Enzymatic bleaching of kraft pulp by xylanase from *Aspergillus sydowii* SBS 45

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Abstract Crude xylanase from *Aspergillus sydowii* SBS 45 was tested for enzymatic bleaching of kraft (Decker) pulp. After optimization of three parameters, consistency of pulp, retention time and enzyme dose, considerable increase in the release of UV and visible absorbance spectra of materials and reducing sugars was observed, which clearly indicated the action of xylanase on pulp. Final brightness of pulp was increased from 29.42 to 70.42% and kappa number was reduced from 15.93 to 1.61, when 25 U of xylanase was given with a retention time of 5 h and at a consistency of 10%. When 10 U g⁻¹ xylanase was given, 14.3% elemental chlorine and 14.3% H₂O₂ could be reduced and when 25 U g⁻¹ xylanase was given 14.3% elemental chlorine and 28.6% H₂O₂ could be reduced thereby retaining the brightness at control level.

Keywords *Aspergillus sydowii* · Xylanase · Kappa number · Kraft pulp · Enzymatic bleaching

Introduction

Xylan is the major hemicellulose constituent of hard wood and soft wood, and is the next most abundant renewable polysaccharide after cellulose. Xylan is a heterogeneous carbohydrate consisting of a homo polymeric backbone of β -1,4 linked D-xylopyranose units and short chain branches consisting of O-acetyl, α -L-arabinofuranosyl and α -D-glucuronyl residues. Endo-xylanase (β -1,4-D-xylan-xylanohydrolase, EC 3.2.1.8) is the key enzyme for xylan depolymerization. A large number of bacteria and fungi are known to produce xylanases [1, 2]. Filamentous fungi are industrially important producers of this enzyme due to extra-cellular release of xylanases, higher yield compared to yeast and bacteria and production of several auxiliary enzymes that are necessary for de-branching of the substituted xylans [3]. However, fungal xylanases are generally associated with concurrent production of cellulases [4]. Xylanases are produced by either solid state or submerged fermentations. Enzyme productivity in solid state fermentation (SSF) is usually much higher than that of submerged fermentation [5]. Therefore solid state fermentation has gained renewed interest from researchers in recent years and has often been employed for the production of xylanases, because of economic and engineering advantages [6, 7]. Microbial xylanases are used in animal feed industry, textile, food processing and in the production of several valuable products like xylitol and ethanol [8]. Bio-bleaching of pulps using xylanase is one of the most suitable applications to be used in the pulp and paper industry to reduce and/or eliminate the use of chlorine and chlorine dioxide.

The most common pulping process is the kraft process or sulfite process where cooking of wood chips is carried

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out in a solution of $\text{Na}_2\text{S}/\text{NaOH}$ at about 170°C for 2 h resulting in the degradation and solubilization of lignin. To obtain pulp of very high brightness and brightness stability all the lignin must be removed from the pulp. This residual lignin is removed by subsequent bleaching process. Organic chlorine by-products formed during these processes are toxic and highly resistant to biodegradation and form one of the major sources of environmental pollution [9]. Xylanase enhances pulp bleaching by breaking covalent linkages between the hemicellulose and lignin moieties, releasing chromophores and lignin from the pulp. In addition, after kraft pulping some of the xylan may be re-precipitated on the surface of the pulp fibre. The xylanase treatment causes de-polymerisation of xylan precipitated on the surface of the fibre, which would facilitate penetration by subsequent bleaching agents [10].

The main objective of the present study is to find out the suitability of xylanase from *Aspergillus sydowii* SBS 45 in the pulp-paper industry as a potential enzymatic bleaching agent.

Materials and methods

Chemicals

Birchwood xylan was purchased from Sigma chemicals Co., USA. Carboxy Methyl Cellulose was purchased from Merck, India.

Organism and growth conditions

Aspergillus sydowii SBS 45 was isolated in our laboratory and was maintained on Sabouraud dextrose agar (SDA) medium containing 0.1% (w/v) birch wood xylan. The plates were incubated at 30°C for seven days for spore production and then stored at 4°C until use.

Xylanase production by *Aspergillus sydowii* SBS 45

Solid state fermentation (SSF) was carried out using wheat bran as the substrate. Fermentation was carried out in 500 ml Erlenmeyer flask containing 20 g wheat bran moistened with 20 ml distilled water. The media were then autoclaved for 20 min at 121°C (15 lbs). After cooling, the flasks were inoculated with 1 ml of spore suspension having 1×10^6 spores ml^{-1} . The flasks were incubated at 30°C under static conditions for 9 days. After incubation, the enzyme was harvested in distilled water. The fermented slurry was filtered through cheese cloth and centrifuged at $10000 \times g$ for 20 min at 4°C and the clear supernatant was used for enzyme assays.

Enzyme assays

Xylanase activity was determined by taking 0.9 ml of 1% (w/v) birch wood xylan (prepared in 50 mM Na-citrate buffer, pH 5.3) and 0.1 ml of suitably diluted enzyme and the mixture was incubated at 50°C for 5 min [11]. The reaction was stopped by the addition of 1.5 ml 3,5-dinitrosalicylic acid (DNS) reagent and the contents were boiled for 5 min [12]. After cooling the colour developed was read at 540 nm. The amount of reducing sugar liberated was quantified using xylose as standard. One unit of xylanase is defined as the amount of enzyme that liberates 1 μmol of xylose equivalents per minute under the assay conditions.

Cellulase (CMCase) activity was determined by incubating 0.9 ml of 1% (w/v) CMC (prepared in 50 mM Na-citrate buffer pH 5.3) and 0.1 ml of suitably diluted enzyme at 50°C for 15 min [13]. The reaction was stopped by the addition of 1.5 ml 3,5-dinitrosalicylic acid (DNS) reagent and the contents were boiled for 5 min [12]. The colour developed was read at 540 nm. The amount of reducing sugar liberated was quantified using glucose as standard. One unit of cellulase is defined as the amount of enzyme that liberates 1 μmol of glucose equivalents per minute under the assay conditions.

Characterisation of xylanase

Effect of temperature on xylanase activity was determined by varying reaction temperature from 30 – 80°C . The thermal stability of xylanase was determined by measuring residual activity after incubating enzymes at temperatures ranging from 30 – 80°C for 5 h. Effect of pH on xylanase activity was determined by incubating enzyme at various pH ranging from 2–12. Various buffers used were 50 mM glycine-HCl (2–3), 50 mM sodium acetate (4–6), 50 mM Tris-HCl (7–9) and 50 mM glycine-NaOH (10–12). The pH stability was determined by incubating enzyme at a pH of 2–12 at room temperature for 5 h and residual activity was determined.

Xylanase bleaching of Kraft (Decker) pulp

The bleaching studies were conducted in the R&D Lab of Hindustan Newsprint Limited, Kottayam, Kerala, India. Enzymatic treatment was carried out in kraft (Decker) pulp (made from 50% Bamboo and 50% Reed) using crude xylanase from *Aspergillus sydowii* SBS 45. The pH of the unbleached pulp was 8.2. Three parameters viz. consistency of pulp, incubation time and xylanase dose, which influence the effectiveness of xylanase treatment, were optimised.

Pulp equivalent to 20 g OD (oven dried) taken in polyethylene bags were charged with xylanase (2, 5, 10, 25 Ug^{-1}

OD pulp). Consistency was adjusted (3, 5, 10%) and the sealed plastic bags were incubated (1, 3, 5 h) in a water bath at 40°C with intermittent kneading. Control samples were treated under same conditions with distilled water. After incubation, pulp slurry was filtered through a Buchner funnel and filtrates were analysed [14]. Now the pulp was washed to remove residual enzyme and chlorination was done at 3% consistency for 45 min at 30°C. After washing H₂O₂ bleaching was carried out at 10% consistency at 78°C for 90 min. After washing pulp was analysed for kappa number and brightness.

Influence of the consistency of the pulp on xylanase bleaching

Consistency of the pulp was set at 3, 5 and 10%. 2 U xylanase gm⁻¹ OD pulp was given. Incubation time given was 1 h. Temperature was set at 40°C. After xylanase treatment routine bleaching procedures were done as described previously.

Influence of incubation time on xylanase bleaching

The incubation time set at 1, 3 and 5 h. 2 U xylanase gm⁻¹ OD pulp was given. Consistency was adjusted to 10%. Temperature was set at 40°C.

Influence of different dose of xylanase on bleaching

Different dose of xylanase such as 2, 5, 10 and 25 Ugm⁻¹ OD pulp was given. Consistency was adjusted to 10% and 5 h of incubation time was given. Incubation was carried out at 40°C.

Analysis of pulp filtrates

Absorbance of filtrate collected after xylanase treatment was taken at 237 nm, 280 nm and 465 nm for determining the release of lignin derived compounds and chromophoric materials from the pulp [15]. Amount of reducing sugars released was determined spectrophotometrically at 540 nm according to DNS method [12]. Residual xylanase activity was measured as previously [11].

Pulp properties

Kappa number of pulp was measured according to Technical Association of Pulp and Paper Industry (TAPPI) Test Methods T236 cm-85. Kappa number is defined as the amount of (ml) 0.1 N KmnO₄ solution consumed by 1g moisture-free pulp under the conditions specified. The brightness was determined in an ISO brightness testing machine (Colour Touch).

Results and discussion

*Xylanase production by *Aspergillus sydowii* SBS 45*

Aspergillus sydowii SBS 45 secreted 87.12 U ml⁻¹ xylanase and 3.05 U ml⁻¹ cellulase when it was grown on wheat bran moistened with distilled water. Addition of organic or inorganic medium components was not necessary for the fungal growth and xylanase production, but the enzyme titer was lower compared to SSF conditions with mineral salt medium, where 102.58 U ml⁻¹ xylanase and 1.29 U ml⁻¹ cellulase was produced. Higher cellulase production in former condition might be due to lack of additional carbon source – birch wood xylan. During preliminary studies (results not shown) some of the medium components found in enzyme preparation were found to be inhibitory for subsequent bleaching stages (chlorination and H₂O₂), and affected the final brightness of pulp. Hence, to avoid the interference of medium components in bleaching process, mineral salt medium was omitted during the production of xylanase. Mineral salts medium contain metal ions like Fe³⁺, Zn²⁺ and Ca²⁺ and these metal ions might show some interference during chlorination and subsequent H₂O₂ stage (H₂O₂ is very sensitive to metal ions such as Fe³⁺).

Characterisation of xylanase

Xylanase was optimally active at 50°C. After 5 h of incubation the enzyme retained 100% activity at 30°C, 90%

Fig. 1 Effect of temperature on the stability of xylanase from *Aspergillus sydowii* SBS 45. Enzyme was incubated at 30–80°C for 5 h. Aliquots withdrawn after each hour up to 5 h and residual activity was measured. (■-Relative activity after 1 h (%); ●-Relative activity after 2 h (%); ▲-Relative activity after 3 h (%); ▼-Relative activity after 4 h (%); ◆- Relative activity after 5 h (%)).

activity at 40°C and more than 40% activity at 50°C (Fig. 1). Activity was negligible at 70°C and 80°C. The optimum pH of xylanase was 10.0. More than 95% activity was retained at pH 5–9, 90% activity was retained at pH 10 and 11 after 5 h of incubation at 30°C (Fig. 2).

Application of xylanase in the pulp and paper industry

Analysis of pulp filtrates

Enzymatic bleaching of kraft (Decker) pulp with xylanase resulted in considerable improvement in the release of

Fig. 2 Effect of pH on the stability of xylanase from *Aspergillus sydowii* SBS 45. Xylanase was incubated in buffers like 50 mM glycine-HCl (2-3), 50 mM sodium acetate (4-6), 50 mM Tris-HCl (7-9) and 50mM glycine-NaOH (10-12) at 30°C. Residual activity was measured after each hour up to 5 h. (■-Relative activity after 1 h (%); ●-Relative activity after 2 h (%); ▲-Relative activity after 3 h (%); ▼-Relative activity after 4 h (%); ◆- Relative activity after 5 h (%))

Fig. 3 Effect of different % of consistency on xylanase bleaching on the release of chromophoric material and reducing sugars from Decker pulp (●- reducing sugar; ▲-absorbance at 237 nm; ▼- absorbance at 280 nm; ■- absorbance at 465 nm).

UV and visible absorbance spectra of materials (237 nm 280 nm 465 nm) (Figs. 3, 4, 5). From optimization studies it was clear that 10% consistency was most suitable to achieve maximum brightness. At this consistency, action of xylanase on pulp fibers was maximum. Release of chromophores and reducing sugars was maximum at 10% consistency. According to Daneault et al. [16] pulp consistency determine effective dispersion of enzyme and improve the efficacy of enzyme treatment.

More than 70% increase in the release of reducing sugars, and 20% increase in the release of UV and visible absorbance spectra of materials was observed when time of incubation was increased from 1 h to 5 h. Li et al. [17] reported that the amount of reducing sugars released from wheat straw pulp by xylanase of *Thermomyces lanugin-*

Fig. 4 Effect of retention time on xylanase bleaching on the release of chromophoric material and reducing sugars from Decker pulp (●- reducing sugar; ▲-absorbance at 237 nm; ▼- absorbance at 280 nm; ■- absorbance at 465 nm).

Fig. 5 Effect of different enzyme dose on xylanase bleaching on the release of chromophoric material and reducing sugars from Decker pulp (●- reducing sugar; ▲-absorbance at 237 nm; ▼- absorbance at 280 nm; ■- absorbance at 465 nm).

nosus CBS 288.54 was significantly greater with increasing time.

When enzyme dose was increased from 2 U to 25 U g⁻¹ more than 40% increase in the release of UV and visible absorbance spectra of materials was observed. The reducing sugar release was maximum at 25 U g⁻¹. Medeiros et al. [18] reported that the release of chromophores is a better indication of xylanase action on pulp than reducing sugar because the reducing sugars can be produced continuously from the xylo-oligomers produced by the initial hydrolysis of xylan from pulp. This might be the reason for the elevated level of reducing sugars in filtrates. Bissoon et al. [14] also reported that when enzyme dose was increased, a corresponding increase in the release of chromophoric materials and reducing sugars was observed. Shobhit et al. [19] reported that the amount of reducing sugars released from the pulp was found to depend on the xylanase dose. According to Salles et al. [20], prolonged time of incubation and higher doses of enzyme lead to an increase in chromophore release and increased absorbance at 237 nm attributed to lignin depolymerisation. Elegir et al. [21] reported that the release of chromophores and absorbing materials at 237 nm increased with enzyme dose and this correlates well with total sugar release. This could be considered as a simple method to determine the efficacy of the enzyme treatment.

Analysis of pulp filtrates shown that the enzyme retained 90% activity, suggesting that xylanase from *Aspergillus sydowii* SBS 45, could withstand the incubation temperature (40°C) for 5 h at a pH of 8.2. Christov et al. [22] reported residual xylanase activity in filtrates of *Aspergillus oryzae* and *Gliocladium viridae*. Filtrate after xylanase treatment could be used for recycling as part of chemical recovery.

Pulp properties

After the optimization steps – consistency of pulp, incubation time and enzyme dose, brightness was increased from 64.43 to 70.42% and kappa number was reduced from 2.91 to 1.61 (Figs. 6–8). Brightness and kappa number of unbleached Decker pulp was 29.42% and 15.93 respectively. Turner et al. [23] reported that brightness of enzyme treated pulps were 5–7 points higher than those for pulp bleached without enzyme treatment and kappa number decrease with enzyme treatment alone did not decreased much, but enzyme treated pulps did respond better to further bleaching than the non-enzyme treated pulps, thus making downstream bleaching more effective. The increase in brightness up to 3.93% in wheat straw pulp was reported when it was treated by xylanase from *Thermomyces lanuginosus* 288.54 by Li et al. [17]. Medeiros et al. [18] reported reduction in kappa number and improvement of brightness

Fig. 6 Effect of different enzyme dose on xylanase bleaching on final brightness and kappa number of Decker pulp (▲ - kappa number; ■ - brightness).

Fig. 7 Effect of retention time on xylanase bleaching on final brightness and kappa number of Decker pulp (▲ - kappa number; ■ - brightness).

Fig. 8 Effect of different enzyme dose on xylanase bleaching on final brightness and kappa number of Decker pulp (▲ - kappa number; ■ - brightness).

in kraft pulps after bleaching with xylanases from *A.niger*, *T.longibrachiatum* and *P.corylophilum*. After treating with xylanase, an increase in brightness and reduction in kappa number in bagasse pulp and kraft pulp was reported by Madlala et al. [24]. Enhanced pulp brightness after xylanase bleaching was also reported in bagasse pulp by Bissoon et al. [14] and Kulkarni and Rao [25].

Reduction of chemicals

When 10 U g⁻¹ xylanase was given, 14.3% elemental chlorine and 14.3% H₂O₂ could be reduced, and when 25 U g⁻¹ xylanase was given 14.3% elemental chlorine and 28.6% H₂O₂ could be reduced – thereby retaining the brightness at control level. Madlala et al. [24] reported that extent of chlorine dioxide reduction during bleaching depended on the type of pulp and enzyme used.

Mathur et al. [26] obtained a 14.6–25.9% reduction in active chlorine, when chemical pulp was bleached using a commercial xylanase (enzyme dose range between 9.35 IU g⁻¹ to 18.2 IU g⁻¹). According to Bissoon et al. [14] it was possible to lower the total charge of active chlorine on bagasse pulp by 18% when bagasse pulp was treated with 150 U g⁻¹ xylanase from *Thermomyces lanuginosus* SSBP. Li et al. [17] reported that the xylanase pre-treatment was able to lower the total chlorine up to 28.3%, when xylanase from *Thermomyces lanuginosus* CBS 288.54 was used for bleaching wheat straw pulp at an enzyme dose of 15 U g⁻¹.

Conclusion

Crude xylanase from culture filtrate of *Aspergillus sydowii* SBS 45 could be used as a potential enzymatic bleaching agent in pulp bleaching process. Even though initial pH of the pulp was 8.2, no pH adjustment was needed, because the enzyme could retain 95% of its activity in a broad pH range (5–9). The enzyme retained more than 90% activity after 5 h of incubation at 40°C at pH 8.2. Reduction in considerable amount of chlorine (14.3%) after xylanase treatment made the bleaching process more environment friendly, and reduction in H₂O₂ (up to 28.6%) made the bleaching process cost-effective. However, physical properties of xylanase treated pulp like burst index, tear index, tensile index, tensile strength etc. are to be tested before commercial application.

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