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Application of *Aspergillus fumigatus* Xylanase for Quality Improvement of Waste Paper Pulp

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Enzymes as an alternative to polluting technologies have now reached a level such that they can be considered for application (Srinivasan and Rele 1999). Due to the ever-increasing demand for paper, the paper pulp industry is rapidly growing and becoming one of the worst offenders in environmental terms. Chlorine is by far the most common halide present in pulp mill effluent. Organic chlorine compounds are formed during the chemical bleaching of pulp. These compounds are formed mainly from the reaction between residual lignin present in the wood fibers and the chlorine used for bleaching (Khandeparkar and Bhosle 2006). Pulp mills are voracious water users and conventional cooking and bleaching processes have caused serious water pollution problems (Xie and Long 2000, Zhao et al. 2006). Their consumption of fresh water can seriously harm habitats near pulp mills, reduce the water levels necessary for fish, and alter the water temperature, which is a critical environmental factor for fish. Mill effluent tends to be deep brown in colour and can thus interfere with aquatic photosynthesis. It also contains compounds that are toxic to fish such as methyl mercaptan, and paper and wood pulp preservatives such as pentachlorophenol and sodium pentachlorophenate. As the use of chlorine-containing paper and products can cause some health problems, there is increasing demand for totally chlorine-free paper and paper products such as baby diapers and food packaging (Shoham et al. 1992; Taspinar and Kolankaya 1998).

It has been proven in numerous published studies that enzyme (mainly xylanase) pre-bleaching is a clean, environmentally friendly, economically attractive technology that can decrease the amount of bleaching chemicals required to attain a given brightness in subsequent chemical bleaching stage (Zhao et al. 2006). Xylanases are widely used in the manufacture of bread and drinks, textiles, waste treatments and as a biological bleacher in the paper industry (Polizeli et al. 2005; Beg et al. 2001). Owing to the increasing biotechnological importance of thermostable xylanases, many thermophilic fungi have been examined for xylanase production (Maheshwari et al. 2000; Singh et al. 2003; Yang et al. 2006).

In this study, the possibility of reducing chlorine usage and its removal from chlorine-bleached waste paper pulp was investigated using xylanase produced from *A. fumigatus*. The purified xylanase was used as a prebleaching agent for waste paper pulp to improve paper quality and reduce chemical usage. Kappa number, brightness, burst capacity, thickness and bulkness of the pulp were used as paper quality parameters.

Materials and Methods

The fungus *Aspergillus fumigatus* was isolated from garden soil by the serial dilution technique (Waksman, 1922) and maintained on Czapek Dox agar (Purvis et al. 1964) slants at 37°C. For inoculum preparation, the fungus was grown on Czapek Dox agar plates for four days at 37°C and the plates were flooded with sterile, distilled water to attain a spore concentration of 10^6 spores/mL.

For xylanase production, the fungus was grown in minimal salt medium amended with 1% (w/v) oat spelt xylan for six days on a rotary shaker (125 rpm) at 27 ± 2°C (Carter and Bull 1969). After six days, the fungal biomass

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was filtered and the filtrate was centrifuged at 10,000 rpm for 20 min at 4°C. Xylanase and cellulase activity was determined in terms of reducing sugar as described by Nelson Somogyi (Somogyi, 1952).

Unbleached waste paper pulp was obtained from the GVG paper mills, Udumalpet, Tamilnadu, India. The optimum conditions for efficient biobleaching process in terms of treatment period, pulp consistency, temperature and enzyme charge were determined. To determine the optimum treatment period, 1g of the pulp was incubated with 5 IU/g of enzyme charge for various treatment periods (6, 12, 18, 24, 30 h) at a standard temperature of 60°C. Similarly, to determine the optimum pulp consistency, temperature and enzyme charge the waste paper pulp was subjected to various pulp consistencies (0.5, 1.0, 1.5 and 2.0%), temperature (40, 50, 60, and 70°C) and enzyme charge (2.5, 5.0, 7.5 IU/g). The efficiency of each treatment process was measured in terms of reduction in kappa number (TAPPI, 1990).

The waste paper pulp (1.5% consistency) was charged with the optimum enzyme concentration of 5 IU/g pulp at 60°C for 18h. After 18 h, the pulp was filtered and the supernatant was assayed for pH and reducing sugar content. A portion of the filtered pulp was made into hand sheets and the quality of the pulp was determined (TAPPI, 1990). After thorough washing in distilled water, the remaining pulp from the enzyme pretreatment was dried and used for alkali and chlorine treatment. Pulp treated with potassium phosphate buffer (50 mM; pH 7.2) served as the control.

In alkali treatment, the enzyme-pretreated pulp (1% consistency) was treated with 3% sodium hydroxide at 80°C for 1 h (Christov and Prior 1994). After treatment, the pulp was washed with excess water, filtered and dried. Hand sheets were prepared from the treated pulp and the quality parameters were determined. For chlorine treatment, a portion of the enzyme-pretreated pulp was subjected to the conventional chlorine extraction sequence proposed by Buchert et al. (1992). Hand sheets were prepared from the treated pulp and the quality parameters were determined. In each treatment an individual control was maintained.

The brightness of the hand sheets was measured at 475 nm in a Perkin Elmer $\lambda 3\beta$ spectrophotometer equipped with a reflectance sphere. Bulkness was measured as the ratio of the volume of the paper under standard atmospheric conditions to the volume of an equal weight of water at 4°C. Burst capacity and thickness was measured using an electronic incinerator and a Vernier caliber, respectively.

All experiments were conducted in triplicate, and their mean values are presented. Kappa number, brightness, burst capacity, thickness and bulkness of the pulp obtained after various treatments were statistically analyzed by Duncan's multiple-range test (DMRT) (Duncan, 1955).

Results and Discussion

Pre-bleaching with xylanase lowered the amount of chlorine compounds used by up to 30% and reduced the level of organochlorines in the effluent by nearly 20%. The use of xylanases could lead to the replacement of 5–7 kg of chlorine dioxide per ton of kraft pulp and an average decrease of 2–4 units in kappa number (Polizeli et al. 2005). The efficacy of microbial xylanases in bleaching process has been studied for *A. oryzae* (Christov et al. 2000), *A. niger* (Zhao et al. 2002) and *A. nidulans* (Taneja et al. 2002).

The use of xylanase in pulping and bleaching facilitates the removal of xylan, which enhances the extractability of lignin from the wood by other pulping and bleaching chemicals. In turn, the chemical requirements and effluent load from the bleach plant are significantly reduced (Senior et al. 1992). The test fungus grown in the minimal salt medium amended with 1% (w/v) oat spelt xylan as the substrate produced a xylanase activity of 7.20 IU/mL. Although the xylanase had a lower cellulose activity, it lacked activity towards substituted xylan.

The results revealed that the xylanase obtained in the present study had an optimum pH and temperature of 6.0 and 60°C, respectively. The enzyme was stable for four days at 30°C and 24 h at 60°C. These results showed that xylanase purified from *A. fumigatus* is highly thermostable. Hence, the xylanase purified in the present study will be stable over a wide range of temperature and will also withstand temperature increases during the pulping and bleaching processes.

The waste paper pulp was pretreated with *A. fumigatus* xylanase. The enzyme-pretreated pulp was then subjected to alkali treatment and the EDED (E: alkali extraction; D: Chlorine dioxide) process. The effect of the treatments on paper quality was analyzed by estimating the amount of pentosans released, kappa number, brightness, burst capacity, thickness and bulkness of the treated pulp.

In the present study, the optimum conditions for effective reduction of kappa number from the waste paper pulp were determined as treatment period of 18 h, pulp consistency of 1.5%, temperature of 60°C and enzyme charge of 5 IU/g pulp (Fig. 1). The amount of pentosans released by enzyme pretreatment, enzyme–alkali treatment and enzyme–EDED treatment processes were 1.99, 0.48 and 0.32%, respectively; whereas in the control treatment 0.55, 0.42 and 0.25% of pentosans were released, respectively. The DMRT analysis revealed that enzyme pretreatment released larger amounts of pentosans than the other two treatments. The enzyme pretreatment process itself reduced

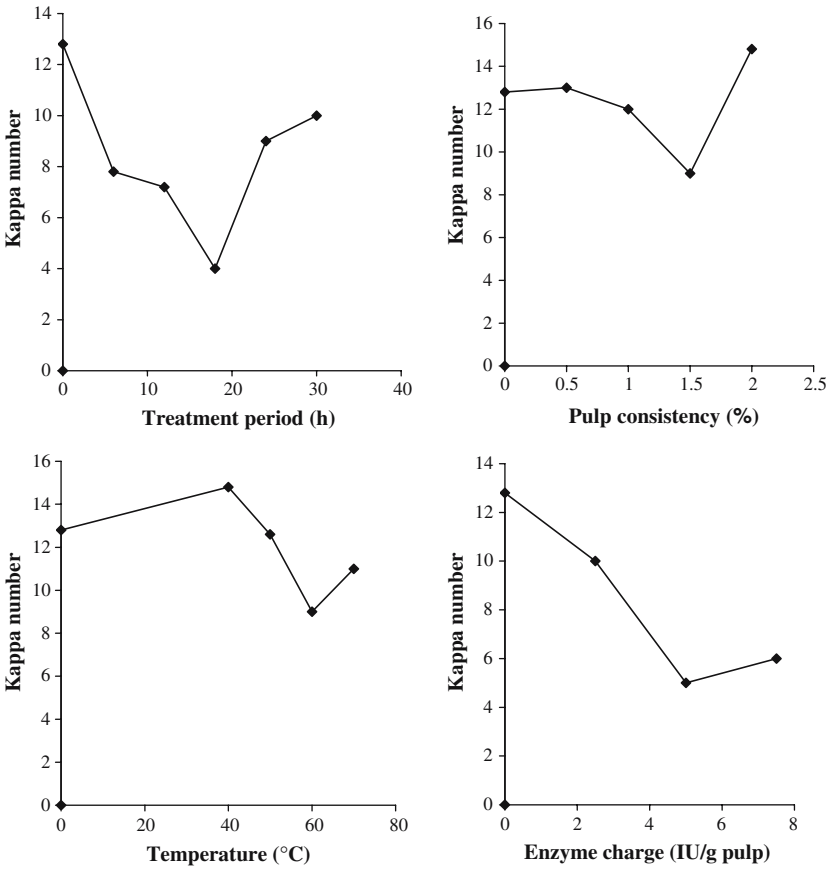

Fig. 1 Optimum conditions for the treatment of waste paper pulp

the kappa number from 19 to 15 and increased the brightness from 30 to 32 ISO units. When the enzyme-pretreated pulp was subjected to alkali treatment, the kappa number was reduced to 13 and the brightness was increased to 34. When the enzyme pretreated pulp was subjected to the EDED process, the kappa number was reduced to 11 and the brightness increased to 36 ISO units. The other parameters such as burst capacity, thickness and bulkness of the hand-made sheets were also measured to check for the quality of the paper.

Pretreatment of pulp with xylanases from *A. awamori, A. tamarii* and *T. Koningii* before the EDED process reduced the kappa number by 19.36% while xylanase from *A. japonicus* reduced the kappa number by 13.98%; *A. ficum* and *A. oryzae* xylanases reduced the kappa number by 8.6%. The brightness was increased to approximately the same level of 45.70–49.67% in each case (Palaniswamy, 1997). Angayarkanni (2006) reported that *Aspergillus* spp. xylanase induced the release of pentosans from the pulp, which indicates the removal of hemicelluloses from the paper pulp with an increase in brightness of 41 ISO units from 19.83 ISO units and reduced kappa number of 6.7–7.2 from 18.60.

The biotreatment of bagasse pulp with xylanase from *Bacillus* sp. NCIM 59 resulted in a 21% reduction in kappa number and a 2.5% increase in brightness (Kulkarni and Rao 1996). Silva et al. (1994) reported that xylanase from *Humicola* sp. reduced the kappa number by 25% and increased the brightness by 10%. A single pretreatment with the α-galactosidase, mannanase and xylanase enzymes from *Pseudomonas fluorescens* spp. yielded a kappa number reduction from 24 to 9.72 (Clarke et al. 2000).

Paper quality parameters were analyzed in the treated waste paper pulp. The burst capacity was increased from 4 units in the control to 7 and 8 units in the enzyme–alkali and enzyme–EDED treatments; the thickness of the paper increased from 360 mm in the enzyme treatment to 422 mm in the enzyme–alkali treatment and 308 mm in the enzyme–EDED treatment. The bulkness of the paper was reduced from 500 units to 474 units by the enzyme–alkali treatment and to 346 by the enzyme–EDED treatment (Table 1). The study revealed that pretreatment of waste paper pulp with xylanase enzyme from *A. fumigatus* at 5 IU/g pulp concentration and 1.5% pulp consistency at 60°C for 18 h followed by the EDED process was effective in increasing the quality of the paper prepared from the waste paper pulp. Xylanase pretreatment of waste paper pulp will facilitate reduced usage of chemical bleaching agents in paper manufacture and effective recycling of waste papers to avoid the accumulation of solid waste in the environment.

Table 1 Effect of xylanase treatment on paper quality

Treatment/Enzyme concentration (IU/g pulp)	Pentosans released (%)	DMRT rank	Kappa number	DMRT rank	Brightness (ISO units)	DMRT rank	Burst capacity	DMRT rank	Thickness (mm)	DMRT rank	Bulkness (mm)	DMRT rank
Enzyme treatment												
Control	0.55	**b, 2**	19	f, 6	30	f, 6	4	d, 5	360	**c, 3**	500	**a, 1**
Enzyme pretreatment	1.99	**a, 1**	15	**c, 3**	32	d, 4	5	**c, 4**	305	e, 5	314	f, 6
Enzyme and alkali treatment												
Control	0.42	**cd, 4**	17	**d, 4**	31	e, 5	5	c, 4	249	f, 6	345	d, 4
Enzyme pretreatment	0.48	**bc, 3**	13	**b, 2**	34	**b, 2**	7	**b, 2**	422	**a ,1**	474	**b, 2**
Enzyme and EDED treatment												
Control	0.25	e, 6	18	e, 5	33	**c, 3**	5.4	**c, 3**	397	**b, 2**	333	e ,5
Enzyme pretreatment	0.32	de, 5	11	**a, 1**	36	**a, 1**	8	**a, 1**	308	d, 4	346	**c, 3**
CV (%)	10.3		3.2		3.2		9.8		0.2		0.1	
P	1		1		1		1		1		1	
LSD (1%)	0.172		1.233		1.384		1.394		1.348		1.203	
SED	0.32		0.404		0.453		0.456		0.441		0.394	

Treatment period: 18 h; pulp consistency: 1.5 %; temperature: 60°C; enzyme charge: 5 IU/g of pulp

CV Coefficient of variation, *LSD* Least Square Difference, *P* Probability, *SED* Standard Error Deviation

The cost of enzyme production can be overcome by finding optimal growth conditions, isolating hyper producing organisms, producing mutants and possibly by other genetic engineering methods.

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