Influence of fungal enzyme pre-treatment on totally chlorine-free (TCF) bleaching of dimethyl formamide bagasse pulp

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Abstract
A study was carried out on totally chlorine-free (TCF) bleachability of dimethyl formamide (DMF) treated bagasse pulps exposed to CZ-3 and FP 90031-sp strains of white-rot fungus *Ceriporiopsis subvermispora*. This process involved a bleaching sequence consisting of oxygen and peroxide treatment stages. The effect of enzymatic stage on bleachability properties was studied and compared with control pulps, processed without enzyme addition. A final brightness of 79-80% International Standard Organization of brightness (ISO) was achieved after complete bleaching. The effects of direct bleaching caused pulp brightening (1.7-1.3% ISO) and delignification (>10%) immediately after the enzymatic stage. Under a peroxide charge of 3% to 9%, the improvements in brightness and the bleachability of these pulps were found to be superior to those of the control during all peroxide stages. The selective bleaching of each process was assessed by changes of intrinsic viscosity. Generally higher bleachability and bleaching selectivity of xylanase-treated pulps and the inevitable maximal gain in pulp brightness (or bleach boosting, as a main objective of xylanase application) were only achieved after the first and second peroxide bleaching stages. These substantially diminished by the end of the sequence.

Keywords: *Ceriporiopsis subvermispora*; Fungal enzyme; Brightness; Xylanase; Dimethyl formamide pulping.

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INTRODUCTION

The current environmental pressure to reduce toxic effluents from pulp and paper mill, particularly chlorinated organics from bleach mill effluents, has led to a substantial interest in technological approaches with minimized ecological impact. Organic solvent-based delignification so-called ‘‘organic solvent’’ and totally chlorine free (TCF) pulp bleaching using oxygen-containing oxidative chemicals such as molecular oxygen ozone and hydrogen peroxide proved to the among the best potential alternatives to conventional sulfur and chlorine based industrial pulping and bleaching technologies Stockburger, 1993; Adachi and Chen, 2007; Han *et al*., 2007; Shatalov and Pereira, 2007 a, b; Reeve, 1996).

Lignocellulose degradation is a multienzymatic process due to the complex nature of lignified plant materials (Eriksson *et al*., 1990). This degradation represents an important step for carbon recycling in terrestrial ecosystems involving both hydrolytic and oxidative enzymes. Such enzymes are also of interest for the industrial use of plant biomass including pulp and paper manufacturing (Bajpai and Bajpai, 1998).

Lignin removal, the key step for natural biodegradation of lignocellulose, is required for both converting raw material into pulp and bleaching pulp fibers. The interest for xylan degrading enzyme and its applications in the pulp and paper industries has advanced significantly over the past few years (Christov *et al*.,

1999; Srinivasan and Rele, 1999; Garg *et al*., 1998).

Xylanase treatment can substantially improve the final brightness of bleached pulps, along with a decrease in bleaching costs, when combined with non-chlorine bleaching chemicals (hydrogen peroxide and ozone) within TCF bleaching sequences (Shen Liu, 2007; Allison and Clark, 1994). *Ceriporiopsis subvermispora*, a white rot basidiomycetous fungus, has gained importance in causing selective specific changes in lignin content and structure, which leads into fiber individualization and decolorization of substrate and produces manganese-dependent peroxidase enzyme (Rajasekar, 2007; Yaghoubi *et al*., 2007; Artik *et al*., 2006; Souza-Cruz *et al*., 2004; Saxena *et al*., 2001).

In addition, the hydrogen peroxide bleaching was chosen as a simple to performance, effective and fairly selective chlorine-free process, which is generally used as a separate bleaching stage incorporated into the multi-stage bleaching sequence for successful bleaching of industrial pulps. Shatalov and Pereira (2007b) described this process for three stages H_2O_2 bleaching and two commercial xylanase of pulps and their final brightness of 86% ISO were achieved after complete bleaching (Shatalov and Pereira, 2007b).

In this research, based on our previous publications, dimethyl formamide (DMF) pulp (Ziaie-Shirkolaee *et al*., 2008 a, b, 2007; Soltanali and Ziaie-Shirkoalee, 2007; Rezayati *et al*., 2006; Navaee-Ardeh *et al*., 2004, 2003) was treated with CZ-3 and FP 90031-sp strains of the white-rot fungus *C. subvermispora* and bleached by the three-stage hydrogen peroxide bleaching sequence. The use of the three peroxide stages was intended to achieve maximum peroxide bleaching of the tested pulps. The results were compared to a control sample (processed without enzyme addition) with respect to optical properties, bleaching selectivity (based on intrinsic viscosity) and pulp bleachability.

MATERIALS AND METHODS

Materials: Processed bagasse used in this study was obtained from a local pulp and paper factory (Pars Paper Company), Iran. Before pulping, the raw material was cleaned, sorted and air-dried.

In this research, the dimethyl formamide (DMF) solvent with a medium boiling point (152-154ºC) was selected for delignification of wheat straw at a high cooking temperature of 200ºC and a maximum pressure of 12 atm. This chemical solvent (DMF) can be considered as an environment for solubilizing fragmentation products of lignin that are produced by the thermohydrolysis reaction (Ziaie-Shirkolaee *et al*., 2007).

Pulping: Pulps were made in a 21 liter batch cylindrical mini-digester (stainless steel 32 liter, Chouka, Iran). The mini-digester includes an electrical heater, a motor actuator and the appropriate instruments for measurement and control of pressure and temperature. The pulping process was made as described previously (Ziaie-Shirkolaee *et al*., 2008 a, b, 2007). The cooking conditions consisted of a cooking time and temperature of 150 min and 200ºC, respectively. A DMF concentration of 50% at a constant liquid to dry bagasse weight ratio of 12:1 was used during the process.

Analysis of raw material and pulps: Analyses of raw material and pulp of wheat straw were carried out according to the Tappi Standard Methods (TAPPI, 2002) with the exception of the holocellulose contents which was determined by Wise's sodium chlorite method (Wise and Murphy, 1946), the cellulose content which was determined according to the Kurscher and Hoffner's nitric acid method (Rowell and Young, 1997) and the viscosity of pulp which was measured in cuprietylenediamine (CED) solution according to the SCAN-CM 15:88 standard (SCAN, 1998). Residual lignin content was determined as a Klason and acid-soluble lignin was measured according to the T 222 om-88 and UM 250 TAPPI standards (TAPPI, 2002). Handsheets weighing 60 g/m^2 were formed which were conditioned at 23ºC and 50% RH (Ziaie-Shirkolaee *et al*., 2008 a) for at least 24 h before testing (Soltanali and Ziaie-Shirkolaee, 2007).

Xylanase pre-treatment: The white-rot fungus *Ceriporiopsis subvermispora* was obtained from Forest Products Laboratory in Madison, WI, USA. Two strains (CZ-3 and FP 90031-sp) of the fungus were compared with various other strains, on the basis of their lignin degrading abilities (Akhtar *et al*., 1997; Blanchette *et al*., 1992). All strains were supplied by the Center for Forest Mycology Research of the USDA Forest Products Laboratory in Madison, WI, USA. Liquid inocula were prepared in Petri dishes as

described (Atik and Imamoglu, 2003). The spent medium in the dish containing the fungal biomass was then decanted; mycelium was washed with sterile distilled water and then blended aseptically in a Waring blender (ASN, Germany). Liquid inocula containing 0.1 mg/ml of fragmented mycelium (dry weight; DW) were used for inoculation of the bagasse (approximately 5mg of mycelium per kg of material was used).

Enzyme activities were determined by the dinitrosalicylic acid (DNS) method (Bailey, 1988). Diluted enzyme solution (30 ml) was incubated with 300 ml of 1% (w/v) birch wood xylan (Sigma, Germany) solution (containing 100 mmol/l of acetate buffer and 0.4% (v/v) Tween 20, pH 5) at 40ºC for 20 min. One unit (U) of xylanase activity was defined as the amount of enzyme that catalyses the release of 1 mmol of xylose per minute. The Pulps was incubated at 27ºC for 15 days. The control samples were treated in exactly the same way, but without enzyme addition.

Totally chlorine free totally chlorine-free (TCF) bleaching: Totally chlorine free (TCF) bleaching of pulp was carried out by an oxygen and three stage peroxide bleaching sequence XOQPPP (where O is the oxygen stage, Q the chelating treatment stage, P the hydrogen peroxide bleaching stage and X the enzymatic procedure). The use of the three stages was intended to achieve maximum bleaching of the tested pulps with peroxide. All tested pulps were bleached under equal conditions at each stage. All bleaching stages, except for the oxygen stage were performed in plastic ziplock bags in a water bath with intermittent kneading. The oxygen stage was carried out in a 320 cm^3 stainless-steel pressurized vessel that was immersed in a water bath. The oxygen stage was performed at 70ºC for 1 h using 1.5% (w/v) NaOH and 0.2% (w/v) $MgSO_4$ and pressurized with oxygen (10 Bar). The EDTA treatment was carried out at 5% (w/v) using 1% (w/v) EDTA for 30 min at 50ºC (Shatalov and Pereira, 2007b, 2005; Soltanali and Ziaie-Shirkolaee, 2007; Atik *et al*., 2006). The peroxide bleaching stage contained 3% (v/v) H_2O_2, 1.5% (w/v) NaOH, 0.2% (w/v) $MgSO_4$, and was conducted on pulps with 10% consistency, for 2 h at 70ºC. Prior to use and after each bleaching step, the pulp was thoroughly washed with one liter of distilled water (Shatalov and Pereira, 2007 b, 2005).

RESULTS

The chemical composition of bagasse was determined on an oven-dry weight basis as follows: 51.72% cellulose, 20.7% lignin, 79.4% holocellulose, 46.2% a-cellulose, 2.8% ash and, 1.87% extractable ethanol/dichloromethane. The variations of their means were <10%.

As would be expected from the known mode of xylanase performance during pulp biobleaching, direct brightening and delignification were observed immediately after the X stage, i.e., before chemical bleaching. The gain in brightness of 1.7 and 1.3% ISO as well as removal of lignin by 12.65 and 10.37% (as compared with the control) was noted for *C. subvermispora FP* 90031-*sp* and *C. subvermispora CZ*-3 treated bagasse organic solvent pulps, respectively (Table 1). The brightness stability (reverted brightness) and

Table 1. Xylanase-aided TCF bleaching of bagasse organosolv pulp.

Enzyme treatment	X			XOQP			XOQPP			XOQPPP		
	C*	X_1**	X_2***	C*	X_1**	X_2***	C*	X_1**	X_2***	C*	X_1**	X_2***
Brightness (%ISO)	41.3	43	42.6	63	68.1	67.6	71.2	75.5	75.1	76.5	80.2	79.5
Yellowness	41.2	39.5	40	15.5	13.3	13.9	10.1	9.0	9.9	7.8	7.5	7.9
Reverted brightness (%ISO)	40.1	42.3	41.5	61.2	67.3	66.7	69.8	74.7	74.6	74.3	79.1	78.3
Lignin (% of oven-dry pulp)	3.95	3.45	3.54	2.9	2.35	2.41	2.55	2.18	2.23	2.43	2.06	2.12
Klason	2.35	1.83	1.87	1.14	1.1	1.33	0.95	0.88	0.94	0.89	0.73	0.8
Acid-soluble	1.6	1.62	1.67	1.76	1.25	1.08	1.6	1.3	1.22	1.54	1.33	1.32
Intrinsic viscosity (ml/g)	1193	1229	1216	1070	1096	1083	999	1030	1015	814	817	815

(X: enzymatic pre-treatment, Q: chelating, O: oxygen stage, P: peroxide bleaching stage).
*C: Control sample
**X_1: C. subvermispora FP 90031-sp
***X_2: C. subvermispora CZ-3

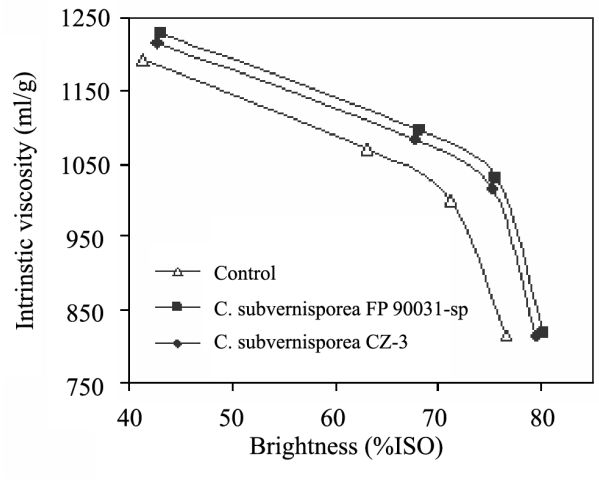

Figure 1. Brightness development with respect to change in intrinsic viscosity of xylanase-treated and control (untreated) pulps during each stage of totally chlorine-free (TCF) bleaching.

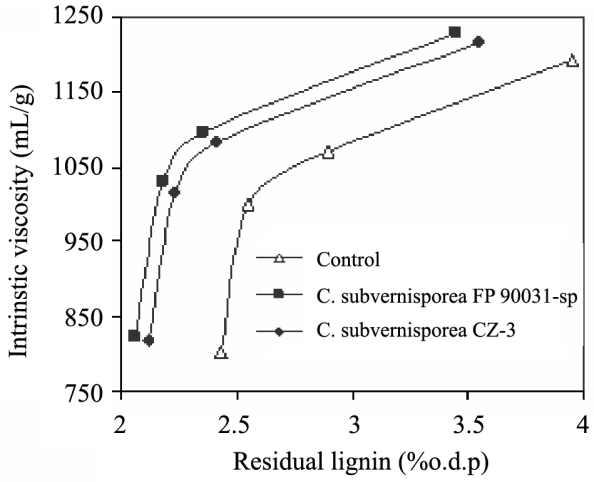

Figure 2. Change in intrinsic viscosity of xylanase-treated and control (untreated) pulps with delignification during each stage of TCF bleaching (o.d.p: oven dry pulp).

intrinsic viscosity of xylanase-treated pulps were also found to be superior to the control.

As shown in Table 1, maximal xylanase bleach boosting was achieved after the first peroxide stage, with equal brightness improvement of 5.1 and 4.6% ISO (in comparison with the control) for *C. subvermispora FP* 90031-*sp* and *C. subvermispora CZ*-3 xylanase preparations. The positive effects of xylanases were then substantially diminished in the two subsequent peroxide stages. The gain in brightness by 3.1 and 2% ISO only (respectively for the *C. subvermispora FP* 90031-*sp* and *C. subvermispora CZ*-3 treated pulps) was noted after complete bleaching, which was even less than that achieved through direct brightening after the enzymatic stage. The negative impact of the consecutive hydrogen peroxide stages on brightness improvement has also been noted for some other pulps treated by fungus xylanase preparations (Yang *et al*., 1992).

The reduced gain in brightness at the end of the bleaching can not be explained by change (or reduction) in lignin removal of enzyme-treated pulps. It is evident from the presented data (Table 1) that both the xylanases enhance pulp delignification during each peroxide stage causing additional lignin loss in comparison with the control. Thus, it is most likely that the polysaccharide-derived chromophores of bagasse organosolv pulps are responsible for the loss in xylanase efficiency during peroxide bleaching.

Bleaching selectivity: On the basis of our previous publications, the advantages of DMF as a solvent in comparison with other pulping processes include more retention of carbohydrates and low degradation of cellulose (as assessed by the yield and viscosity in comparison to other processes) (Ziaie-Shirkolaee *et al*., 2008 a,b; 2007). The results of viscosity can be applied in estimating the extent of cellulose degradation during cooking process (SCAN, 1998). It can also be observed from Table 1, that xylanase treatment of organosolv pulps is the influential factor with respect to their viscosities as compared with the control pulp.

In Figures 1 and 2, the intrinsic viscosity of enzyme-treated and control pulps, measured after each bleaching stage is shown as a function of residual lignin content and pulp brightness, respectively. Obviously, the xylanases substantially improve the peroxide bleaching selectivity of bagasse organosolv pulp. In the selected range of residual lignin content of 2.3-4.0% and pulp brightness of 44-78% ISO, the intrinsic viscosity of both the *C. subvermispora FP* 90031-*sp* and *C. subvermispora CZ-3* treated pulps is always higher than those of the control. At the same time, the dramatic drop in viscosity (over the control) of both enzyme-treated pulps was observed after more profound bleaching (ca. 2% lignin and ca. 79-80% ISO brightness, Figs. 1 and 2) at the end of the last peroxide stage, thereby giving somewhat inferior final viscosity values for fully bleached enzyme-treated pulps as compared to those of the control (Table 1). Thus, the positive effect of xylanase treatment on pulp viscosity shown after an enzymatic and the first two chemical bleaching stages is lost by the end of the complete bleaching. The enhanced degradation of ligninassoci-

ated carbohydrates and cellulose under deep delignification of enzyme-treated pulps within the last peroxide stage caused the decrease in pulp (Shatalov and Pereira, 2005).

Pulp bleachability: The pulp bleachability can be numerically expressed by the amount of active bleaching chemical consumed in order to get the specified value of brightness. Alternatively, the pulp bleachability can be considered as an efficiency of the active bleaching chemical to improve the quality parameters (brightness and lignin content) of bleached pulps.

In Figure 3, the brightness improvement (%) and lignin removal (%) from enzyme-treatment and control samples of bagasse organosolv pulps are plotted against values of hydrogen peroxide consumption during each bleaching stage. The values of relative brightness improvement (ΔB) and lignin removal (ΔL) were calculated according to following equations:

$$\Delta B = [(B_i - B_o) / B_o] \, 100 \, (\%),$$
$$\Delta L = [(L_i - L_o) / L_o] \, 100 \, (\%),$$

Where B_o and L_o represent the starting values of brightness and lignin content of unbleached pulps; B_i and L_i are the current values of brightness and lignin content of bleached pulps; i = 1, 2, 3 represent the number of bleaching stages (Shatalov and Pereira, 2005). From Figure 3, it is evident that in terms of lignin removal, the bleachability of xylanase treated pulps at each stage of the bleaching sequence is substantially higher than that of the control. Lignin removal by 40.28% and 40.11% and 38.48 was noted for *C. subvermispora FP* 90031-*sp* and *C. subvermispora CZ*-3 and the control sample, respectively, within the specified range of peroxide charge of 3-9%. Also, according to Figure 3, in terms of brightness improvement and the bleachability of these pulps were superior to the those of the control during all peroxide stages.

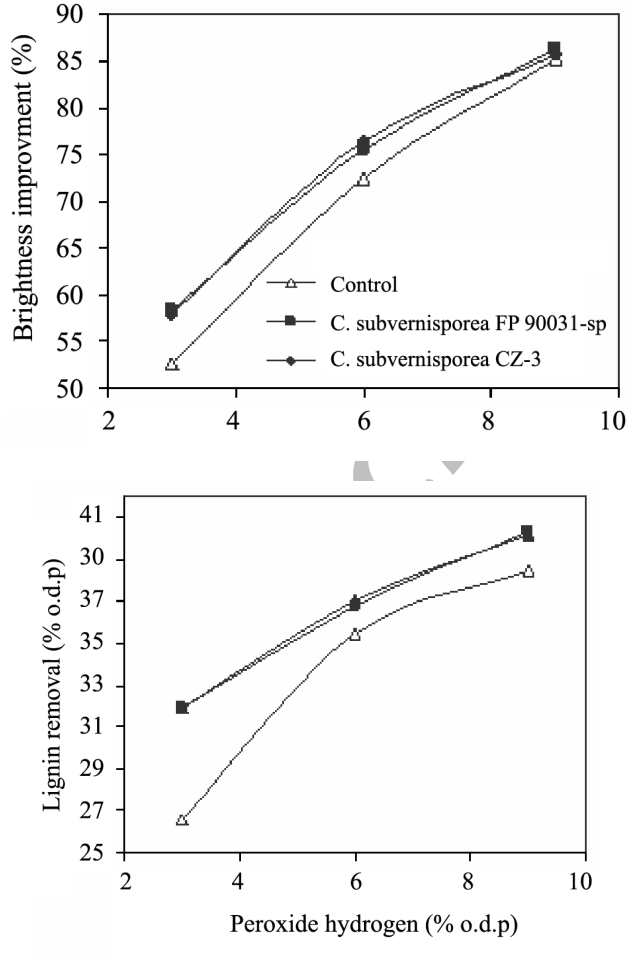

Figure 3. Residual lignin removal (bottom) and brightness improvement (top) of xylanase-treated and control (untreated) pulps during TCF bleaching (o.d.p: oven dry pulp).

DISCUSSION

From Figure 3, it is evident that in terms of lignin removal, the bleachability of xylanase treated pulps at each stage of the bleaching sequence is substantially higher in comparison to that of the control. Lignin removal by 40.28%, 40.11% and 38.48 was observed for *C. subvermispora FP* 90031-*sp*, *C. subvermispora CZ*-3 and the control sample, respectively, within the specified peroxide charge range of 3-9%. Also, according to Figure 3, under a peroxide charge of 3% to 9%, the brightness improvement and the bleachability of these pulps were superior to those of the control during all peroxide stages.

The properties of *C. subvermispora FP* 90031-*sp* treated pulps much better than those of *C. subvermispora CZ*-3 and the control sample. Generally, The beneficial effect of fungal enzymes on bleachability of bagasse DMF pulp was shown to be limited when enzymatic pre-treatment was combined with the extended oxygen and hydrogen peroxide bleaching sequence. A similar effect of direct bleaching has been reported for some other pulps (Shatalov and Pereira, 2007 a; Suurnakki *et al*., 1994; Yang *et al*., 1992) which has been attributed to the enzymatic attack of lignin-carbohydrate complexes (LCC) resulting in

removal of certain lignin fragments and lignin-associated chromophores (Jong *et al*., 1997; Yang and Eriksson 1992). The enzyme-assisted removal of xylan-derived chromophores (e.g., hexenuronic acids) and dissolved xylooligosaccharide fractions (Shatalov and Pereira, 2007a) can also contribute to brightness as well as improved brightness stability (Buchert *et al*., 1997). The dissolution of lowmolecular weight (oligosaccharide) xylan fractions is an obvious reason for elevated pulp viscosity of enzyme-treated pulps (Suurnakki *et al*., 1994).

A generally higher bleachability and bleaching selectivity of xylanase-treated pulps and maximal gain in pulp brightness (or bleach boosting, as a main objective of xylanase application) could be achieved only after the first peroxide bleaching stage which substantially diminished by the end of the sequence. The final gain in brightness of fully bleached pulps was close to that achieved by direct brightening during an enzymatic stage, i.e., before proper chemical bleaching. In addition, pulp viscosity is a basic as well as one of the most important pulp properties that makes it possible to check the extent of carbohydrate degradation caused by pulping and bleaching and thereby predict the quality of the final fiber products. The change in pulp viscosity with brightness development and lignin removal defines the selectivity of the bleaching process with respect to the main bleaching objectives, brightening and delignification (Shatalov and Pereira, 2005).

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