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Short communication

Application of xylanase enzyme of *Bacillus coagulans* as a prebleaching agent on non-woody pulps

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Abstract

The effectiveness of xylanase as a prebleaching agent has been established on a commercial scale. The efficiency of bleaching improves with the enzyme treatment but it varies with the pulping method and pulp raw material. In the present work, crude xylanase from *Bacillus coagulans* has been characterized with respect to the operational parameters and the stabilities at various pH values. Xylanase from *B. coagulans* has been tested on three non-woody pulps as a prebleaching agent. The effects of crude enzyme on wheat straw, rice straw and jute pulps at two different initial pH of pulp have been studied and a maximum brightness gain of 5.1 points has been achieved with rice straw pulp at an initial pH value of 8.5. In the case of wheat straw and rice straw pulps, maximum brightness gain has been obtained at the higher pH values.

Keywords: Xylanase; *Bacillus coagulans*; Enzyme stability; Non-woody pulp; TCF

1. Introduction

In response to the growing concerns for the environment, restriction on the release of waste bleach waters from the pulp and paper industry are becoming stringent. Thus, necessitating an urgent need to lower the impact of pulp bleaching on the environment. A suggested alternative for the reduction in chlorine consumption is to use biological agents like hemicelullase. This has been first demonstrated by Viikari et al. [1] in 1986 and it has been reported that the efficiency of hemicellulases varies with hardwood and softwood pulps [2]. Xylanase and mannanase have been used as a prebleaching agent but the efficiency of xylanase was superior in comparison to mannanase alone [3,4]. Realizing the importance of xylanase, major attention has been paid on developing xylanase as a prebleaching agent [5–8]. In the initial years of use of xylanase as a prebleaching agent, fungal source has been explored but due to the presence of cellulase, low optimum pH and less stability of enzyme, this source of enzyme finds limited acceptability on a commercial scale [7,8]. For the development of suitable xylanase as a prebleaching agent, the stability of enzyme at higher optimum pH and temperature is desirable [7,8]. Extremophiles have provided number of stable xylanases for biobleaching applications [7,8]. The stability of enzyme at a temperature of 105 °C and pH value as high as 11–12 has been reported in the case of *Thermogota* and *Bacillus* enzyme, respectively [7].

Commercially, numbers of enzyme are available but due to the variations in the pulping process and raw materials used, the efficiency of these enzymes also differs for a particular enzyme [9]. In this context, the present study explored the xylanase of *Bacillus coagulans* on three non-woody pulps (wheat straw, rice straw and jute) in a total chlorine free bleaching sequence (TCF). In TCF bleaching sequences, the addition of enzymes increases the final brightness value, which is a key parameter in marketing the chlorine free pulps [10]. In the present study, the dependence of the enzyme efficiency on the pulp raw materials and the initial pH of the pulp have been accessed under the similar experimental conditions.

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2. Materials and methods

In this work, the xylanase has been used as a prebleaching agent and the enzyme has been obtained from *B. coagulans*, which was kindly provided by Dr. K.J. Mukherjee of Center for Biotechnology, Jawaharlal Nehru University, New Delhi, India. The Birchwood wood xylan was purchased from the Sigma Chemicals Co. (USA) and the yeast extract and peptone powder was procured from Merck Co. (Mumbai, India). For *B. coagulans* culture, medium with the following composition has been used for the subculturing and the enzyme production. Birchwood xylan or wheat straw powder: 10 g/l; yeast extract: 5 g/l; peptone: 5 g/l; K_2HPO_4: 1 g/l; $MgSO_4$: 0.2 g/l; pH 7. The production of xylanase has been carried out with *B. coagulans* in a fermentor under controlled optimum conditions (temperature 45 °C, pH 7, rpm: 200–400, aeration: 1 vvm). The cell-free broth has been used for the enzyme activity determination and optimization studies.

2.1. Enzyme activity determination

The activity of the xylanase has been determined with 1% Birchwood xylan in 50 mM phosphate buffer of pH 7 at 55 °C using the method described by Bailey et al. [11]. The enzymatic reaction has been carried out for 15 min and the reducing sugar has been determined using the DNS method [12]. Filter paper activity of the crude enzyme was measured by using the method recommended by IUPAC using filter paper as a substrate [13]. The reaction was carried out at 50 °C for 60 min.

2.2. Pulp-making process

The pulps were produced by the typical method of ''open hot digestion'' where raw materials were cooked with 8% NaOH for a period of 3 h at the boiling temperature.

2.3. Enzymatic prebleaching

The pulps were subjected to the enzyme treatment in polythene bags. For this, two lots of each pulp were taken. One lot was added with enzyme and the other lot as a control was subjected to the similar conditions without adding enzyme. The pulp consistency was 6% and the enzyme treatment of pulp has been carried out at two pH values (7.0 and 8.5) at 55 °C. For maintaining the initial pH of the pulp to the desired value, 4N NaOH and 4N H_2SO_4 were used. Enzyme dose of 20 IU/g OD pulp was used for all the studies and incubated for 3 h. After 3 h, pulps were then squeezed to collect the water extracts and then washed with hot water. The enzyme filtrates collected were then analyzed for the color and reducing sugar (as xylose) to find out the efficacy of the enzyme. Color of filtrate was analyzed by measuring the absorbance at 480 nm and kappa number of pulp has been evaluated to access the effect of enzyme treatment on lignin using TAPPI test methods, T236cm-85. The values of reducing sugar, color of filtrate and kappa number presented here are the average value of three independent analyses.

2.4. Bleaching methods

Both the treated and control pulps were subjected to the peroxide bleaching after enzyme treatment. All the pulps were given an additional EDTA-treatment (0.2% EDTA; pH 4–5; temperature: ambient; time: 45 min; consistency: 5%) before peroxide bleaching so as to increase the effect of peroxide, because EDTA is a chelating agent, which traps the hindering metal ions in the pulps. Peroxide bleaching was carried out at a consistency of 8%; NaOH: 1%; hydrogen peroxide: 2%; temperature: 70 °C; time: 2 h; final pH >9.0. In the case of wheat straw and rice straw pulp, additional step of peroxide bleaching has been carried out with 1 and 2% hydrogen peroxide, respectively, to improve the final brightness of the pulps.

2.5. Evaluation of optical and strength properties

The bleached pulps were used to make hand sheets (T-205om-88, [14]) so as to evaluate their optical (brightness: ISO-2471, [15]) and strength properties (tensile strength: ISO-1924, [15], tear strength: ISO-1974, [15]) using the standard procedures. From each pulp, nine sheets have been made and three sheets of each pulp have been used for determining an individual parameter. Brightness, tensile and tear index values presented in the next section are the average of three experimental values.

3. Results and discussion

The major developments in the biobleaching process have been observed with hardwood kraft pulps [1,2,4,9,10,22–25] but few studies have also concentrated on the non-woody pulps like bagasse [22] and wheat straw pulps [16–21], etc. In this context, the present study has attempted to study the effect of crude xylanase from *B. coagulans* on three non-woody (wheat straw, rice straw and jute) pulps in a total chlorine free bleaching sequences. These pulp raw materials are mostly in use in handmade paper industry where high brightness of final product is not desirable. However, the attainment of high brightness of the final product from wheat straw pulp has been reported with new bleaching sequences [16]. The evaluation of xylanase as a prebleaching agent has been carried out at two different initial pH of pulp (optimum pH of the enzyme and at higher pH of 8.5). The evaluation of optimum parameters (pH and temperature) of the enzyme has been carried out with the crude enzyme, which is the preferred form for the pulp treatment. The results of optimization studies and evaluation of enzyme as a prebleaching agent are presented in the following sections.

3.1. Optimum temperature of crude xylanase of *B. coagulans*

The activity of the enzyme has been determined in the temperature range of 40–80 °C at a pH of 7. It has been observed that the xylanase of *B. coagulans* has an optimum temperature of 55 °C with corresponding activity of 18 IU/ml and lower activity at other temperatures: (temperature, activity IU/ml) 40 °C, 11.04; 50 °C, 11.61; 60 °C, 13.41; 70 °C, 5.91; 80 °C, 5.21. The activity profiles of enzyme at different temperatures have been determined in three independent experiments and the values presented here are the average value having standard deviations within the range of 5–7%. Literature-reported value for the optimum temperature of enzyme activity lies in the range of 50–110 °C [7,8].

3.2. Optimum pH of crude xylanase of *B. coagulans*

In biobleaching applications using xylanase, major important parameters are pH optima and enzyme stability at higher pH (>7), etc. The enzyme activities at various pH (6–11) were determined using 50 mM various pH buffers having appropriate pH values. The result (Fig. 1) shows that the enzyme from *B. coagulans* was active in the pH range of 6–9. The enzyme has pH optima around 7 but the activity at pH value of 8–9 makes it suitable for biobleaching applications. Among the xylanases from *Bacillus* species, highest pH optima has been reported for *Bacillus* Tar-1, C-125 and *Bacillus* sp. NCL-86-6-10 [7,8].

3.3. Enzyme stability

In the previous section, it has already been mentioned that stability of enzyme at operating conditions is important. So the stability of enzyme has been studied at optimum temperature (=55 °C) and pH (=7). Besides, the stability of enzyme has also been determined at other pH (5.5 and 8) at 55 °C to find out its suitability. The stability study has been carried out for 60–120 min and samples have been taken at a regular interval of time for the activity determination. Results (Fig. 2) indicate that at a pH value of 7, the enzyme is

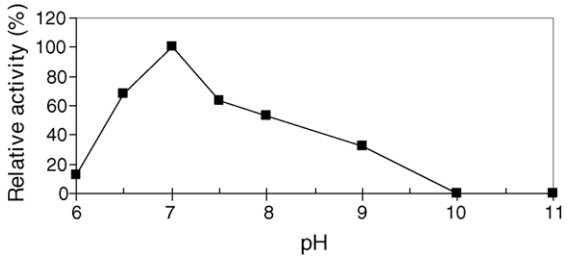

Fig. 1. Effect of pH on the activity of crude xylanase of *B. coagulans* at 55 °C. Various pH buffers of 50 mM have been used for the activity determination at different pH values. Data presented here are the average of three independent experiments having standard deviation within the range of 4–6%.

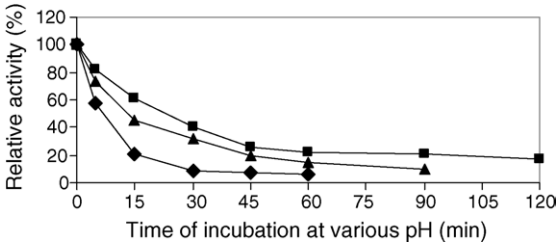

Fig. 2. Stability of crude xylanase of *B. coagulans* at 55 °C and three different pH values (pH 5.5 (◆), pH 7 (■), pH 8 (▲)). Data presented here are the average of three independent experiments having standard deviation within the range of 5–8%.

relatively stable as compared to the pH value of 5.5. *B. coagulans* enzyme loses 85% of its activity within 2 h at a pH value of 7. From the literature it can be found that the xylanase has stability in the pH range of 3.9–8 [7,8]. However, *Bacillus* sp. Bp-23 has been reported to be stable a pH value of 10 and that of *Bacillus* sp. W1 and W2 in the broad pH range of 4.5–10 [7]. Even, stability at one of the highest pH value of 11–12 has been reported for *Bacillus* sp. [7,8].

3.4. Evaluation of enzyme as a prebleaching agent

In the present study, the efficiency of the crude xylanase of *B. coagulans* has been evaluated on three different non-woody pulps at two different initial pH of pulp.

The cell-free crude enzyme used in the biobleaching applications has been obtained from the fermentor and it has been further concentrated in a 10 kDa ultrafiltration membrane. The final xylanase activity was 330 IU/ml with a corresponding filter paper activity of 0.12 IU/ml. Three different pulps produced from wheat straw, rice straw and jute were treated with the concentrated enzyme at a temperature of 55 °C and at two different initial pH of pulp (7 and 8.5). The water extracts or pulp filtrates collected from the treated and control pulps were than analyzed for color and reducing sugar (as xylose release) (Table 1). From Table 1, it can be seen that maximum reducing sugar has been released from rice straw pulp followed by wheat straw pulp. The effect of initial pH of the pulp on reducing sugar release was almost insignificant for all three pulps.

In case of color released from the pulp due to the enzymatic treatment, similar trend was observed in all three pulps (Table 1). The maximum color has been released in case of rice straw pulp at pH 7. The effect of enzyme treatment on pulps has also been accessed by evaluating the kappa number of pulps. The effect of pH was prominent in the case of rice straw pulp and maximum kappa number reduction was observed at higher pH of 8.5 (Table 1). In case of wheat straw pulp, higher pH favors maximum kappa number reduction but the difference in kappa number reduction at two pH values was not significant. However, for jute pulp the maximum kappa number reduction has been observed at a pH value of 7.

Table 1
Reducing sugar and color release from pulps and kappa number of pulps at two different initial pH of pulp

Pulp raw material	pH	Reducing sugar as xylose (g/l)		Color of pulp filtrates in PCU		Kappa number	
		Control	Enzyme	Control	Enzyme	Control	Enzyme
Wheat straw	7	2.26 ± 0.12	4.06 ± 0.21	131.7 ± 17	226.8 ± 19	31.4 ± 0.42	30.4 ± 0.41
	8.5	2.27 ± 0.15	4.21 ± 0.12	140.2 ± 12	254.8 ± 15	32.0 ± 0.49	30.6 ± 0.47
Rice straw	7	2.28 ± 0.16	4.87 ± 0.19	339 ± 18	709.7 ± 22	19.15 ± 0.5	19.0 ± 0.52
	8.5	2.4 ± 0.19	4.87 ± 0.24	226 ± 28	474.4 ± 31	20.4 ± 0.43	19.1 ± 0.45
Jute	7	0.82 ± 0.07	1.99 ± 0.13	36.5 ± 2.9	76.8 ± 3.9	31.7 ± 0.56	30.1 ± 0.59
	8.5	0.65 ± 0.054	2.3 ± 0.16	40.2 ± 3.2	108.5 ± 5.9	33.0 ± 0.52	33.4 ± 0.51

After the enzymatic treatment of pulp, subsequent EDTA treatment and bleaching with hydrogen peroxide has been carried out. The bleached and unbleached pulps were used to make sheets for analyzing optical properties. Brightness gain of the enzyme-treated pulp as compared to the control pulp has been the major parameter of interest during evaluating the efficiency of the enzyme. The comparison of brightness gain value of pulp after the enzymatic treatment and bleaching has been undertaken to evaluate the bleach boosting effect of enzyme on three different pulps.

From the results (Table 2), it can be seen that after the enzyme treatment (prior to bleaching stage), brightness of the pulp improved only for rice straw pulp (at both pH values) and jute pulp at pH 7. Similar phenomena have also been reported for the enzymatic treated kraft pulp with brightness gain of 2–4 points prior to bleaching stage [24]. After the bleaching stage, the maximum brightness gain has been improved to 7.9 points with the same kraft pulp [24]. In this study, it has been observed that at a pH value of 7, brightness gain for the bleached rice straw pulp has remained constant at around 2.4 points (Table 2). However, the brightness gain has been improved for the bleached rice straw pulp at pH 8.5 and bleached jute pulp at pH 7 as compared to the corresponding unbleached enzyme treated pulps. In case of wheat straw pulp, brightness gains of 1.8 and 4.4 points have been obtained only after bleaching stage with the enzyme-treated pulp at a pH of 7 and 8.5, respectively. Maximum brightness gain of 5.1 points has been observed with rice straw pulp at a pH value of 8.5. Excepting jute pulp, maximum brightness gain has been observed at higher pH values for wheat straw and rice straw pulps. The beneficial effect of higher pH in improving brightness gain has also been reported for wheat straw pulp treated with xylanase of *Thermomyces lanuginosus* CBS 288.54 [20]. However, in their case, effect has been studied till a pH value of 11 and maximum brightness gain has been observed at a pH value of 9. Shah et al. [23] also reported that XynA from *Thermogota maritime* is capable of releasing lignin, sugars at pH 5–10, and is effective at pH 10 in enhancing pulp bleaching. As far as studies on enzymatic treatment of non-woody pulps are concerned, there is rarely any report on rice straw and jute pulps. Jimenez et al. [21] have reported on biobleaching of wheat straw pulp and they have reported a brightness gain of 2.4 points for enzyme peroxide bleaching and 3 points gain in case of enzyme peroxide-active chlorine bleaching. Herpoel et al. [17] have used laccase mediator system along with xylanase for treatment of wheat straw pulp in a total ECF sequences resulted in 69% ISO brightness but there was reduction in the degree of cellulose polymerization. Jimenez et al. [19] have optimized the experimental conditions for enzyme treatment of wheat straw pulp with cartazyme enzyme followed by hydrogen peroxide bleaching. Although enzymatic treatment reduces the pulp yield but there was increase in final brightness value of pulp. Roncero et al. [18] have presented the kinetic study of ozone treatment of wheat straw pulp and it has been compared with eucalyptus pulp. The kinetics of the enzyme pre-treatment effect showed similar behavior in both raw materials, although the constants of delignification and elimination of chromophore are higher in straw pulp. Thus brightness gain observed in this study with xylanase treated wheat straw pulp is consistent with other studies.

Table 2
Brightness value of bleached and unbleached pulp of three different raw materials at two different initial pH of pulp

Pulp	pH	Brightness (%ISO) of unbleached pulp		Brightness (%ISO) of bleached pulp	
		Control	Enzyme	Control	Enzyme
Wheat straw	7	25.6% ± 1.35	25.5% ± 1.42	30.2% ± 0.95	32% ± 0.91
	8.5	24.7% ± 1.21	24.5% ± 1.12	30.3% ± 1.31	34.7% ± 1.24
Rice straw	7	27.5% ± 1.31	30.0% ± 1.26	33% ± 1.36	35.4% ± 1.27
	8.5	27.5% ± 1.19	29.0% ± 1.64	42.14% ± 1.57	47.3% ± 1.29
Jute[a]	7	32.3% ± 1.25	34% ± 1.31	44.9% ± 1.53	48.9% ± 1.28
	8.5	33.5% ± 1.64	33.0% ± 1.51	56.4% ± 1.67	57.2% ± 1.58

[a] In case of jute pulp, only one stage of bleaching has been carried out.

Table 3
Effects of xylanase treatment on tensile and tear indexes of three different pulps at two different initial pH of pulp

Pulp raw material	Tensile index (Nm/g)		Tear index (mNm^2/g)	
	Control pulp	Enzyme-treated pulp	Control pulp	Enzyme-treated pulp
Wheat straw at pH 7	36.4 ± 1.32	37 ± 1.27	9.77 ± 0.21	10 ± 0.19
Wheat straw at pH 8.5	36.7 ± 1.18	36.2 ± 1.06	9.73 ± 0.26	10 ± 0.23
Rice straw at pH 7	41.8 ± 1.41	41.5 ± 1.39	7.6 ± 0.14	7.2 ± 0.12
Rice straw at pH 8.5	30.3 ± 1.22	31 ± 1.16	8.2 ± 0.16	7.54 ± 0.15
Jute at pH 7	35.5 ± 1.27	36.9 ± 1.29	17.3 ± 0.27	16.2 ± 0.25
Jute at pH 8.5	48.7 ± 1.61	47.2 ± 1.57	16.9 ± 0.23	16.9 ± 0.26

Sheets made from bleached pulp were also used to determine the strength properties of enzymatic treated bleached pulps and control pulps (Table 3). Tensile and tear indexes have been measured to determine the effect of enzyme treatment on pulp. In case of tensile index, there was a marginal decrease in value for jute pulp at higher pH values (=8.5). The effect of enzymatic treatment on tear index has been more prominent only in case of jute and rice straw pulps. However, it should be noted that the differences in the values of either tear or tensile index of enzyme treated and control pulps are not statistically very significant. In the present study, enzyme dosage has been chosen according to the literature reported value and further optimization of enzyme dosage yet to be carried out.

4. Conclusions

The present work suggests that the effect of enzyme varies with the type of pulp material. The xylanase of *B. coagulans* found to have positive effect on three pulps with significant brightness gain in case of wheat straw and rice straw at higher pH values. In the case of jute pulp, maximum gain of 4 points has been obtained at a pH value of 7, which is significantly higher than the value obtained at a pH value of 8.5. Although the crude enzyme has optimum pH of 7 but the maximum gain of 5.1 points has been obtained with rice straw pulp at a pH of 8.5. In case of rice straw and jute pulp, brightness gain has been observed even after enzyme treatment stage (prior to bleaching stage). Brightness gain in wheat straw pulp has been observed only after the bleaching stage. This is probably the first report on the use of rice straw and jute pulps in biobleaching applications.

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