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Oxalic acid: a microbial metabolite of interest for the pulping industry [☆]

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Abstract

Oxalate is a common metabolite produced by almost all plant-pathogenic fungi. The degradation of cell wall from poplar chips and poplar sawdust by oxalate is reviewed here. Oxalate treatments decrease slightly the amount of sugars constituting hemicelluloses, but only in fibres and not in sawdust or wood chips. The examination of the cell wall ultrastructure of wood chips by transmission electron microscopy (TEM) after polysaccharide staining showed a characteristic fading of the staining of the S1/S2 and S2/S3 transition areas, supporting the idea that reactivity and organization of polysaccharides had changed after the oxalate treatments. Finally, all these changes enhanced the ability of the wood chips to be defibrated by a thermomechanical (TMP) process, as well as the further refining of the pulps. Looking at the fiber surface, it became apparent that fracture areas during the TMP pulping had moved toward the S2 layer, explaining why defibrating and refining occurred more easily, with less energy input in the process. ***To cite this article: V. Meyer-Pinson et al., C. R. Biologies 327 (2004).***
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Résumé

L'acide oxalique : un métabolite microbien d'intérêt pour l'industrie papetière. L'acide oxalique est un métabolite produit par la plupart des microorganismes pathogènes des plantes. Son effet déstructurant sur les parois de bois de peuplier est résumé dans cet article. Les traitements à l'oxalate de sodium ont provoqué une légère baisse des teneurs en sucres constitutifs des hémicelluloses, mais uniquement sur les fibres ; la cellulose ne semble cependant pas altérée. Au niveau ultrastructural, une modification de l'aspect des zones de transition entre les sous-couches S1/S2 et S2/S3 des parois a été mise en évidence

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[☆] This article is dedicated to Prof. Bernard Monties on the occasion of his retirement.

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par microscopie électronique à transmission après contraste des polysaccharides. Ces modifications affectent directement la manière dont les fibres se séparent dans un procédé thermomécanique papetier. On observe ainsi un déplacement de la zone de fracture vers l'intérieur de la sous-couche S2 lors du défibrage du copeau, avec une réduction importante de l'énergie totale requise pour la séparation des fibres et leur raffinage ultérieur. Les mécanismes exacts restent maintenant à élucider, notamment le lien qui existe entre la biochimie des polymères pariétaux modifiés et les propriétés physico-chimiques et mécaniques qui en découlent. ***Pour citer cet article : V. Meyer-Pinson et al., C. R. Biologies 327 (2004).***

1. Introduction

The pulp and paper industry is faced to a global environmental challenge, which implies the reduction of the energy consumption needed during the processes and the reduction of the total rejects in the environment.

Thus, as much as 45 to 65% of the total energy needed in a mill is consumed during the pulping and refining steps [1]. This may explain the active research currently underway on the basics of energy savings mechanisms and on process development, ranging from machinery to biotechnology [1,2].

We recently demonstrated that the solubilisation of MnO_2 by oxalate at pH 2.5 could form Mn-chelates able to oxidize lignin within the cell wall of wheat straw, poplar and spruce sawdust [3–5]. The biochemical analysis performed on plant cell wall revealed two separate effects of the oxalate and of the Mn-oxalate chelates: the first was mainly targeted toward polysaccharides, whereas the second was mainly targeted toward lignins, mimicking to some extend the ligninolytic Mn peroxidase enzyme [6,7].

Thus, we decided to test if such biochemical and abiotic Mn chelates could modify the cell wall structure and enhance the fibre separation efficiency during a thermomechanical (TMP) high-yield pulping process [8].

For this purpose, wood chips were treated by either MnO_2/oxalate or oxalate alone. The energy needed to produce the pulps was determined, as well as their technical properties [9]. The impacts on the cell-wall ultrastructure and on the polymer structure were further investigated, in order to understand the underlying mechanisms [10].

This paper aims at describing a first picture of the impact of oxalate salts at acidic pH on the conversion process of poplar wood chips into fibres.
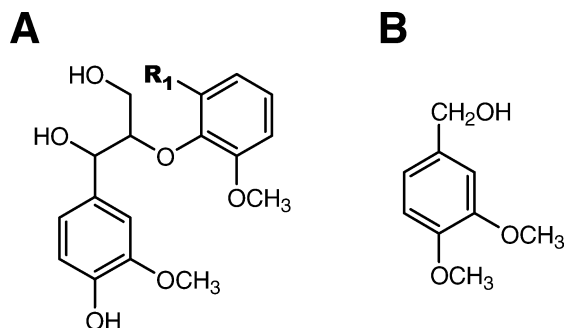

Fig. 1. Structures of models described in the text: (**A**) dimers representing the main β-O-4 linkage present in lignins; G = guaiacyl; S = syringyl; G-G type: R_1 = H; G-S type: R_1 = OCH_3 [11]; (**B**) 3,4-dimethoxybenzyl alcohol (veratryl alcohol).

2. Experimental

2.1. Plant material and substrates

Poplar wood was used as extractive free sawdust (*Populus trichocarpa,* cv Fritzi Pauley) or industrial chips, from the STORA-ENSO plant (Corbéhem, France) [3,10]. Lignin model compounds, of the G-G (Guaiacyl) and G-S (Guaiacyl-Syringyl) types, were synthesized according to the previously published methods [11] (Fig. 1). Other substrates (veratryl alcohol, birch xylans) were commercial products of analytical grade (Sigma Aldrich; Fluka).

2.2. Chemicals

Sodium–oxalate and oxalic acid were purchased from Sigma Aldrich (France). Other reagents and solvents used were of analytical grade.

2.3. Treatment of wood by oxalate

The poplar wood chips (500 g) were incubated during 6 h at 25 °C in 5 l of a 200-mM Na-oxalate buffer

(pH 2.5); the poplar sawdust (50 mg) was incubated during 20 h at 25 °C in 10 ml of a 100-mM Na-oxalate buffer (pH 2.5). The control experiments consisted in the incubation of the samples in water, during 6 or 20 h, at 25 °C.

At the end of the reaction period, the samples were recovered by vacuum filtration and washed with hot water (tap water for chips, deionised for sawdust) to remove oxalate salts. The samples were then freeze-dried before chemical analysis. For microscopic analysis, the samples were prepared as previously described [4,12].

2.4. Treatment of model compounds by oxalate

The lignin model compounds of the G-G and G-S type (30 mg) were incubated in 5 ml of a 200-mM Na-oxalate buffer (pH 2.5) during 6 h at 25 °C (Fig. 1A). Veratryl alcohol (∼3 g) was incubated in 50 ml of 300-mM oxalic acid (pH ∼ 1.0) during 4 h at 21 °C (Fig. 1B). Birch xylans (3 g) were incubated overnight in 40 ml of 200-mM Na-oxalate (pH 2.5) at 25 °C. All control experiments consisted in incubations of the substrate in deionised water only.

2.5. Chemical analysis

2.5.1. Lignin content and characterization

The lignin content in the sample was determined according to a procedure similar to that described by Effland [13] or by the TAPPI standard method (#T-222; http://www.tappi.org). The lignin fraction is accounted for as the acid insoluble fraction depleted from its ashes.

The content of monomer structures released by the thioacidolysis method was determined according to the published procedures [14]. The yields of the guaiacyl (G) and syringyl (S) monomers reflect the amount of such units only involved in α or β-O-4 bonds.

2.5.2. Polysaccharide composition

Polysaccharides in samples were hydrolysed by H_2SO_4 according to [15]. The monosaccharides released by the treatments were analysed by high-performance anion-exchange chromatography (HP-AEC) with pulsed amperometry detection, as described previously [16]. Pentosan content in pulps

was determined according to the standard NFT 12-008 method (http://www.boutique.afnor.fr/).

2.5.3. Characterization of reaction products issued from lignin model compounds and veratryl alcohol

Reaction products issued from parent lignin model compounds were extracted by ethyl acetate or dichloromethane, or directly recovered as a hydrophobic resin coming out from the water phase of the reaction medium. The products were analysed by size-exclusion chromatography (HP-SEC) and/or reverse phase liquid chromatography (HPLC, C18 phase, CH_3CN/H_2O eluents), as previously described [10, 16]. Preparative HPLC was used to fractionate and purify selected dimeric constituents, which were after characterized by ^1H and ^{13}C NMR, GC-MS and elemental composition (unpublished; communicated at the 8th ICBPPI, Helsinki, Finland, 2001; see also [17]).

2.5.4. Viscosimetric measurement of xylan solutions

Viscosimetry of xylans solutions was determined by capillary flow-rate measurement according to the ISO 5351:2004 protocol (http://www.iso.ch/iso/fr). The calibration of the system was performed by determining the viscosities (*i*) of the oxalate buffer alone and (*ii*) of a reference solution of xylan in water.

2.6. Microscopy

Low-field emission scanning electron microscopy (SEM) at medium magnification level was used to analyse the surface aspects of the freeze-dried samples. Transmission electron microscopy (TEM) was performed on ultrathin sections of dehydrated samples embedded in LR-White resin (London Resin Company). The tissues were stained for polysaccharides with the periodic acid-silver reagent (PATAg) of Thiery adapted by Ruel [18].

2.7. Thermomechanical pulping of poplar

Industrial poplar chips from the STORA-ENSO plant were used. The chips underwent first a mechanical pre-treatment in a Modular Screw Device (MSD) 6″ pressafiner (plug screw feeder) to reduce the heterogeneity among chips size and to enhance the absorption capacity of the oxalate liquor [8]. The chips were

then defibrated and refined at atmospheric pressure in a 12″ single disk Sprout Waldron refiner equipped with C2976 disks. The electric power needed in both stages of the TMP process was measured by means of a wattmeter.

2.8. Pulp quality evaluation

Fibre morphological characteristics were determined by analysis with PQM 1000 (Metso, Finland). The physical and optical properties of pulps were evaluated on paper handsheets according to ISO standards (http://www.iso.ch/iso/fr).

3. Results and discussion

This paper aims at combining information obtained at different scale levels of the cell wall on the impacts of oxalate buffer on the conversion of wood material into fibres.

3.1. Modification of cell wall ultrastructure by oxalate

An intermediate level of observation of the cell wall complexity is its ultrastructure. In TEM, PATAg staining for polysaccharides in poplar wood chips showed that the contrasted areas delimiting the different layers S1, S2 and S3 in normal wood appeared less marked in oxalate treated samples (Fig. 2A and B); the limit between S2 and S3 also sometimes completely disappeared (Fig. 2C and D). Such areas are junctions between layers with different organizations (i.e., cellulose microfibrils orientations, relative abundance of hemicelluloses and lignins). The fading of the staining by PATAg suggests that oxalate may change the reactivity of polysaccharides toward PATAg within the S2 layer. One can suggest that extraction or alteration of hemicellulose-rich compounds in the transition areas S1/S2 are at the origin of this apparent structural homogenisation of the S1/S2 layers. The biochemical analysis supports the hypothesis that polysaccharides are one of the targets of oxalate (see below).

At the level of the fibres produced from oxalate-treated chips by the TMP process (Ox-TMP), an exfoliation of the S1 layer is observed in SEM, with the

unmasking of the S2 layer, exposing its characteristic fibrillar and oriented organization, compared to the reference fibre produced from chips treated by water only (Fig. 3A and B). The observation of pulps refined at 100 ml CSF (Canadian Standard Freeness) shows an increased fibrillation of the S2 layers (Fig. 3C and D), accompanied with an internal fibrillation and delamination [9] (inset in Fig. 3D), not detected in the reference samples.

Thus, all these observations indicate that oxalate salts at pH 2.5 are modifying the cell-wall architecture. Among others, possible reactive sites would be the junction areas between the different layers composing the cell wall in poplar wood. Such modifications may affect either the mode of fibre separation from chips during an industrial thermomechanical (TMP) process, as shown by the different surface aspects of the obtained fibres, as well as the behaviour of the fibres during beating (internal delamination within the S2 layer and secondary fibrillation enhancement).

3.2. Chemical modifications of the cell-wall polymers by oxalate

Despite visual changes in chips' or fibres' ultrastructure related to the biochemical treatments, no lignin and polysaccharide removal could be quantified in wood sawdust nor in solid wood samples that have been treated by oxalate [6,10] (Table 1, data not shown, Gaudard and Kurek, unpublished); the only significant impact was a loss of xylose, arabinose and galactose, but on fibres issued from oxalate-treated chips (Table 1; Gaudard and Kurek, unpublished). Concomitantly, a slight increase (∼10%) in syringyl-type lignin structures released by thioacidolysis was observed [9,10]. Thus, it seems that the fibre separation removed and/or extracted some components from the chips, which remained otherwise in place in the non-divided material. This is consistent with the fact that fibres produced from oxalate treated chips exhibit different surface morphology from that of control fibres, indicating a distinct separation pattern during the process (Fig. 2). One can suggest that the removed material is composed of hemicelluloses, maybe originating from fines of the S1 layer, and otherwise resistant to the thermal and/or mechanical treatments (Fig. 2). The kind of chemical modifications (degradation, depolymerisation, extraction) associated to this slight but
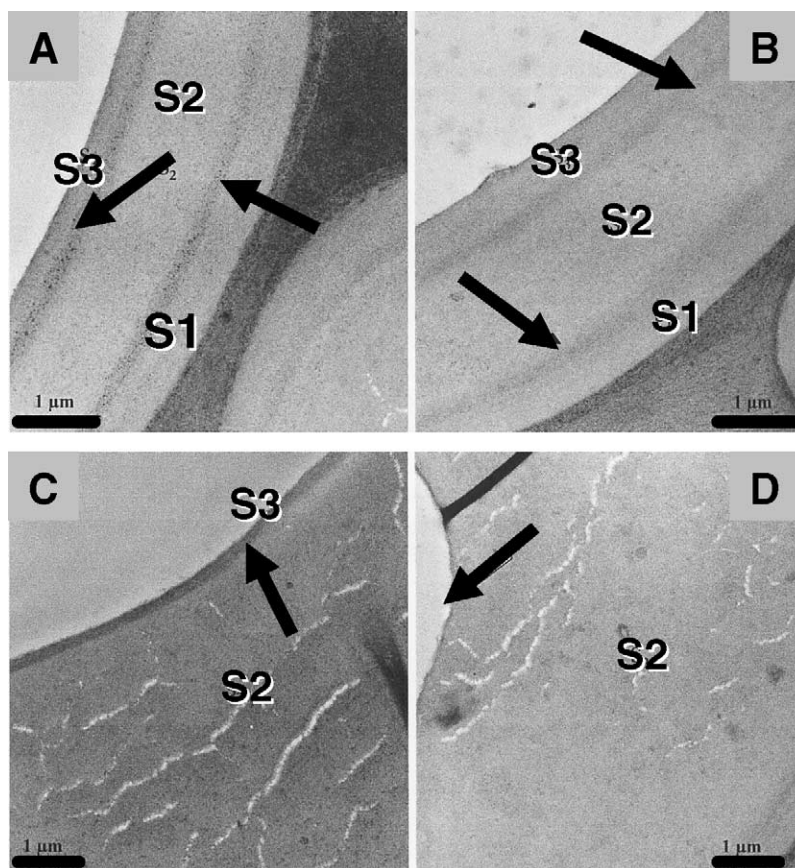
Fig. 2. Impact of oxalate treatment on poplar wood chips (TEM observations, PATAg): (**A**) and (**C**) chips treated by water; (**B**) and (**D**) chips treated by oxalate, as described in experimental section; note that no MSD destructuration was applied on the chips before impregnation (see experimental). The fading of PATAg staining is visible at the transition areas between S1 and S2 and S2 and S3 (**A** and **B**; arrows). The disappearance of the S3 layer was also observed in some cases (**C** and **D**; arrow). Scale bar: 1 μm.

Table 1
Chemical composition of poplar wood chips, sawdust and fibres

	Poplar sawdust	Fibres originating from industrial poplar wood chips	
	Control[a] (mg g^{-1})	Control[a] (mg g^{-1})	Treated[b] (mg g^{-1})
Lignin[c]	223.8 ± 4	245	246 (NS)[d,e]
Glucose	471.2 ± 23	487	476 (NS)
Mannose	19.1 ± 0.8	26	17.5 (S)
Xylose	145.4 ± 7	157	135 (S)
Galactose	42 ± 0.6	51	39 (S)
Pentosan[f] (Xyl + Ara)	Nd	171	143 (S)

Nd: not determined.

[a] Sawdust or chips incubated in water;

[b] from chips treated by oxalate pH 2.5;

[c] acid insoluble lignins; see experimental section for the methods used;

[d] NS: difference between treated and control samples not significant at $p < 0.05$; S: difference significant at $p < 0.05$;

[e] in this case, a significant ~10% increase of the release yields of S structure by thioacidolysis was measured [10];
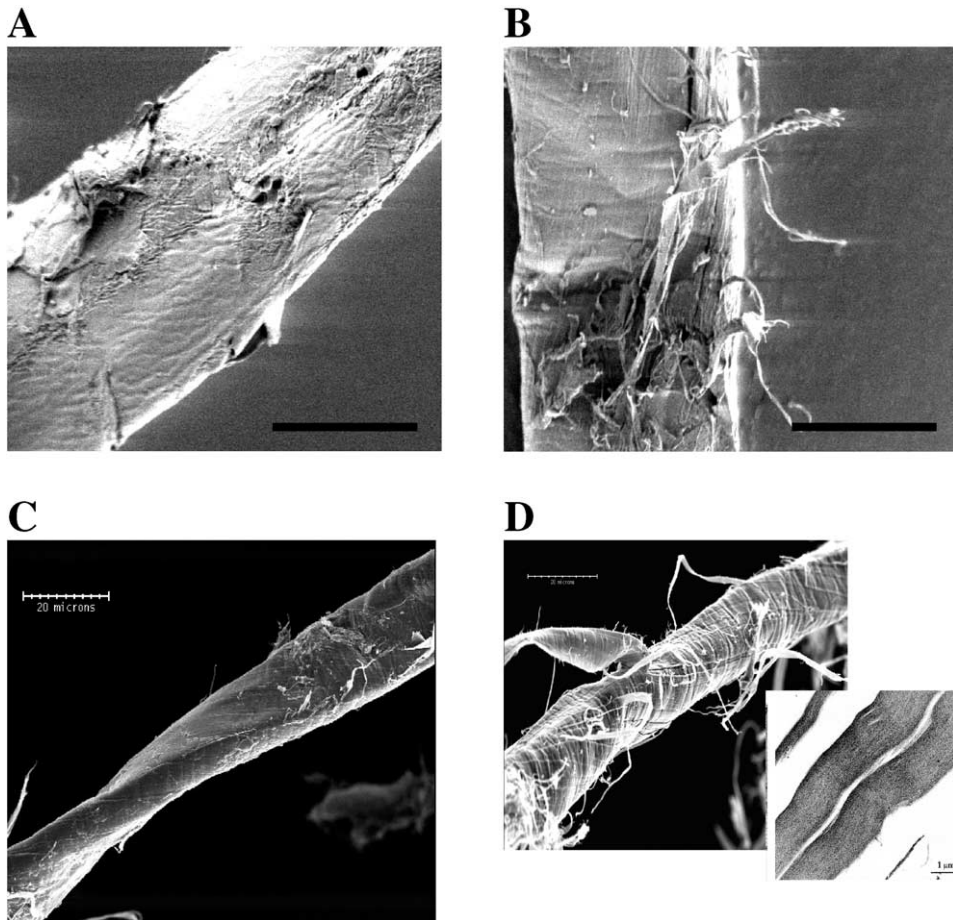
[f] xylose plus arabinose.

Fig. 3. SEM observation of poplar fibres obtained by thermomechanical pulping after water (**A** and **C**) or oxalate (**B** and **D**) impregnation. (**A**) and (**B**): Fibres obtained after defibration; the surface of the water treated sample; (**A**) exhibits a typical smooth surface, indicating that rupture in the chips occurred between compound middle lamellae and the S1 layer; (**B**) the apparent ribbon-like structures removed from the surface of oxalate treated sample indicated that the rupture during TMP process was mainly between S1 and S2 or within the S2 layer. (**C**) and (**D**): Fibres refined at 100 ml CSF (Tappi-T-227 method); fibrillation is much more important in the oxalate-treated sample (**D**) than in the reference water-treated one (**C**). Inset (**D**): TEM picture showing internal fibrillation and delamination of the fibre (PATAg staining). All scale bars: 20 μm.

significant loss of polysaccharides during TMP oxalate process remains to be determined.

3.3. Modelling the action of oxalate on isolated cell wall components

The combination of microscopy and biochemical analysis suggests that non-cellulosic polysaccharides are one of the targets of oxalate in cell wall. To test further this hypothesis, we incubated solubilized birch xylans with oxalate salts (overnight incubation in a Na-oxalate buffer, pH 2.5). This leads to a 15–20% decrease of the viscosity [10]. Thus, xylan depolymerisation occurred and such effect could also take place within the cell wall, resulting in loosening of the polysaccharidic network. Considering that oxalic acid is a strong metal chelatant, binding of calcium from pectins may also loosen the cell-wall structure at the middle lamella–primary wall level accordingly [19, 20].

Another action of oxalic acid and/or Na-oxalate was also detected on lignin structures. Incubation of
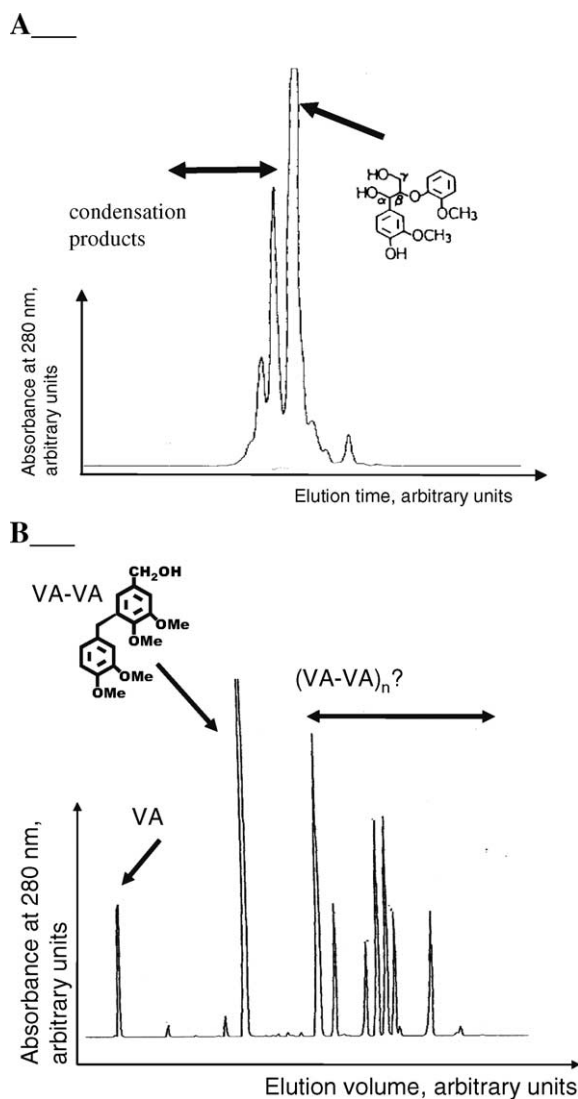
A

condensation products

Absorbance at 280 nm, arbitrary units

Elution time, arbitrary units

B

VA-VA

(VA-VA)$_n$?

VA

Absorbance at 280 nm, arbitrary units

Elution volume, arbitrary units

Fig. 4. Condensation of (**A**) lignin dimers (G-G) by Na-oxalate at pH = 2.5 and of (**B**) veratryl alcohol (VA) by oxalic acid at pH < 1.5 (refer to Fig. 1 for structures). **A**: HP-SEC profile of the lignin dimer after reaction (PL-gel 100A, Polymer Labs, with THF as the eluent); **B**: HPLC profile of the products formed from VA and structure of the dimer VA-VA identified (C18 column, CH$_3$CN/H$_2$O eluent; unpublished data; see also [17,21]); (VA-VA)$_n$: unidentified oligomeric structures.

lignin model compounds of the G-G and G-S types in conditions close from that used for chips' treatments yielded a slight, but significant polymerisation of the dimers into oligomeric structures [10] (Fig. 4A). The reactivity of the non-phenolic monomer veratryl
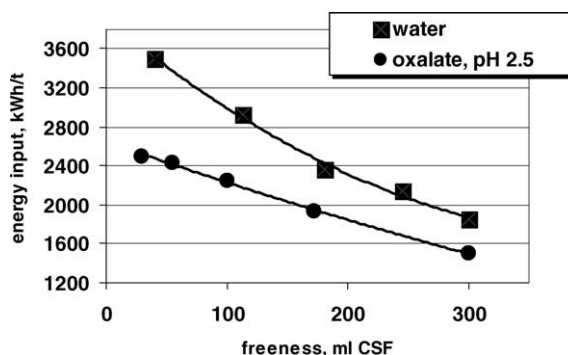

Fig. 5. Evolution of the energy consumption during the refining of poplar pulp (adapted from [9]); CSF: Canadian Standard Freeness.

alcohol (VA; Fig. 1) was also tested. During its incubation at a more acidic pH (oxalic acid 100 mM; pH < 1.5), a resin was rapidly formed, from which a characteristic dimeric structure was isolated and purified. The structure found was similar to that reported for acidic condensation of veratryl alcohol in H$_2$SO$_4$ at high temperature under reflux [17,21] (Fig. 4B; Kurek, Gaudard and Cathala, unpublished). Of course, the idea that lignins or some lignin oligomers are mobile within the intact cell wall and ready to condense is highly questionable [22,23]. However, if such polymerisation actually occurred in situ, some structuration of the cell wall could proceed together with xylans depolymerisation. Thus, a combination of local loosening and stiffening of the cell wall would well explain the modifications of its ultrastructure, as well as of the mechanisms by which fibres are separated during oxalate-TMP pulping (see below).

3.4. Defibration of wood chips and properties of the fibres

From a technical point of view, oxalate treatment of poplar wood chips resulted in a strong decrease of the total energy needed during defibration and refining processes (savings up to 1000 kWh t^{-1} at high freeness/low CSF index in the refining curve; Fig. 5).

The mechanical and optical properties of the resulting pulps were not altered nor really enhanced (Table 2). Statistical analysis reveal that differences are only significant at a low discriminatory level ($p < 0.65$; [10]); the different pulps can then be described as being similar. Nevertheless, the shive content in oxalate-treated screened pulps shows a tendency to de-

Table 2
Properties of pulps produced from polar chips impregnated by water and by oxalate

Treatment[a]	Water	Oxalate
Tensile index ($Nm\,g^{-1}$)	21.0	19.1 (S)[b]
Tear index ($mN\,m^2\,g^{-1}$)	2.6	2.7 (S)
Breaking length (km)	2.1	1.9 (S)
Brightness (% ISO)	61.6	62.7
Opacity (%)	96.7	97.0
k coefficient ($m^2\,kg^{-1}$)[c]	62.1	61.7
s coefficient ($m^2\,kg^{-1}$)[d]	2.9	3.2
Fibre length (mm)	0.8	0.8
Fibre width (μm)	32.6	33.8 (S)
Fibre curl (%)	16.2	17.8 (S)
Coarseness ($mg\,m^{-1}$)	0.11	0.10
Shive content (%)	2.1	1.9 (S)
Bulk ($cm^3\,g^{-1}$)	2.4	2.4

[a] All pulps were refined at 100 ml CSF;
[b] S: difference relative to water-treated sample significant, but at $p < 0.65$; the numbers of replicates was too low to separate significantly groups within the slight variations measured for the pulp properties;
[c] light specific absorption coefficient;
[d] light scattering coefficient.

crease, suggesting a higher defibration efficiency of the material during the primary and/or secondary refining steps (Table 2). As a consequence, a slightly higher tear index was obtained for oxalate-TMP pulps. At the opposite, the presence of oxalic acid during defibration and refining process may hinder fibre hydration, thereby diminishing fibre flexibility [24] (Table 2). Combined with the removal of the hemicelluloses and pentosans, a higher stiffness of the fibres may explain the slight decrease of the burst and tensile indexes (Table 2). These tendencies suggested that a potential exists to improve fibre properties. However, the process parameter(s) to adjust for this purpose remain(s) to be identified.

The main technical impact of the oxalate treatment of wood chips during TMP pulping is then the significant energy savings measured during the two critical defibration and refining steps. However, separate measurements indicated that the defibration energy remained essentially the same for oxalate- and water-treated chips, despite clear differences existing in the resulting fibre surfaces [10] (Fig. 3). The refining was however greatly facilitated as higher fibrillation extend at a same CSF point was reached with lower energy input. How to combine the energy savings with en-

hancement of the fibre properties is now the challenging technical question to solve.

4. Conclusions

The treatment of wood chips by oxalate leads to various modifications within the cell wall. The targets identified so far are the non-cellulosic polysaccharides (xylans). However, model compounds studies indicated that xylans depolymerisation might possibly occur simultaneously with some lignin condensations. All these reactions and modifications are leading to the energy savings obtained during the refining processes of the TMP pulps. However, the underlying mechanisms are not known for the moment. In particular, the in situ behaviour of the modified cell wall polymers after oxalate treatments under thermo-mechanical stress seems to be an important topic to address in the future [25,26].

Acknowledgements

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