CLEAVAGE OF SOFTWOOD KRAFT PULP FIBRES BY HCL AND CELLULASES

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A new pulp fibre testing procedure called the HCl method was used to compare different spruce and pine fibres and mixtures of these fibres to calculate number of fibre cleavages in dislocations and other weak points. This method was compared with treatment of softwood kraft pulp fibres using different cellulase mixtures. The HCl method can distinguish between mill- and laboratory-made softwood kraft pulp fibres from the same wood batch. The sugar release is characterized by xylose and other hemicellulose sugars and little glucose. This is in contrast to cellulases, which despite strong fibre cleavage, did not distinguish between mill- and laboratory-made pulp fibres and released large amounts of glucose from the fibres. Hemicellulose degradation by HCl and deep penetration of the acid into the primary and secondary fibre cell walls at 80°C seems to be of major importance for the differentiation between mill and laboratory pulp fibres. Cellulases, in contrast, act mostly on the fibre surfaces, and deep penetration only takes place in amorphous regions of dislocations.

Keywords: Softwood kraft pulp; Dislocations; HCl, Cellulase; Endoglucanase; Hemicellulose; Fibre length; Polarized light, SEM

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INTRODUCTION

A dislocation, discussed in detail in metallurgy (Reed-Hill and Abbaschian 1992), is a structure containing slip lines and slip planes that are easily seen in cellulose fibres in polarised light microscopy (PLM). They are defined here as the result of a localised change or distortion of the crystalline cellulose microfibrils in either the S1 or both S1 and S2 secondary cell wall layers. Fibre deformations in the form of dislocations are present already in small amounts in growing trees due to wind action, increase in size during pulp cooking and bleaching, and if large enough can decrease paper strength (Nyholm et al. 2001). Small dislocations, sometimes called slip planes in the cellulose fibre literature, affect fibre flexibility and are not considered detrimental for paper-making. Large dislocations also give fibre flexibility, but due to the less ordered or more open amorphous cellulose structure in the dislocations, they can be the target for chemical, mechanical or enzymatic attack, for example by cellulases or acids such as HCl, H_2SO_4, or acid sulphite, giving fibre shortening and less paper strength, at least when measured as wet zero span (cf. Iribarne and Schroeder 1999).

Acid conditions were used already 70 years ago, and Dadswell and colleagues (Dadswell and Langlands 1938; Wardrop and Dadswell 1947) in a "broken fibre test" used a mixture of $KClO_3$ and HNO_2 to test "brittle heart" in Australian timbers. Wardrop and Dadswell (1947) also used boiling H_2SO_4 and calculated the ratio of broken fragments to unbroken fibres as a diagnostic test. Stone (1961), in an excellent paper, stated that latent damage (formed during tree growth) is developed by acid hydrolysis during acid pulping. Comprehensive investigations on wood and pulp fibres under acid conditions or during pulping with acid sulfite and acid bisulfite have also been done by Green and coworkers (Green 1962; Green and Yorston 1939) and by Hartler and coworkers (Frölander et al. 1969; Hartler 1963, 1969, 1995). In general, these investigations show that all acid conditions partly cleave fibres in dislocations (slip planes, nodes, minute compression failures, misaligned zones), and this leads to inferior strength.

During recent years not many papers concerning the importance of dislocations have been published. Wilkins (1986) in a review dealing with the nomenclature of cell wall deformations suggested that the historically accepted term slip plane should be used for the white bands seen in polarized light microscopy across the fibre width and also seen in scanning electron microscopy. Currently, most researchers use the name dislocation for the compressed cellulose fibril structure (Nyholm et al. 2001). The term deformation includes also curl, kinks, and crimps (Molin and Alfredsson 1990; Page et al. 1985).

Recently, Terziev et al. (2005) and Eder et al. (2008) studied the effects of dislocations in Norway spruce fibres. They used spruce wood blocks and applied tangential loading under dry or wet conditions to induce dislocations. Control and compressed wood blocks were chipped and paper produced. A significantly lower zero span tensile index was observed for both compressed woods, with the wet wood giving the lowest zero span value and the largest amounts of dislocations. Compressed fibres also gave a paper with lower tensile-tear index than paper made from control samples. Tensile strength of isolated earlywood and latewood single fibres from the same wood as above were lower for the compressed fibres as compared with non-compressed fibres when tested in a micro-mechanical testing device (Eder et al. 2008). These results strongly indicate that dislocations in wood chips and in pulp fibres may decrease pulp and paper mechanical properties measured as zero span and tensile-tear indexes. Recent results by Terziev et al. (2008) show that spruce fibres from irrigated and fertilised trees as well as from compression wood all had more dislocations, as compared with fibres from "normally" grown trees, and gave paper with reduced mechanical properties. Thus, dislocations make the cell wall more susceptible to enzymatic and chemical attack, allowing for the stronger penetration of cooking and bleaching chemicals during pulping that can lead to inferior pulp and paper.

Pulp fibres are not only degraded and cleaved in dislocations by hydrochloric acid, but also by cellulases (Gurnagul et al. 1992; Iribarne and Schroeder 1999; Nyholm et al. 2001; Ander and Daniel 2006). One important difference between the action of HCl and cellulase is that HCl can distinguish between spruce kraft pulps made in the laboratory or under mill conditions, even though the same batch of spruce wood chips are used in cooking (Ander et al. 2005; Ander and Daniel 2007). This is not done with cellulase. In the present paper, differences and similarities in the effects on softwood

kraft pulp fibres by HCl and cellulase treatments are described. As an aid in this evaluation, results using the HCl method (Ander and Daniel 2004a,b; 2006; 2007; Ander et al. 2005), that are related to the number of dislocations in pulp fibres are presented.

EXPERIMENTAL

Materials

Pulps

Wood chips for kraft cooking were from Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.).

Exp. I. Two unbleached kraft pulp samples for HCl and cellulase experiments were taken from a pulp fibre line from a Scandinavian kraft pulp mill. For pulp properties see Sjöström et al. (2005). The raw materials for both pulps were spruce, one prepared from final cutting (Mill F) and one from thinning wood (Mill T). From the same raw materials, two pulps were produced in the laboratory (Lab F and Lab T). They were produced in a circulation digester using similar chemical profiles and were cooked to about the same kappa number as the respective Mill pulps. All the above fibres were enriched in a laboratory hydrocyclon at M-real, Örnsköldsvik to obtain short- (SF) and long-fibre fractions (LF). Each fraction contained 10-20% of the other fraction. The use of the same wood chip batch and similar chemical profiles in cooking means that the Mill and Lab pulps can be used in Strength Delivery investigations (Berggren 2003; Sjöström et al. 2005). Strength Delivery (SD) is defined as the ratio between tear indexes of mill and laboratory pulps at tensile index 90, and has been used to evaluate and improve pulping conditions (MacLeod et al. 1995; Iribarne and Schroeder 1999; Tikka and Sundquist 2001).

Exp. II. Four Swedish unbleached softwood kraft pulps (Sjöström et al. 2007): Mill 3 and Lab. 3 with a spruce:pine ratio of 33:67 and 34:66 respectively, were produced in a continuous cook in the mill and in the laboratory. Mill 4 was made in batch cook and Lab 4 in basket cook. These two pulps had a spruce:pine ratio of 84:16.

Methods

HCl method

- All pulp fibres (never-dried with dry-weights 100-250 mg) were swelled for 10-15 minutes in 20 ml water during stirring with a 25 mm stirring bar in a 100 ml Erlenmeyer flask.
- Treatment with 40 ml 1 M HCl pH 0 at 80-82°C for 4h in a reciprocal water bath without using the stirring bar. Water baths: SALVIS and from November 2006 Julabo SW22 with 16 places.
- Completion of cleavage with 25 mm stirring bar at 150 rpm for 30 minutes during cooling to near room temperature. Incubation platform: Polymix PX-MS15.
- Fibre washing with 0.2 M phosphate buffer (PB) pH 7 on Munktell filters 1002, 1003 or glass filters G2 or G3. Before electron microscopy, the fibres were washed with water to remove buffer.

- Fibre lengths of fibres from HCl treatment in Exp. I were determined using the FibreMaster at Södra Cell, Mörrum, while fibres from Exp. II were length determined at the FibreMaster at Södra Cell, Värö.

Cellulase treatment

Exp I. Endoglucanase II (EG II; EC 3.2.1.4) and cellobiohydrolase I (CBH I; EC 3.2.1.91) from *Trichoderma reesei* were obtained from Jerry Ståhlberg, SLU, Uppsala. Purification was described in Ståhlberg et al. (1988; 1996). EG II, earlier called EG III, had OD 28 at 280 nm and was diluted 10x and mixed with CBH I (16,75 mg/l, diluted 10x) in equal parts. One ml of the enzyme mixture was added to 225 mg swelled enriched short fibre fraction (Final cutting of Lab F and Mill F, SF) or to 225 mg long fibre fraction (Final cutting Lab F and Mill F, LF) in 40 ml 0.1 M acetate buffer pH 5 in duplicates. Incubation was for 4½ h at 40°C followed by final cleavage for 60 min using a stirring bar. After treatment the fibres were washed with 0.1 M PB-buffer pH 7.5 and 0.5 ml PB-buffer was added. The FibreMaster at Södra Cell, Mörrum was used for fibre length determination.

Exp. II. Cellulase treatment of pulps Mill 3 and Lab 3 and Mill 4 and Lab 4: Fibre dry-weight 200 mg (duplicates) and temperature 50°C. Cellulases from Novozymes: Novozym 476, 2 x 10^6 ppm (monocomponent endoglucanase EG from *Aspergillus oryzae*) used at pH 7 in 0.1 M phosphate buffer. Novozym 342, 2 x 10^6 ppm (multicomponent endoglucanase + cellobiohydrolase CBH from *Humicola insolens*) used at pH 5 in 0.1 M acetate buffer. With Celluclast 1.5L (EG + CBH from *Trichoderma reesei*) two concentrations 0.75 x 10^5 (0.3 ml) and 1.5 x 10^5 ppm (0.6 ml) at pH 5 were used as shown in Table 3. Enzyme incubation time: 195 min + 45 min stirring during cooling. Microlitre (mg) original enzyme solution per kg pulp dry weight = ppm.

Enzymes were from Novozymes, Bagsvaerd, Denmark. The multicomponent cellulase preparations N342 and Celluclast 1.5L contained many EGs and more than one CBH. According to the manufacturer, the activity of Novozym 476 was 4500 ECU/g at pH 7.5; for Novozym 342 it was 90 ECU/g at pH 6; and for Celluclast 1.5L it was 700 EGU/g at pH 6 – all determined using CMC and viscosimetry (Product data sheet and Eugen Müller, Novozymes Deutschland GMBH, personal commun., January 2008).

FibreMaster: Helena Tufvesson/Siv Skoglund, Korsnäs AB. L_0-values were from the FibreMaster at Södra Cell, Värö.

Fibre length analyses and cleavage per fibre

Length-weighted fibre lengths and other fibre properties such as fibre width, fibre form, fines content, curl and coarseness were analysed using the FibreMaster instruments at Södra Cell AB, Mörrum and Värö, and at Korsnäs AB (see Exps. I & II). StoraEnso, Karlstad analyzed the spruce:pine relation; Cleavage per fibre = $(L_0 / L) - 1$ where L_0 is length weighted fibre length distribution in mm for control in water (or for untreated fibres), and L is length weighted fibre length distribution in mm for HCl-treated fibres.

Sugar analyses

Total reducing sugars (monosugars + oligosaccharides) in the fibre filtrates were determined with the BCA-method at 560 nm (Garcia et al. 1993). Arabinose, galactose,

glucose, mannose, xylose were determined directly in fibre filtrates. Acidic filtrates were adjusted to pH 6-7 with 0.2 M PB-buffer. No other pre-treatment was done before analyses at M-real/MoRe using ion chromatography and pulsed amperometric detection (Jacobs et al. 2003). Since β-glucosidase or trifluoracetic acid treatment was not used, this method does not give oligosaccharides. Some degradation of oligosaccharides to monosugars by the HCl treatment at pH 0 and 81°C may have occurred.

Light microscopy

For polarized light microscopy a Leica DMLB or DMLS coupled to an Image-Pro Plus image analysis program was used. Dislocations were most easily seen in latewood fibres.

Scanning electron microscopy (SEM)

Pulp fibres and fragments (free of buffer) following HCl or cellulase treatments were freeze-dried over night, mounted on double bonding tape on metal discs, and sputtered with gold for 3 min at 50 mA (sputtering device K550X from Emitech, UK). Thereafter the samples were observed using a XL30 ESEM from Philips. Pulp samples were from Mill 4. Ten micrographs were taken of each treatment.

RESULTS AND DISCUSSION

Initial Studies and Reproducibility of the HCl Method

In preliminary investigations using HCl at pH 1.8 or pH 1 at 80°C, almost no cleavage of spruce kraft pulp fibres was obtained (Ander 2002; Ander and Daniel 2004a,b). However, with 1 M HCl at pH 0, ca 3 cleavages per fibre were obtained, with cleavage increasing to 6 cleavages per fibre with 2 M HCl. Based on these results and on length-weighted fibre length distribution curves, 1 M HCl at 80°C was chosen for further studies (Ander and Daniel 2004a). Original fibres or those incubated in water at 80°C or 40°C had the same fibre lengths. In Ander et al. (2005) a time study for 2, 4, and 6 h was reported, supporting a suitable incubation time of 4h followed by 30 min stirring to

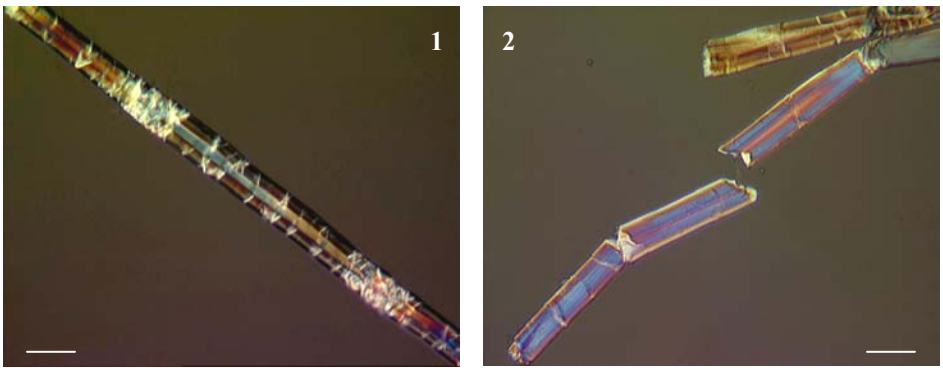


Fig. 1. Small and large dislocations in a spruce latewood fibre (Ander and Marklund 2003).
Fig. 2. Two spruce latewood fibres cleaved in dislocations by HCl (Ander and Daniel 2006). Bars: 30 μm.

give final cleavage in dislocations and other weak points. Most importantly, it was also shown that HC-induced cleavage was much stronger in Mill pulps compared with Lab pulps, demonstrating that the HCl method could provide an important tool in Strength Delivery investigations (see Materials) and also in other pulp studies. Currently the HCl method outlined here and published in Ander and Daniel (2006) is as follows:

Dislocations were most easily observed in latewood fibres in polarized light microscopy, and a typical example for a spruce latewood fibre is shown in Fig. 1. After HCl-induced cleavage in dislocations, the fibres may appear as shown in Fig. 2. The breakage points can be studied by SEM, which can give valuable information on the micro- and ultrastructure of dislocations, and the sites attacked by HCl. As reported earlier (Ander and Daniel 2006; Ander et al. 2007), one Lab and two Mill pulps were tested in triplicates at 82°C on four different days to determine the reproducibility of the method. Cleavage per fibre for Lab 1, and Mill 4 and 5 pulps were 0.95 ± 0.20, 3.35 ± 0.34, and 3.73 ± 0.39, respectively, at 95% confidence level. Thus the HCl method has good reproducibility. Lab fibres were cleaved less than Mill fibres confirming earlier results (Ander et al. 2005).

HCl treatment caused ca 2 % of the fibre amount to be released as mono-saccharides, mainly as xylose and mannose. However, no difference was found between sugar amounts released from Mill and Lab fibres. As shown above, Lab fibres gave about 1 cleavage per fibre, while the Mill fibres gave 3-4 cleavages. This means that sugar release from Mill fibres should theoretically be 3-4 times higher if dislocations and weak points are the only places of HCl attack. As this was not the case, sugars are mostly released from the fibre cell walls, and sugar analysis is not a viable method for calculating type or amount of sugars in the breakage points, or for comparing Mill and Lab. fibres (see also Tables 1-3).

Results with HCl and Cellulases (Exp. I)

Ander et al. (2005), reported that cellulase (EG I + CBH I) had rather small fibre cleaving activity and briefly mentioned that EG II + CBH I strongly cleaved spruce fibres but did not distinguish between Mill and Lab fibres. These unpublished results are now given in more detail for pulp fibres made from final cuttings and from thinnings of Norway spruce trees. Table 1 shows that a Mill long fibre fraction was cleaved by both HCl and the cellulase mixture EG II + CBH I. However, the cellulase mixture cleaved Mill and Lab pulp fibres similarly, while HCl-cleaved Mill fibres more than Lab fibres. Due to the length of only slightly more than one mm, the short SF fibres were cleaved less than the LF fibres that were almost 3 mm long.

Release of reducing sugars (BCA method) was the same if comparing Mill and Lab fibres for both HCl and cellulase. However, cellulase gave more sugars (4.7-4.9 weight %, based on added fibres; Table 1) as compared with HCl, which gave 3.4-3.8 %. Not surprisingly, the "weaker" short SF fibre fraction with more earlywood fibres gave slightly more sugars than the longer and stronger LF fibres. This distinction was not attributed to cellulase.

Table 1. Length Weighted Fibre Lengths (LWFL), Cleavage per Fibre and Release of Reducing Sugar by **HCl** (100 mg fibre) and by **Cellulase** (225 mg fibre). Length values are averages of duplicates.

Spruce fibres	LWFL for control to HCl/Cellulase (mm)	LWFL after HCl (mm)	Cleavage per fibre after HCl	Reducing sugars after HCl (BCA mg or %)	LWFL after Cellulase (mm)	Cleavage per fibre after Cellulase	Reducing sugars after Cellulase (BCA mg)
Mill F, SF	1.14	0.615	0.85	3.8	0.76	0.49	10.9 (4.8%)
Mill F, LF	2.95	0.620	3.77	3.5	0.76	2.91	11.0 (4.9%)
Mill T, SF	1.25	0.740	0.69	3.9	0.78	0.60	10.6 (4.7%)
Mill T, LF	2.63	0.750	2.51	3.4	0.72	2.65	10.9 (4.8%)
Lab F, SF	1.30	0.890	0.47	3.7	0.67	0.94	10.8 (4.8%)
Lab F, LF	2.93	1.080	1.72	3.6	0.63	3.08	10.9 (4.8%)
Lab T, SF	1.31	0.920	0.42	3.7	0.73	0.79	10.9 (4.8%)
Lab T, LF	2.73	1.320	1.06	3.4	0.80	2.42	10.9 (4.8%)

Results with Pulps 3 & 4 using HCl and Three Cellulase Mixtures (Exp. II)

The four pulps, Mill 3 and Lab 3 (spruce:pine ratio of 33:67 & 34:66 resp.) and Mill 4 and Lab 4 (spruce:pine ratio of 84:16) were tested with the HCl method. For the Mill 3 pulps (continuous cook), Table 2 shows that cleavage per fibre for Mill 3 was 1.93 and for Lab. 3 0.935. The difference was smaller than for the Mill 4 pulps, resulting in 4.16 cleavages for Mill and only 1.23 for Lab pulp. The strong cleavage of Mill 4 pulp may be due to the large 84 % proportion of more acid-sensitive spruce in this pulp. A similar result was also obtained for bleached spruce and pine kraft pulps or for spruce and pine thermomechanical pulps (Ander and Daniel 2006, 2007) and may reflect differences in the S1 cell wall in pine. This is the first time a comparison regarding HCl sensitivity of pine-containing Mill and Lab pulps has been reported. Once again Mill fibres gave stronger acid cleavage than Lab pulp fibres.

Table 2. Cleavage per Fibre by **HCl** and Monosugar Release for Mill and Lab (3 and 4) Unbleached Kraft Pulps. (Fibre dry-weights: 100 mg for fibre lengths and 200 mg for monosugar determination at MoRe. Shaking water bath Julabo SW22.)

Pulp	Ratio Spruce:Pine	Cleavage/fibre *(mean)*	Arabinose (g/l)	Galactose (g/l)	Glucose (g/l)	Mannose (g/l)	Xylose (g/l)
Mill 3	33:67	1.93/1.93 ***1.93***	0.039/0.041	0.024/0.025	0.014/0.014	0.024/0.026	0.10/0.11
Lab 3	34:66	1.07/0.80 ***0.935***	0.040/0.038	0.026/0.025	0.012/0.014	0.028/0.026	0.11/0.105
Mill 4	84:16	4.14/4.18 ***4.16***	0.038/0.038	0.021/0.021	0.015/0.014	0.024/0.024	0.11/0.11
Lab 4	84:16	1.22/1.24 ***1.23***	0.038/0.037	0.021/0.020	0.015/0.014	0.024/0.024	0.10/0.10

Novozym 476 (monocomponent endoglucanase) at pH 7 without synergistic effect gave very little cleavage (0.22-0.42 in cleavage/fibre; not shown) but has been used by Hildén et al. (2005) to study surface properties of kraft pulp fibres. It is not discussed

further here. As shown in Table 3, Cellulase N342 gave 8.4-10, Celluclast 5.6-5.9 for 0.3 ml enzyme, and 9.6-9.9 cleavages/fibre for 0.6 ml enzyme. This was much more than for HCl at pH 0 and 81°C (Table 2). Thus, despite strong cellulase-catalyzed cellulose cleavage with N342 and Celluclast giving 5.5–10 cleavages/fibre, no distinction between Mill and Lab fibres was obtained. Using Celluclast and mill- and laboratory-cooked pulps from Southern yellow pine chips, Iribarne and Schroeder (1999) came to a similar conclusion regarding fibre cutting.

Comparing monosugar release, it is shown in Table 2 that HCl gave mostly xylose (0.1 g/l) and 0.021-0.040 g/l of arabinose, galactose and mannose and very little glucose (0.015 g/l). This indicates that degradation of xylan and glucomannan is of importance for the differences obtained between Mill and Lab pulp fibres. The results are comparable to earlier results (Ander et al. 2005), and it is thought that also measuring small amounts of oligosaccharides would have given a similar result. Furthermore, the results are in line with BCA sugar determination giving mono- plus oligosaccharides. For Cellulase (Table 3) the situation was different and glucose was the major sugar released, reflecting the strong catalytic activity of endoglucanase + cellobiohydrolases, giving up to 0.8 g/l of glucose plus some xylose (0.07-0.15 g/l) depending on the pulp type; the latter possibly was attributed to xylanase activity at least in N342 (Richardson et al. 1998; Lumme et al. 1999). All other hemicellulose sugars were released in very small amounts. HCl swelled the above fibres between 0.7-1.7 μm, while N342 and Celluclast decreased fibre width by 1-3 μm, suggesting removal of S1 and the outer part of the S2 fibre cell walls by cellulase (Ander and Daniel 2007). The conclusion of these results is that cellulase cleavage in dislocations and the simultaneous degradation of fibre surfaces is not reflecting possible differences in cell wall structures and/or carbohydrate composition in Mill and Lab pulp fibres.

Table 3. Cleavage per Fibre by **Cellulase** at 50°C and Monosugar Release for Mill and Lab (3 and 4) Unbleached Kraft Pulps (200 mg) as in Table 2.

Pulp	Ratio Spruce:Pine	Cellulase	Cleavage per fibre	Glucose (g/l)	Xylose (g/l)
Mill 3	33:67	N342 pH 5	8.40	0.67/0.68	0.14/0.15
Lab 3	34:66	N342 pH 5	9.31	0.75/0.72	0.15/0.14
Mill 4	84:16	N342 pH 5	9.98	0.76/0.67	0.14/0.14
Lab 4	84:16	N342 pH 5	9.95	0.60/0.67	0.12/0.12
Mill 3	33:67	Celluclast*	5.59	0.54/0.53	0.13/0.12
Lab 3	34:66	Celluclast*	5.73	0.55/0.44	0.11/0.09
Mill 4	84:16	Celluclast*	5.90	0.47/0.47	0.08/0.08
Lab 4	84:16	Celluclast*	5.51	0.43/0.52	0.07/0.08
Mill 4	84:16	Celluclast**	9.90	0.80/0.82	0.11/0.12
Lab 4	84:16	Celluclast**	9.61	0.77/0.76	0.10/0.11

* 0.3 ml; **0.6 ml; see Methods.

Cellulases may penetrate deep into the dislocations and bind to amorphous parts, causing strong cleavage. However, penetration into the fibre surfaces may be low except in some pores or other fibre defects that are large enough to allow enzyme penetration. According to our unpublished results, the difference between pores in the above Mill and

Lab fibres were however small, meaning that the possibility to distinguish between Mill and Lab fibres are also small when using cellulases. The small HCl molecules at 80°C easily penetrate through all fibre cell walls during cleavage of cellulose β-1,4-glucosidic bonds and degradation of hemicelluloses, which provides a differentiation between Mill and Lab fibres. Cellulase only penetrates the fibre walls in-depth at sites of dislocations.

In general, maximum fibre cleavage for HCl was 5-6 cleavages per fibre, while strong cellulase activity gave up to 10 cleavages. Compared to dislocation calculations in polarized light using kraft cooked latewood fibres (Ander et al. 2005; Thygesen and Ander 2005) that gave 12-17 dislocations per fibre, the values for HCl or cellulase cleavage were lower. This difference may be due to incomplete cleavage even after 6 h, or at 85°C or with the strongest cellulase activity used. Stronger degradation, however, will give more very short fibres. Fibre fragments with fibre lengths less than 4 times the fibre width cannot be measured by the FibreMaster (Karlsson et al. 1999). Similarly, fibres and fines shorter than 50 μm are not measured in Kajaani instruments (Mooney et al. 1999). Based on inspection of fibre length distribution curves (Ander, unpublished results, not shown), it appears that 10 cleavages per fibre is rather near the highest cleavage value that is possible to calculate correctly after HCl or cellulase cleavage. Nevertheless, the 12-17 dislocations per fibre obtained for LW fibres fits well with number of fibre cleavages obtained maximally with HCl or cellulase, assuming that fibre lengths are possible to measure accurately after slightly stronger cleavage. This in turn indicates that the number of dislocations and cleavage per fibre are related phenomena.

Light Microscopy and Effects of Cellulase on Early- and Latewood Spruce Fibres

Following Celluclast (and Novozym 342) incubation there was strong erosion and degradation of earlywood fibre surfaces in addition to fibre cleavage as shown by polarized light microscopy (Fig. 3A). Deep fibre surface erosion and fibre cleavage of an earlywood (EW) fibre is shown. For latewood fibres (LW; Fig. 3B) this type of erosion/degradation was not discerned. Instead, polarized light colouration patterns

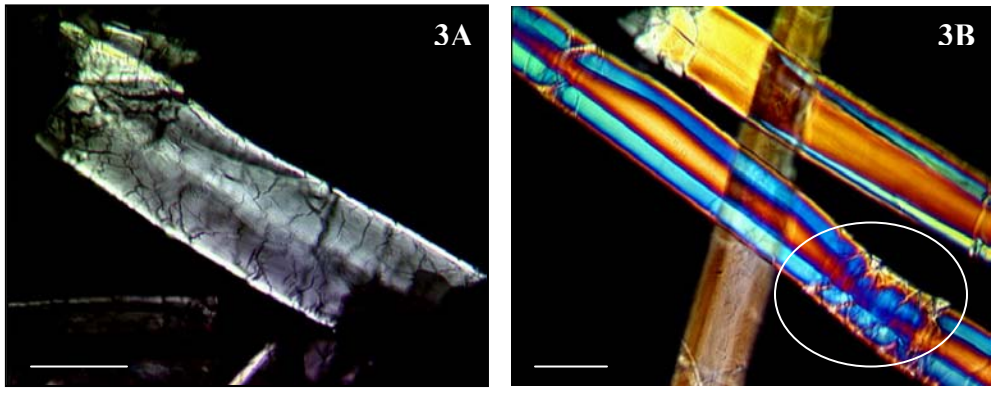
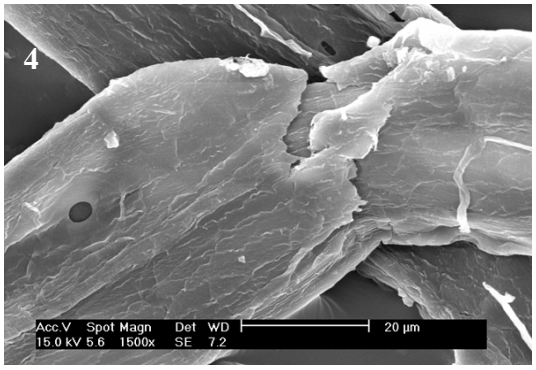


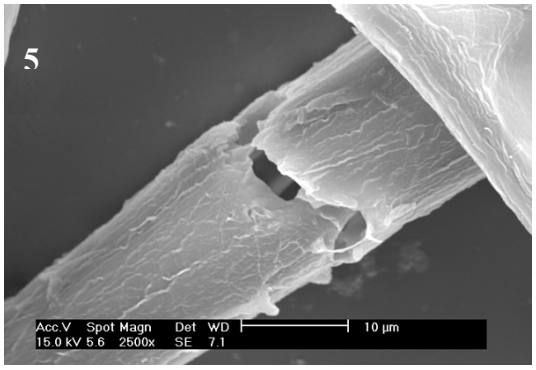
Fig. 3A and B. Appearance of unbleached kraft pulp fibres in polarized light after degradation by Celluclast (endoglucanase + cellobiohydrolase). Fig. 3A: Mill 4 earlywood fibre and Fig. 3B: Lab. 4 latewood fibres. Bars are 35 and 30 μm respectively. A large dislocation is seen in the latewood fibre in the ellipse.

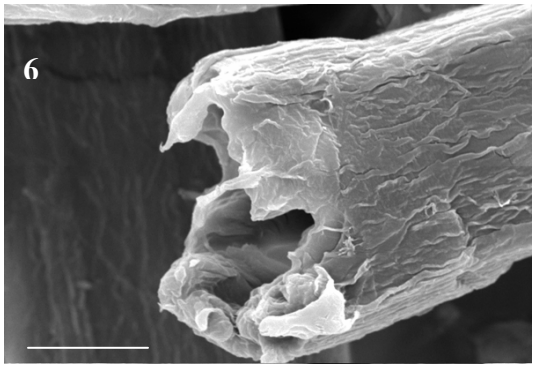
typical for LW fibres were seen. A typical large dislocation is seen in the ellipse. Fibre surface erosion was not visible. A higher cellulose crystallinity and a thicker secondary cell wall render the LW fibres more birefringent (anisotropic), and different colours appear. Thus, EW fibres were found to be less crystalline and more sensitive to cellulases (and degradative bleaching chemicals).

Scanning Electron Microscopy and Comparison of HCl and Cellulase Effects on the Fibres

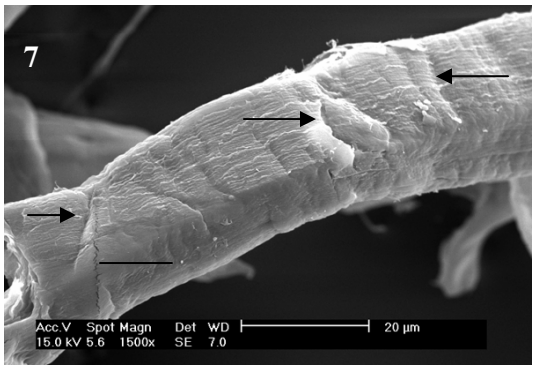
Mill 4 fibres after HCl and Celluclast treatments were studied using SEM.







Figs 4-6. Three stages in HCl attack on softwood spruce fibres. Fig. 4: Initial degradation of the S1 wall. Fig. 5: Further attack cleaving both the S1 and S2 fibre walls. Fig. 6: Complete cleavage of the fibre, also revealing the cell lumen. Bar: 10 µm.



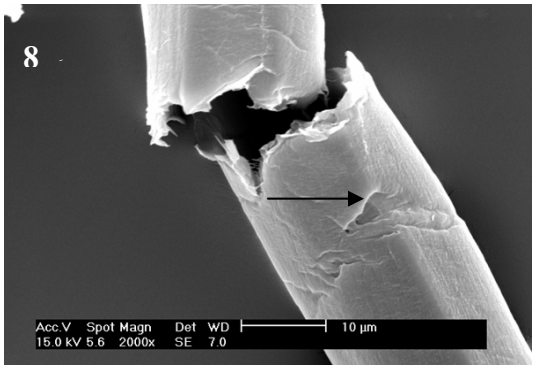


Fig. 7. Early phase of cellulase (0.3 ml Celluclast) action producing numerous clefts/ grooves in the outer fibre wall (see black arrows). An artefact crack is shown at the line to the left.
Fig. 8. Advanced stage of cellulase (0.6 ml Celluclast) action causing a deep perpendicular angular-shaped cleft (black arrow) and typical fibre cleavage.

Cleavage per fibre for these fibres was 4.16 for HCl and 5.71 and 9.76 for the two Celluclast concentrations (see Tables 2 and 3). Apart from fibre cleavage revealing the cell lumen, there appeared to be very little change in the surface morphological structure of fibres after HCl treatment, and the ridges and grooves shown in Figs 4-6 most probably reflect drying artefacts. Cellulase activity often produced a smooth fibre surface (i.e. biopolished), and fine cavities at an angle to the fibre axis (i.e. along the S1 layer) were common. Such cavities had angular or diamond-like shapes (Figs 7 and 8), a feature seen more clearly after extended cellulase incubation (Daniel and Pettersson, unpublished results). Such cavities may be the result of initial dislocations in which the large cellulase molecules bind to and degrade amorphous and/or crystalline cellulose (Wardrop and Jutte 1968; Chanzy and Henrissat 1985). It seems likely the small acid molecules are not dependent on dislocation structures or large weak defects to cleave the fibres – thus general hemicellulose degradation occurs in this case.

CONCLUSIONS

1. The HCl method can be used to determine dislocations and other weak points in different pulp fibre types and may be a complement to wet zero span measurements and other paper tests, making improvements in pulp and paper production possible.
2. The HCl method can differentiate between mill- and laboratory-produced kraft pulps from the same wood batch in Strength Delivery investigations, which is not possible using cellulase activity.
3. Hemicellulose degradation by HCl seems to be of importance for the differentiation between Mill and Lab pulp fibres, while cellulases mainly release glucose from the fibre surfaces, causing decreased fibre width.
4. Dislocation cleavage and/or cleavage of β-1,4-glucosidic bonds in cellulose by HCl or Cellulase give little sugar release, and the amount of sugars cannot be used to differentiate between Mill and Lab pulp fibres.
5. Spruce pulp fibres are more sensitive to HCl than pine pulp fibres.

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