed by ESCA/XPS analysis, vessels surface has lower O/C ratio and higher C_1 carbon, which indicates higher surface coverage of extractives and/or lignin. Xylanase reduces this ratio for both fibers and vessels, hinting extractives surface content reduction, while the enzymatic cocktail provokes distinct effects on fibers and vessels.

Vessel picking counts reduced after enzymatic treatments, hinting an increase of vessel-fiber bond strength after treatment with xylanase, and further increase for the enzymatic cocktail (cellulases and laccase) treated samples.

Fiber sheet samples had lower water contact angle than the vessel rich sheets, supporting the hypothesis that vessels are more hydrophobic than fibers. Both enzymes reduced vessels hydrophobicity, creating the necessary conditions to minimize the ink refusal phenomena during the printing process.

It is proposed that differences in vessel and fiber porosity structures lead to differential enzymatic attacks, eventually causing vessel passivation. The results showed that enzymatic treatments affect pulp vessel elements, making them more susceptible to breakage during beating, affecting their bonding to the paper structure and decreasing their hydrophobicity, revealing the potential of combining mechanical and enzyme activities.

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