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Biobleaching of Kraft Pulp by Xylanase Produced by *Bacillus subtilis*

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ABSTRACT

Xylanase treatment of kraft pulp resulted in increased percentage loss in weight with increasing incubation period. The reducing sugars in the hydrolysates increased from 53.0 to 193 μM with the passage of time. Kappa number decreased from 70.0 to 53.2 demonstrating the removal of lignin contents and also indicating the removal of xylan attached to lignin in kraft pulp samples. The lignin decreased from 9.2 to 7.6% when the incubation period increased from 2 to 6 h. Weight losses of pulp samples was observed when the amount of xylanase increased from 20 to 100U in 25 mL reaction mixture at 50°C for 6 h. Kappa number decreased from 70.0 to 41.0 with the loss of lignin contents from 9.0 to 5.8% when the amount of xylanase increased from 20 to 100U. As the amount of pulp increased from 1.0 to 2.0 g in 25 mL reaction mixture, the percentage losses increased from 5.2 to 7.0. An increase in reducing sugars was also observed in the hydrolysates. Lignin contents were reduced accompanied by decrease in kappa number from 49.0 to 34.3.

Key Words: Bleaching; Pulp; Xylanase; *Bacillus subtilis*

INTRODUCTION

The most promising application of xylanases is in the prebleaching of kraft pulp. Other applications proposed for xylanases include debarking, refining pulp fibers and preparing dissolving pulps. The pulp and paper industry is modifying its pulping, bleaching and effluent treatment technologies to reduce the environmental impact of mill effluents. If kraft pulps are prebleached with xylanases, then lower chlorine charges are required to bleach the kraft pulps, which reduce chloro-organic discharges (Koponen, 1991; Viikari *et al*., 1991). The concept of using enzymes to enhance the delignification of pulp was first presented by Viikari *et al*. (1986). Tremblay and Archibald (1993) reported the delignification of unbleached softwood and hardwood kraft pulps thus reducing the Cl_2 required to achieve a given degree of bleaching. Oksanen *et al*. (2000) has reported the changes in fiber properties by treating recycled pulps with purified *T. reesei* cellulases and hemicellulases. Qy *et al*. (1996) reported the enzymatic treatment of birch kraft pulp, which resulted in a brightness of 6.8% SBD more than untreated one using the same chlorine dosage.

This paper reports the prebleaching of kraft pulp by the xylanase produced by a locally isolated strain of *B. subtilis.*

MATERIALS AND METHODS

Enzyme production. *B. subtilis* was grown in minimal salt medium and enzyme was produced as described previously (Saleem *et al*. 1997).

Effect of incubation time on pulp bleaching. Unbleached kraft pulp prepared from wheat straw by kraft process was supplied by Packages Ltd., Lahore. 1.0 g of unbleached kraft pulp was treated with 40U of xylanase activity for different time intervals i.e. 2, 4 and 6 h at 50°C. After enzymatic treatment, the pulp samples were filtered and washed with distilled water and then dried in an oven at 50°C to a constant weight. The weight loss of pulp was calculated by difference method. The effect of enzymatic treatment on pulp was determined by the Kappa number and Kappa number was calculated as described by Technical Association of the Pulp and Paper Industry (Anonymous, 1984). This value describes the residual lignin contents of the pulp. Kappa number is defined as the volume (in mL) of 0.1N potassium permanganate solution consumed by one gram of moisture free pulp under standard conditions and is equivalent to approximately seven times the mass percentage of lignin.

Effect of enzyme concentration on pulp bleaching. Pulp samples were treated with different enzyme concentration 20, 40, 60 and 100U for 6 h at 50°C. The treated samples were filtered, washed and dried in an oven to obtain a constant weight. Their weight losses, reducing sugars in hydrolysates and kappa number were determined.

Effect of amount of pulp. Reaction mixtures containing 60U of xylanase activity in 25 mL Mcllvaine buffer (pH 6.0) and different amounts of prebleached pulp (1.0, 1.5, 2.0 g) were incubated at 50°C for 6 h. The residual pulp samples were washed, dried and weight losses were determined by difference method. Kappa number, amount of lignin in the pulp samples, reducing sugars and residual xylanase activity in the hydrolysates were determined.

RESULTS AND DISCUSSION

Xylanases were used as process aids in industrial scale bleaching processes. Progress towards the replacement of elemental chlorine by chlorine dioxide and oxygen based bleaching chemicals has resulted in the introduction of several modified and new kraft cooking concepts. The potential of xylanase of *B. subtilis* for pretreatment in kraft pulp bleaching have been eveluated.

Effect of incubation time on pulp bleaching. 1.0 g of prebleached pulp (prepared from wheat straw by kraft process) was treated with 40 U of xylanase activity for 2, 4 and 6 h. Enzyme treated samples were washed and dried in an oven and the weight losses were determined and results are shown in Table I. It is clear from the table that weight loss in pulp increased gradually with increasing incubation time. Enzymatic hydrolysis of hemicellulosic contents contributed to this weight loss.

Kappa number decreased gradually from 70.0 to 56.0 on treatment with xylanase for 6 h indicating increasing lignin loss with increase in xylanase treatment time. From an initial lignin content of 10% in untreated pulp sample, it decreased to 8% when treated with xylanase for 6 h. Reducing sugars in the hydrolysates increased from 40.0 to 100 µM with increase in the xylanase treatment period upto 6 h indicating hydrolysis of xylan attached to lignin in the pulp. The physical appearance of the xylanase treated kraft pulp was generally brighter than the control pulp.

Table I. Effect of incubation period on weight loss and lignin contents of kraft pulp

Incubation period (h)	%age loss in weight	Reducing sugars µmole	Kappa number	%age of lignin in pulp
Untreated	-	-	70.0	10.0
2	1.6	40.0	64.4	9.2
4	2.6	60.0	59.5	8.5
6	3.6	100	56.0	8.0

Effect of enzyme concentration. 1.0 g of the kraft pulp samples were treated with different amounts of xylanase activity ranging 20-100U by incubating at 50°C for 6 h. The residual pulp after treatment was filtered, washed and dried. Weight losses of the pulp samples are shown in Table II. The weight loss increased with increasing xylanase concentration. Similarly reducing sugars were increased with increasing enzyme concentration. The kappa number of the pulp was 63.0 when treated with 20U of xylanase activity and decreased with increasing xylanase. The lignin contents decreased from 9.0 to 6.2% in the kraft pulp samples when xylanase activity increased from 20U to 100U. The pulp brightness increased with increasing enzyme concentration.

Effect of amount of pulp. Effect of different amounts of pretreated kraft pulp on kappa number and lignin contents was studied and results are shown in Table III. Reaction mixtures contained 60 units of xylanase activity in 25 mL Mcllvaine buffer (pH 6.0) and different amounts of pulp samples (0.5, 1.0, 1.5, 2.0 g) were added and incubated at 50°C for 6 h. The residual pulp samples were washed, dried and weight losses were determined by difference method. With increasing amount of pulp in the reaction mixture, the percentage losses decreased. Lignin contents reduced from 8.5 to 6.0, accompanied by decrease in kappa number from 60.0 to 42.0.

Table II. Effect of amount of xylanase on weight loss and lignin contents of pulp

Xylanase activity (U)	%age loss in weight	Reducing sugars µmole	Kappa number	%age of lignin in pulp
Untreated	-	-	70.0	10.0
20	2.0	50.0	63.0	9.0
40	3.5	90.0	56.7	8.1
60	5.3	126.0	49.7	7.1
100	6.8	167.0	43.4	6.2

Table III. Effect of amount of pulp on weight loss and lignin contents of pulp

Amount of pulp (g)	%age loss	Kappa number	%age of lignin	Residual xylanase activity (U)	Reducing sugars µmole
Untreated	-	70.0	10.0	-	-
0.5	4.5	60.0	8.5	60	95
1.0	5.2	49.0	7.0	55	118
1.5	4.7	45.0	6.4	50	200
2.0	4.0	42.0	6.0	30	233

The residual activity after the reaction was estimated and about 91% xylanase activity was found in the hydrolysate when 1.0 g pulp was incubated at 50°C for 6 h in 25 mL assay mixture. In contrast, 50% residual xylanase activity was observed in the hydrolysate containing 2.0 g pulp. The residual xylanase activity in the hydrolysates decreased with increasing the amount of substrate which is most probably due to the adsorption of xylanase activity on the substrates. The amount of reducing sugars increased from 95 to 233 µmole in the hydrolysates when the amount of pulp increased from 0.5 to 2.0 g in the reaction mixture. The use of xylanases in the pulp and paper industry will open the door further for the use of other enzymes, such as lipases for reducing pitch, triglycerides and resin acids (Fujita *et al*., 1992). Other biotechnological developments, such as biobleaching and biopulping using white rot fungi (Bar-Lev *et al*., 1982; Kirk & Yang, 1979) will be established.

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