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Educational initiatives

Synergistic effect of xylano-pectinolytic enzymes produced by a bacterial isolate in bleaching of plywood industrial waste

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ABSTRACT

Bleach boosting effect of xylano-pectinolytic enzymes produced by *Bacillus pumilus* AJK was evaluated on plywood veneer soda anthraquinone pulp. Enzyme dose of 6 IU xylanase and 2.4 IU pectinase per g pulp at pH 8.5, temperature 55 °C and retention time of 120 min mitigated kappa number of pulp by 9.375%, thus exhibiting remarkable delignification efficiency. The consumption of chlorine and chlorine dioxide diminished by 25% and 23.8% respectively in subsequent bleaching stages with no detrimental effect on the brightness of pulp. Enzyme treated pulp exhibited improved physical properties viz. breaking length, burst factor, tear factor and viscosity. Furthermore, this approach resulted in 16.67% and 18.03% decrease in the BOD and COD values of effluents. This is the first report representing the use of xylano-pectinolytic enzymes, produced concurrently by a cellulase free bacterial strain utilizing agro-residues based production media for bleaching of softwood soda anthraquinone pulp.

1. Introduction

As an outcome of our social development, environmental pollution has become one of the major issues for the human race on this planet. Paper industries are one of the major contributors to the overall pollution load. The chlorinated organic by-products including dioxins generated in bleaching effluents cause severe environmental damage including larger COD values of water, ultimately resulting in expensive waste water treatment (Zhang et al., 2008) and are also responsible for causing cancers, endocrine disorders and serious health effects (Fiedler et al., 1990). This necessitates the search for a biologically safe eco-friendly alternative which was first reported by Viikari et al. (1986) for bleaching of hardwood and softwood kraft pulp using endo-xylanases produced by *Aspergillus awamori* VTT-D-79125 and *Streptomyces olivochromogens* VTT-E-82157 strains. With the increasing concern towards the environment, research has been focused on the usage of several other enzymes besides xylanase, such as pectinase, galactosidase, mannase, laccase and ligninase (Bhoria et al., 2012; Martin-Sampedro et al., 2012; Virk et al., 2012; Valls et al., 2010; Clarke et al., 2000) to improve the quality of paper in paper and pulp industries.

Lignin, hemicelluloses and celluloses are the major constituents of plant cell wall. Lignin has a complex polyphenolic structure that is strongly intermeshed and chemically associated by covalent linkages and non-covalent interactions with hemicelluloses (Perez et al., 2002) and celluloses and thereby protecting these polysaccharides from microbial degradation and hence strengthens the cell wall. Xylan, being the major component of hemicelluloses, acts as an interface between lignin and cellulose. The layer of xylan reprecipitates over surface of pulp fibers after alkaline cooking, hence prevents the easy removal of lignin by creating a physical barrier to the oxidizing chemicals. This xylan can be enzymatically hydrolyzed by cellulase-free xylanase, leaving the cellulose fibers undisrupted. Xylanases cleave the β-1, 4 bonds of xylan, thus generating pores in the xylan backbone and allow easy access of oxidizing agents for the degradation of residual lignin in paper pulp (Kantelinen et al., 1991). Enzymatic treatment of the pulp opens the pulp structure and releases lignin–carbohydrate complexes (Yang and Eriksson, 1992) and also causes the hydrolysis of alkali insoluble hemicelluloses and releases the chromophores associated with carbohydrates (Patel et al., 1993). The US patent number 5487812A discloses the fact that the aqueous phase of alkaline treated pulp contains considerable amount of pectins (Thornton et al., 1996). The presence of pectinases in aqueous phase of pulp

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causes the degradation of harmful pectins and thus improves the paper making process.

Reports are available on bio-bleaching of woody and non woody SAQ pulp (Lin et al., 2013; Garg et al., 2011; Singh et al., 2011; Khristova et al., 2006). The beneficial perspective of non sulfur process is mainly attributed to its improved energy efficiency over kraft pulp (Francis et al., 2008). Anthraquinone is mainly responsible for preservation of polysaccharides as illustrated by higher pulp viscosity depicting less damage of cellulose fibers (Khristova et al., 2006). Concurrent production of xylano-pectinolytic enzymes using agricultural wastes in a fermenter is beneficial for industries from economic point of view. This is the first report showing the use of xylano-pectinolytic synergism from *Bacillus pumilus* AJK in bleaching of plywood veneer SAQ pulp.

2. Materials and methods

2.1. Microorganism

Bacterial strain *B. pumilus* AJK (MTCC Accession No. 10414) was used for the production of cellulase free xylano-pectinolytic enzymes.

2.2. Chemicals and raw materials

All the chemicals used were of analytical grade and were purchased from Himedia. Birchwood xylan was purchased from Sigma Aldrich. Wheat bran and citrus peel were purchased from local market.

2.3. Enzymes production

Submerged fermentation was used for producing xylanase and pectinase concurrently. The 50 ml basal media containing peptone, 5 g/l; $MgSO_4.7H_2O$, 10 mM; pH 7.0 along with wheat bran and citrus peel (2% each) in 250 ml Erlenmeyer flask was used as production media. The flasks were inoculated with 21 h old of 2% inoculum. After 60 h of incubation period at a shaking speed of 200 rpm, the media was centrifuged at 10,000 g for 15 min to remove the debris and the clear supernatant was used as a source of xylano-pectinolytic enzymes.

2.4. Assay conditions

For the estimation of enzyme activity, method given by Miller (1959) was followed. Assay conditions were similar to as reported by Kaur et al. (2010). The lot of enzymes used in this study contained 200 IU/ml of xylanase and 80 IU/ml of pectinase.

2.5. Pulp sample

Unbleached SAQ pulp consisting of 90% veneer (plywood industrial waste), 5% bamboo and 5% mixed hardwood was used for the present study. Concentration of anthraquinone used was 0.08%. All the experiments were performed at 10% pulp consistency.

2.6. Optimization of reaction conditions for enzymatic bleaching of soda-anthraquinone pulp

The four main conditions namely temperature, pH, enzyme dose and treatment time (Table 1) were optimized to select the most appropriate condition for bio-bleaching of SAQ pulp. The pulp samples were initially treated at variable pH from 7.5 to 9.5. Enzyme dose was optimized by carrying out the reaction in a range of 2.0–7.0 IU of xylanase and 0.8–2.8 IU of pectinase per g of oven dried pulp. Effect of different treatment time and temperature conditions was also studied by conducting the experiments in the range from 60 to 210 min and temperature range from 45 °C to 70 °C. Control samples were also run simultaneously under same treatment conditions by adding heat inactivated enzymes.

Handsheets were prepared according to standard TAPPI (Technical Association of Pulp and Paper Industry) methods (TAPPI test methods, 1996) after washing pulp samples thoroughly with distilled water. Kappa number (TAPPI test method T236) of the pulp handsheets was calculated for determining the best bleaching conditions.

2.7. Bleaching

Enzymatically treated pulp samples (under optimized conditions) were bleached along with control pulp samples (without any enzyme treatment) following a sequence of steps abbreviated as $CDEPD_1D_2$ (CD – Chlorination; EP – Alkali extraction; D_1 – Chlorine dioxide treatment 1; D_2 – Chlorine dioxide treatment 2) (Table 1). Percentage reduction in the amount of chlorine consumed after enzymatic treatment of the pulp was evaluated by conducting experiments using reduced amount of bleaching chemicals, while keeping all other parameters and treatment conditions unchanged and analyzing the extent of brightness obtained by enzyme treated pulp as compared to control pulp.

This was then followed by alkaline extraction step. The pulp was washed with distilled water and treated with 2.5% NaOH and 0.8% H_2O_2 at 80 °C for 2 h. After treatment with alkali, pulp was thoroughly washed with water to remove any left alkali and a known amount of pulp was taken to prepare handsheets for evaluating brightness, while the remaining pulp was treated with chlorine dioxide in two stages, D1 and D2 (1.0% and 0.3% ClO_2 was used in D1 and D2 stage) respectively at 70 °C for 3 h to remove the residual lignin. Percentage reduction in the consumption of chlorine dioxide at D1 and D2 stage was also determined. After the final bleaching step, pulp free filtrates were obtained by squeezing the pulp samples to determine their BOD and COD values and were compared with control.

2.8. Sheet preparation and analysis of physical properties of pulp

Laboratory handsheets were prepared. As per standard TAPPI test methods, these handsheets were examined for various physical properties of the pulp viz., Kappa number, (Test Method TAPPI T 236), Brightness, (Test Method TAPPI T 452), Breaking length, (Test Method TAPPI T 404), Burst factor, (Test Method TAPPI T403), Tear factor, (Test Method TAPPI T 496) and Viscosity, (Test Method TAPPI T 230). All the experiments were carried out in triplicates and the results mentioned are the mean of three experiments.

3. Results and discussion

Simultaneous production of two industrially important enzymes in the same production media utilizing inexpensive agro-residues is a workable strategy to lower the production cost of enzymes, thereby reduces the cost of bleaching process and hence makes the process suitable for commercialization.

3.1. Optimization of enzymatic reactions conditions

Fig. 1 indicates the effect of pH and enzyme dose on SAQ pulp unveiling the most favorable pH of 8.5 and enzyme dose of 6.0 IU xylanase and 2.4 IU pectinase per g of pulp for bleaching purpose. Increase in the enzyme dose above these values could not further