MODIFICATION OF SPRUCE SULPHITE PULP BY CELLULASE TREATMENT

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Enzymatic treatment of different sulphite pulps with cellulases has been studied in this work. Endoglucanases were shown to beneficially alter the properties of both paper-grade and dissolving-grade pulps. In the case of the paper-grade pulp, the treatment with endoglucanase resulted in a considerable reduction of the pulp refining time without decrease in strength properties. While for the dissolving pulp, a viscosity decrease was achieved by cellulase treatments, which might be useful in upgrading pulp bleaching processes. The structure and composition diversity of sulphite and kraft pulps was found to define different employment of cellulases for their treatment.

Keywords: sulphite pulp, paper-grade pulp, dissolving pulp, cellulases, endoglucanases, pulp viscosity, mechanical properties, refining velocity

INTRODUCTION

Enzyme application in the pulp and paper industry has been a subject of intensive research over the last few decades. The high selectivity of the enzymatic action on the substrate, as well as environmental safety issues, makes enzymes superior reagents compared to conventional chemicals. These advantages define the commercial feasibility of developing enzyme technologies in the pulp and paper industry, and the production of new high performance enzymes.

Hydrolytical enzymes, cellulases and xylanases, can enhance the fiber characteristics of both paper-grade and dissolving-grade pulps.[1,2] Fiber structure alterations at initial stages of enzyme hydrolysis are still poorly understood. However, such alterations may provide beneficial changes of fiber properties. Enzymes can cause separation of fibers and fibrils, as well as disruption of highly ordered cellulose structure followed by reduction of crystallinity. These phenomena are used for various applications, such as to facilitate the refining process, improve inter-fiber bonding, accessibility and reactivity in etherification processes, and correspondingly enhance final product properties.

The various applications for paper-grade pulp include reduction of refining energy requirements, improvement of beatability and fibrillation of chemical pulps and increase in the freeness of secondary fibers.[1] Most of the recent investigations have been focused on the modification of kraft pulps, whereas, only relatively few studies have been dealing with enzymatic treatment of sulphite paper-grade pulp.

Sulphite and kraft pulps significantly differ in content, structure and dispersion of the main components. In kraft pulp, xylan is localized preferentially on the fiber surface and outer layers of the cell wall, as a result of its redeposition at the end of the kraft cooking process.[3] Therefore, xylanase treatment is considered to be the most effective for the kraft pulps. It was reported that small dosages of cellulases and xylanases can improve the refining ability of the kraft fibers, while pulp strength properties remain almost unchanged.[4-6]

The sulphite cooking process is the second most important treatment, after the kraft process. The sulphite pulp is defined by high yield, superior bleachability and satisfactory mechanical properties. The high swelling capacity and accessibility of hemicelluloses in the sulphite pulps facilitate their use as dissolving pulp. Dissolving pulp is produced by both sulphite and

prehydrolysis kraft processes. During sulphite cooking, process conditions, such as acid medium and high temperature, provide a high degree of hemicellulose removal, deep and homogeneous delignification, as well as low viscosity of the pulp.

Xylanases are also suggested for the treatment of dissolving pulps. They are tested for the removal of hemicelluloses from different types of pulps.[7,8] L. P. Christov and co-workers examined in detail the xylanase enzyme treatment of eucalyptus sulphite dissolving pulp. The xylanases increased brightness and α-cellulose content of the tested pulp by single and repeated treatments,[7,9] as well as reduced the active chlorine charge for bleaching.[10] The removal of pentosans from the pulp appeared to have a limit of less than 50%. The removal of hemicelluloses from the kraft softwood dissolving pulp also had a limit, which was reported to be related to the presence of lignin-carbohydrate complexes (LCC).[8,11,12]

Cellulases, especially endoglucanases, were shown to be highly effective in increasing the reactivity of kraft and sulphite dissolving pulps for viscose production.[13-15] Several researchers related this reactivity improvement to the enhancement in fiber swelling in enzymatically treated pulps.[16] These treatments were carried out using commercial pulps. The enzyme applications mentioned above allowed the development of treatment sequences for conversion of non-dissolving pulps into dissolving, mainly for hardwood kraft paper-grade pulp.[13]

Therefore, the possibilities of sulphite pulp modification seem to be related to cellulase treatment. The aim of this study was to investigate the effect of cellulase modification on sulphite fibers in achieving the benefits previously demonstrated for the kraft pulps. The treatment is applied to both paper-grade and dissolving-grade pulps. To elucidate the differences of the enzyme action on sulphite and kraft fibers, treatments of different pulp samples with the same enzyme products were carried out.

EXPERIMENTAL

Materials

Pulp samples

Sulphite softwood dissolving pulp was provided by the Kotlas Pulp and Paper Mill (presently Ilim Group Koryazhma Subsidiary, Russia). Three types of pulp samples were used: unbleached never-dried, bleached never-dried and commercial dried dissolving pulp. Paper-grade unbleached never-dried bisulphite pulp was obtained from Sokol Pulp and Paper Mill, Russia. Unbleached dissolving and paper-grade pulps exhibited kappa numbers of 12 and 48 units, respectively. The unbleached hardwood kraft pulp (kappa number 67) was provided by the Archangelsk Pulp and Paper Mill, Russia.

Enzymes

Commercially available monocomponent endoglucanases were provided by Novozymes. The catalytic activity of the enzymes towards carboxymethylcellulose (CMC, 1% solution) was measured by monitoring the rate of sugar accumulation using the Somogyi-Nelsons test. Table 1 shows the values for activities and optimal reaction conditions of the enzymes used. The Pulpfor enzyme complex had a number of activities (except CMC-ase activity), such as xylanase activity of 2250 units g^{-1}, filter paper activity of 76 units g^{-1}, and β-glucosidase activity of 5000 units g^{-1}. Relatively high activity toward filter paper indicates the presence of cellobiohydrolases.

Table 1
Enzymatic activities and optimal reaction conditions

Enzyme	Activity according to manufacturer data, ECU g^{-1}	CMC-ase activity, CMC-units ml^{-1}	Reaction conditions	
			Temperature, °C	pH
Fiber Care D	9800	273	40-60	6-9
Novozym 476	5000	230	40-65	6-9
Novozym 613	2500	not determined	40-60	6-8
Novozym 51059 (Renozyme)	4500	not determined	40-70	5-8
Novozym 51008	1000	68	40-70	5-9
Pulpfor	15000 *	110 *	40-70	4-7

*Corresponding activity units per g

Methods

Enzymatic treatment

Enzymatic treatment of 3% consistency pulp slurries was carried out at 50 °C in phosphate buffer (pH 7.0) or acetate buffer (pH 4.5) depending on the enzyme pH-optimum. Followed by enzyme addition, pulp slurries were intensely mixed and left to stand for 2 hours at 50 °C. Different enzyme dosages were used. After the treatment, the pulp slurry was filtered on a Buchner funnel, using a vacuum pump. Reference pulps were treated under similar conditions without enzyme addition. Filtrates were tested for chemical oxygen demand (COD). The pulp samples were dried at room temperature and used for α-cellulose content and viscosity analysis.

For refining experiments, the pulp was treated at 30 °C and pH 7.0 for 2 hours with Fiber Care D dosage of 60 g g^{-1} of oven dry pulp.

Determination of pulp enzymatic degradation

The effect of enzymes on pulp degradation was determined by the rapid method of chemical oxygen demand quantification (COD). 0.25 N potassium dichromate (5 ml) and concentrated sulfuric acid (15 ml) were added to the filtrate (10 ml) in an Erlenmeyer flask. After that, the sample was subjected to boiling for 1 minute and was diluted with distilled water (20 ml). The amount of unreacted dichromate was determined by titration against 0.25 N Mohr's salt. The reference experiment was carried out with distilled water.

α-Cellulose content was measured by the standard GOST 6840-78 as the amount of cellulose residues undissolved in 17.5% NaOH solution. This method is an equivalent of TAPPI procedure T 203 cm-99. The viscosity of 1% cellulose copper-ammonia solution was determined according to the standard method GOST 14363.2-83 (equivalent of T 230 om-08) using a capillary viscometer.

Pulp refining and mechanical testing

The 6% consistency pulp (16 g dry weight) was refined up to 70 °SR using a Jokro mill. Pulp breaking length was measured according to ISO 1924-2:2008 and burst index according to ISO 2758:2001.

RESULTS AND DISCUSSION

Action of different enzyme compositions on pulp fibers

Both endoglucanases (EG) and cellobiohydrolases (CBH) are able to provide enhancement in pulp fiber properties. The influence of cellulase components on pulp properties has been studied separately.[17] Although CBH treatment of mechanical pulps revealed some benefits,[18] most of the applications are related with EG action. Thus, EG monocomponents are more commonly used. In earlier works, pulp treatments were carried out using crude enzyme preparations containing several enzymatic activities, including xylanases.[19] Xylanases have been reported to act together with cellulases improving pulp refining ability, as well as flexibility of the fibers.[19]

In this work, the treatment of sulphite and kraft pulp samples was carried out using two enzymes: fiber Care D, a monocomponent EG preparation, and Pulpfor, a crude *Myceliophthora fergusii* enzyme complex. Pulpfor had a relatively high CBH and xylanase activity, its CMC-activity was approximately 2.5 times lower when compared to Fiber Care D. These enzymes were used to elucidate the differences between sulphite and kraft pulps behavior during the treatment.

The COD of the pulp filtrates was determined to evaluate the extent of pulp fiber degradation after enzymatic treatment. The method is sufficient for characterization of a cumulative amount of low molecular hydrolysis products released after enzymatic action. This technique also allows the observation of the hydrolysis products accumulation with increased enzyme dosage.

The treatments were carried out with an enzyme dosage of 5-50 mg g^{-1}. The highest dosage is not commercially viable. However, it was chosen for the current study to insure the maximum effect. The hydrolysis extent of the kraft pulp was greater after the treatment with Pulpfor, compared to Fiber Care D. The opposite effect for both enzymes was observed in the case of the sulphite pulp (Fig. 1).

Cellulases can considerably destruct cellulosic fibers, thus they must be used carefully for fiber modification.[19,17] The degradation effect can be independent from the hydrolysis rate, as it was shown by Pere *et al.*,[17] when the handsheet mechanical strength and extent of the fiber hydrolysis were evaluated on the same sample. As the hydrolysis rate was not sufficient for a comprehensive analysis of fiber degradation, α-cellulose content was determined to assess the enzymatic influence on the treated fiber. The α-cellulose content analysis is employed to characterize the relatively high molecular weight cellulose component and, therefore, can be used to assess the EG action on fibers.

The main reduction of α-cellulose level by Fiber Care D occurred at the minimum enzyme dosage of 10 mg g^{-1} (Fig. 1). The treatment with FCD resulted in 2.0 and 1.5% decrease of α-cellulose content for the sulphite and kraft

pulps, respectively. When Pulpfor and the same CMC-activity (enzyme dosage 3 times higher) were used, the α-cellulose contents decreased by 0.5 and 4.5%, respectively.

The greater extent of kraft pulp hydrolysis and the corresponding decrease in α-cellulose content by the action of Pulpfor might be attributed to the xylanase action on precipitated xylan. The localization of xylan in kraft fiber makes it accessible for enzymatic treatment. This xylan was proposed to be the primary substrate for xylanases in the process of enzymatic prebleaching[3]. However, the latest study has confirmed that highly substituted xylan in the inner layer of the kraft fiber wall, as well as sulphite fiber xylan, can also be affected by enzymes.[7]

On the other hand, xylan may protect the cellulose of the kraft fiber against the action of monocomponent EG (Fiber Care D). This indicates that xylanases are synergistically involved in the degradation process. Therefore, the cellulase effect on the kraft pulp fibers could be greatly enhanced by xylanases. This observation is in agreement with earlier works.[19,12] The sulphite fibers are less protected from cellulase enzymatic attack, so that the treatment of sulphite pulp should be carried out carefully with lower enzyme dosage.

The degradation curve of unbleached dissolving pulp is presented in Figure 2. Oppositely to paper pulps, the curve is straight. It means that the hydrolysis products accumulation is in direct proportion to the enzymatic dosage, indicating the absence of any interfering factors. One of these factors could be the presence of lignin, as the lignin content for the paper-grade pulp was considerably higher than for the dissolving pulp. The degradation of cellulose by Fiber Care D was twice more efficient than that obtained by Pulpfor, with the corresponding differences in the CMC-activity of the enzymes.

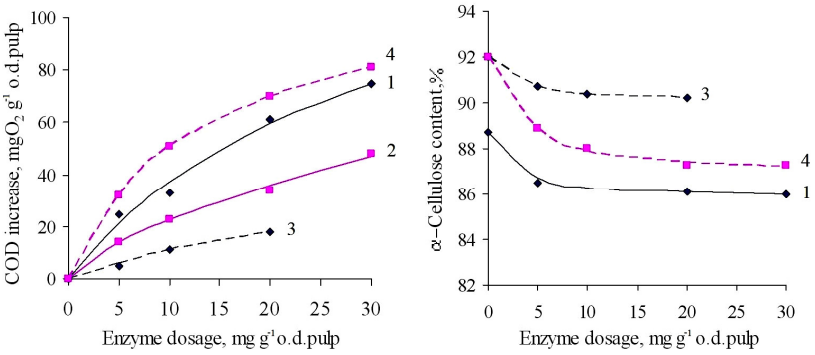

Figure 1: Enzyme destruction of unbleached paper-grade pulp: (a) hydrolysis product accumulation measured by COD and (b) α-cellulose content after enzymatic treatment (1 – sulphite pulp treated with Fiber Care D; 2 – sulphite pulp treated with Pulpfor; 3 – kraft pulp treated with Fiber Care D; 4 – kraft pulp treated with Pulpfor)

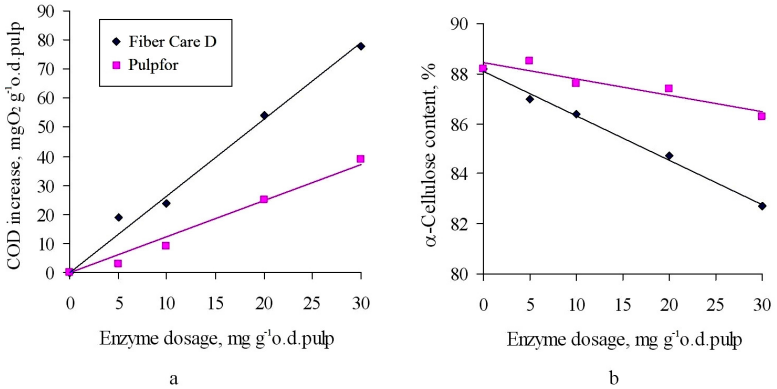

Figure 2: Enzyme destruction of unbleached dissolving pulp: (a) hydrolysis product accumulation measured by COD and (b) α-cellulose content after enzymatic treatment

The decrease in α-cellulose content showed the same tendency. The α-cellulose of the dissolving pulp is a widely used characteristic of pulp quality corresponding to chemical yield and fiber strength. The α-cellulose level of the treated pulp was reduced as a result of the increase in enzyme dosage. The extent of reduction by Fiber Care D was almost 3-4 times higher compared to Pulpfor.

Therefore, the effect of Pulpfor on sulphite fibers could be ascribed to cellulase rather than xylanase action. The hydrolysis extent was in good correlation with the CMC-activity of the enzymes. The degradation of sulphite dissolving pulp fibers by Fiber Care D was 2-4 times higher than by Pulpfor. Also, it had an activity 2.5 times higher than that of the latter. The degradation effect was unsubstantial at low enzyme dosages, which are usually used in various applications. This allows the use of enzymes for fiber modification.

Refining of sulphite pulp treated with enzymes

Cellulases and xylanases have been shown to improve the refining ability of kraft pulps,[4-6] resulting in the decrease of energy consumption. The refining of a sulphite pulp after enzymatic treatment was carried out in this work, using a Jokro mill. The refining rate was significantly increased after the treatment with Fiber Care D, compared to a reference experiment (Fig. 3). The reduction of the refining time was registered to be of about 20%, which involves significant energy savings. The refining rate (expressed as SR-value increase per minute) for the Fiber Care D was almost 2.3 compared to 1.5 for the reference pulp sample. The refining rates for the other tested enzymes were 1.6 and 1.5 for Novozym 476 and Pulpfor, respectively. Therefore, the Fiber Care D appeared to be the most effective enzyme, providing significant acceleration of the refining process. It is interesting that EG Fiber Care D and Novozym 476 acted in different ways on the pulp refining ability, possibly due to structure differences, which determine various performance of the enzymes. Novozym 476 presumably has a lower degradation effect on sulphite fibers.

The Pulpfor enzyme complex, containing xylanases, had no effect on sulphite pulp refining. However, xylanases were shown to improve the refining ability of kraft fibers.[6] Therefore, xylan degradation in sulphite fiber was less important for the refining process or was difficult due to xylan localization.

As cellulases can decrease pulp mechanical properties, the refining acceleration can be caused by the strong degradation of fibers. The basic mechanical characteristics of the pulps after the enzymatic treatment were measured using standard techniques (Table 2). The strength properties remained almost unchanged, except for a slight increase of burst index after the treatment with Pulpfor.

During cellulase treatment, two opposite processes are known to occur:[19] on the one hand, enzyme degradation reduces fiber intrinsic strength; on the other hand, raised fibrillation and development of inter-fiber bonding are able to provide an increase in the overall pulp handsheet strength. Fiber fibrillation was reported to reach a maximum depending on enzyme dosage, and at higher dosages enzyme defibrillation was observed.[19]

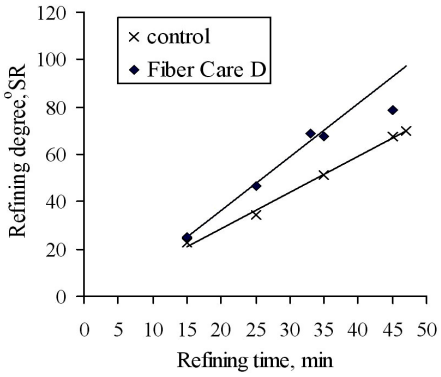

Figure 3: Refining of control and enzyme treated pulps

Table 2
Mechanical properties of treated pulps

Pulp sample	Refining time, min	Refining degree, ºSR	Breaking length, m	Burst index, kPa·m^2 g^{-1}
Control pulp	46	69	11860	5.60
Pulp treated with Fiber Care D	34	69	10970	5.55
Pulp treated with Pulpfor	46	68	11980	6.20

Enzyme charge 0.06 mg g^{-1} o.d. pulp, handsheet grammage 75 g m^{-2}

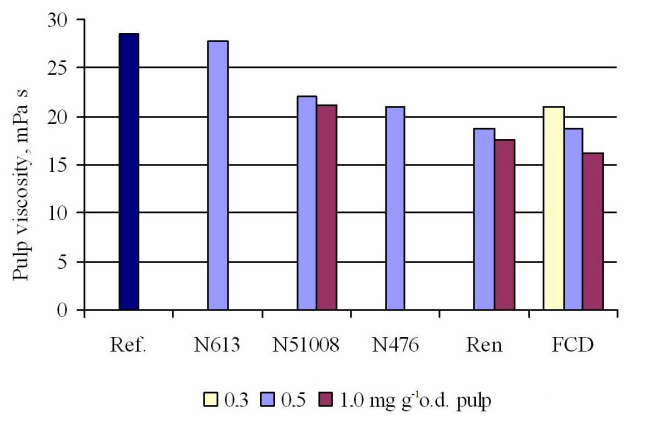

Figure 4: Decrease in dissolving pulp viscosity after treatment with enzymes

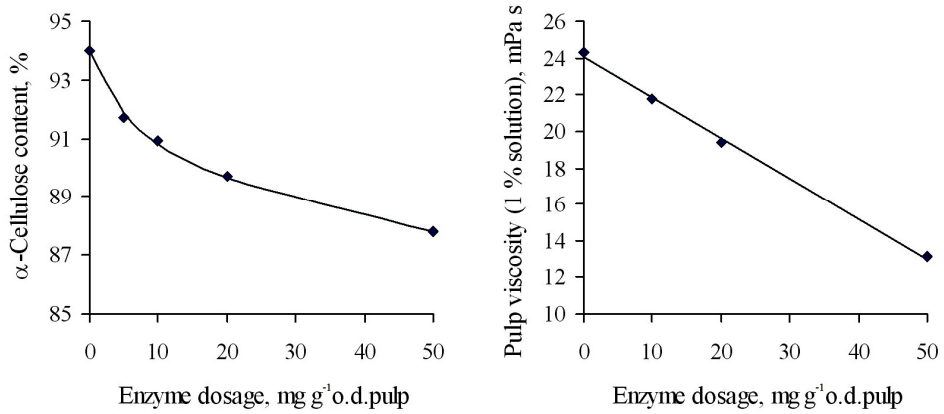

Figure 5: Enzyme destruction of commercial dissolving pulp: (a) α-cellulose content and (b) viscosity after enzymatic treatment

Therefore, it is possible to achieve the refining acceleration of sulphite pulps with the aid of cellulases, as it has been demonstrated for kraft pulp. The enzymatic action may differ significantly, so it is necessary to test the enzyme preparation based on the general characteristics of the composition and activity. The Fiber Care D was proved to be the most effective in increasing the refining rat, while preserving the mechanical properties of the sulphite pulp.

Reduction of dissolving pulp viscosity with EG

EG are able to reduce the cellulose chain length and, therefore, may efficiently provide a pulp viscosity decrease. The CBH are less effective. Several enzymes were tested for their ability to reduce pulp viscosity (Fig. 4). As can be seen, all the enzymes significantly reduced pulp viscosity, but to a various extent.

Novozym 476 is most commonly used as an EG source.[12-14] It represents well purified fungus

Aspergillus EGV cellulase with relatively high activity. However, new enzymes obtained from other sources, can show better performance on the pulp fibers due to specific structure features. In this study, the Fiber Care D was tested for the first time.

Novozym 613 is a monocomponent EGI derived from genetically modified *Aspergillus* species containing no CBD. Its effect on pulp viscosity was almost unessential. It was used in several works to demonstrate the role of CBD in EG application.[20] The CBD was reported to play a significant role in enzyme sorption on the fiber surface and contribute to effective hydrolysis of the fibers.[21] Our results also indicated the importance of CBD in EG performance. A distinct viscosity decrease by CBD containing enzymes was registered, supporting previous findings.

The effect of other EG in this work seemed to be more evident with CMC-activity increase. Therefore, the CMC-activity of EG may, to some degree, indicate the enzyme productivity for viscosity decrease. However, enzymes with close activities, Novozym 476 and Fiber Care D, differed significantly in their performance. It is known that the effect of the enzymatic action depends on the enzyme structure rather than other properties, so every EG enzyme should be tested for its ability to decrease pulp viscosity. The Fiber Care D was registered to be the most effective and can be used in lower dosages.

The treatment of commercial dried dissolving pulp with Fiber Care D (Fig. 5) at high dosage caused deep degradation, resulting in a significant decrease of α-cellulose content and viscosity of the pulp. At low dosage, when the enzyme is already able to decrease the viscosity to the required value, the decrease of α-cellulose content is expected to be insignificant. Thus, cellulases application for viscosity control would not cause any loss of the dissolving pulp quality.

The viscosity decrease is commonly achieved in the final stages of the bleaching sequences. However, chemical reagents provide non-selective reduction in the degree of polymerization of cellulose and can cause the generation of toxic waste compounds. The replacement of chemicals, especially chlorine containing reagents, with enzymes can reveal some benefits, including environmental safety and the possibility of switching to ECF-bleaching of dissolving pulp.

CONCLUSION

While the enzymatic degradation of the kraft pulp is determined by effective xylan hydrolysis, the sulphite pulp is affected only by cellulases. In order to achieve the desired alteration of properties, the kraft pulp should be treated with xylanases together with cellulases. Opposite to it, the sulphite pulp should be treated with lower dosage of cellulases to gain the same benefits.

The acceleration of sulphite pulp refining is possible using enzymes. It is related to significant energy savings, while the fiber strength properties are preserved. The composition and activity of the enzymes have to be tested prior to application, in order to define the differences in their efficiency. The Fiber Care D was found to be the most efficient enzyme preparation among the tested products.

Dissolving pulp is more accessible to enzymes due to the more extensive treatment during the cooking process. The viscosity reduction with enzymes may be a valuable tool, since it increases the environmental safety of pulp bleaching. The most effective enzyme preparation must be chosen taking into account its CMC-activity and structure features.

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