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Full Length Research Paper

Biobleaching of wheat straw-rich-soda pulp by the application of alkalophilic and thermophilic mannanase from *Streptomyces* sp. PG-08-3

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An alkalophilic and thermophilic mannanase from *Streptomyces* sp. PG-08-3 was applied to wheat straw-rich-soda pulp to check its bleaching potential. Optimum conditions for bio-bleaching of pulp were as follows: Mannanase 5 Ug^{-1} of pulp at pH 8.5 with temperature 55ºC that enhanced the brightness by 7.3% and reduced the kappa number by 24.6% within 4 h of incubation. Tear index (20%) and burst index (11.2%) were also improved by mannanase-treated pulp as compared to the untreated pulp. Treatment of chemically (CEH_1H_2) bleached pulp with enzyme showed significant effect on release of chromophores, hydrophobic and reducing compounds. Mannanase-prebleaching of raw pulp reduced the use of hypochlorite by 16% to achieve brightness of resultant hand sheets similar to the fully chemically bleached pulp. Scanning electron microscopy of wheat straw rich soda-pulp after treatment with denatured and active mannanase was performed. There was appearance of micro-fibers on the surface of pulp that was treated with active mannanase.

Key words: Biobleaching, mannanase, wheat straw-rich-soda pulp.

INTRODUCTION

Bleaching of pulp is necessary for whitening of paper and is based on removal of residual lignin from the cellulose fibers but should have no adverse effect on cellulose fiber quality (Unal and Kolankaya, 2001). However, use of chlorine-based bleaching process tends to generate toxic and highly persistent chlorinated organic by-products, which eventually pollute water bodies (Singh et al., 2008). Also, the use of such chemically-treated pulp/paper in the manufacturing of direct-body-contact consumables like baby diapers and food packaging is of major concern as it is associated with chlorinated compounds including the animal carcinogen, dioxin (Shoham et al., 1992). Due to increasing health awareness and growing public sensitivity towards the negative environmental impact of chlorinated pulp, there is an ever growing demand for use of chlorine-free paper products all over the world. In this regard, the world is focusing research on the development of newer healthy and environmentally friendly technologies. Among these, biobleaching with enzymes has shown immense potential in minimizing use of bleaching chemicals containing chlorine (Singh et al., 2008).

Endomannanases (EC 3.2.1.78; 1,4–β-D-mannan mannanohydrolases) catalyse the random hydrolysis of mannan distributed in nature. The industrial application of these enzymes include clarification of fruit juices, biotransformation of plant material, processing of coffee, manufacture of oligosaccharides and rarely in the bleaching of pulps.

During chlorine bleaching of pulp, about 10% of the chlorine used gets bound to the residual lignin and forms chlorinated organic compounds. The rest of the chlorine (about 90%) ends up as common salt. Chlorination of pulp results in an increase in free phenolic hydroxyl groups and carboxyl groups, which decreases the molecular weight of lignin making the lignin easily soluble in water and alkaline solution. Various kinds of chlorinated phenols, phenolic, carboxylic acids, resin acids and

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Authors equally contributed in present study hydrocarbons originating from lignin or extraction of wood are found to be present in the bleach effluent.

Currently, it is essential to minimize the use of chlorine-based chemicals used in bleaching of pulps. The available options are oxygen delignification extended cooking and substitution of chlorine dioxide for chlorine, hydrogen peroxide and ozone treatment. But most of these methods involved are high capital investment for process change. Thus, an alternative and cost effective method that is, use of enzymes have provided a very simple and economic way to reduce the use of chlorine and other bleaching chemicals. The present study is an attempt to apply alkalophilic mannanase from *Streptomyces* sp. PG-08-3 for the chlorine-free bleaching of wheat straw-rich soda pulp. It is an effort to develop an eco-friendly and reduced chemical consumption process for the bleaching of wheat straw rich-soda pulp. Based on present knowledge,this work is the first report on enzymatic bleaching of agrobased pulp by *Streptomyces* mannanse. It is a known fact that reports on prokaryotic mannanses are not common.

MATERIALS AND METHODS

Microorganism and cultural conditions

Streptomyces sp. PG-08-3 was isolated from naturally degraded biomaterials rich in hemicellulose that was collected from Rajasthan desert, India (Bhoria et al., 2009) and was maintained as a suspension in 20% glycerol at -70°C and was routinely cultured on the medium containing locust bean gum 0.5%; Na_2HPO_4 0.7%; KH_2PO_4 3%; NH_4Cl 0.1% and NaCl 0.05%. Final pH was adjusted to 8.0 with 0.1 N NaOH and incubated at 37°C under shaking conditions (150 rpm). After every 24 h, a loopful of inoculum was streaked onto nutrient agar (pH 8.0) and actinomycetes isolation agar (pH 8.0) composed of sodium consinate 0.2%; asparagine 0.01%; sodium propionate 0.4%; K_2HPO_4 0.05%; $MgSO_4 \cdot 7H_2O$ 0.01%; $FeSO_4 \cdot 7H_2O$ 0.0001% and agar 2.0% was subsequently incubated at 37°C.

Enzyme assay

Mannanase activity was determined by using locust bean gum (0.5% w/v) as substrate. The mannanase activity was measured in terms of the amount of reducing sugars released from locust bean gum by the action of enzyme. Reducing sugars were measured using 3, 5-dinitrosalicylic acid (DNSA) method of Miller, (1959). The amount of sugars released (mg ml^{-1}) were determined by using mannose as a standard. The one international unit of mannanase activity was defined as the amount of enzyme that is required to release 1 µmole of mannose under standard (pH 8.0 and temp. 75ºC) assay conditions. Mannanase activity was expressed in terms of Uml^{-1}

Pulp samples

Wheat straw-rich soda pulp with 10% consistency (% age of pulp in water) used in bleaching experiments, was obtained from Shreyan paper mill (Punjab, India). The pulp was composed of (w/w) wheat straw (*Triticum astivum*) (78.8%), sarkanda (*Saccharum spontaneum*) (10.6%) and candy (*Eragrostis* sp.) (10.6%) cooked at 165 - 175°C for 30 min at a pressure of 7.0 - 7.5 kg m^{-3}.

Pulp tests

Kappa number and brightness of pulp were determined according to Tappi (Technical Association of pulp and paper industry) test methods T 236 and T 452, respectively. Tear index (resistance of paper fibers to tear, $Nm^3 g^{-1}$) and burst index (pressure required to rupture a standard sample of paper divided by its basis weight, KPa $m^2 g^{-1}$) were determined using facilities available at mill. Denatured and active mannase-treated pulp samples were filtered through muslin cloth and respective filtrates were studied for release of chromophores, hydrophobic compounds and release of reducing sugars (Ninawe and Kuhad, 2006). Pulp was washed several times with tap water till proper sheets could be prepared with a Buchner funnel (diameter, 16 cm). Sheets were air dried before testing for brightness and kappa number (defined as the ml of 0.1 N $KMnO_4$ solution consumed per gram of moisture-free pulp under standard conditions). All pulp bleaching experiments were conducted at least in triplicates. Results presented are the average of these values.

Optimization of enzyme dose and reaction time for the biobleaching of wheat straw rich-soda pulp

The optimization of enzyme dose and reaction time for biobleaching was carried out (at 55ºC, pH 8.5) by processing the pulp with varying doses of mannanase, ranging between 0 to 8 Ug^{-1} of moisture free pulp for variable time intervals up to 6 h and pulp properties were studied at regular interval.

Effect of mannanase on chemically (CEH_1H_2) bleached pulp

Fresh pulp samples were treated in CEH_1H_2 sequence ((C-chlorine treatment with 4.5% Cl_2 (70°C, 30 min, pH 2.0); E-caustic extraction with 0.8% NaOH (70°C, 1 h, pH 10 - 10.5); H_1-hypochlorite treatment 1 with 6% hypochlorite (50°C, 1.5 h, pH 8.0); H_2-hypochlorite treatment II with 4.5% hypochlorite (45°C, 2.5 h, pH 7.0)). Pulp from each stage was washed twice with tap water to assure the removal of residual chlorine/hypochlorites before treating with optimal mannanase dose at pH 7.5 and 50°C. Thereafter pulp properties were determined for each sample.

Evaluation of enzyme pre-treatment vs. chemical treatment of wheat straw-rich soda pulp

The first stage raw pulp samples were treated with mannanase under optimal conditions followed by hypochlorite (4 - 6%) treatment. Samples without enzyme treatment were also processed for chemical treatment under the same conditions as control to analyze the effect of mannanase pre-treatment on reduction of use of hypochlorite and quality of paper produced.

Scanning electron microscopy of mannanase treated and un-treated pulp samples

Samples of pulp fibers were processed for scanning electron microscopy. The fibers were washed thrice with deionised water and fixed with 2.5% glutaraldehyde solution prepared in phosphate buffer, pH 7.2 for 1 h. Fibers were separated from glutaraldehyde and washed thrice with same buffer and were gradually dehydrated with acetone gradient between 30 to 90% and finally suspended in 100% acetone. Small pieces of fibers were air-dried and placed on the stubs mounted with silver tape and sputter coated with gold using fine coat, JEOL ion sputter model JFC – 1100. The samples were examined at 15 KV under scanning electron microscope (Model JSM 6100, JEOL) at various magnifications.

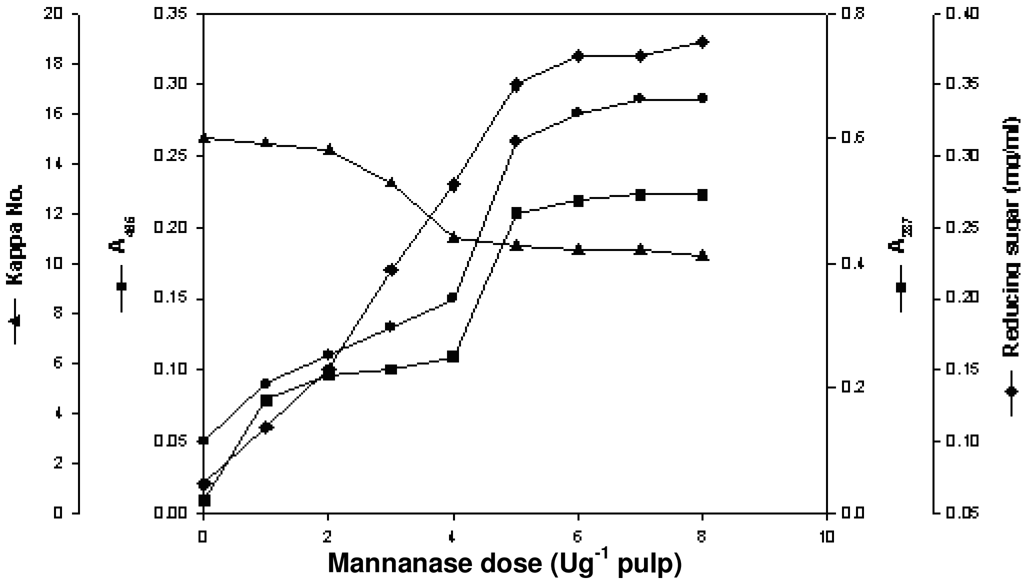


Figure 1. Optimization of mannanase dose (Ug^{-1} pulp) for the biobleaching of wheat straw rich soda pulp (pH 8.5 and 55ºC after 4 h).

RESULTS AND DISCUSSION

Biobleaching of wheat straw rich-soda pulp

In the pulp and paper industries, bleaching of different types of pulp is generally performed by two methods. One is degradation/removal of liginin by chemical treatment or by lignin degrading enzymes (laccases, lignin peroxidases) and the other is the removal of lignin together with hemicelluloses by hemicellulases such as xylanases (Jimenez et al., 1997). Among hemicellulases, xylanases are widely used and rarely, mannanases have been tested and found to be effective for pulp bleaching (Patel et al., 1993; Beg et al., 2000). In the present study, pretreatment of wheat straw-rich soda pulp with mannanase has been shown to assist in delignification of pulp by chlorine containing bleaching agents.

Streptomyces sp. PG-08-3 produced mannanase without xylanase and cellulase when checked by liquid assay in 10% concentrated enzyme broth (data not shown). The mannanase dose for the biobleaching of wheat straw rich-soda pulp at 55°C was optimized as 5.0 Ug^{-1} of moisture free pulp (Figure 1). Enzyme was effective in releasing chromophores, hydrophobic compounds and reducing sugars from pulp. There was linear increase in reducing sugars and hydrophobic compounds, further increase in enzyme concentration did not enhanced the release of reducing sugars and hydrophobic compounds. The biobleaching efficiency of mannanase was found to be maximum after 4 h with a reduction in kappa number from 15 to 11.3 and the release of reducing sugar increased from 0.07 to 0.33 mg g^{-1} of dry pulp (Figure 2). Release of chromophores and hydrophobic compounds were 0.48 and 0.26 (A_{237}nm), respectively. Further increase in either enzyme dosage or prolonged treatment period had no additional effect on biobleaching benefits.

Effect of mannanase on chemically (CEH_1H_2) bleached pulp

There was addition of mannanase after each step of CEH_1H_2 based conventional chemical bleaching process. Thereafter, the influence of enzyme on pulp properties was observed after each stage of the process. It was noted that the maximum effect of enzyme was at the first stage where pulp exhibited maximum reduction in kappa number (24%) (Table 1). First stage pulp contained maximum lignin so that enzymatic prebleaching of pulp can be established as the most suitable step to facilitate bleach boosting of pulp.

Evaluation of enzyme pre-treatment vs. chemical treatment of wheat straw-rich soda pulp

In this experiment wheat straw rich-soda pulp was pre-bleached with 5 U g^{-1} of mannanase and then subjected to 6% chlorine treatment at 55°C for 45 min. The physico-chemical properties of the pulp revealed an increase in brightness index by 8.7% ISO and reduction in kappa number by 16% (11.3 - 9.5) as evident from the release of chromophores and hydrophobic compounds (Table 2). A reduction in chlorine consumption up to 4% could be achieved with biobleached pulp when subsequently subjected to chorine treatment that could facilitate not only the economic feasibility of the process but also a lower environmental impact.

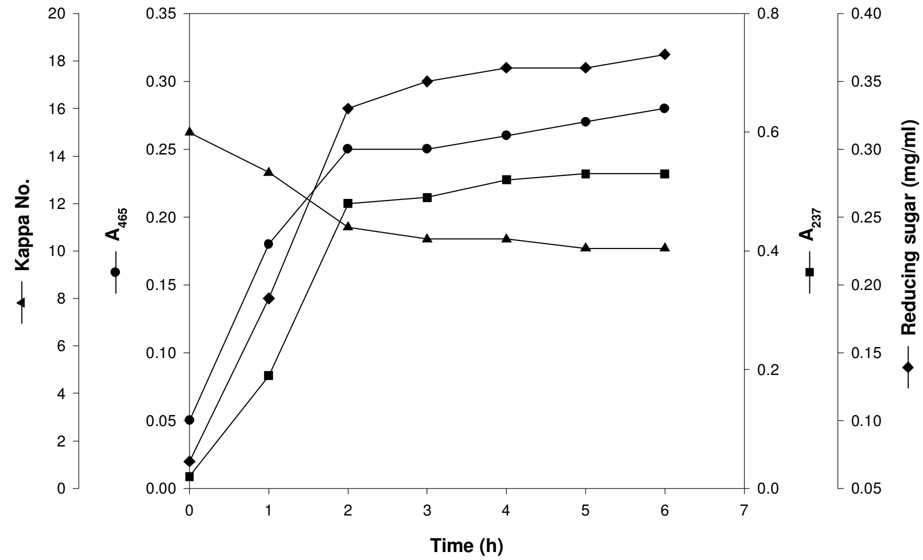


Figure 2. Optimization of time (h) for biobleaching of kraft pulp (pH- 8.5) with $5Ug^{-1}$ of mannanase dose at 55°C.

Table 1. Effect of mannanase treatment on a multi-step chemical (CEH_1H_2) based bleaching of wheat straw rich soda pulp.

S/N	Bleaching state	Pulp properties			
		Kappa number	Reducing sugars released (mg/g pulp)	A_{237}nm Hydrophobic compounds	A_{465} nm Chromophoric compounds
1.	Untreated	15.0	0.07	0.020	0.050
2.	X	11.3	0.35	0.480	0.260
3.	H_1	10.0	0.31	0.360	0.080
4.	H_1X	8.5	0.45	0.473	0.110
5.	H_1E	5.5	0.45	0.601	0.135
6.	H_1EX	5.3	0.51	0.611	0.152
7.	CE H_1	4.1	0.58	0.753	0.199
8.	CEX	4.0	0.59	0.754	0.199
9.	CEH_1H_2	4.1	0.60	0.798	0.258

X = Mannanase treatment with 5 Ug^{-1} moisture free pulp (50°C for 2h: pH 7.0 - 7.5); C = chlorine treatment with 6% Cl_2 (50°C for 45 min; pH 1.6); E = Caustic extraction with 35% Caustic (55°C for 90 min; pH-9.8); H_1 = Hypochlorite I treatment with 4% Cl_2 (80°C for 90 min; pH 6.1); H_2 = Hypochlorite II treatment with 0.2% chlorine (80°C for 90 min: pH –4.9).

In mannanase treatment followed by chemical treat-ment (XC), it was found that the XC treated pulp exhibited 9% higher reduction in kappa number. There was also improvement in tensile strength and brightness by 21 and 14.7%, respectively, as compared to CX treatment. However there was not much difference in the burst factor and tensile strength in both treatment. Similar results was also reported by xylanase from *Streptomyces* sp. QG-11-3 for pretreatment of pulp with xylanase and its subsequent treatment with 4.5% chlorine, which exhibited 14% higher reduction in kappa number and a 31 and 30% higher improvement in tensile strength and brightness, respectively (Beg et al., 2000). Mannanase from *Streptomyces ipomoea* CECT 3341 applied to pine, kraft pulp increased the pulp brightness by 1.7% (Montiel et al., 2002).

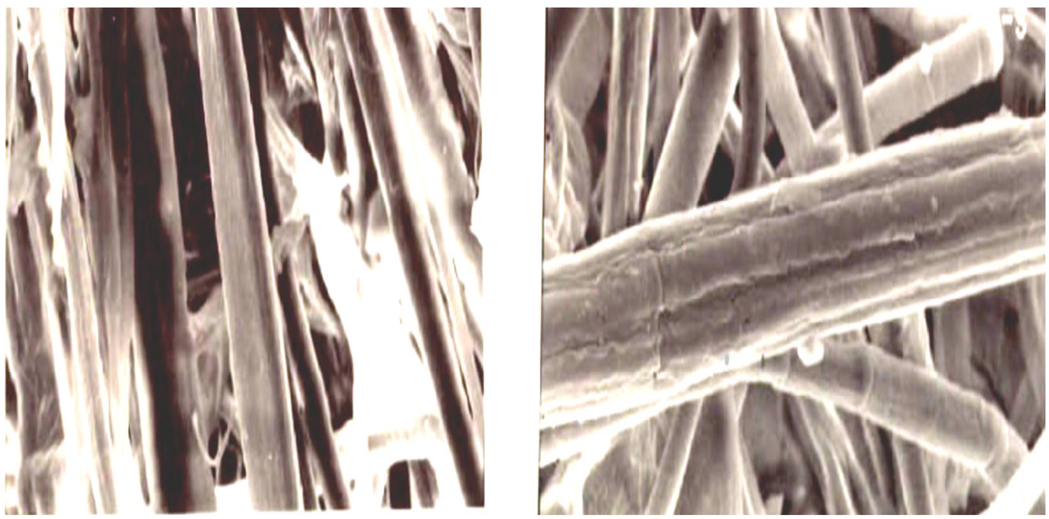
Scanning electron microscopy (SEM) of pulp

Scanning electron microscopy of unbleached (with denatured mannanase) pulp sample showed the smooth surface of pulp, that is, no change in pulp texture Figure 3A. Figure 3B revealed the active mannanase treated pulp with remarkable peeling effect on its surface which exhibited many cracks, swelling and separation in the

Table 2. Physiochemical properties of mannanase and chemically treated wheat straw- rich-soda pulp.

Bleaching process	Pulp properties						
	Kappa number	Tensile Strength	Brightness (%ISO)	Burst factor (KN/g)	Reducing Sugars (mg/g pulp)	A_{237} nm Hydrophobic compounds	A_{465} nm Chromophoric compounds
Untreated (control)	15.0	9.0	34.0	0.98	0.07	0.02	0.05
X	11.3	10.8	36.8	1.07	0.35	0.48	0.26
XH	9.5	15.5	45.5	1.09	0.41	0.33	0.12
H	10.0	13.2	38.0	1.09	0.31	0.365	0.08
HX	9.8	13.6	40.5	1.10	0.45	0.473	0.11

X = mannanase treatment with 5 Ug^{-1} moisture free pulp (50°C for 4h: pH 7.0-7.5); H = Hypochlorite treatment with 6% Cl_2 (50°C for 45 min; pH 1.6); XH = Mannanase treatment followed by hypochlorite treatment; HX = Hypochlorite treatment followed by mannanase treatment.



A B

Figure 3. Scanning electron micrograph of (A) boiled mannanse treated (B) activated mannanase treated samples of wheat straw rich-soda pulp.

pulp fibers. Results of scanning electron microscopy confirmed that mannanase removes the hemicellulose from the pulp fiber which was helpful in bleaching and has equivalent effect to chemicals.

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