Holzforschung
50 (1996) 579–582

Short Note

Impact of Xylanase and Fungal Pretreatment on Alkali Solubility and Brightness of Dissolving Pulp

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Keywords: Sulphite pulp · Dissolving pulp · Brightness · Alkali solubility · Xylanases · White-rot fungi · Biobleaching · Chemical savings

Introduction

Chemical cellulose is widely used for production of viscose rayon and cellulose esters and ethers (Sjöström 1981). Both brightness and alkali solubilities S_{10} and S_{18} are important parameters of dissolving pulp providing information on the bleachability, and degradation of cellulose or hemicellulose during pulping and bleaching, respectively. High hemicellulose content (S_{18}, DP of up to 50) and degraded cellulose content (S_{10}-S_{18}, DP of 50–150) affect the degree of swelling of pulp, xanthation reaction, and other required characteristics in the viscose process (Hinck *et al.* 1985). Therefore, new environmentally benign elemental chlorine-free (ECF) and totally chlorine-free (TCF) bleaching technologies are necessary for minimizing the hemicellulose content in dissolving pulp, adjusting the brightness at a high level and improving simultaneously the quality of the effluents in terms of toxicity and adsorbable organic halogen (AOX).

Biological methods of pulp prebleaching using xylanases provide the possibility of selectively removing up to 20% of xylan from pulp (Kantelinen *et al.* 1993) and saving up to 25% of chlorine-containing bleaching chemicals (Viikari *et al.* 1986). Alternatively, pulp can be bleached with white-rot fungi and their lignolytic enzymes, enabling chemical savings to be achieved (Reid and Paice 1994) and a chlorine-free bleaching process to be established (Murata *et al.* 1992).

Previously we reported the application of *Aureobasidium pullulans* xylanases for direct removal of xylan from dissolving pulp (Christov and Prior 1993) and enzymatic prebleaching of sulphite pulps (Christov and Prior 1994), thereby reducing the pentosan content as much as 50% (Christov and Prior 1996). The white-rot fungus *Ceriporiopsis subvermispora* was shown to be very effective in biomechanical pulping (Akhtar *et al.* 1993) and biosulphite pulping (Fischer *et al.* 1994) for papermaking. The aim of the present study was to investigate whether the pretreatment of sulphite pulp with *C. subvermispora* and/or *A. pullulans* xylanases would improve the quality of dissolving pulp in terms of alkali volubility and brightness.

Materials and Methods

Treatment of pulp with A. pullulans enzyme preparation

The maintenance of *Aureobasidium pullulans* NRRL Y-2311-1, enzyme production and preparation was reported before (Christov and Prior 1994). No cellulase activity was detected in the enzyme preparation (Christov and Prior 1996). Xylanase activity in the enzyme preparation was assayed using oat spelts xylan as substrate according to the Somogyi-Nelson method (Nelson 1944; Somogyi 1952). One unit (IU) of xylanase activity was defined as that amount of enzyme which catalyzes the release of 1 micromole of xylose equivalents per minute of reaction. Pulp (250g dry weight) was treated with the enzyme preparation (15 IU xylanase/g pulp) at 55°C. pH 4.7 (sodium acetate buffer) and pulp consistency of 9% for 1 h. After treatment, enzyme reaction was stopped by boiling the samples for 15 min.

Treatment of pulp with C. subvermispora

The white-rot fungus *Ceriporiopsis subvermispora* (strain L-14807 SS-3 and CZ-3) was obtained from the Center for Forest Mycology Research, USDA Forest Products Laboratory, Madison, WI, USA. The maintenance and inoculum preparation for treatment of pulp were previously described (Fischer *et al*, 1994). Pulp samples (each 500g dry weight) were autoclaved in autoclavable bags for 30min at 121°C. After cooling to room temperature, a 0.1% inoculum of *C. subvermispora* (dry weight basis), and autoclaved modified chemically defined medium (Leatham 1983) were added to the pulp and held at room temperature for 2 hours with mixing every 30min for better distribution of inoculum and medium. The pulp moisture content was adjusted to 80% (wet weight basis). The fungal treatment of pulp was performed in static bed bioreactors at 27°C for 2 weeks. During incubation, humidified air was aerated aseptically through the bioreactor with

a specific aeration rate of 0.05L $L^{-1}min^{-1}$ to maintain a relative humidity of about 65%. Treatments were performed in duplicate.

Chemical Bleaching of pulp

Chemical bleaching of pulp was carried out after the fungal and/or enzyme treatments using oxygen (O), chlorine dioxide (D), NaOH in oxygen atmosphere (E_0), and sodium hypochlorite (H) in sequence $OD_1E_0D_2H$. The charges of chemicals (% of dry weight pulp) were as follows: O, oxygen 0.8 and NaOH 1.5; D_1. 1.1 as active chlorine (act.Cl): E_0. NaOH 4 and oxygen 0.8; D_2, 0.6 as act. Cl: H, 0.65 as act. Cl. The pulp consistency and temperature used were respectively 11% and 100°C. for O; 10% and 65°C, for D_1; 11% and 100°C, for E_0; 11% and 65°C. for D_2; 12% and 55°C, for H. The control samples were bleached without prior biotreatments under the same conditions.

Tests on pulp properties

Unbleached pulp produced by the acid sulphite method from *Eucalyptus grandis* wood was obtained from SAPPI SAICCOR (Pty) Ltd., South Africa. The cooking liquor contained 8.5% total SO_2 and 1.1% CaO. The total pulping time was 7h with the maximum temperature of 147°C being reached in 6h. Prior to use and after each treatment step with biological agents (fungi and enzymes) or chemicals, pulp was thorougly washed with distilled water until a neutral pH of the wash waters was obtained. Brightness and alkali solubilities S_{10}) and S_{18} were tested according to the Standard Methods of the Technical Association of the Pulp and Paper Industry (TAPPI, Atlanta, GA, USA): TAPPI T 452 om-87 and TAPPI T 235 cm-85, respectively.

Results and Discussion

Biobleaching of sulphite pulp with A. pullulans xylanases

The xylanase-aided bleaching had a well pronounced effect on the reduction of the hemicellulose content of pulp as compared to the control and both S_{10} and S_{18} were lowered by 10% (Table 1). The decrease in S_{10}-S_{18} may indicate that a fraction of relatively higher molecular weight xylan, which contributed together with the short-chain cellulose fraction in pulp to the value of the S_{10} measurement, was hydrolyzed and removed from pulp. Brightness of xylanase-prebleached dissolving pulp was slightly improved over the control (Table 1). The cause of the bleaching effect by xylanases on sulphite pulps differs from that of kraft pulps (Kantelinen *et al.* 1993). The remaining xylan in sulphite pulps after pulping resembles the native xylan of wood with less substituents on the xylan backbone (Gamerith and Strutzenberger 1992). The most resistant side-chain groups to acid hydrolysis are those of acetic acid and glucuronic acid (Rydholm 1965). Apparently, the sulphite cooking solubilizes and removes preferentially the accessible fraction of xylan thereby leaving the most recalcitrant part of it intact. The latter is suspected to be mainly covalently bound to other polysaccharides and lignin (via glucuronic acid groups), apart from other types of possible associations, since no reprecipitation of xylan onto the cellulose fibres occurs in the course of the acid sulphite pulping process (Annergren and Rydholm 1959). Therefore, major factors influencing the degree of enzymatic removal of xylan from sulphite pulp would be the accessibility of the xylan substrate in pulp, on one hand, and the penetration capabilities and substrate specificity of xylanases, on the other (Christov and Prior 1993, 1994). Hence, the mechanism of xylanase bleaching of sulphite pulp would involve hydrolysis of xylan localized in the primary and mainly secondary cell walls. In addition, that part of xylan that is involved in lignin-carbohydrate complexes would be partially degraded thereby reducing the size of these complexes and improving their mobility and extractability from the cell walls (Paice *et al.* 1992).

Table 1. Bleaching of sulphite pulp in sequence $OD_1E_0D_2H$ following pretreatment with *C. subvermispora* (F) and/or *A. pullulans* xylanases (X)

Pulp treatment	S_{10}	S_{18}	S_{10}-S_{18}	Brightness (%, ISO)
	(%, w/w)			
$OD_1E_0D_2H$ (control)	9.5	5.2	4.3	87.8
X-$OD_1E_0D_2H$[a]	8.5	4.7	3.8	88.6
FS-$OD_1E_0D_2H$[b]	17.2	7.2	10.0	94.0
FZ-$OD_1E_0D_2H$	13.7	6.4	7.3	92.5
FS-X-$OD_1E_0D_2H$[c]	15.1	6.1	9.0	94.2
FZ-X-$OD_1E_0D_2H$	12.4	5.6	6.8	93.0

[a] X-$OD_1E_0D_2H$: Xylanase-pretreated and $OD_1E_0D_2H$-bleached sulphite pulp. [b] FS/FZ-$OD_1E_0D_2H$: Fungus strain SS-3-/CZ-3-pretreated and $OD_1E_0D_2H$-bleached sulphite pulp. [c] FS/FZ-X-$OD_1E_0D_2H$: Fungus SS-3/CZ-3-xylanase pretreated and $OD_1E_0D_2H$-bleached sulphite pulp.

Biobleaching of sulphite pulp with C. subvermispora

Pretreatment of pulp with both strains of *C. subvermispora* resulted in production of dissolving pulp with superior brightness (Table 1). However, alkali solubilities S_{10} and S_{18} increased significantly, indicating non-selective cellulose degradation. Cellulose was more affected when pulp was prebleached with strain SS-3 than CZ-3: the degraded cellulose content (S_{10}- S_{18}) as well as S_{18} increased almost 2.3 times and 41%, respectively, as compared to the control. Apparently, some cellulose chains of pulp fibrils were exposed to a more severe dismemberment by the fungus than others to yield glucopolysaccharides with a DP less than 50. This would cause an increase of S_{18}, when analyzed. This also confirms previous morphological and ultrastructural observations that some parts of the cell wall can be preferentially attacked and modified (Otjen and Blanchette 1987). Furthermore, the selectivity of the white-rot fungi in terms of lignin degradation tends to decline as the incubation periods progress (Otjen and Blanchette 1984).

Combined biobleaching of sulphite pulp

Enzyme bleaching following *C. subvermispora* treatment of pulp did not improve substantially the alkali

Table 2. Biobleaching of sulphite pulp with *C. subvermispora* SS-3 (F)[a], *A. pullulans* hemicellulases (X)[b] and reduced doses of active chlorine in sequence F-X-OD$_1$E$_0$D$_2$H

% Active Cl reduction			S_{10} (%, w/w)	S_{18} (%, w/w)	Brightness (%, ISO)
D_1	D_2	Total			
0	50	13	15.1	5.9	94.1
0	100	26	14.9	5.9	93.6
50	0	23	14.9	6.1	93.6
50	50	36	14.8	6.1	93.5
50	100	49	15.0	6.0	93.4
75	0	37	15.2	6.0	93.5
75	50	50	15.1	5.9	93.4
75	100	63	14.8	5.9	93.0

[a] Fungal treatment conditions: 27 °C; 14 days. [b] Xylanase treatment conditions: 15 IU xylanase/g pulp; 55 °C; pH 4.7; 1 h.

solubilities S_{10} and S_{18} of the fungal-prebleached dissolving pulp (Table 1) to satisfy the requirements in the viscose process. However, brightness was further enhanced. When the combined pretreatment of sulphite pulp was coupled to a chemical bleaching using reduced active chlorine doses, alkali volubility of pulp S_{10} and S_{18} did not undergo significant changes (Table 2). Moreover, no correlation was observed between the reduction of active chlorine and alkali volubility of pulp. However, the brightness values of the dissolving pulp still remained above the 93% level (Table 2). Similar results were obtained when strain CZ-3 was used (data not shown). It was possible to eliminate completely the second chlorine dioxide stage (D_2) and save up to 63% active chlorine without affecting significantly the brightness. Similarly, Fujita *et al.* (1993) reported a 73% reduction of active chlorine at both C and D stages when softwood kraft pulp was prebleached with the white-rot fungus IZU-154. These results suggest that some selected strains of white-rot fungi would be able to degrade and/or modify lignin during the prebleaching step to such an extent that the final delignification and bleaching of pulp could be accomplished with lower amounts of chemicals. Thus, a future optimization of the fungal treatment conditions would possibly diminish the negative impact of *C. subvermispora* on the cellulose in sulphite pulp.

Conclusion

The results of this work imply that pretreatment of sulphite pulp with *A. pullulans* xylanases could improve alkali volubility and brightness, important parameters of dissolving pulp for producing viscose rayon. The major effect caused by xylanases is a selective reduction of the hemicellulose content of dissolving pulp. On the other hand, biobleaching with the white-rot fungus *C. subvermispora* could enhance significantly the brightness, however, affecting the cellulose content of dissolving pulp. A combined fungus-enzyme pretreatment of sulphite pulp leads to production of dissolving pulp with 63% less active chlorine and a brightness of over 93% ISO. A TCF bleaching for dissolving pulp production would be feasible once the fungal treatment conditions are optimized in terms of preventing the non-selective degradation of cellulose by *C. subvermispora.*

Acknowledgements

The authors wish to thank D. van den Berg, M. Cawood, and M. Lentz for technical assistance. This work was supported by FRD and SAPPI SAICCOR (Pty) Ltd.

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Received June 16th, 1995

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