The Effect of Enzymatic Treatments of Pulps on Fiber and Paper Properties

P. Žnidaršič-Plazl,[a,*] V. Rutar,[b] and D. Ravnjak[c]

[a] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Aškerčeva 5, 1000 Ljubljana, Slovenia

[b] Pulp and Paper Institute, Bogišičeva 8, 1000 Ljubljana, Slovenia

[c] Papirnica Vevče d.o.o., Papirniška pot 25, 1261 Ljubljana Dobrunje, Slovenia

Original scientific paper
Received: May 29, 2009
Accepted: September 30, 2009

Dedicated to the memory of Professor Dr. Valentin Koloini

Biotechnological treatment of pulps provides great potential for the reduction of energy consumption and greenhouse gas emissions. In the present work, the influence of different commercial cellulases and xylanases or their mixtures on the quality of different bleached kraft pulps was investigated. The effects of enzymes on reducing sugars release and changes in fiber length were assessed for 3 different eucalyptus pulps, for a pulp consisting of a mixture of hardwoods and for softwood pulp. Despite the extremely high enzyme concentrations used in this part of the study, almost no change in fiber length was observed for pulps treated with enzyme preparations alone, while all combinations of cellulases and xylanases resulted in significant changes in average fiber length, portion of fines and reducing sugars release. Besides, vessel cell deformation was observed as a consequence of all enzymes applications. Furthermore, physical and mechanical properties of laboratory sheets made with enzymatically treated pulps were evaluated for various refining conditions and compared to those of untreated pulps. Potential energy savings up to 17 % were achieved with enzyme treatment, but some decrease in pulp quality was also observed.

Key words:
Cellulases, xylanase, kraft pulp, fiber length, paper properties, vessel cells

Introduction

Paper production is an energy-intensive process and the improved energy efficiency is therefore a matter of high priority for the paper industry. Besides, reduction of the effluents from combustion of fossil fuels is the priority goal for sustainable development. In the era of very high economic and environmental concerns, minimizing the environmental impacts of paper manufacturing is of key importance.

Based on several literature reports, summarized in review articles,[1–5] biotechnological treatments of pulp provide great potential for the reduction of energy consumption and greenhouse gas emissions, as well as for the improvement in the efficiency of unit operations and quality of final products. The advantages of biotechnology implementation include the specificity of reactions which can be performed at mild conditions, environmentally friendly processes, only minor changes in the existing industrial processes, energy saving and, ultimately, costs reduction.[3,5] The use of different enzymes in pulping, pulp bleaching and deinking of recycled pulps, as well as in pitch degradation is nowadays well established. In addition, there has been a growing interest in enzyme-assisted fiber modifications, necessary for improving pulp properties, including decreased vessel picking from tropical hardwood pulps.[1,3]

Pulp quality has no general definition and depends on the end product quality demands. Its assessment is mainly focused on the dewatering properties of the pulp, and physical and mechanical properties of paper sheets including tensile, burst and tear index. In the actual process of paper production, the drainage resistance of pulp, which depends on the fibrillation grade, is one of the most important parameters in paper sheet formation and influences the runnability of the paper machine. The most common method used for the determination of pulp's dewatering properties is the Schopper-Riegler degree (°SR), which enables us to control the refining process. The main purpose of refining is to increase the internal fibrillation and consequently fiber swelling and flexibility by cell wall degradation, and external fibrillation which results in increased outer surface area of fibers.[1]

* Corresponding author: Tel: +386 1 2419 533; e-mail: polona.znidarsic@fkkt.uni-lj.si

Physical characteristics and surface chemistry are both of great importance for the behavior of fibers and fines in papermaking and for the properties of final paper products.

Pulp fibrillation by cellulases for enhancing strength properties was patented already in 1959.[6] The effect of different hydrolases on the improvement of the refining process by means of reducing energy consumption, has been a matter of intensive research in the last two decades.[7-20] Different cellulases and hemicellulases have been proved to have a beneficial effect on refining and on surface fiber morphology, giving rise to better bonding properties and a closer structure of paper.[7,10-20] Furthermore, cellulase-free xylanase application has improved pulp fibrillation and water retention, shortened the time of refining in virgin pulps, enhanced the restoration of bonding and increased both the freeness in recycled fibers and the selective removal of xylan from dissolving pulps.[8,9]

Although the positive effects of using carbohydrases on both economy and environmental impact of the paper industry are well-documented, the exact mechanism by which enzymes act on fibers and affect paper properties is still not understood, and further basic research in this area is needed.[1] Besides, a detrimental effect of enzymatic hydrolysis on fibers and thereby final paper quality might limit the application of this sustainable technology. Excessive enzyme treatment might erode the fiber surface and reduce the strength of pulp.[3] For a particular pulp or fiber, the use of specific enzymes, their doses, and conditions regarding pH, temperature, retention time of enzyme treatment and refining conditions should be specified in order to maintain or even improve the desired paper quality at lower energy consumption for refining.[3]

In the present work, the influence of enzymatic treatment on the quality of various pulp types was studied. The effect of some commercial cellulases and xylanases on reducing sugars release, fiber length, and paper quality assessed by tear, burst and tensile index was evaluated under different treatment conditions.

Materials and methods

Enzymes

The following commercial enzyme preparations were tested: Novozym 342, produced by *Humicola* sp., comprising mainly endo-1,4-β-glucanase activity (3.2.1.4), as well as cellubiohydrolase, cellubiase, xylanase and other hemicellulases with declared activity of 90 EGU g^{-1}; Novozym 476, a monocomponent endo-1,4-β-glucanase produced by *Aspergillus* sp. with declared activity of 4500 ECU g^{-1}; Novozym 51024, a thermo-stable endo-1,4-β-xylanase (3.2.1.8) from *Thermomyces lanuginosus,* produced by genetically modified *Aspergillus oryzae* with declared activity of 650 FXU g^{-1}; and Pulpzyme HC, an endo-1,4-β-xylanase produced by *Bacillus* sp. with declared activity of 1000 AXU g^{-1}. All enzyme preparations were kindly donated by Novozymes Deutschland GmbH (Nieder-Olm, Germany). Further denomination of enzymes is based on the number (342, 476, 51024 for Novozymes) and HC for Pulpzyme HC.

Cellulose pulps

Five different commercial bleached kraft pulps were used throughout this study. Three different eucalyptus pulps or their mixtures were used, here denoted as EUC 1-pulp (HUELVA, 100 % *Eucalyptus globulus* from Spain), EUC 2-pulp (ARAUCO, 70 % *E. globulus* and 30 % *Eucalyptus nitens,* both from Chile) and EUC 3-pulp (SANTA FE, 30 % *E. globulus* and 70 % *E. nitens,* both from Chile), one short-fiber pulp denoted as SF-pulp (KOTLAS, 50 % poplar and 50 % birch), and one long-fiber pulp (BOHEMIA, 100 % spruce), denoted as LF-pulp.

Enzymatic treatment of pulps

Test-tube experiments

Small-scale experiments were performed in test tubes containing 11 mL of 1 % pulp suspensions with pH adjusted to 7.0 by means of 0.1 mol L^{-1} H_3PO_4 addition. Individual enzymes were added to give final concentrations of 90 g kg^{-1} of oven-dried (o.d.) pulp and the mixtures were thoroughly mixed by means of a magnetic stirrer. After 1, 2 or 3 hours of incubation at 40 °C, the content of the test tubes was filtered through a 0.45 μm PTFE filter. The filtrates were analyzed for reducing sugars contents, while in the solid phase (cellulosic pulp), which was washed with water in order to remove the enzyme, the fiber length was determined.

Experiments in a laboratory-scale stirred tank reactor

For further laboratory testing of pulps and laboratory sheet preparation, 3 L of 5 % pulp suspensions in water with pH adjusted to 7.0 were mixed in a laboratory-scale mixer with the Rushton turbine. Enzymes were added in the amounts specified in the results, giving final concentrations between 0.9 and 90 g kg^{-1} of (o.d.) pulp. The mix-

tures were stirred at 510 min^{-1} and thermostated at 40 °C for 1 or 2 h as specified in the results. Again, water was removed by means of filtration and fibers were washed with water in several consecutive steps by using multiple sample volumes of water in order to remove the enzymes as much as possible.

Sheet preparation and paper testing

For the mechanical treatment of untreated and enzymatically treated fibers, a PFI laboratory beater mill was used and the samples were refined according to ISO 5264/2 from 500 to 6000 PFI revolutions as specified in the results. Drainability, expressed as the Schopper-Riegler degree (°SR), was determined according to standard ISO 5267/1, while paper sheets were produced according to standard ISO 5269/2. For drainability measurements, the standard deviations were within 1 °SR. Paper strength properties were characterized by standardized methods: tensile properties were measured according to ISO 1924/2, bursting strength according to ISO 2759 and tear resistance according to standard ISO 1974. The standard deviations of measurements were below 4 N m g^{-1}, 0.25 kPa m^2 g^{-1} and 0.4 mN m^2 g^{-1} for tensile and bursting strength, and for tear resistance, respectively.

Analyses

Determination of reducing sugars

The amount of reducing sugars in filtrates obtained by filtration of enzymatically treated or untreated pulps was estimated using DNS reagent according to the Miller's method[21] and the results were expressed in the weight of glucose equivalents released per weight of o.d. pulp. The analyses were performed in parallels and the average was calculated. The standard deviation for all samples was below 1 g kg^{-1}.

Fiber analysis

Fiber length distribution and fiber length were measured according to standard T 271 om-91 on Kajaani FS-200. Mechanical properties of fibers in terms of fiber length were expressed as the average value of 30 – 35000 particles counted in the sample (L_{AV}, mm), and the fraction of fines shorter than 0.2 mm (P, %). The analyses were performed in 3 parallels and the average was calculated. The standard deviations were below 0.02 mm for L_{AV} value and below 2.67 % for P value.

Results and discussion

The effect of enzymatic treatment on reducing sugars release and fiber length reduction

Preliminary tests on the effect of particular enzyme preparations or combinations of preparations containing cellulases and xylanases on chosen pulps were accomplished at enzyme concentrations of 90 g kg^{-1} o.d. pulp. Such extremely high dosages, which are 2 orders of magnitude higher than recommended by the producer, were used in order to clearly see the differences in their effect on pulps and to minimize experimental errors possibly due to the adsorption of enzymes on glass surfaces of the test tubes where enzyme preparations are added on a μL scale. The hydrolytic action of enzymes was followed by the quantification of reducing sugars released into the filtrates, and the changes in fiber length and in the portion of fines.

The reducing sugars concentrations were monitored during the first 3 h of incubation of pulp suspensions at 40 °C in the presence or absence of enzymes. In all cases, no reducing sugars were present in controls without enzymes, while small amounts present at the beginning of the experiments due to the presence of sugars in enzyme preparations were subtracted from the measurements. The released sugars concentrations increased with time in the first 3 h and differed significantly among the enzymes used. The results for eucalyptus kraft EUC 1-pulp, summarized in Fig. 1, confirmed varied hydrolytic action of different enzymes on fibers. Despite the immense concentrations used, individual commercial enzymes did not have a pronounced influence on fiber average length, while the portion of fines in this pulp slightly increased. The highest concentrations of reducing sugars were obtained as a consequence of the treatment with 342, which consists of a mixture of cellulases and hemicellulases. The monocomponent 476 alone, how-

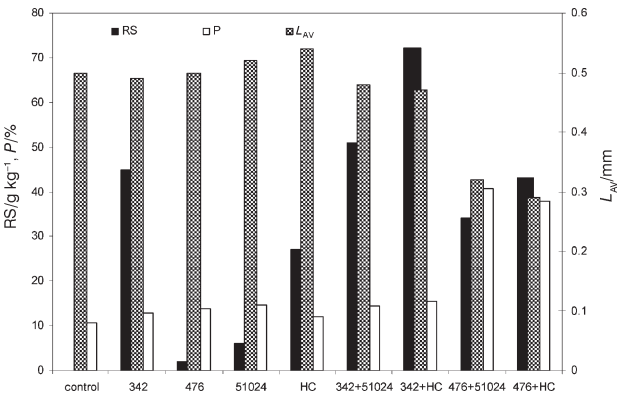


Fig. 1 – *Released sugars concentrations (RS), average fiber length (L_{AV}) and percentage of fine fraction (P, < 0.2 mm fiber length) after 3 h treatment of 1 % EUC 1-pulp with different enzymes in concentration of 90 g kg^{-1} pulp without refining*

ever, was several times less efficient in reducing sugars release, confirming the synergistic action of different cellulases and hemicellulases on the hydrolysis of complex fiber structure. On the other hand, since their action resulted in only slight changes in average fiber length, these results imply that only the most accessible parts of the cellulose and hemicellulose present in fibers and fines were hydrolysed to simple sugars. These findings are in accordance with the report of Gil *et al.*[11] on bleached *E. globulus* fibers treated with mixtures of cellulases, observing insignificant changes in length while expressing a pronounced (22.2 %) reduction in pulp viscosity and 1.1 % extent in pulp total hydrolysis to sugars. Among xylanases, HC was very effective in reducing sugars release, generated by xylanase hydrolysis of the soluble oligossacharides released by the initial depolymerisation of the xylan on the fiber surface,[8] while the other xylanase 51024 was less efficient, which is in accordance with the differences in the specific activity of both preparations (1000 AXU g^{-1} and 650 FXU g^{-1} for HC and 51024, respectively).

On the contrary, a combined action of cellulases and xylanases significantly lowered the average length and increased the portion of fibers shorter than 0.2 mm to up to 40 % of total fibers in the sample. The most exhibited fiber degradation to fines in this eucalyptus pulp was achieved by a synergistic effect of 476 with a very high specific endoglucanase activity (4500 ECU g^{-1} as compared to 90 EGU in 342) together with xylanase HC, while the hydrolysis to reduced sugars was again the most pronounced in combination of 342 with HC. This again confirmed the difference in the total degrading capability between both cellulase preparations, where endoglucanase 476 presumably acted on the amorphous regions of cellulose and therefore produced more fines[11] which were not completely degraded to simple sugars due to the absence of other hydrolases.

In general, similar effects of the actions of individual enzyme preparations or their combinations were observed also for other pulps used in this study. The results, summarized in Table 1, confirmed a very small effect of individual enzymes on fiber length, while a huge decrease in average length accompanied with an increase in fines portion was observed again with both eucalyptus pulps, treated with 476 in combination with both xylanases. For the other two pulps treated only with individual enzyme preparations, no such effect was discovered. Interestingly, very low reducing sugar release was noticed with the long-fiber pulp treated

Table 1 – *The effect of different enzymes and their combinations on average fiber length (L_{AV}), percentage of fine fraction (P, < 0.2 mm fiber length) and released sugars concentrations (RS) after 3 h treatment of 1 % pulp without refining*

Enzyme	Enzyme concentration/g kg^{-1}	EUC 2-pulp			EUC 3-pulp		
		L_{AV}/mm	*P*/%	RS/g kg^{-1}	L_{AV}/mm	*P*/%	RS/g kg^{-1}
control	0	0.56	10.51	0	0.49	12.12	0
342	90	0.54	12.83	38.1	0.49	12.81	45.3
476	90	0.54	14.08	0.5	0.48	14.68	0.7
51024	90	0.57	12.27	2.4	0.51	14.41	4.1
HC	90	0.56	11.66	40.1	0.46	18.35	23.1
342 + 51024	90 + 90	0.53	13.54	65.1	0.49	12.26	68.2
342 + HC	90 + 90	0.53	16.07	66.3	0.49	12.78	76.1
476 + 51024	90 + 90	0.40	35.46	24.6	0.37	24.16	25.1
476 + HC	90 + 90	0.31	40.11	45.4	0.27	38.46	39.1

Enzyme	Enzyme concentration/g kg^{-1}	SF-pulp			LF-pulp		
		L_{AV}/mm	*P*/%	RS/g kg^{-1}	L_{AV}/mm	*P*/%	RS/g kg^{-1}
control	0	0.67	15.13	0	1.03	37.56	0
342	90	0.64	14.84	35.2	1.00	37.56	31.0
476	90	0.66	14.05	0.2	0.98	34.26	1.1
51024	90	0.66	14.60	0.9	0.99	35.05	0.9
HC	90	0.66	14.50	10.9	0.95	36.98	3.7

with HC as compared to other pulps used in this study, implying the presence of a less accessible xylan in this pulp.

Despite a very small effect of individual enzymes on the reduction of fiber length, a successful degradation of vessel cells was observed in all tested eucalyptus pulps, which is a very challenging result due to the extensive problem of vessel picking experienced with these pulps.[3] Microscopic observations of damaged vessel cells in EUC 1-pulp are presented in Fig. 2. As expected, a very efficient degradation of vessel cells was observed also when using mixtures of enzymes, especially 476 and 51024 (Fig. 2b). These findings are in accordance with some patents on the reduction of vessel element picking after the treatment of eucalyptus pulps by cellulase/xylanase mixtures.[22,23] In order to clearly see the relation of vessel cell damage caused by enzymes on the minimization of the vessel picking problem, further investigations are required.

The effect of enzymatic treatment with refining on fiber length

While some effect of enzyme treatment on fiber length was observed already before mechanical treatment of pulps, the influence of enzyme treatment followed by a refining process was also studied under different treatment conditions. Refining has a large effect on the pulp properties as mechanical treatment in the refiner changes the single fiber properties. The primary structural effects of refining on fiber properties are internal and external fibrillation, fines formation and fiber cutting.[13]

Pulps were subjected to various refining intensity levels – from 500 to 4500 revolutions for the same period of time. As seen from Fig. 3, refining of two different eucalyptus pulps, EUC 1-pulp and EUC 3-pulp, under used conditions without an enzyme treatment had almost no effect on the average fiber length for both pulps, while the increase in percentage of fines slightly increased, more evidently in the case of EUC 1-pulp. When the same pulps were treated for 2 hours prior to refining with xylanase HC at an extremely high concentration of 90 g kg^{-1} o.d. pulp, up to 20 % differences in terms of decreased average fiber length were noticed for EUC 1-pulp. Taking into account that untreated pulp samples contained 10–12 % of fines, the reduction of fiber length was accompanied by an increase in fine fraction portion with the level of refining of up to 30 % regarding control (Fig. 3). For the other xylanase-treated eucalyptus pulp EUC 3-pulp, the changes in fiber length after the refining of fibers were much less evident, which was also the case without the enzyme treatment, suggesting that this eucalyptus was less sensitive to mechanical and chemical treatment. These findings confirmed that xylanase treatments alone had only a slight effect on pulp properties compared to the results obtained when mixtures where used, which has also been reported by other researchers.[11–14]

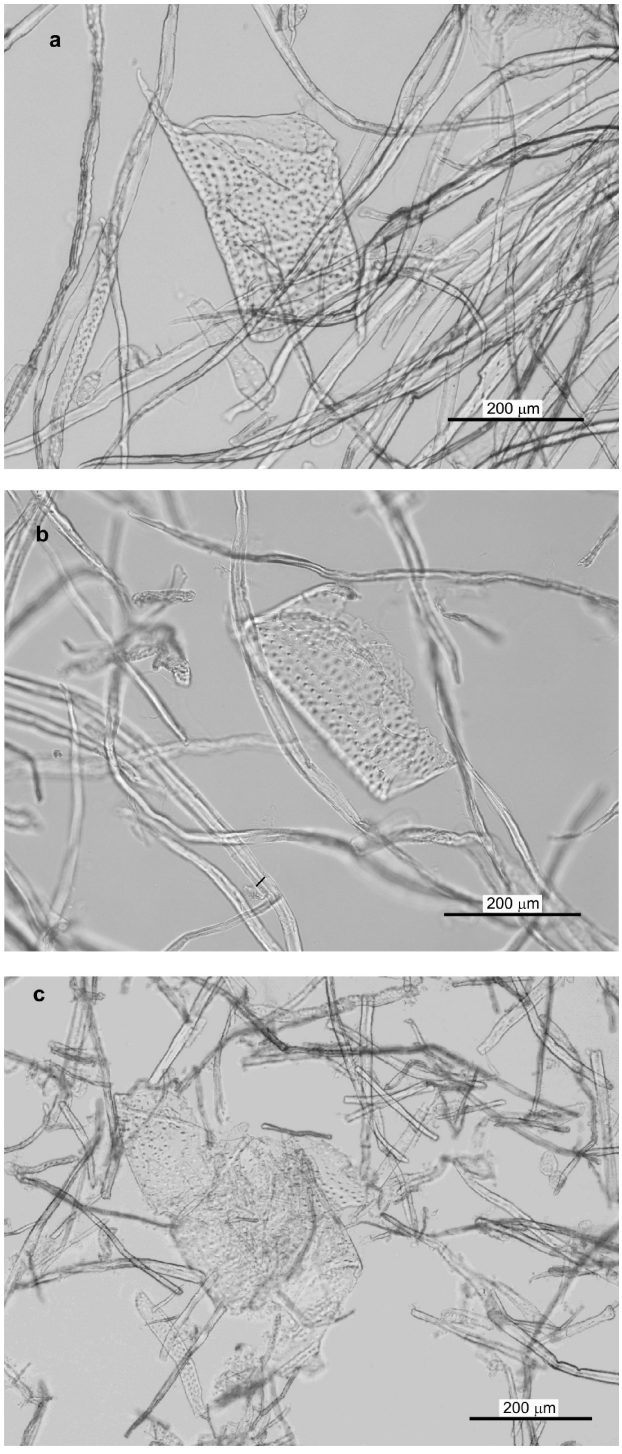
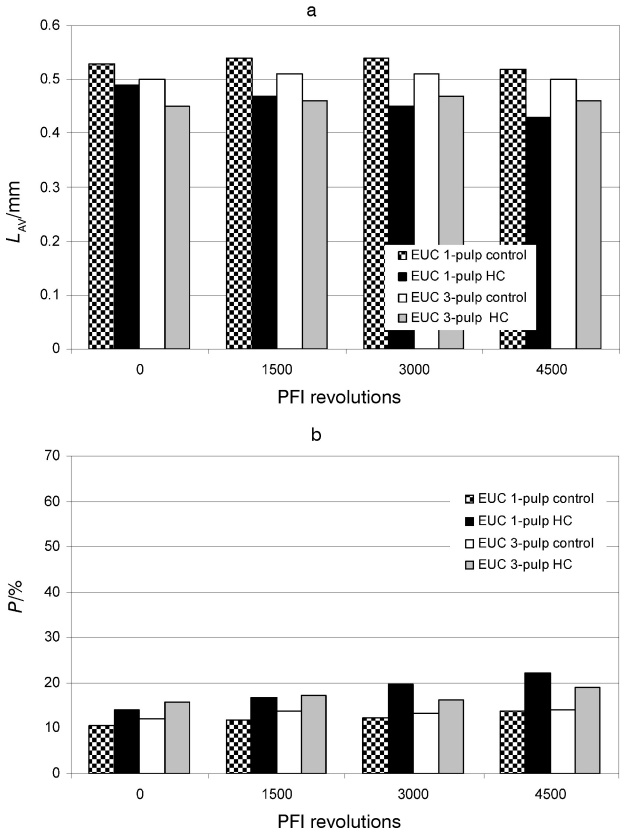
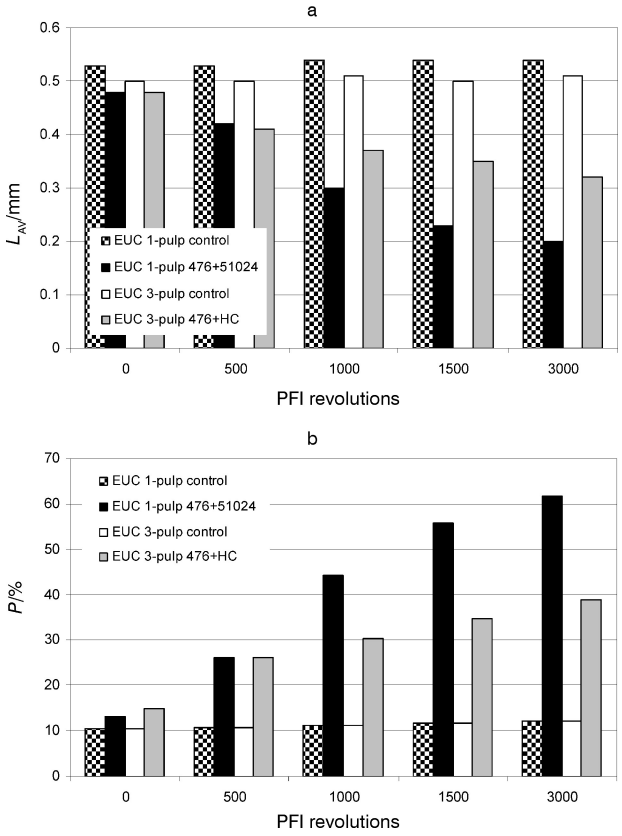


Fig. 2 – *Microscopic observations of EUC 1-pulp a) before treatment, b) after treatment with endoxylanase (HC), and c) after treatment with 476 combined with 51024 under conditions specified in Table 1*

On the contrary, when a combination of cellulase 476 and thermally stable endoxylanase



F i g . 3 – *The effect of endoxylanase (HC) treatment of EUC 1-pulp and EUC 3-pulp, followed by refining at various intensity levels, on: a) L_{AV} and b) P. The enzyme treatments were performed for 2 h with enzyme concentration of 90 g kg^{-1} o.d. pulp, while the controls were refined without enzyme treatment.*



F i g . 4 – *The effect of combined action of cellulase 476 together with xylanases (5124 or HC) on different eucalyptus pulps, followed by refining at various intensity levels on: a) L_{AV} and b) P. The treatments were performed for 2 h with an enzyme concentration of 1.8 g kg^{-1} pulp of each in the case of EUC 1-pulp and for 1 h at 0.9 g kg^{-1} pulp of each enzyme dose for EUC 3-pulp, while controls were refined without the enzyme treatment.*

51024 was used for the treatment of EUC 1-pulp in a fifty times smaller concentration (1.8 g kg^{-1} pulp of each), a significant reduction in average fiber length and a several-fold increase in fine fraction portion correlated with applied refining intensities were observed already after one hour of enzyme treatment prior to refining (Fig. 4). A similar although less pronounced effect was observed when a combination of 476 with xylanase HC was used for the treatment of EUC 3-pulp in even smaller concentrations (0.9 g kg^{-1} pulp of each). These results imply that the amount of hemicellulose (including xylan) present on the fiber surface is generally not very high, the latter being mostly a consequence of cellulose cooking and bleaching. On the other hand, a significant amount of hemicellulose present inside the fiber structure is accessible to the endoxylanase yet after the cellulosic fiber structure has been opened due to cellulase treatment. Nevertheless, when this process is initiated, the refining-induced fiber disintegration proceeds much faster than without enzyme treatment. It seems that a selective treatment with endoxylanase alone that would have no detrimental effect on the quality of cellulose fibers cannot be successful, because the hemicellulose structures inside the fibers were inaccessible to this enzyme. The results presented here confirm the explanation suggested by other authors[11,12,15] – due to the relatively complex structure of pulp fibers, a significant change (reduction) in fiber length or an increase in the amount of fines after refining generally requires a combination of two or more enzyme.

The effects of HC on hardwood SF-pulp and softwood LF-pulp regarding the changes in fiber length during refining are shown in Table 2. The reduction of fiber length and an increase in fines fraction after refining of SF-pulp was almost negligible when compared to controls without enzymes, while the effect of this enzyme preparation on long fiber pulp was more pronounced. With the evolution of the refining process of LF-pulp, the portion of fines even decreased, which was also the case when 476 was used for the treatment. This implies that the refining of enzymatically treated LF-pulps caused the fibrillation and/or shortening of fibers while producing more or less constant amounts of fines,

Table 2 – *The effect of refining on L_{AV} and P of hardwood SF-pulp and softwood LF-pulp: control without enzyme and comparison with pulp after 1 h treatment with HC at enzyme concentration of 0.9 g kg^{-1} pulp*

SF-pulp	L_{AV}/mm		*P*/%	
PFI revolutions	control	HC	control	HC
0	0.67	0.63	15.13	15.97
1500	0.66	0.60	20.34	19.58
3000	0.59	0.58	20.03	21.54
4500	0.55	0.55	23.13	24.15
LF-pulp	L_{AV}/mm		*P*/%	
PFI revolutions	control	HC	control	HC
0	1.03	0.87	37.56	43.75
1000	0.95	0.87	39.62	33.10
2000	0.95	0.85	39.22	31.84
4000	0.95	0.84	37.03	29.33

yielding a lower portion of fines in a specified pulp volume. The effects of 342 and 476 on SF-pulp and LF-pulp fiber length during refining were also relatively small; however the changes in LF-pulps were again slightly more pronounced (data not shown). These findings are in accordance with the differences in chemical composition of hardwoods and softwoods, resulting in higher amounts of cellulose and hemicelluloses in LF-pulps as compared to SF-pulps.

The effect of enzymatic treatment with refining on mechanical properties of paper

As mechanical properties of paper are very important for its use, the influence of enzyme treatment followed by refining on tensile, burst and tear strength was also investigated on the pulps treated with enzymes as specified in the previous section. Table 3 shows the mechanical properties of selected pulps before and after enzyme treatment, where the properties of unrefined pulps as well as of pulps refined at 1500 revolutions are compared. These results confirm that treatment with different enzymes or their combination before refining has a diverse effect on the mechanical properties of pulps. Endoxylanase HC alone has only slightly deteriorated the mechanical properties of all pulp types in unrefined pulps as well as in pulps refined at 1500 revolutions despite the extremely high concentrations used, which is in agreement with the results of fiber length determination. The use of cellulase 476 alone or in combination with endoxylanase HC resulted in an increase of mechanical strength (especially tear and tensile), however only for hardwood pulps (EUC 3-pulp and SF-pulp). In addition, similar results were obtained for EUC 1-pulp and EUC

Table 3 – *Mechanical properties of unrefined pulps (0) and pulps refined at 1500 revolutions after 1 h treatment with different enzymes in concentration of 0.9 g kg^{-1} o.d. pulp compared to controls without enzyme treatment, except for EUC 3-pulp, where the treatment with HC lasted 2 h with enzyme concentration of 90 g kg^{-1} o.d. pulp*

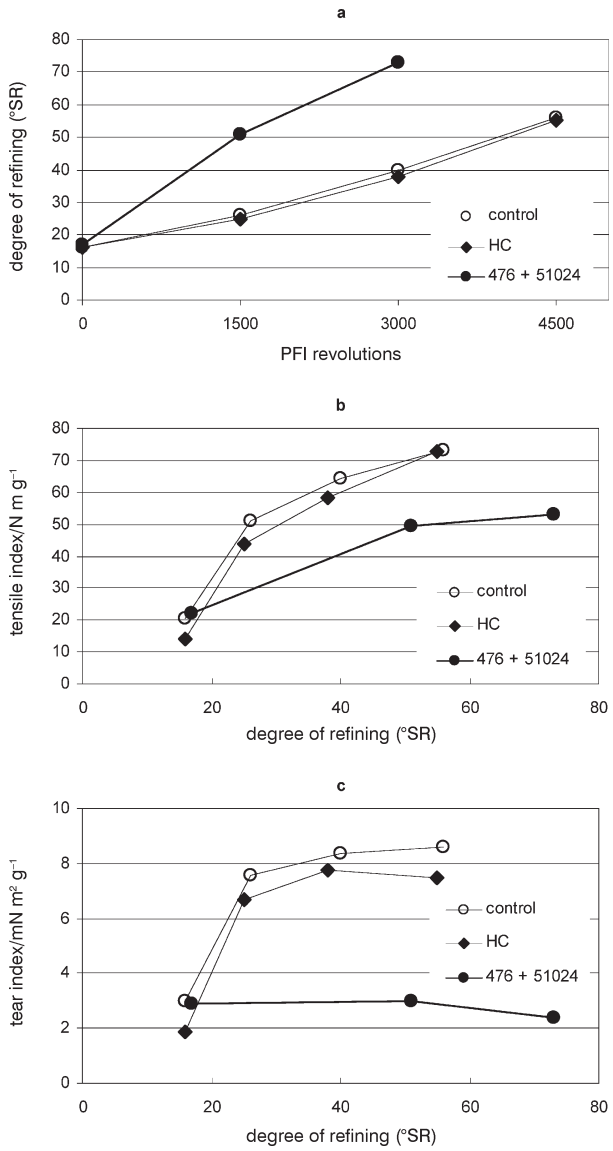
EUC 3-pulp	°SR		Tensile index/N m g^{-1}		Tear index/mN m^2 g^{-1}		Burst index/kPa m^2 g^{-1}	
PFI revolutions	0	1500	0	1500	0	1500	0	1500
control	17	22	23.16	53.99	4.03	8	1.55	4.24
HC	17	24	14.63	41.7	3.1	7.4	0.8	2.75
HC + 476	18	34	25.69	60.82	4.3	4.54	1.59	4.36
SF-pulp	°SR		Tensile index/N m g^{-1}		Tear index/mN m^2 g^{-1}		Burst index/kPa m^2 g^{-1}	
PFI revolutions	0	1500	0	1500	0	1500	0	1500
control	14	18	15.94	47.5	2.85	7.42	0.85	3.27
HC	16	21	13.93	42.71	2.48	8.57	0.57	2.66
476	16	31	18.69	50.32	3.53	4.37	0.86	3.17
342	16	21	14.29	41.23	3.07	7.06	0.66	2.63
LF-pulp	°SR		Tensile index/N m g^{-1}		Tear index/mN m^2 g^{-1}		Burst index/kPa m^2 g^{-1}	
PFI revolutions	0	1500	0	1500	0	1500	0	1500
control	13	19	17.62	53.99	17.72	18.65	1.33	3.99
HC	13	16	16.53	44.72	5.11	8.42	1.61	4.08
476	13	19	17.33	51.76	13.74	9.6	1.46	3.99
342	13	16	16.05	44.24	15.09	17.18	1.32	3.74

2-pulp (data not shown). The extent of this improvement ranges between 7 to 17 % for unrefined pulps and from 6 to 12 % for refined pulps. Under specified refining conditions, higher drainability values (°SR) were generally achieved with enzymatically treated pulps. In terms of refining energy, necessary for the desired improvement of pulp properties, this indicates potential energy savings up to 17 %, achieved with enzyme treatment. These results are also in accordance with other studies, where potentials for energy savings on pulp refining as a consequence of enzyme (pre)treatment were investigated.[10,13,18,20]

The mechanical properties of EUC 1-pulp after refining, presented in Fig. 5, were in accordance with previous findings, again confirming the negligible effect of HC on fibers albeit used in very high dosages. However, treatment with this enzyme in a fifty times lower concentration together with cellulase 476 resulted in a significant loss of tensile and tear strength at a certain Schopper-Riegler degree when compared with pulp treated only with xylanase or untreated pulp. This was predominantly a consequence of the fiber structure destruction due to the enzyme treatment, which weakens the fiber structure and consequently increases the effects of mechanical refining, resulting in fiber shortening and production of fine fraction.

While other researchers[10,13,16,17,18] reported similar effects of enzyme treatments on pulp drainability during refining, generally no significant loss in the mechanical strength of pulp was described when using enzymes with either xylanase or cellulase activity. However, when mixtures of cellulases and hemicellulases were tested, some deteriorations of mechanical strength were also observed.[11,12,14,15,19]

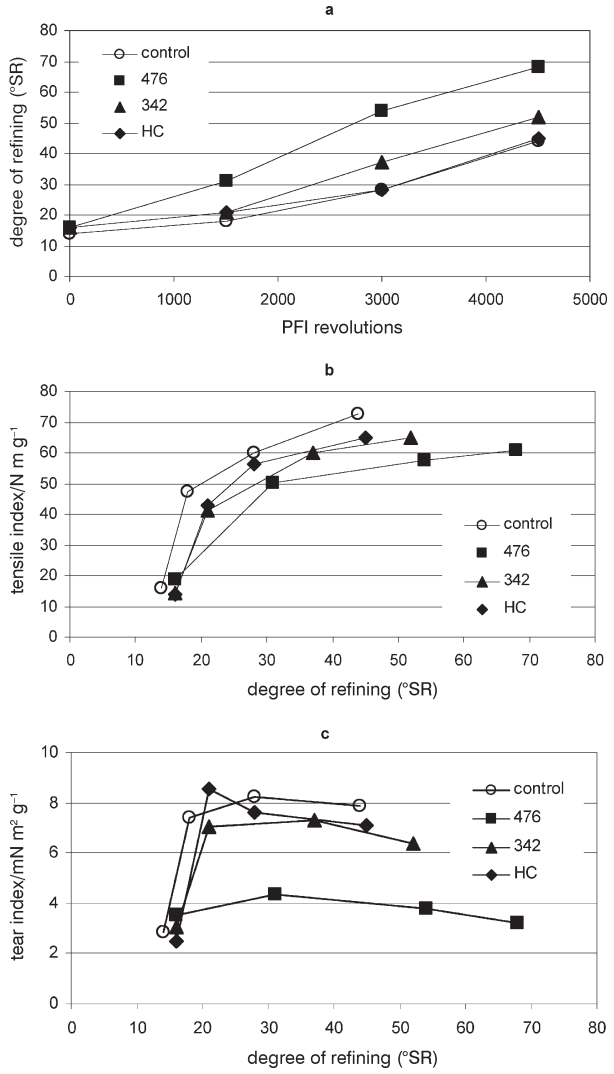
The enzyme treatment of hardwood SF-pulp prior to refining resulted in a smaller loss of fiber strength (Fig. 6). The use of cellulase 476 alone improved drainability during refining considerably, while this increase was only moderate or almost negligible when 342, a mixture of cellulases and hemicellulases, or endoxylanase HC were used, respectively (Fig. 6a). However, the reduction of fiber strength during refining correlated with the improved °SR – the higher the increase in drainability in comparison to untreated pulp, the greater the decrease in fiber strength (Fig. 6b). On the other hand, a significant increase in tear strength of SF-pulp treated with HC was observed at approx. 20 °SR when compared with untreated refined pulp (Fig. 6c). This result implies that although fibers were only moderately shortened during refining, the treatment with endoxylanase (HC) somewhat degraded or "softened" the fiber surface and thus facilitated the fibrillation of fibers.[11,17] As a consequence, stronger fibrillation then resulted in better



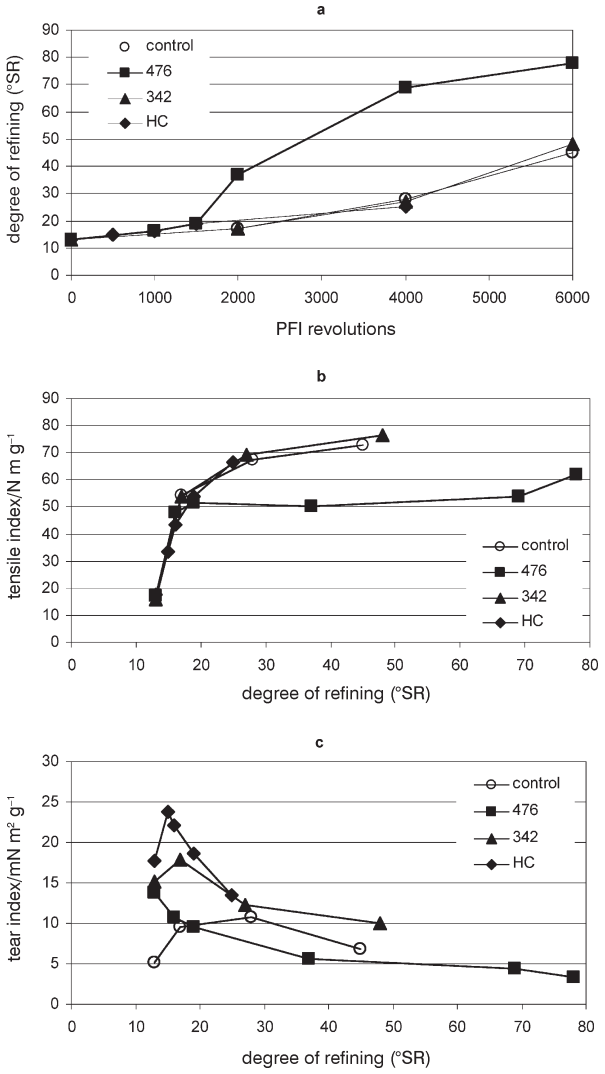
F i g . 5 – *The effect of enzyme treatment of EUC 1-pulp followed by refining on a) drainability expressed as °SR in dependence of evolution of refining process, b) dependence of tensile index on °SR and c) dependence of tear index on °SR. The conditions of enzyme treatments are specified in Fig. 2 and 3.*

or stronger interfiber bonding and consequently in higher tear resistance. With further refining, these fibrils were then probably more or less removed from the fiber surface, causing a weaker tear resistance. The structure of softwood fibers differs significantly from the structure of hardwood fibers (including eucalyptus), which is the main reason why they behave differently during the enzyme treatment followed by refining.

The observations concerning hardwood pulp are even more pronounced in the case of softwood LF-pulp. As shown in Fig. 7a, treatment with the abovementioned enzymes again increased in drainability during refining – cellulase 476 showing the strongest effect, while with other enzymes the ef-



F i g . 6 – *The effect of enzyme treatment of SF-pulp followed by refining on a) drainability expressed as °SR in dependence of evolution of refining process, b) dependence of tensile index on °SR and c) dependence of tear index on °SR. The conditions of enzyme treatments are specified in Table 3.*



F i g . 7 – *The effect of enzyme treatment of LF-pulp followed by refining on a) drainability in dependence of evolution of refining process, b) dependence of tensile index on °SR and c) dependence of tear index on °SR. The conditions of enzyme treatments are specified in Table 3.*

fect was almost insignificant. With this enzyme, the reduction of mechanical strength of pulp during refining was observed as well (Fig. 7b). On the other hand, in the case of enzyme treatment with 342 or with HC, tensile strength was slightly improved, while tear strength improved significantly in comparison to untreated pulp, especially in the range between 15 and 30 °SR. In addition, it was interesting to see that tear strength improved already with unrefined pulp and even with the monocomponent cellulase treatment (Fig. 7c). Again this behavior can be explained with the "softening" of the fiber surface due to enzyme treatment, which facilitates the formation of fibrils during refining. It seems that after the enzyme treatment, the fiber surface is "opened" to the extent that increases tear strength already with unrefined pulp. Nevertheless, further intensive refining of LF-pulp again reduced the amount of fibrils resulting in the reduced possibility for interfiber bonding. Consequently, tear strength was reduced to a level comparable with the untreated pulp, indicating also the comparable amount of fibrils on the fiber surface.[11,17]

Conclusions

Despite the large concentrations used in initial studies on fiber hydrolysis, individual commercial enzymes had no pronounced influence on fiber average length, while the portion of fines in tested pulps slightly increased. However, a combined action of cellulases and xylanases significantly lowered the average length and increased the portion of fibers lower than 0.2 mm to up to 40 % of total fibers in the sample. The highest hydrolytic action was ob-

served with the multicomponent cellulase 342, especially when combined with xylanases, whereas the cellulase preparation 476 with a very high endoglucanase activity alone could not release high concentrations of reducing sugars from pulps used in this study, confirming the need for synergistic action of the enzyme for fiber complete hydrolysis.

Although some influence on fiber length was noticed with unrefined pulps treated with enzymes only, the main changes in fiber length were observed only when the enzyme treatment was followed by refining. Generally, the use of enzyme combinations resulted in a significant change (reduction) in fiber length and in an increase in the amount of fines after refining. The effects of cellulases and hemicellulases were generally more pronounced on hardwood (LF) than on softwood (SF) pulps, which were expected due to higher portions of cellulose and hemicelluloses in LF-pulps.

The refining energy needed for obtaining the desired pulp properties was reduced, although the possibility of pulp strength deterioration was also proven. The use of various enzyme combinations showed the strongest influence on paper strength, although the significance of pulp origin (type) should not be neglected. The latter was especially obvious in terms of the tear strength of LF-pulp, which was significantly improved after enzyme treatment and refining, while there was only a minor or even no improvement with SF-pulp and EUC-pulps, respectively. This behavior can be explained by the different degrees of "softening" of the fiber surface due to the enzyme treatment, consequently facilitating the formation of fibrils during refining and thus improving tear strength.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Technology of Slovenia, Grant P2-0191. The authors thank Mrs. T. Tancek and Mr. E. Sarhatlić for their help in experimental work and Mrs. N. Pregl for the editing.

Nomenclature

Abbreviations

AXU – xylanase (AnhydroXylose) Unit

ECU – Endo-Cellulase Unit

EGU – Endo-Glucanase Unit

EUC 1-pulp – eucalyptus pulp, 100 % *E. globulus* from Spain

EUC 2-pulp – eucalyptus pulp, 70 % *E. globulus* and 30 % *E. nitens,* both from Chile

EUC 3-pulp – eucalyptus pulp, 30 % *E. globulus* and 70 % *E. nitens,* both from Chile

FXU – Farbe Xylanase Unit

HC – Pulpzyme HC

L_{AV} – average length

LF-pulp – softwood pulp, 100 % spruce

P – percentage of fines with length below 0.2 mm

RS – reducing sugars

SF-pulp – hardwood pulp, 50 % poplar and 50 % birch

°SR – Schopper-Riegler degree

342 – Novozym 342

476 – Novozym 476

51024 – Novozym 51024

References

1. *Kirk, T. K., Jeffries, T. W.,* ACS Symposium Series 655, Enzymes for Pulp and Paper Processing (1996) 2.
2. *Ragauskas, A. J.,* Prog. Biotechnol. **21** (2002) 7.
3. *Kenealy, W. R., Jeffries, T. W.,* ACS Symposium Series 845; Wood Deterioration and Preservation Advances in Our Changing World (2003) 210.
4. *Patidar, J., Sharma, A. K., Sharma, S.,* Pollution Research **27** (2008) 451.
5. *Covarrubias, R.,* FindArticles.com. (available on 28th September 2009) http://findarticles.com/p/articles/mi_qa3636/ /is_200606/ai_n17178312/
6. *Bolaski, W., Gallatin, A., Gallatin, J. C.,* US Patent 3,041,246 (1959).
7. *Pommier, J. C., Fuentes, J. L., Goma, G.,* Tappi J. **72** (1989) 187.
8. *Salles, B. C., Medeiros, R. G., Báo, S. N., Silva, F. G. Jr, Filho, E. X. F.,* Process Biochem. **40** (2005) 343.
9. *Battan, B., Sharma, J., Dhiman, S. S., Kuhad, R. C.,* Enzyme Microb. Technol. **41** (2007) 733.
10. *Ahmad, S., Dutt, D., Upadhyaya, J. S., Tyagi, C. H., Kumar, V.,* Papir a Celuloza **62** (2007) 238.
11. *Gil, N., Gil, C., Amaral, M. E., Costa, A. P., Duarte, A. P.,* Biochem. Eng. J. **46** (2009) 89.
12. *Oksanen, T., Pere, J., Buchert, J., Viikari, L.,* Cellulose **4** (1997) 329.
13. *Bhardwaj, N. K., Bajpai, P., Bajpai, P. K.,* J. Biotechnol. **51** (1996) 21.
14. *Kibblewhite, P. R., Clark, T. A.,* Appita J. **49** (1996) 390.
15. *Oksanen, T., Pere, J., Paavilainen, L., J., Buchert, J., Viikari, L.,* J. Biotechnology **78** (2000) 39.
16. *Wong, K. K. Y., Richardson, J. D., Mansfield, S. D.,* Biotechnol. Prog. **16** (2000) 1025.
17. *García, O., Torres, A. L., Colom, J. F., Pastor, F. I. J., Díaz, P., Vidal, T.,* Cellulose **9** (2002) 115.
18. *Mohlin, U.-B., Pettersson, B.,* Prog. Biotechnol. **21** (2002) 291.
19. *Pastor, F. I. J., Pujol, X., Blanco, A., Vidal, T., Torres, A. L., Díaz, P.,* Appl. Microbiol. Biotechnol. **55** (2001) 61.
20. *Bajpai, P., Mishra, S. P., Mishra, O. P., Kumar, S., Bajpai, P. K., Varadhan, R.,* 7th International conference on pulp, paper and conversion industry, New Delhi, India, 3-5 Dec. 2005 (2005) 143.
21. *Miller, G. L.,* Anal. Chem. **31** (1959) 426.
22. *Nakamura, K.* (Mitsubishi Paper Mills, Ltd., Japan) JP 2004149948, 27 May 2004, Jpn. Kokai Tokkyo Koho (2004).
23. *Cooper, E. W.,* III. (P. H. Glatfelter Co., USA). US 5770012, 23 June 1998, Cont.-in-part of U.S. 5,725,732, U.S. (1998).