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The beneficial effect of relatively small amounts of ZnO to concentrated NaOH solutions has been observed by, among others, Borgin et al. (1950) and Vehvilainen et al. (2008). Probably, sodium zincate ion, Zn(OH)$_4^{2-}$, is formed as a reaction product of ZnO with NaOH and its association to cellulose introduces a negative charge into the molecules (Kihlman et al. 2013).

To reduce the chemical consumption, enzymatic pre-treatments prior to the actual dissolving stage has been suggested. For example, pretreatments of pulps with xylanase have been investigated because the reduction of hemicellulose content improves the cellulose solubility (Guan et al. 1998; Zhou and Chen 1998; Köpcke et al. 2008). Treatment of both dissolving and kraft pulps with mono-component endoglucanases improves the reactivity, according to the Fock reactivity test (Fock 1959), which have been reported by Henriksson (Henriksson et al. 2005; Engström et al. 2006; Kvarnlöf et al. 2007a; Köpcke et al. 2008; Ibarra et al. 2010). Treatment with cellulose degrading enzymes, i.e. cellulases, has also been shown to increase the solubility of cellulose in strong alkali (Rahmkamo et al. 1996; Rahmkamo et al. 1998).

An activation of the cellulose to increase its reactivity is a necessary step for a successful dissolution (Turbak 1983). Therefore, a pretreatment of the pulp in a sequence of xylanase/NaOH extraction/endoglucanase, could be a way to remove the majority of the hemicelluloses from the pulp, to decrease the DP, and to make the cellulose more accessible to the subsequent dissolving in a following NaOH/ZnO stage. The objective of this study was to investigate the possibilities and limitations of this approach.

Material and methods

Dissolving pulp: A commercial fully bleached and dried prehydrolyzed kraft Southern pine dissolving pulp – supplied by Buckeye Tech. Inc., Memphis, TN, USA – was investigated. Intrinsic viscosity: 460 cm$^3$ g$^{-1}$ (ISO 5351:2004), cellulose content: 94.7%, total hemicellulose content: 4.5% (2.5% xylan and 2.0% glucomannan) (SCAN-CM 71:09), WRV-value: 0.69 (ISO 23714), fibre length: 2.7 mm, fibre width: 32 μm. NaOH (purity 99 % from VWR International, Radnor, PA, USA) and zink oxide (ZnO) (purity 99.9% from Sigma-Aldrich, Buchs, Switzerland).

Enzymes: Mono-component xylanase (Pulpzyme HC®, from a genetically modified Bacillus species) and mono-component endoglucanase (FiberCare R®, from a genetically modified Aspergillus species), both provided by Novozymes AS, Bagsvaerd, (Denmark), were used. The xylanase activity was determined by the manufacturer and expressed in Active Xylanase Units (AXU) as 1000 AXU g$^{-1}$. The endoglucanase activity was determined by the manufacturer and expressed in Endo Cellulase Units (ECU) as 4500 ECU g$^{-1}$.

Enzymatic and chemical treatments: Enzymatic treatments were performed at 3% pulp consistency and pH 7 (buffer: 11 mM NaH$_2$PO$_4$ and 9 mM Na$_2$HPO$_4$). The enzymes were added to the buffer and then to the pulp, to achieve a homogeneous distribution. The incubation was carried out in plastic bags in a water bath at 60°C for 2 h with the xylanase, and at 50°C for 1 h with the endoglucanase. The pulps were kneaded every 2 h, and then filtered through 70 μm sieve. The pulp was then re-wetted with 0.5% zink oxide and 1.5% NaOH and placed in plastic bags and kept in a water bath at 60°C for 2 h. The pulps were then re-filtered and the pH was adjusted at 8 with 50% NaOH.
For inactivation of the enzymes, the pulp was filtered and washed with hot water at 90°C (vacuum filtration on a RBU glass filter of VitraPOR® Borosilicate 3.3 Por. 3 with a pore size of 15 to 40 μm). Thereafter the pulps were mixed with hot water at 90°C and placed in a 90°C water bath for 30 min. Subsequently, each pulp sample was filtered and washed with 1000 mL of deionized water. The enzyme dosages: xylanase 0, 3.3, 16.7, and 50 AXU g⁻¹ and endoglucanase 0, 5, and 10 ECU g⁻¹ (b.o. dry weight pulp). Alkali extraction: at 4% pulp consistency with 7% NaOH solution at room temperature (r.t.) 1 h. Then the pulps were filtered and washed with deionized water to a neutral pH. After endoglucanase treatment the pulps were dried at 105°C for 3 h. As a control, pulps were treated under identical conditions without enzymes.

**Experimental design:** Umetrics software, Umeå, Sweden, MODDE 7 was applied for systematic data collection under different reaction conditions. The parameters were factorized at four dosage levels for xylanase (Xyla) and at three levels for endoglucanase (Endo). The factor settings were based on the criterion of D-optimality (Eriksson et al. 2000). The responses were fitted by the multiple linear regression (MLR) model, namely: the pulp yield, the DPₙ, the dissolution, the contents of cellulose, hemicelluloses, xylan, and glucomannan.

**DPₙ of enzymatic treated pulps** was determined according to ISO 5351:2004 standard. The method measures the viscosity of cupriethylenediamine (Cuen) solution of cellulose in a capillary viscometer. Then the DP was calculated via the intrinsic viscosity $[\eta]$ according to Evans and Wallis (1989):

$$DP_{90}^{0.90} = 1.65 \times [\eta] \quad \text{Eq. [1]}$$

**Carbohydrate analysis and R₁₈ determination** were done according to the SCAN-CM 71:09 standard and ISO 699-1982 standard, respectively.

**Preparation of cellulose solution in the solvent system NaOH/ZnO (8.5:0.8 by wt.):** Each solution had a total weight of 100 g at a pulp content of 3%. This corresponds to 11.5 mol NaOH mol⁻¹ AGU, and 0.5 mol ZnO mol⁻¹ AGU (anhydroglucose unit). The freshly prepared solvent systems were pre-cooled to -1°C, and the cellulose to around 0°C. When the enzymatically treated cellulose was added, the temperature of the mixture was maintained at approx. -1°C during the first couple of minutes, and then increased to +4°C. The solutions were stirred for 20 min at 500 rpm with a robust stirrer equipped with two counter-rotating propellers. The different steps of the pulp sample treatment are explained in a simple manner in Figure 1.

**Figure 1**

**Polarized optical microscopy:** Transparency, birefringence, and undissolved fragments were observed (at magnification of 200x) by an Olympus BX51 microscope (Hamburg, Germany) equipped with a ColorView 111 soft imaging system (Münster, Germany); the samples were placed on a microscopic glass slide and covered with a cover glass slide.

**Determination of the dissolved material:** The cellulose solutions were centrifuged at 5000 rpm, i.e. 3000 ± 50 g, for 1 h at -2°C to separate the undissolved cellulose residuals. The sediment was washed by vacuum filtration on a RBU glass filter of
VitraPOR® Borosilicate 3.3 (Por. 3 with a pore size of 15 to 40 μm), with 500 mL of deionized water. The washed material was dried at 105°C for 3 h.

\[
\text{Dissolved cellulose (\%) = 100 x (1 - C_i/C_f)}
\]

where \(C_i\) is the initial weight of the dry pulp, and \(C_f\) is the weight of the dry insoluble part after extraction with water and a following filtration stage.

NIR FT Raman spectroscopy on a Bruker RFS 100 spectrometer equipped with a liquid N₂-cooled Ge diode as the detector. An Nd:YAG-laser, operating at \(\lambda_0 = 1064\) nm and a maximum power of 1500 mW, served as the light source for Raman scattering (180° backscattering geometry; 350 mW laser power output). Frequency range: 3400 to 100 cm\(^{-1}\), resolution: 4 cm\(^{-1}\); 400 scans were averaged. The cellulosic samples were placed in small aluminum cups of the sampling accessory. These measurements were repeated twice for each sample under the same conditions and an average spectrum was calculated. A quantitative estimation of crystallinity was performed based on the Raman regression models developed by Schenzel and Fischer (2005).

Specific surface area from CP/MAS \(^1\)C-NMR spectroscopy: Selected samples were wetted with deionized water (40 - 60% water content) and packed uniformly in a zirconium oxide rotor. Instrument: Bruker Avance AQS 300 WB instrument operating at 7.04 T. All measurements were performed at 290 (±1) K (double air-bearing probe), MAS rate: 5 kHz. Data acquisition: CP pulse sequence by a 4.3-μs proton 90° pulse, 800-μs ramped (100-50%) falling contact pulse, a 2.5-s delay between repetitions. A T PPM15 pulse sequence was used for \(^1\)H decoupling. The Hartman–Hahn matching procedure is based on α-glycerine. The chemical shift is related to TMS, (CH\(_3\))\(_4\)Si. The data point of maximum intensity in α-glycerine carbonyl line was assigned to a chemical shift of 173.03 ppm. The software for spectral fitting was developed at STFI-Packforsk AB and it is based on a Levenberg-Marquardt algorithm (Larsson et al. 1997). The estimates of lateral fibril aggregate dimension (LFAD), that are obtained from the model developed by Chunilall et al. (2010) by NMR, served for estimation of the specific surface area of cellulose I samples in a liquid-swelled state. Within this model framework the side length of a fibril aggregate (α) is related to the specific surface area (σ):

\[
\sigma = 4/(\rho \times \alpha)
\]

where \(\rho\) is the cellulose density.

Results and discussion

As revealed in Table 1, the yield loss of the pulp samples during the combined xylanase, alkali and endoglucanase treatment was relatively low, always less than 4.2% and less than 3.2% if compared with the reference sample where no enzyme treatment had been used. The table also shows that in the best case of the pretreatments studied the solubility of the pulp increased to 81% in the NaOH/ZnO solution which can be compared with 29% when no pretreatment at all was used. When the pretreatment of the pulps was done with only an alkali extraction (E, with 7% NaOH), i.e. without any enzymatic treatment, this resulted in a pulp solubility of 34%. The pulps treated with
only endoglucanase showed a significantly higher solubility in the NaOH/ZnO solution compared to pulps treated with only xylanase which probably was a result of the much lower xylanase content v.s. the cellulose content in the staring pulp. It is interesting to note that all pulp samples, according to NIR FT Raman spectroscopy, still had cellulose I structure after the combined enzymatic and alkali treatments although the pretreatment improved the solubility considerably.

Table 1. Figure 2.

Figure 2 shows the optical images of NaOH/ZnO solutions of untreated original pulp (a) and the pulp treated with the sequence 16.7 AXU g\(^{-1}\)/E/10 ECU g\(^{-1}\) (b). The lot of undissolved fibres and fibres with ballooned and swollen parts in (a) disappeared in (b) and only a few transparent discs are visible. The more or less total absence of ballooning in (b) can be interpreted as a result of a digestion of the primary wall carried out by the enzymes. Moigne et al. (2010) interpreted their results in a similar way when they observed swelled fibres in 8% NaOH solution and when a subsequent enzymatic peeling led to cutting of the fibres into discs as a result of enzymatic peeling.

Our results showing that the solubility of the pretreated pulps increased considerably (Table 1), while the crystallinity and the specific surface area of the pulp did not change. This is in accordance with Wang et al. (2008), who observed something similar: i.e. the solubility of cotton linters in cold NaOH/urea solution increased from 30% to 65% after an endoglucanase treatment, but the crystallinity of the samples remained almost unchanged. Wollboldt et al. (2010) showed in a later study that the cellulose crystallinity did not affect the dissolving pulp reactivity as determined by the filterability and the particle spectrum of viscose dope. The specific surface area of cotton linters is reported to be 53 m\(^2\) g\(^{-1}\) (Chunilall et al. 2010).

Expectedly, a higher degree of dissolution and a lower DP\(_n\) seemed to correlate although not to a high extent. Thus, the solubility of the pulp in the NaOH/ZnO solution was in one experiment as high as 81% although the DP\(_n\) of the same sample was surprisingly intact i.e. 1074. One interpretation is that the degradation of the primary wall also has an effect on the solubility. Our results can be understood from the findings of Trygg and Fardim (2011) who suggested that the enhancement of the dissolution in NaOH-urea-water is due to a combination of a degradation of primary fiber wall layers and a reduction of the cellulose DP.

The combined treatment with xylanase, alkali and endoglucanase caused more degradation of the DP than an endoglucanase treatment alone and it has been reported that enzymes are active both in the outside and the inside of the fibres (Moigne et al. 2010). This is in agreement with Henriksson et al. (2005) who have observed that a decrease in the degree of polymerization is not the explanation behind the activating effect of the endoglucanase treatment. Endoglucanase are known to preferably degrade amorphous cellulose rather than highly crystalline cellulose (Rabinovich 2002). Since less crystalline cellulose seems to occur between, and on the surface of cellulose fibrils (Vietor 2002; Wickholm 2001), endoglucanase action might therefore lead to a swelling of the cell wall, resulting in increased exposure to solvents and reagents of the dissolving pulp cellulose (Henriksson et al. 2005). A mechanism of the activating effect of the mono-component cellulase has been suggested by Henriksson et al. (2005), and the results from our study support this mechanism, Figure 3.
An increasing charge of xylanase from 16.7 to 50 AXU g\(^{-1}\) did not further reduce the xylan or the glucomannan contents. Unexpectedly, the xylan content in the enzyme treated pulp even increased compared to the treatment at 16.7 AXU g\(^{-1}\). This unexpected result may be due to a disruption of the cellulosic matrix leading to an internal collapse of the fibers, if the enzyme charge is too high. Thus, a more dense fiber with reduced accessibility for the endoglucanase in the following stage will be the result. This hypothesis can also explain the higher DP\(\eta\). That a too high xylanase charge can lead to an increased xylan content has recently also been shown by Liu et al. (2013).

The pulps treated with 50 AXU g\(^{-1}\) had approximately 6% lower R\(18\) than the samples that had been treated with lower enzyme charges. This was unexpected but the interpretation of the R\(18\) data is challenging (Table 1) as the samples with R\(18\)-values of 100% still contained 2.6% and 3.0% hemicelluloses respectively. Accordingly, the R\(18\) method gives probably only a rough estimation of the cellulose content of pulps.

Correlation between degree of dissolution and DP\(\eta\)

Quadratic terms were obtained for the effects of both the xylanase and the endoglucanase treatment, implying the existence of a function with a maximum or minimum. The dependence of the amount of dissolved cellulose and the DP\(\eta\) on the xylanase and endoglucanase dosages was predicted by the models and is shown in Figure 4.

Figure 4

The impact of xylanase and endoglucanase on the degree of dissolution of cellulose in the NaOH/ZnO stage and on the DP\(\eta\) are shown on the figure. It can be seen that for both enzymes, there is charge ratio where the enzyme effect is at its maximum for both solubility and DP\(\eta\). There is a correlation between the DP\(\eta\) and the degree of dissolution, because both results are influenced by the enzyme action. It is possible that at higher dosage of enzymes the amount of amorphous cellulose is decreased, and if such cellulose acts as spacers in the fiber structure this leads to a denser fiber structure with lower accessibility for enzymes in the following stage. Kvarnlöf et al. (2007b 2007) speculated that an extended enzyme treatment would disrupt the cellulosic matrices, which could lead to an internal collapse of the fibre architecture after which they are inaccessible to chemicals.

Conclusions

A treatment with xylanase, followed by an alkaline extraction, and a final treatment with endoglucanase is effective to increase the solubility of a dissolving pulp in a NaOH/ZnO stage. An optimum degree of enzyme dosage leading to the best dissolution has been found. In the best case, the solubility of pulp fibres in a cold NaOH/ZnO stage increased from 29% to 81% while the DP\(\eta\) was surprisingly high i.e.1074.
References


Liu, W., Zhou, S., Qi, X. and Pu, J. (2013) Preparation of acetate-grade dissolving pulp from eucalyptus by processes including alkaline pretreatment and combined post-treatments with xylanase and alkali. Tappi (12) 6, 19-24


Figures and Table

**Figure 1** An algorithm that describes the different steps of the experiments.

**Southern pine prehydrolyzed kraft pulp** (fully bleached and dried)

**Treatment with xylanase**
(3.3; 16.7; 50 AXU g⁻¹ pulp)
at 3% consistency, pH 7, 60°C, 2 h

**Alkali extraction (E)**
with 7% NaOH at room temp., 1 h

**Treatment with endoglucanase**
(5 and 10 ECU g⁻¹ pulp)
at 3% consistency, pH 7, 50°C, 1 h

**Treated pulps** (dried)

**Dissolution in NaOH/ZnO (8.5; 0.8)**
under stirring for 20 min, 3% consistency

**Solution**

**Centrifugation**

**Sediment**

**Solvent**

**Filtration**

**Dissolved**

Determ. of yield; DPₙ, CH comp.; R₁₅, Raman and CP/MAS ¹³C NMR

Microscopic observation
Figure 2 Images from pulps dissolved in NaOH/ZnO (8.5% / 0.8%) where the pulp consistency was 3%. (a) No enzyme treatment was done prior to the NaOH/ZnO stage, (b) Prior to dissolution with NaOH/ZnO the pulp was treated with: Xylanase (16.7 AXU g⁻¹), 7% NaOH extraction and finally Endoglucanase (10 ECU g⁻¹)
Figure 3 a-c The activating effect of enzymes (adopted from Henriksson et al. 2005). a) The cellulose in the pulp fibres consists mainly of crystalline fibrils and paracrystalline segments between fibrils ( ). b) The endoglucanase ( ) cleave preferentially the paracrystalline moieties. c) Degradation leads to separation of the fibrils, i.e. to swelling that increases its reactivity. The DP is not reduced drastically.

Figure 4 Response contour plots of the fraction dissolved in NaOH/ZnO (8.5%/0.8%) and its DP as a function of the factors xylanase and endoglucanase.
Table 1. Various characteristics of the pulps as a function of the applied enzyme dosage of xylanase and endoglucanase.

<table>
<thead>
<tr>
<th>Dos. (g·pulp)</th>
<th>Characterisation of the treated pulps treated by Xy/E/Endogl</th>
<th>Properties of treated pulps after dissolution in NaOH/ZnO</th>
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* Specific surface area, ** in NaOH/ZnO