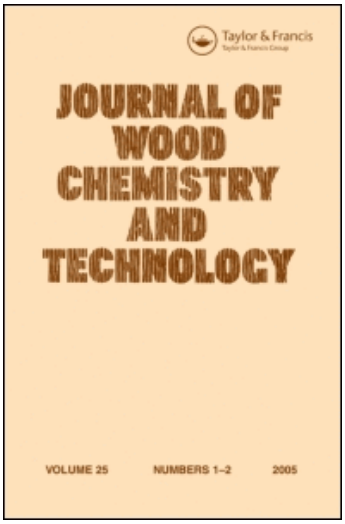

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Action of Xylanases on Chemical Pulp Fibers Part II : Enzymatic Beating

P. Noé[a]; J. Chevalier[a]; F. Mors[b]; J. Comtat[b]
[a] Centre Technique de l'Industrie des Papiers, Cartons et Celluloses, Grenoble cedex, France [b] Centre dt. Recherches sur les Macromolécules Végétales, France

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ACTION OF XYLANASES ON CHEMICAL PULP FIBERS

PART II : ENZYMATIC BEATING

P. Noë*, J. Chevalier*, F. Mora** and J.Comtat**
*Centre Technique de l'Industrie des Papiers,
Cartons et Celluloses,
B.P. 7110 - 38020 Grenoble cedex (France)

**Centre de Recherches sur les Macromolécules Végétales, C.N.R.S.
B.P. 68 - 38402 Saint Martin d'Hères cedex (France).

ABSTRACT

Two chemical bleached pulps were treated with a crude enzyme mixture in the presence of $HgCl_2$ (1 mM). Such treatment inhibited endo-cellulases. Xylans were thus subjected to selective in-situ hydrolysis. The enzyme-treated pulps can be compared to slightly beaten pulps. The modified fibers show external fibrillation and reveal strong beatability. Therefore, a decrease in energy demand can be gained in the papermaking process.

A reduction of fiber intrinsic strength is, however, observed mainly due to the hydrolysis of xylan macromolecules which confirms their important role in fiber wall cohesion.

** Laboratoire propre du C.N.R.S., associé à l'Université Scientifique, Technologique et Médicale de GRENOBLE.

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INTRODUCTION

The pulp and paper industry is concerned in many respects with biotechnology research and development. According to Kirk[1], biopulping, biobleaching, and biotechnical improvement of pulp have a potential impact on the industrial processing of the future. Most works on these subjects deal with the action of fungi on wood and mechanical pulp[2-5]. In a recent work, Paice and Jurasek[6] have used an enzymatic complex enriched in xylanases to obtain a dissolving pulp through a biological process, instead of the usual extraction of xylans by ultra-hot alkaline process ; but the target of digesting all but 2 % of the total pentosans could not be obtained. As can be understood from these examples, research in biotechnology is devoted to replacing energy and chemical consuming processes by less costly and more versatile enzymatic treatments.

To our knowledge, refining of chemical pulps is a field where biotechnology has not yet been applied. It has been shown, however, that the amount of theoretical energy needed to obtain a complete separation of the microfibrils is only about one-tenth of the total energy demand[7], therefore, most of the work is dissipated as heat released to the surrounding fluid.

Beating and refining are papermaking processes elaborated to create desirable structural changes in the fiber cell wall[8]. The visible aspect of fiber modifications is the external fibrillation, but the delamination of internal cell wall layers, or fiber swelling, is the most important feature for paper strength, since it brings flexibility to fibers and thereby bonding ability. That is why any means of loosening the internal cohesion of the cell wall will decrease the fiber resistance to swelling and help reduce the energy demand in beating.

As demonstrated in a previous paper[9] (Part I), xylans can be hydrolyzed inside the fiber wall, resulting in the formation of

micropores which increase the specific area of pulp. The wall cohesion must certainly be affected ;the present work is devoted to the study of the expected wall loosening in relation to beatability and papermaking properties of the enzymatically treated pulps.

MATERIALS

Enzymes and pulps (See Part I).

The crude enzymatic mixture used throughout this work is the culture filtrate of the Basidiomycete Sporotrichum dimorphosporum.

Two chemical pulps received as dried sheets were used : a bleached kraft pulp from birchwood and a bleached sulfite pulp from sprucewood.They were acid hydrolyzed according to Saeman et al.[10] and their neutral sugar composition has been previously given[9].

METHODS

Enzymatic treatment of pulps.

The dry pulp was soaked overnight at room temperature in an aqueous solution of 1 mM $HgCl_2$. It was then filtered, washed with the 1 mM $HgCl_2$ solution and stirred to obtain a pulp slurry. The crude enzymatic complex was shaken for 15 min in the 1 mM $HgCl_2$ solution and then poured into the slurry.The final volume was 1L and the pulp consistency was 3 %. The crude enzyme concentration was 60 $mg.L^{-1}$and 120 $mg.L^{-1}$ for Kraft birch and sulfite spruce pulps respectively. The incubation was performed at 40°C under gentle stirring. No mechanical treatment causing breakage or damage to the fibers was used at this stage of the experiment. The treatment time was varied from 2 to 54 h. The pulp was then

filtered, washed, and soaked for 30 min at 25°C in a solution of HCl (0.05N) to stop the enzyme activities. The reference pulp was obtained by the same treatment except that no enzymes were used. The filtrate and the washings were collected and heated for 20 min in a boiling water bath. The soluble sugars were measured by the reducing end-group determination[11], and expressed as xylotriose, the most abundant soluble sugar. The percent mass loss was the ratio of the weight of xylotriose to the weight of initial pulp.

Pulp and paper characterizations

Schopper Riegler and water retention values were measured for both the control and the enzymatically treated pulps and handsheets were formed on a Rapid-Köthen sheet machine. Density, tensile strength and wet zero-span tensile strength were measured. A part of the treated pulp was also beaten in a JOKRO mill until 60°SR were reached. Sheets were formed after beating and mechanically tested.

The Schopper Riegler values:°SR(ISO standard 5 267/1-1979) measure the drainage ability of a pulp related to its hydrophilic properties. This index is mainly influenced by fiber fibrillation and by the amount of fine elements. It is used as a measure of the beating progress.

The water retention value (WRV) measures the amount of water retained inside the fiber wall after centrifugations[12]. It is correlated to the extent of fiber swelling due to the internal fibrillation.

The fiber flexibility producing the strength of the fiber network in the paper sheet was measured by the apparent density on eight handsheets according to ISO standard 438-1980.

The tensile strength was registered on an Instron Extensometer 1122(ISO Standard 1924-1983) and breaking lengths computed. The wet zero-span tensile strength was measured on a Pulmac zero-span Tensile Tester (Model ZST-15). Wet samples were

prepared by pressing between blotters a dry sheet after being thoroughly soaked. The value obtained is assumed to be the intrinsic fiber strength.

Pulp viscosities were measured in cuene according to TAPPI method T 230.

A special test was set up to check the beatability of the pulp : the time-to-reach 60° SR in the JOKRO mill. The JOKRO mill was run according to standard conditions (ISO Standard 5264/3-1979).

RESULTS

Inhibition efficiency.

From filtrate analyses and molecular weight distribution curves (See Part I), we have concluded that endo-cellulases were appropriately inhibited in the 1mM $HgCl_2$ medium. The activity of the enzymatic complex is thus directed towards hemicelluloses and particularly towards xylans in the case of the hardwood pulp.

Enzymatic treatment effects on pulp characteristics.

Tables 1 and 2 collect the results related to the two pulps.

The mass loss increases slightly, then remains constant at a low level even for long periods of attack.

The Schopper Riegler index continuously increases under enzymatic treatment up to a value of 32°SR in the case of birch pulp;a more limited value of 19°SR is obtained for spruce pulp. The enzymatic treatment yields externally fibrillated fibers. This effect is clearly visible on micrographs[9] (Part I). It had been already observed by Bolaski[13] on cotton fibers treated with cellulases.

WRV increases too, showing a significant fiber swelling. This modification occurs however to a lesser extent in the case of spruce pulp when compared against birch pulp.

TABLE 1

Pulp Characteristics of the Enzyme-Treated Pulp from Birch.

Treatment time (h)	0	2	6	9	15	21.5	24
Mass loss (%)	0	0.7	1.1	1.6	1.4	1.7	1.8
Schopper-Riegler index	18	17	17	20	20	24	32
WRV ($g.g^{-1}$)	1.10	1.33	1.50	1.58	1.60	1.90	1.98
Viscosity (cP)	15.7	11.2	12.1	10.9	12.4	10.5	10.2
Apparent density ($Kg.\ m^{-3}$)	580	580	580	620	600	650	680
Breaking length (Km)	2.2	2.3	2.8	3.8	3.5	3.7	4
Wet zero-span Breaking length (Km)	9.6	3.5	3.8	4.9	4.1	3	2.1
Time-to-reach 60°SR (min)	50	35	20	18	19	10	5
Apparent density($Kg.m^{-3}$) after beating at 60°SR	900	900	890	870	880	800	760
Breaking length after beating at 60°SR (Km)	8.3	5.1	5.3	4.6	4.9	3.9	4

TABLE 2

Pulp Characteristics of the Enzyme-Treated Pulp from Birch.

Treatment time (h)	0	5	9	24	30	54
Mass loss (%)	0	0.5	0.9	1.0	0.8	1.0
Schopper-Riegler Index	13	14	14	14	19	19
WRV ($g.g^{-1}$)	1.00	1.20	1.32	1.37	1.58	1.63
Viscosity (cP)	28.2	17.6	17.7	19.2	19.5	17.6
Apparent density ($Kg.m^{-3}$)	520	550	570	610	610	650
Breaking length (Km)	0.9	2	2.4	2.5	2.9	3.5
Wet zero-span breaking length (Km)	7.5	5.5	4.3	6.5	6.7	6
Time-to-reach 60°SR (min)	55	25	35	35	22	20
Apparent density after beating at 60° SR	910	970	940	870	850	870
Breaking length after beating at 60° SR (Km)	6.7	5	4.5	5	4.4	4.9

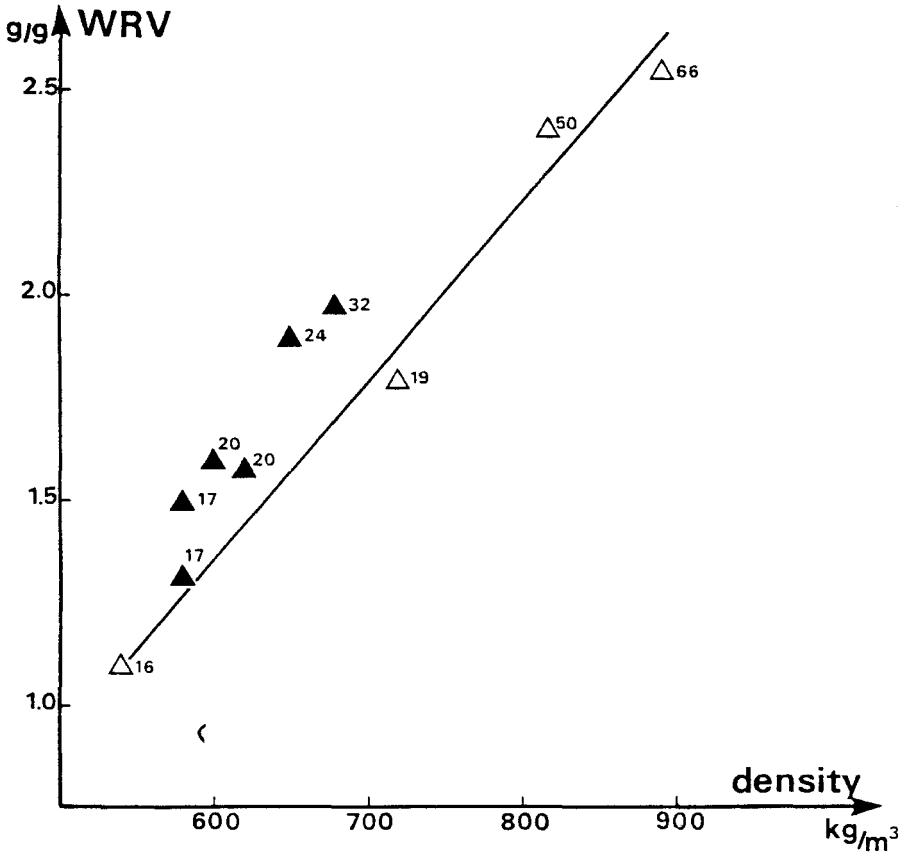

FIGURE 1A. Correlation between WRV and sheet apparent density for birch pulp. (△) Control pulp untreated, beaten in a JOKRO mill.(▲) Enzyme-treated pulp, unbeaten. The figures beside the plots indicate °SR values.

Viscosity rapidly decreases then levels off at values of 10.5 and 18.0 cP for birch and spruce pulps respectively. Hydrolysis of the polysaccharide chains seems to occur at the beginning of the enzymatic attack and then stops. We know however from the molecular weight distribution curves that cellulose is not affected by this hydrolysis. The decrease in viscosity in CUENE is thus solely due to hemicellulose chains rupturing.

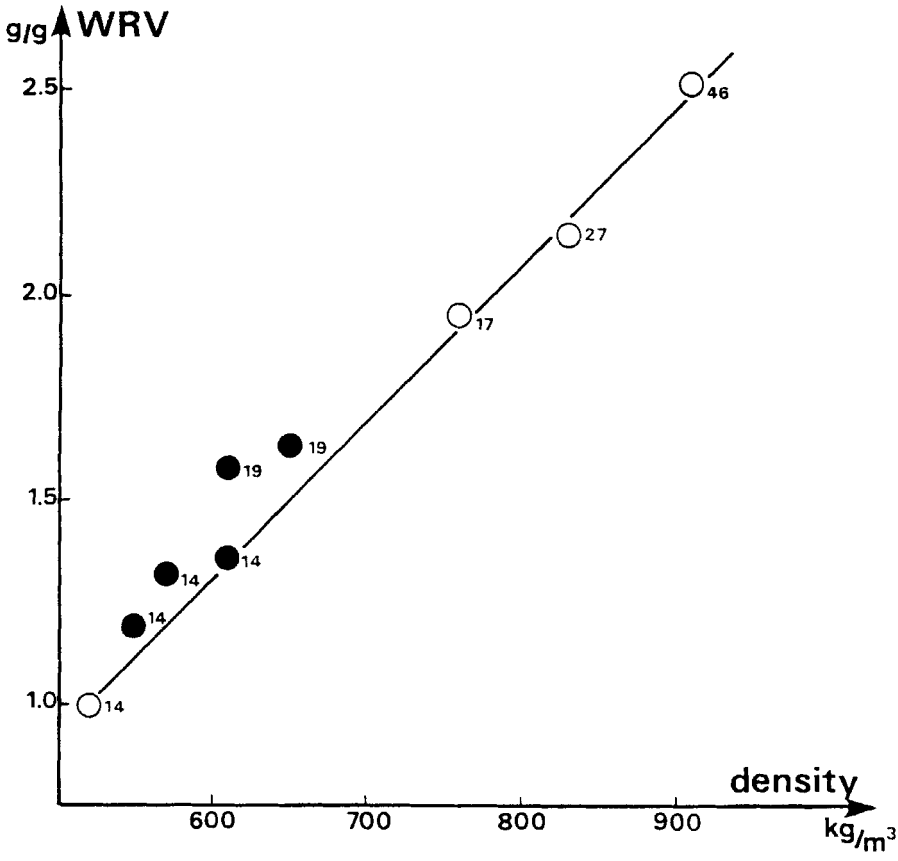

FIGURE 1B. Correlation between WRV and sheet apparent density for spruce pulp.(○) Control pulp, untreated, beaten in a JOKRO mill. (●) Enzyme-treated pulp, unbeaten. The figures beside the plots indicate °SR values.

Enzymatic treatment effects on papermaking properties.

After treatment, a part of the pulp was used for handsheet forming. Density, breaking length and zero-span breaking length were measured.

The density, breaking length, and WRV of the enzymatically treated pulps compare equally to slightly beaten pulps.

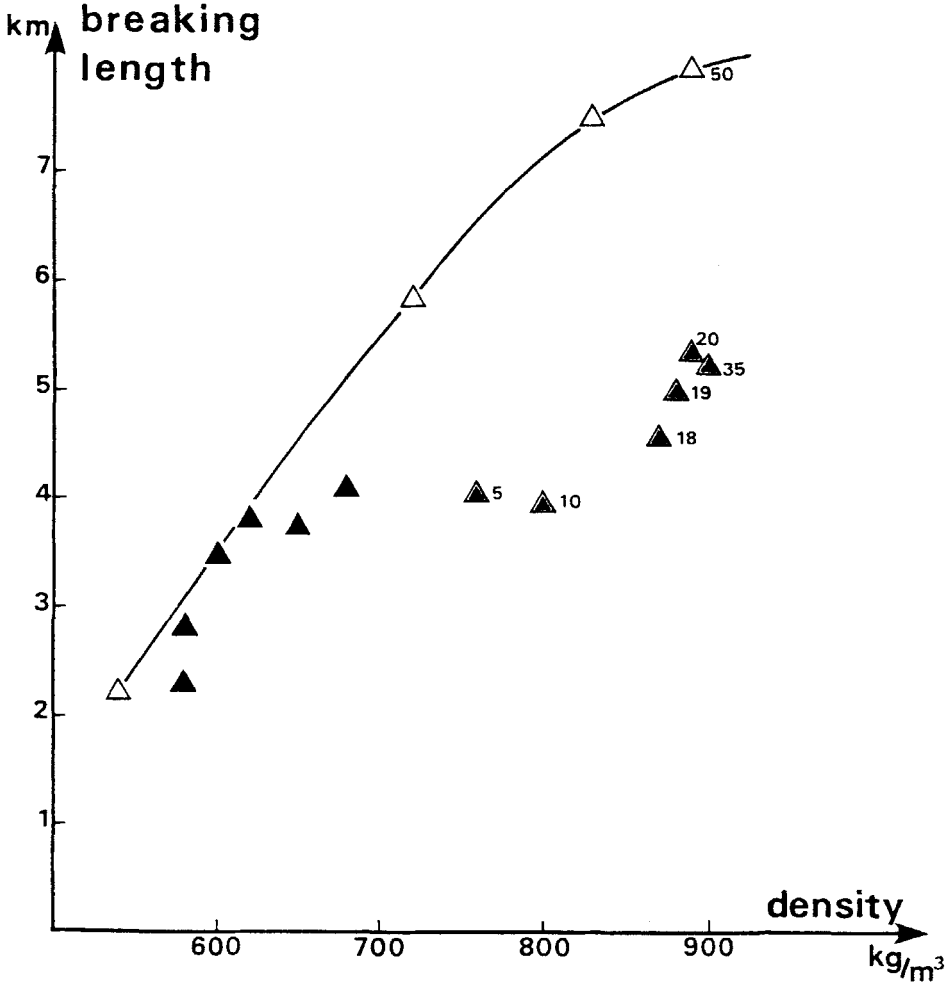

FIGURE 2A. Correlation between breaking length and sheet apparent density for birch pulp. (△) Control pulp,untreated, beaten in a JOKRO mill. (▲) Enzyme-treated pulp, unbeaten. (▲) Enzyme-treated pulp after beating at 60°SR. The figures beside the plots indicate the time (min) to reach 60°SR.

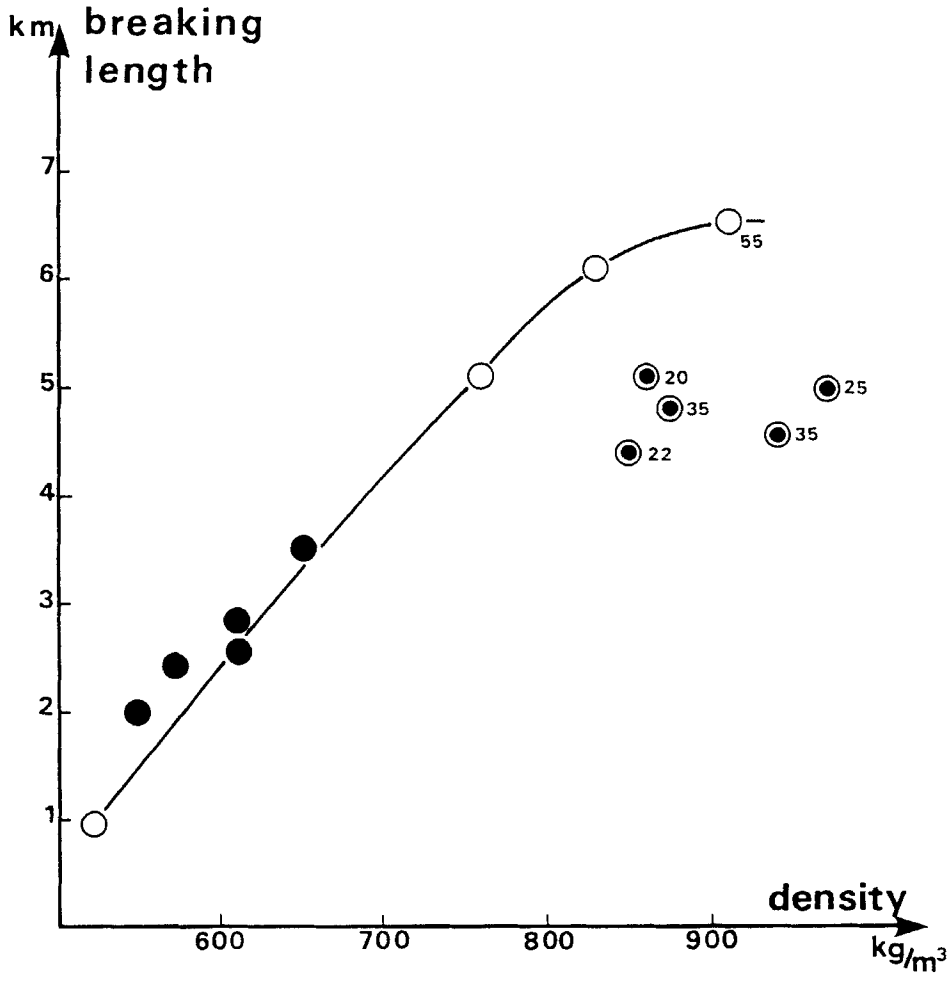

FIGURE 2B. Correlation between breaking length and sheet apparent density for spruce pulp. (○) Control pulp, untreated, beaten in a JOKRO mill. (●) Enzyme-treated pulp, unbeaten. (◉) Enzyme-treated pulp after beating at 60°SR. The figures beside the plots indicate the time (min) to reach 60°SR.

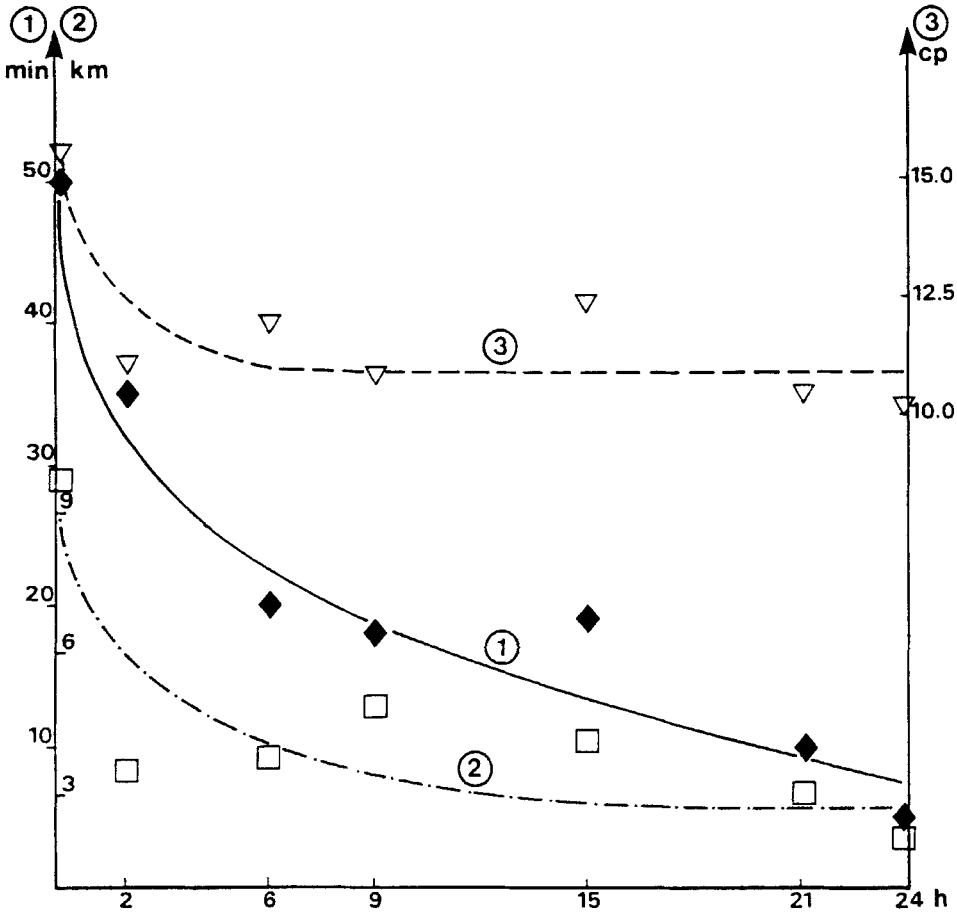

FIGURE 3A. Influence of enzyme treatment time on : (1) time-to-reach 60°SR (min), (2) wet zero-span breaking length (Km), (3) pulp viscosity (cP), for birch pulp.

The density increases, as expected from the increase in WRV (Tables 1-2), the more flexible the fibers the more densely packed sheets are created. Figs. 1A, 1B give the correlation between density and WRV, they show higher WRV at equal density for the enzyme-treated pulps than for control pulps. This can be attributed to pores formation and to changes in the morphology of

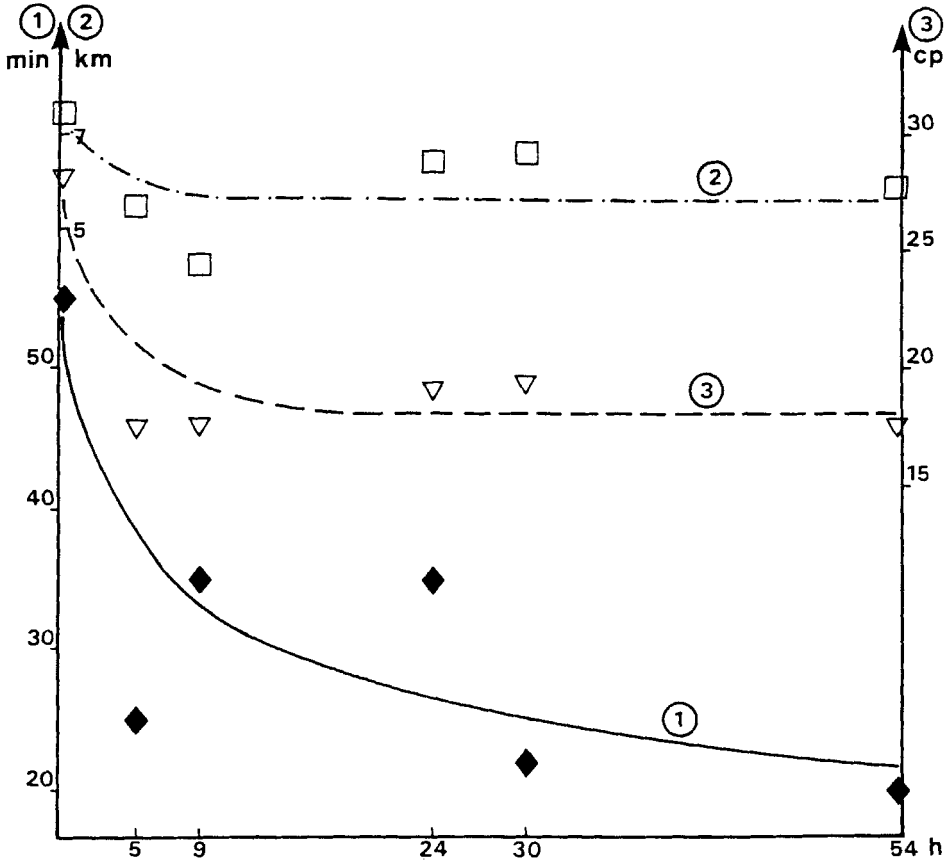

FIGURE 38. Influence of enzyme treatment time on : (1) time-to-reach 60°SR (min), (2) wet zero-span breaking length (Km), (3) pulp viscosity (cP), for spruce pulp.

the outer part of the fiber, the so-called extra-fiber WRV[12] in relation with higher °SR values.

Breaking length vs. density is plotted on Figs. 2A - 2B. The increase in density as well as external fibrillation resulting from enzyme-treatment only, without further beating, brings about an improvement in bonding strength. The number of bonds increases owing to higher flexibility and to larger bonded areas.

The curves of the zero-span breaking length vs. treatment time (Figs. 3A - 3B) have the same profile as that of the viscosity curves,it may be observed a rapid fall and a subsequent levelling-off. The paramount importance of carbohydrate chain length for fiber intrinsic strength has been previously demonstrated[14], although, in that case, degradation of all the carbohydrates (including cellulose) was carried out, whereas in the present work only hemicelluloses, mainly xylans, are severed by the enzymatic hydrolysis.

Enzymatic treatment effects on beatability and papermaking properties.

The beatability of pulps was tested as the time-to-reach 60 °SR under standard conditions in a JOKRO mill. Afterwards sheets were formed on the sheet-machine and density and breaking length measured (Tables 1-2).

The different times-to-reach 60° SR were plotted vs.enzymatic treatment time. The curves show a continuous decrease (curve 1, Figs. 3A and 3B), a beating time as low as 5 min was measured for birch pulp treated during 24 h . The cell wall loosening action of the xylanases that was found at this stage, was the most impressive effect.

The sheet densities obtained after beating of the treated pulps to 60°SR compare to those of the beaten control pulps, except for birch pulp samples which were subjected to enzymatic hydrolysis during the longest times and which were beaten during 5 min or 10 min only (Figs. 2A-2B).

The breaking length of each pulp after beating at 60°SR is smaller than that for the control sample (Tables 1-2). This is due to lower intrinsic fiber strength. Breaking length, as well as zero-span breaking length and viscosity, falls at the beginning of the treatment then levels-off at 4.2 and 4.9 km for enzyme-treated birch and spruce pulps respectively.

DISCUSSION

The action on pulps of the crude enzymatic complex in which endo-cellulases were inhibited can be summarized in terms of external and internal fibrillation. These effects are desirable for papermaking properties. Breaking lengths as high as 4 km are obtained with birch and spruce pulps after the enzymatic treatment only. However, this value remains lower than those obtained with the control pulps which are slightly beaten, for example a 10 min beating treatment gives 5 and 6 km for spruce and birch pulps respectively. It can be concluded that the enzymatic treatment, by itself, is not sufficient to create the necessary loosening of the cell wall structure and the fiber swelling suitable for good fiber flexibility and high bonding strength.

The fibrillation seems favored, especially for birch pulp, since a value as high as 32°SR was obtained after a 24 h enzyme treatment.The beating of enzyme-treated pulps can be performed in very short times as far as °SR is concerned. In this respect, it has been demonstrated that beatability was greatly enhanced by the enzymatic treatment. However, densities of such beaten pulps are lower than those of the control pulps at the same °SR. Since density measures the amount of the specific refining energy[15], this index has to be taken into account when economic assessment is desired. On the basis of equal density, it can be deduced from these results that the energy demand is reduced about three-fold.

A patent[16] has been taken out following the results of the selective action of xylanases on pulps. Under the running title "Enzymatic Beating", it concerns the use of xylanases in papermaking procedures and the pulps so obtained.

Two features related to the xylan "in situ" hydrolysis are interesting and need more discussion : the improved beatability of pulps and the reduction of fiber intrinsic strength. The zero-span breaking length vs.enzyme-treatment time shows a rapid

decrease, then a levelling-off. This behavior is clearly correlated with the viscosity of the dissolved pulps. Since viscosity involves essentially high molecular weight molecules of cellulose, hydrolysis of xylan can continue without being further detected by viscosity. Thus, assuming that the decrease of xylan molecular weights occurs throughout the entire enzymatic attack, chain ends are continously produced, which improves water accessibility, fiber wall loosening and fiber swelling. Furthermore, this increasing release inside cell wall of hydrophilic hydrolysis products from xylan enhances beatability[17].

The xylan molecular weight seems also to be of great importance for fiber intrinsic strength. Actually, the fiber is described as a composite material[18], made of a framework (cellulose microfibrils) and a matrix (hemicelluloses). When a stress is applied to one fiber, the load is carried by both components. As xylan chains are cleaved, they do not contribute any more to fiber strength. Then, the levelling-off value of the intrinsic strength must involve the cellulose microfibrils strength only. Indeed, the little exo-cellulase (cellobiohydrolase) activity which can influence beatability, w.r.v. and perhaps reduces the fiber intrinsic strength to a slight extent, cannot account for the important modifications of the pulp properties. Thus, we conclude that the hydrolysis of xylans is the main cause of the observed changes in pulp and papermaking properties.

CONCLUSION

This work is concerned with the role of xylans in cell wall cohesion and papermaking properties. The use of a crude enzymatic mixture in which endo-cellulases have been inhibited has allowed an important attack of the xylan chains inside the fiber cell-wall, yielding only little mass loss.

Enzyme-treated pulps have shown enhanced beatability and better bonding ability due to increased fiber flexibility. A lessening of fiber intrinsic strength has also occured, pointing out the importance of xylans having chains with a high degree of polymerization for fiber mechanical resistance.

These results are promising and deserve attention from the technologists. Furthermore, if the inhibition with $HgCl_2$ is totally unrealistic in industrial processes, a solution can be realized by continued research in the biotechnological development of cellulase-less mutants in fungi[19] or by the production of pure xylanases by genetically modified bacteria[20]. Alternatively, we are presently investigating a very simple treatment which brings about the total selective elimination of cellulase activities from a crude enzymatic mixture in a way which would avoid pollution.

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REFERENCES

1. T.K. Kirk, T.W. Jeffries, and G.F. Leatham, Tappi, 66 (5), 45 (1983).
2. K-E. Eriksson and L. Vallander, Svensk Papperstidn. 85 (6), R 33 (1982).
3. S.S. Bar-Lev, T.K. Kirk, and H-M Chang, Tappi, 65 (10), 111 (1982).
4. L. Pilon, M. Desrochers, L. Jurasek, and P.J. Neumann, Tappi, 65 (6), 93 (1982).
5. L. Pilon, M.C. Barbe, M. Desrochers, L. Jurasek, and P.J. Neumann, Biotechnol. Bioeng., 24, 2063 (1982).
6. M.G. Paice and L. Jurasek, J. Wood Chem. Technol., 4 (2), 187 (1984).
7. R.H. Attala and C. Wahren, Tappi, 63 (6), 121 (1980).
8. K. Ebeling, International Symposium on Fundamental Concepts of Refining, IPC, Appleton, 1 (1980).
9. F. Mora, J. Comtat, F. Barnoud, F. Pla, and P. Noé, this journal Part I.

10. J.F. Saeman,, W.E. Moore, R.L. Mitchell, and M.A. Millet, Tappi, 37, 336 (1954).
11. M. Somogyi, J. Biol. Chem., 195, 19 (1952).
12. J. Silvy, G. Sarret, and F. Jestin, Proc. of the European Congress on Pulp and Paper Technology, Venice, 169 (1964).
13. W. Bolaski and J.C. Gallatin, U.S. Patent 3,041,246, (1962).
14. H.A. Swenson, Svensk Papperstidn., 78 (2), 61 (1975).
15. B.A. Amero, Tappi, 65 (2), 57 (1982).
16. J. Comtat, F. Mora, and P. Noé, French patent n° 84/00448.
17. A.J. Watson, Appita, 17 (2), 54 (1963).
18. F. El-Hosseiny and D.H. Page, Fibre Sci. Technol., 8, 21 (1975).
19. P. Ander and K-E Eriksson, Svensk Papperstidn., 78, 643 (1975).
20. R. Bernier Jr., H. Driguez, and M. Desrochers, Gene, 26, 59 (1983).