Improved Accessibility and Reactivity of Dissolving Pulp for the Viscose Process: Pretreatment with Monocomponent Endoglucanase

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A high accessibility is an essential prerequisite for a homogeneous substitution of cellulose material. In this study, chemical and enzymatic pretreatments to increase the accessibility of cellulose materials have been investigated. Dissolving pulp has been treated with a monocomponent endoglucanase. Fock's method, a microscale process similar to the viscose process, showed an increase in cellulose yield. Simultaneously, the viscosity decreased. To clarify whether the increase in reactivity was due solely to the decrease in the degree of polymerization, the dissolving pulp was also subjected to acid hydrolysis. At a given viscosity level, the enzymatic pretreated pulp had a higher reactivity than the pulp subjected to acid hydrolysis. To achieve 100% reactivity, according to Fock, the acid-treated pulp showed a lower molecular weight compared to the enzymatic-treated pulp. A monocomponent endoglucanase can thus be used to increase the reactivity and accessibility of dissolving pulp in the viscose process.

1. Introduction

Cellulose is the most abundant of all biomolecules, and all plants on earth synthesize cellulose, as do some kinds of algae, bacteria, and tunicates. This polysaccharide is the main constituent of wood and is a homopolysaccharide composed of β-D-glucopyranose units linked together by $1 \rightarrow 4$ glucosidic bonds. It has a strong tendency to form intra- and intermolecular hydrogen bonds.[1]

Cellulose forms a network structure of fibrils and fibrilar aggregations. Intermolecular hydrogen bonds, dipole interactions, and van der Waals interactions bind the cellulose molecules intensively together. The accessibility of the cellulose in wood pulps to solvents and reactive agents is usually limited. Only the cellulose molecules on the surfaces of the fibrils or fibril aggregates and those between the crystallites in the cell walls are accessible. During different stages in the pulping process, lignin, hemicellulose, and also cellulose are degraded. The remaining structures are then able to bind closer to each other which results in aggregation and a less accessible structure, Figure 1.[2] This limitation in accessibility leads to inhomogeneity in cellulose derivative manufacturing.

Dissolving pulp is produced for the manufacture of cellulose derivatives and regenerated cellulose. Today, dissolving pulp is mainly produced by the acid sulfite and prehydrolysis kraft processes, of which the first is the dominant. The pulp has low hemicellulose content and very low lignin and extractives contents. The reactivity is often the most significant quality parameter of dissolving pulp. The structure and morphology of the fiber determine the reactivity and accessibility of the cellulose to chemicals.[3]

Even though dissolving pulp is a highly purified pulp, it possesses some disadvantages, for example a broad molecular weight distribution and a low viscosity at a given purity level.[4] Organosolve processes have been suggested as alternative pulping methods. The organosolve and the sulfite processes have been compared in several studies,[4−6] and some of the processes, such as Acetosolv, Formacell, and Milox, have shown promising results in terms of purification selectivity.[4]

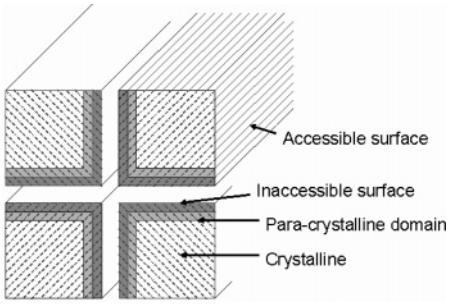

Figure 1. Cellulose structure in wood pulp. Schematic picture of a cellulose microfibril aggregate.[2]

To increase the reactivity of the pulp, it must be possible to measure the accessibility or reactivity. There is today a lack of rapid and general methods to measure reactivity, since the definition of accessibility or reactivity varies depending on the topic of discussion, i.e., accessible to what? Over the years, many attempts have been made to find a correlation between the hydroxyl group reactivity and the microstructure of the cellulose. Both X-ray diffraction and iodine sorption measurements are commonly used to determine the crystallinity and accessibility, respectively. The iodine sorption method measures the amorphous part or the accessible hydroxyl groups, whereas X-ray diffraction measures the crystalline fraction.[7,8] It is not clear how well these methods correlate with the reactivity in industrial production.

In this study, the reactivity of cellulose has been determined by a modification of the method described by Fock which is a microscale process similar to the viscose process.[9] This method measures the amount of cellulose that is not dissolved in sodium hydroxide when viscose is prepared. Fock's method has been used in several reported studies to describe the reactivity of dissolving pulps from different raw materials and pulps produced

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with different pulping conditions.[10,11] This analysis procedure has also been used to determine the applicability of using a multivariate analysis of chemical data obtained from dissolving pulp to find the reactivity of the pulp.[12]

Different degradative, mechanical, and swelling treatments have previously shown to activate cellulose. For example, Tang et al. found that ultrasonic treatment increased the accessibility of cellulose fibers and a large number of pores accessible to water were opened up.[13] By using a solution consisting of soda and urea, Kunze and Fink have shown that the cellulose I structure partially or completely can be destroyed.[14] Furthermore, hydrothermal processes have been used to enhance the reactivity of pulp for the viscose process. Koutu et al. reported that hydrothermal treatment gives a lowering in DP and an increased reactivity toward CS_2 and alkali, which improved the homogeneity of the viscose solution and reduced the chemical consumption.[15]

Different kinds of enzymes have also previously been used to increase the reactivity of cellulose. Rahkamo et al. have studied the modification of hardwood and softwood dissolving pulp with purified endoglucanase and cellobiohydrolases from *Trichoderma reesei*. The results have shown that, as the alkali solubility increases the viscosity decreases.[16,17] Combinations of enzymatic, mechanical, and chemical treatments of dissolving pulps have been used in an attempt to make cellulose directly soluble in aqueous sodium hydroxide. Both hardwood and softwood pulps became, according to Vehvilänen et al., highly soluble in 9% aqueous sodium hydroxide after combined treatment and could be regenerated to films and fibers.[18] Henriksson et al. showed a considerable increase in reactivity for the viscose process after treating dissolving pulp with a monocomponent endoglucanase.[19]

In this paper, the increase in reactivity after endoglucanase treatment has been further studied, and the effects of both enzyme dosage and time have been investigated. Treatments with acid hydrolysis have also been studied in order to investigate the influence of the degree of polymerization on the reactivity as revealed by Fock's method.

2. Experimental Section

Material. The pulp used in this study was an industrial dissolving pulp from Domsjö Fabriker AB, Sweden. This dissolving pulp was an acid sulfite pulp cooked from a mixture of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). The starting raw material was produced with optimized parameters with respect to cellulose accessibility; that is, the lignin content is very low (0.6%) and the R18 is 94.5%. In 18% NaOH (R18), the hemicellulose, and some low molecular weight cellulose, is soluble. A high R18 implies a low hemicellulose content of the dissolving pulp.

The enzyme used was a monocomponent C-type endoglucanase preparation, Novozyme 476, which was a kind gift from Novozyme, Denmark. The enzyme is produced from a genetically modified *Aspergillus* fungus, with an inserted gene from *Humicola insolens*. The cellulolytic activity was determined by the manufacturer and is expressed in Endo Cellulase Units, ECU, per unit mass of material. The enzymatic activity of the preparation was 5000 ECU/g. All other chemicals were of analytical grade.

Enzymatic Treatment of the Pulp. Before the enzymatic treatment, the never-dried pulp was adjusted to pH 7 with a phosphate buffer (11 mM NaH_2PO_4 and 9 mM Na_2HPO_4). To obtain a homogeneous distribution of the enzyme, the enzyme was first added to the buffer before the buffer was added to the pulp, to a final pulp concentration of 3%. The enzymatic incubation was carried out in special plastic bags in a water bath at 50 °C, each pulp sample weighted 20−25 g dry weight. The samples were periodically taken out of the bath and kneaded for 10−15 s to mix the sample. After incubation, the samples were washed on a Büchner funnel with deionized water (90 °C) and transferred to an 80 °C water bath for 30 min to deactivate the enzyme. The treated pulp was then filtered in a Büchner funnel and washed with 1000 mL of deionized water.

Two separate series of enzyme treatments were performed, one where the dosage was varied. Five different enzyme dosages were tested: 0.05, 0.5, 5, 30, and 50 ECU/g dry weight pulp. The incubation time was 3.5 h for all samples, and they were kneaded every 30 min. In the second trial, the time dependency was investigated. The incubation times were 10, 30, and 90 min. The enzyme dosage was 27 ECU/g dry weight pulp. The 10 min sample was not kneaded during the incubation, the 30 min samples were kneaded every 10 min, and the 90 min sample was kneaded every 30 min. In each trial, a reference sample without enzyme was treated in the same way as the other samples. In the trial with varying time, the reference pulp was incubated for 3 h.

Acid Hydrolysis of the Pulp. Preheated, never-dried dissolving pulp (5 g dry weight) was put into an Erlenmeyer flask and diluted with hydrochloric acid (2.5 M) to a pulp consistency of 4%. The pulp and acid mixture was then heated in a water bath, at 90 °C, for 2−60 min and stirred repeatedly. The hydrolysis reaction was terminated by cooling and neutralization of the sample using NaOH, to a pH between 6.5 and 9. Thereafter, the sample was filtered twice and washed on a Büchner funnel, first with phosphate buffer (11 mM NaH_2PO_4 and 9 mM Na_2HPO_4) and thereafter with deionized water.

Reactivity Measurements. The reactivity of the cellulose was determined by a slightly modified Fock's method.[9] The sample was stirred together with sodium hydroxide and carbon disulfide. Cellulose xanthate was formed and a certain amount of the xanthate was thereafter regenerated. Finally the cellulose yield was determined. According to Fock the reactivity can be expressed both as residual cellulose and regenerated cellulose yield; in this study the latter is used.

Step 1. Preparation of Viscose from Dissolving Pulp and Collection of the Regenerated Cellulose. Pulp samples of 0.5 g were weighed into 100 mL Erlenmeyer flasks with a stopper. NaOH (50 mL, 90 g/L) and CS_2 (1.3 mL) were added, and the solution was stirred with a magnetic stirrer (300 L/min) for 4 h at room temperature. The solution was diluted to 100 g using deionized water and carefully shaken. The solution was then left for 2 h in order to allow any undissolved cellulose to settle. An aliquot (10 mL) from the upper clear solution was then transferred to another 100 mL Erlenmeyer flask and neutralized using 29% H_2SO_4. The yellow solution turned transparent and was left overnight in a fume cupboard.

Step 2. Oxidation and Titration of the Regenerated Cellulose. H_2SO_4 (20 mL, 68%) was added to the regenerated cellulose, and the mixture was stirred with a magnetic stirrer for 1 h. The milky solution was diluted to 50 mL with deionized water. $K_2Cr_2O_7$ (10 mL, 1/6 M) was added, and the solution was refluxed for 1 h to fully oxidize the regenerated cellulose and thereby clear the solution. The solution was transferred to a 100 mL measuring flask and diluted with deionized water. A portion of the solution (40 mL) was then transferred to a 250 mL beaker containing KI (0.5 g), stirred with a magnetic stirrer, and titrated with $Na_2S_2O_3$ (0.1 N). When the brown solution started to change color, starch (1.5 g) was added, and the solution turned blue-violet. The titration continued until all of the I_2 was reduced and the solution turned pale blue. The volume of $Na_2S_2O_3$ was then recorded.

Step 3. Calculation of Reacted Cellulose. The percentage of reacted cellulose in the sample was calculated as

$$X = (100)9.62^{a}\frac{M(V_1C_1 - (V_2C_2100/40^{b})/6)}{4Y} \qquad (1)$$

where X = reacted cellulose (%), Y = weight of sample (g), M = molecular mass of glucopyranosyl residue, $C_6H_{10}O_5$ (162 g/mol), V_1 = volume of added $K_2Cr_2O_7$ (L), V_2 = volume of titrated $Na_2S_2O_3$ (L), C_1 = concentration of $K_2Cr_2O_7$ (mol/L), C_2 = concentration of

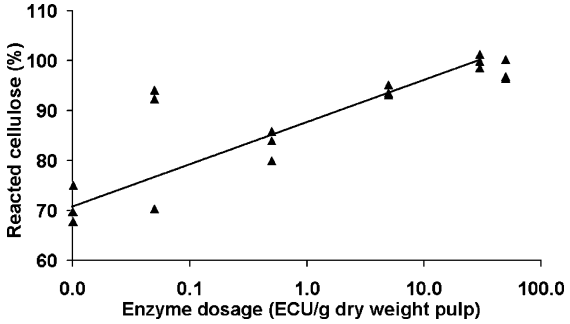

Figure 2. Reactivity, of enzyme treated dissolving pulp, according to Fock versus endoglucanase dosage: 0, 0.05, 0.5, 5, 30, and 50 ECU/g dry weight pulp. The incubation time of the enzyme treatment was 3.5 h.

$Na_2S_2O_3$ (mol/L), a = the first dilution to 100 g and outtake of 10 mL (10.4 g) = 100/10.4 = 9.62, and b = the second dilution of the sample to 100 mL and outtake of 40 mL = 100/40

GPC Analysis. The molecular weight distribution of the pulp samples were determined by GPC in a liquid chromatograph, PL210, with a refractive index detector. The mobile phase of 0.5% LiCl/DMAc was pumped into the system at a flow rate of 1 mL/min. Columns were 2xPL mixedA preceded by a guard column. The system was operating at 70 °C. The samples were dissolved in LiCl/DMAc 8% with derivatization, and the injection volume was 50 μL.

Yield Determination. The pulp yield of the enzymatic and acid treated pulp samples was measured by determination of released reducing carbohydrates in the filtrate after incubations using dinitrosalicylic acid (DNS) assay method.[20] The DNS reagent contained 1% dinitrosalicylic acid, 1% NaOH, 10% NaK tartate, 0.05% Na_2SO_3, and 0.2% phenol. Glucose standards (concentration range 0.5−2.0 mM) were prepared. Samples and glucose standards of 500 μL were mixed with 500 μL of DNS reagent and boiled for 30 min. The boiling initiates the DNS-reducing end reaction. The mixtures were cooled and centrifuged at 10 000 rpm for 5 min. The concentration of the reduced DNS is proportional to the concentration of reducing end groups and can be determined by UV−vis spectroscopy at 516 nm. The concentrations were calculated from the standard calibration curve.

Determination of Viscosity. The viscosities of the enzyme-treated samples and of the references were determined according to SCAN−CM 15:99.

3. Results and Discussion

When the dissolving pulp is treated with a monocomponent endoglucanase, the reactivity according to Fock's method is considerable increased, as shown in Figure 2. There is a linear relationship between the reactivity and the logarithm of the enzyme dosage up to 30 ECU/g dry weight pulp. The reactivity has reached a maximum of 100% already with the addition of 30 ECU/g dry weight pulp.

Figure 3 shows that the reactivity of the endoglucanase-treated dissolving pulp increases rapidly, the reactivity having reached almost 100% within 10 min of incubation.

What is the mechanism behind the increase in reactivity? Cellulases are divided into cellobiohydrolases (CBHs) and endoglucanases (EGs), and the most efficient cellulolytic organism produces several of each type.[21] Today, pure monocomponent cellulases of the endoglucanase type are commercially available. The product used in this experiment, Novozyme 476, is an endoglucanase gene from *Humicola insolens* transferred to an *Aspergillus* species under control by a strong promoter. According to the manufacturer the preparation consists of almost pure endoglucanase with no contamination of other cellulases. Thus, we assume that the effects of the product are due to this enzyme and not of any possible contaminant. The endoglucanase preferably degrades amorphous rather than crystalline cellulose and cleaves the cellulose randomly within the chain.[21,22]

Since less ordered or amorphous regions occur on the surface and between the microfibrils[2,23] endoglucanse treatment may

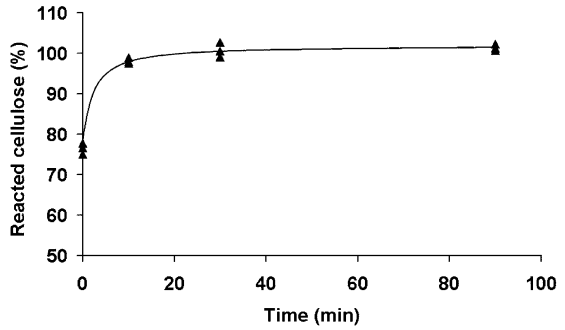

Figure 3. Reactivity, of enzyme treated dissolving pulp, according to Fock at different incubation times: 0, 10, 30, and 90 min. The enzyme dosage was 27 ECU/g dry weight pulp.

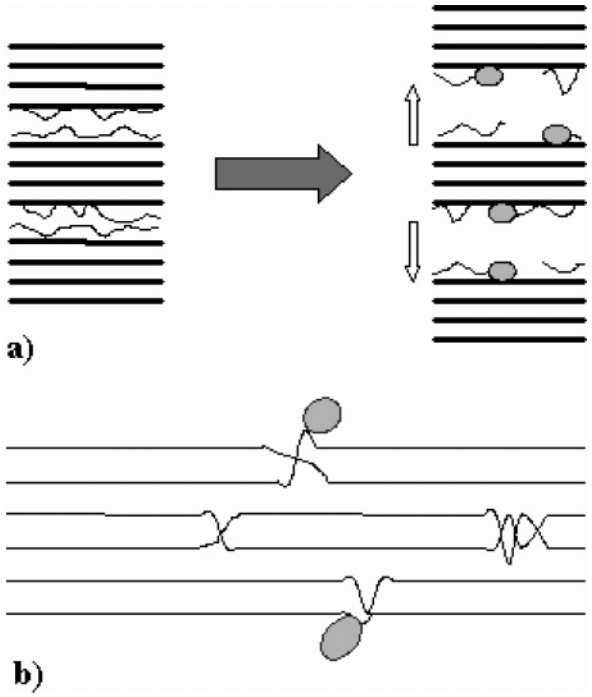

Figure 4. Hypothesis for endoglucanase attacks. (a) The enzyme attack on amorphous regions between the crystalline microfibrils, which leads to swelling. Gray oval = enzyme, black bar = crystalline regions, lighter jagged lines = amorphous regions. (b) Endoglucanase attack on less ordered region of the fibril surface. The gray ovals represents the enzyme.

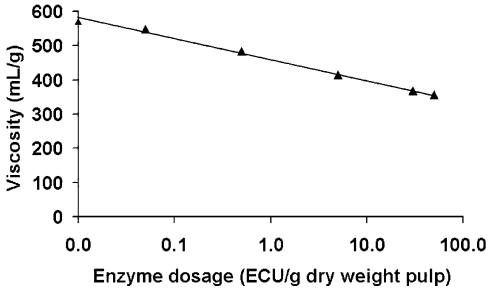

Figure 5. Viscosity of the dissolving pulp samples after enzyme treatment with different endoglucanase dosages: 0, 0.05, 0.5, 5, 30, and 50 ECU/g dry weight pulp.

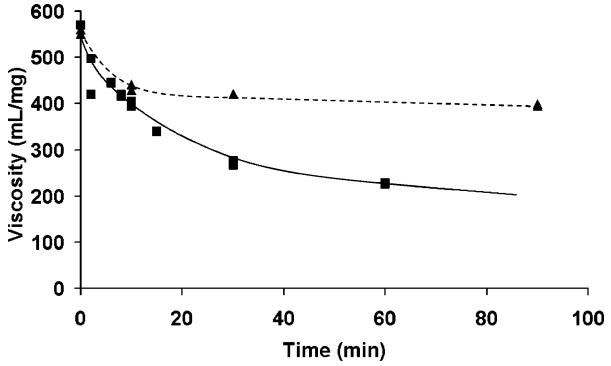

Figure 6. Viscosity of the dissolving pulp samples after enzyme treatment (▲, - - -) with endoglucanase 27 ECU/g dry weight pulp at different incubation times, 0, 10, 30, and 90 min. Viscosity of the dissolving pulp samples after different acid hydrolyze times (■, —). The hydrolyze times were 0, 2, 6, 8, 10, 15, 30, and 60 min.

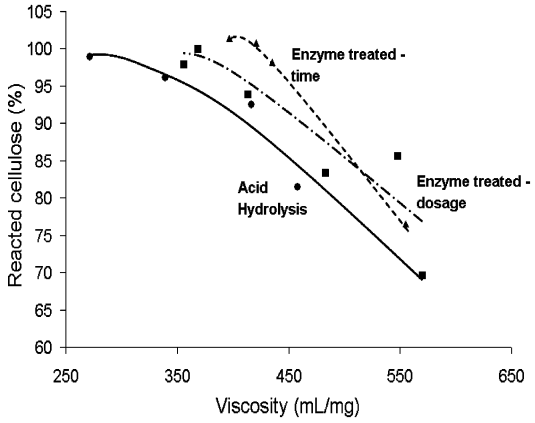

Figure 7. Reactivity according to Fock versus viscosity of dissolving pulp samples subjected to different treatments: (▲, - - -) treated with endoglucanase with different incubation times (from the left in the figure, 90, 30, and 10 min and the reference), (■, — · —) treated with endoglucanase with different enzyme dosage (from the left in the figure, 50, 30, 5, 0.5, 0.05, and 0 ECU/g dry weight pulp), (●, —) treated by acid hydrolysis for 30, 15, 8, 2, and 0 min (from the left in the figure).

lead to a swelling of the cell wall and thus an increase in accessibility to solvents and reagents, as shown in Figure 4.

Table 1. Pulp Yield of the Enzymatic and Acid Treatments Using Dinitrosalicylic Acid (DNS) Assay Method

treatment	yield (%)
varying enzyme dosage; 0−50 ECU/g dry weight pulp	99.1−99.5
varying incubation time of the enzyme; 0−90 min	99.1−99.4
acid hydrolyze with different incubation times; 0−60 min	99.3−99.6

Another possibility is that the microfibrils contain some cellulose II that the endoglucanase can attack and thereby increase the reactivity.[19] Dissolving pulp have been shown to contain more cellulose II than most pulps.[24,25] This polymorph is most probably formed during alkaline condition of the bleaching process. Cellulose II is considered to be less reactive due to a tighter chain packing compared to cellulose I.[12] If the endoglucanases have a higher affinity to cellulose II, the enzyme treatments could result in a decreased amount of cellulose II followed by an increase in reactivity.

However, endoglucanase activity also lowers the degree of polymerization, and that cannot be ruled out as an explanation of the increase in reactivity. The viscosity has been measured, and the results presented in Figures 5 and 6 show that a lower viscosity is associated with higher enzyme dosages and longer reaction times. The yield losses, presented in Table 1, are insignificant in all of the pretreatments. To study the relationship between viscosity and reactivity, some acid hydrolyses were performed on the dissolving pulp. The viscosity (Figure 6) and the reactivity according to Fock were then determined.

The result of the reactivity measurement was interesting since it showed some differences between samples treated with enzymes and those subjected to acid hydrolysis. At a given viscosity, the enzyme-treated samples had a higher reactivity than the samples subjected to acid hydrolysis, Figure 7. The time dependence showed a greater increase in reactivity for a given loss in viscosity. These results indicate that the increase in reactivity according to Fock is not only due to a decrease in the degree of polymerization. The results of the molecular weight distribution support this assumption. In Figure 8, the molecular weight distribution for samples (one from each of

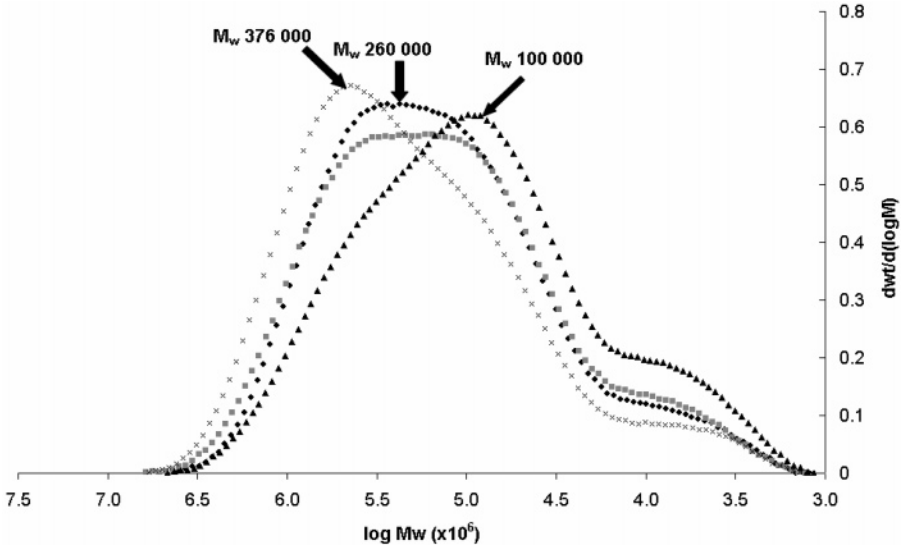

Figure 8. Molecular weight distribution for samples with 100% reactivity according to Fock. (■) Endoglucanse treatment for 30 min and 30 ECU/g dry weight pulp, (●) endoglucanse treatment for 30 min and 27 ECU/g dry weight pulp, (▲) acid hydrolyses for 30 min, (×) the reference pulp.

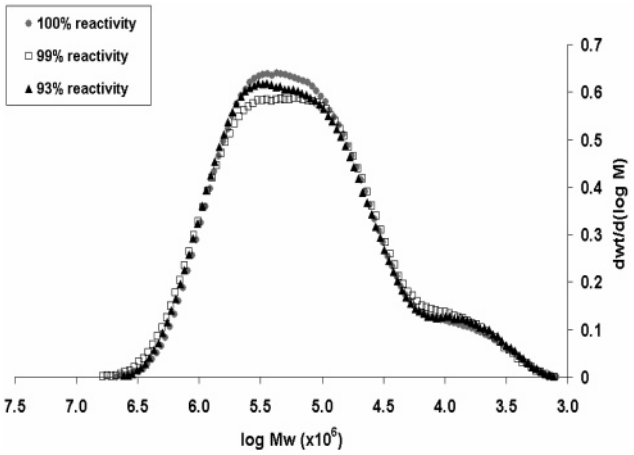

Figure 9. Molecular weight distribution of three different samples with similar distributions. (□) Endoglucanse treatment for 30 min and 30 ECU/g dry weight pulp, (●) endoglucanse treatment for 30 min and 27 ECU/g dry weight pulp, (▲) acid hydrolyses for 8 min.

the three different treatments) that gave 100% reactivity, with as short incubation time respective low dosage as possible, is presented. To achieve maximum reactivity for the pulp treated with acid hydrolyses the molecular weight distribution has to be changed more, and the molecular weight has to be lower compared to the enzymatic-treated pulp. When samples, with similar molecular weight distribution, from each group of pretreatments are compared, the reactivity according to Fock differs, Figure 9. The pulp treated with enzyme has a reactivity of 100% while the pulp treated with acid only has a reactivity of 94%, a considerable difference.

4. Conclusions

- Treatment with the monocomponent endoglucanase Novozyme 476 increases the reactivity of dissolving pulp for the viscose process. Optimal effect can be obtained with less than 20 min incubation time and moderate dosage.
- The activation effect seems not to be correlated only to a decrease in viscosity based on comparisons with strong acid hydrolyzed pulp.
- Neither gives the molecular weight distribution of the cellulose the explanation for the activation effect, thus some other mechanism must be involved as enhancement of swelling of the cellulose structure.
- The possibilities for applying the method industrially seem promising.

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