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Note from the field

Cost-effective scouring of flax fibers using cellulase-free xylano-pectinolytic synergism from a bacterial isolate

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ABSTRACT

Bioscouring of flax fibers was done using concurrently produced alkalo-thermotolerant cellulase-free xylano-pectinolytic enzymes. It resulted in 1.84% release of sugars, 4% weight loss, 19.46%, 8.2% increase in brightness and whiteness respectively and 5.14% decrease in yellowness of the fibers. The enzymatic pretreatment of flax fibers showed 70% reduction in consumption of scouring chemicals or 30% reduction in consumption of bleaching chemicals to obtain the same optical properties of the fibers as achieved by control fibers using conventional scouring and bleaching approach. The merging of bioscouring with chemical scouring reduced the use of environment polluting chemicals and also provided high tensile strength fabric. This is the first report showing the use of xylano-pectinolytic enzymes produced simultaneously by an isolate *Bacillus pumilus* in bioscouring of flax fibers and detailed analysis on the serial reduction in the consumption of scouring and bleaching chemicals in order to make the process ecofriendly and energy saver.

1. Introduction

Use of xylanases and pectinases for textile fiber processing has increased significantly because of their ability to degrade the hemicellulosic and pectin content present in the plant fibers, respectively. Mature flax cell wall consists of 70–75% cellulose, 15% hemicelluloses and 10–15% pectic materials (Abdel-Halim et al., 2008). Thus, by removing the non-cellulosic impurities, xylano-pectinolytic enzymes can increase the access of scouring/bleaching chemicals to the lignin layer by opening up the fiber structure and can reduce the use of harsh chemicals in the textile industries. This enzymatic pretreatment improves the water absorbing capacity, brightness and whiteness of the plant fibers as well as preserves the fiber's structure and strength. Very few reports of the microbes producing xylanase in combination with pectinase are available till date in the literature. Xylanase in combination with pectinase and other enzymes (Ossola and Galante, 2004; Sharma et al., 2005; Dong et al., 2014; Garg et al., 2016) have been studied for the processing of flax fibers. But, in this study, detailed optimization of enzymatic pretreatment reaction conditions has been done for effective bioscouring of flax fibers, comparison of bioscouring with chemical scouring and merging of bioscouring with chemical scouring, with stepwise reduction in the amount of toxic scouring or bleaching chemicals in order to see the exact reduction in the consumption.

2. Materials and methods

2.1. Production of xylano-pectinolytic enzymes

A concurrent producer of xylanase and pectinase, *Bacillus pumilus* AJK (MTCC 10414) was isolated from the soil contaminated with the effluents of paper and pulp industry (Kaur et al., 2011). Xylano-pectinolytic enzymes were produced under submerged fermentation in 50 ml of basal medium (g/l: peptone, 5.0; $MgSO_4.7H_2O$, 2.46; pH 7) supplemented with 2% (w/v) wheat bran and 2% (w/v) citrus peel. The flasks were inoculated with 2% inoculum of 21 h old and incubated at 37 °C for 60 h at 200 rpm. Crude Xylano-pectinolytic enzymes were harvested by centrifugation.

2.2. Enzyme assay

Xylanase (Bailey et al., 1992) and pectinase activities were assayed by measuring the amount of reducing sugars liberated

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from birchwood xylan (2%, prepared in 0.1 M glycine-NaOH buffer, pH 8.5) and polygalacturonic acid (0.5%, prepared in 0.1 M glycine-NaOH buffer, pH 9.0), respectively, using 3, 5-dinitrosalicylic acid (Miller, 1959). Cellulase activity (CMCase and FPase) was determined according to the method of Ghosh (Ghosh, 1987).

2.3. Optimization of enzymatic pretreatment reaction conditions

For effective removal of non-cellulosic impurities and to improve the whiteness and brightness of the fibers for textile utilities, different bioscouring parameters were optimized. The optimization of reaction conditions was done by employing one variable at a time approach. Fiber samples (5 g) were treated with xylano-pectinolytic enzymes at different pH values (6.0–10.0) and buffer molarity (10 mM-100 mM). The enzymatic treatment was carried out by varying the material to liquor ratio in the range of 1:10–1:35. A range of enzyme doses (5.0–30 IU of xylanase and 1.6–9.6 IU of pectinase) were used for different treatment time period (30–180 min), temperature (40–65 °C) and rpm (50–80). Various concentrations (1.0–5.0 mM) of EDTA were used to improve fiber properties. To improve the water absorbency power, different wetting agents Tween-20, Tween-80, Triton X-100, Lissapol and Brij were used at a concentration of 1%. After each treatment, the fibers were washed three times in boiling water to deactivate the enzymes followed by two washings in cold water and then oven dried at 45 °C. For control treatments, the fibers were treated under the same reaction conditions but in the presence of inactivated enzymes (wherever there was variation in control, it is mentioned).

2.4. Conventional alkaline scouring and bleaching

Chemical scouring and bleaching were done using the modified method of Abdel-Halim et al. (2008). Alkaline scouring was carried out using a solution containing sodium hydroxide (5 g/l), sodium carbonate (3 g/l) and non ionic wetting agent Tween-80 (1.5 ml/l) at 95 °C for 60 min at a material to liquor ratio of 1:20. After the alkaline treatment, the fiber samples were washed three times with boiling water, twice with cold water and finally oven dried at 45 °C. Control samples (fibers in the deionized water) were subjected to the same treatment conditions, without addition of chemicals.

Both the conventionally and the enzymatically scoured fiber samples (100 g) were bleached separately, in an aqueous solution containing H_2O_2 (3 g/l), sodium hydroxide (1.5 g/l), sodium silicate (6 g/l), $MgSO_4$ (0.2 g/l) and nonionic wetting agent (Tween-80, 0.2 ml/l) at 95 °C for 60 min at a material to liquor ratio of 1:20. After bleaching, the fiber samples were washed and dried as described above.

2.5. Evaluation of the fiber and fiber free filtrate

To evaluate the removal of xylan and pectin, reducing sugars were measured in the fiber free filtrate after bioscouring and chemical scouring using 3, 5-dinitrosalicylic acid (Miller, 1959). Release of lignin in the fiber free filtrate obtained after the enzymatic and chemical scouring was determined by measuring absorption at λ 280 nm. Fiber weight loss was calculated on the basis of dry weight using the following equation: %Weight loss = (W1 − W2) × 100/W1, where, W1 and W2 are the dry weights of the fiber samples before and after treatment, respectively (Aly et al., 2004). The whiteness index on the Hunter scale (Hunter, 1975), brightness on the ISO 2470 scale (ISO, 1977) and yellowness on the ASTM-E−313 scale (ASTM-E−313, 1974) were measured by Macbeth Color- Eye® spectrophotometer. Wetting ability of the fabric was measured using the drop penetration test before and after the scouring process, according to an AATCC (American Association of Textile Chemists and Colorists) Test method 39−1980 evaluation of wetting ability (AATCC, 1980). Tensile strength of the fabric samples was determined using digital tensile strength tester. Microscopic characterization of raw, bioscoured and scoured fibers was done using light microscope at 400× magnification. The fibers of all treatment stages were also visually analyzed for their surface and optical properties.

3. Results and discussion

Parametric optimization of various conditions was done for effective removal of non-cellulosic impurities from flax fibers. Maximum release of sugars and weight loss with best optical properties was obtained at pH 8.5 (Fig. 1a) of 50 mM glycine-NaOH buffer (Fig. 1b) and material to liquor ratio of 1:15 (Fig. 1c). An enzyme dose of 15 IU of xylanase and 4.8 IU of pectinase (Fig. 1d) showed the optimal efficiency after a treatment period of 60 min at 50 °C and 50 rpm (data not shown). Ossola and Galante (2004) performed the enzymatic scouring of flax fibers at 55 °C for 30 min, at a material to liquor ratio of 1:10 (w/v) in the presence of a surfactant (1 g/l) and mild chelator (2 g/l) while Abdel-Halim et al. (2010) have done the enzymatic scouring of flax fabric for 2 h at 60 °C using a material to liquor ratio of 1:20 (w/v) in the presence of a surfactant (0.1%). Csiszar et al. (2004) scoured linen fabric with pectinase at pH 5.0 (50 mM acetate buffer) for 1 h at 50 °C, 40 rpm and material to liquor ratio of 1:50 (w/v) in the presence of non-ionic surfactant (1 ml/l). Sharma et al. (2005) used the enzyme formulations containing pectinase, xylanase and laccase for the enzymatic treatment of flax fibers at the roving stage for 3 h at 40 °C. EDTA at a concentration of 3 mM was sufficient for the effective removal of impurities (Fig. 2a). EDTA modifies the substrate structure by removing the calcium ions from the cross bridges that link the macromolecules in pectin to one another or pectin to other polysaccharides, therefore when applied simultaneously with the enzyme seems to assist in the creation of free and accessible areas for the enzyme present (Losonczi et al., 2005). Many researchers confirmed that the addition of EDTA into the enzyme solution during bioscouring or during microbial-retting accelerated the degree of hydrolysis significantly (Csiszar et al., 2004; Akin, 2013). Dong et al. (2015) also used EDTA (4 g/l) during the biological scouring of flax roves. Among all the wetting agents used to improve the water absorbency of the fibers, Triton X-100 was found to be best closely followed by Tween-80 (Fig. 2b). Triton X-100 at a concentration of 1% was found to be optimum (Fig. 2c). Wetting agents act by reducing the surface tension on the fibers enabling improved penetration of the enzymes into the fiber microspores (Losonczi et al., 2005). Enzymatic treatment of flax fibers under optimized conditions resulted in 19.46% increase in brightness, 8.2% increase in whiteness and 5.14% decrease in yellowness.

The bioscouring was compared with chemical scouring and then both the chemically and enzymatically scoured fibers were commonly bleached. Bioscouring resulted in release of 1.84 g sugar/100 g fiber with 55.65 whiteness index, 27.74 brightness index and 25.64 yellowness index, while the chemical scouring resulted in release of 3.36 g sugar/100 g fiber with 57.59 whiteness index, 29.92 brightness index and 24.82 yellowness index. Wetting time of both bioscoured and chemically scoured fibers was less than 1 s. Presence of a characteristic absorbance of the fiber free filtrate at 280 nm indicated the release of lignin. Csiszar et al. (2006) also analyzed the lignin content of the enzyme and alkaline solutions after treatment of cotton by measuring absorbance at 280 nm. Bioscouring of flax fibers gave 0.7 absorbance value of fiber free

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