Enzyme and Microbial Technology 43 (2008) 262–269

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Pretreatment processing of fabrics by alkalothermophilic xylanase from *Bacillus stearothermophilus* SDX

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ARTICLE INFO

Article history:
Received 29 December 2007
Received in revised form 23 March 2008
Accepted 29 March 2008

Keywords:
Alkalothermophilic
Bioscouring
Cotton
Desizing
Micropoly
Weight loss
Whiteness index
Xylanase

ABSTRACT

A highly active alkalothermophilic cellulase free xylanase (3446 U/g of dry substrate) has been produced from newly isolated strain of *Bacillus stearothermophilus* under solid-state fermentation using wheat bran (2.5%, w/v) pH 7.0 and at 37 °C. The xylanase was stable over broad range of pH (6.0–12.5) and temperature (37–85 °C) and hence was used in the bioprocessing of fabrics as an alternative to conventional chemical method. The desizing and bioscouring treatments were most efficient at 70 °C after 90 and 180 min of incubation respectively using 5 g/l of xylanase dose in the medium of pH 9.5. Enzymatically desized fabrics when subjected to bioscouring resulted in 0.91%, 0.88% weight loss for micropoly and 0.83%, 0.80% for cotton respectively under agitated (50 ± 2 rpm) and non-agitated conditions. Consequently Whiteness index for cotton (11.81%) and micropoly (52.15%) fabrics was increased. Induction in the release of reducing sugars was also observed for cotton (8.37%) and micropoly (16.79%) fabric over conventional method. Enzymatically processed fabric samples possessed 1.12% and 1.95% more tensile strength than reference. Tearness value also increased for bio-treated cotton (1.57%) and micropoly (2.37%) fabric samples as compared to control.

1. Introduction

Endoxylanases (β-1,4-D-xylanohydrolase, EC 3.2.1.8) are a group of enzymes that catalyze the hydrolysis of xylan. Xylanases have been reported mainly from bacteria [1–3], fungi [2], actinomycetes [4] and yeast [5,6]. The main reason for investigating its production by bacteria is its wide variety of biotechnological applications. Therefore efforts are being made to find out environment-friendly mechanistic approach to improve the processing and finishing of fabrics by using xylanase. Recent research has shown that specific enzymes such as cellulase, xylanase, pectinase, lyases and several non-cellulolytic enzymes can be employed for cleaning, dying and wet processing of the fabrics.

Processing of the fabric includes desizing; removal of adhesive sizing material, scouring; improving absorbency and whiteness of the textile material and bleaching; imparting fixed standard whiteness to the fabric [7,8]. Sizing materials (i.e. starch and waxes, etc.) are used to strengthen the fabric against the mechanical abrasions generated during the weaving. In desizing, wrapped adhesive material is removed to make fabric more accessible to the subsequent stages of the processing. It is carried out at higher temperature with strong oxidizing agents in alkaline solution. After desizing, the fabric needs to be scoured to remove inhibitory materials for its efficient finishing, wetting and dying. Conventional scouring is a chemical intensive, non-optimal process that is unquestionably expensive in terms of energy and water. Concentrated sodium hydroxide (scouring) and hydrogen peroxide (H_2O_2)/sodium hypochlorite (bleaching) solutions are used to eliminate the inhibitory non-cellulosic impurities of wax (0.4–1.2%), pectic substances (0.4–1.2%), ashes (0.7–1.6%), and lignin containing proteins (1.0–1.9%), etc., which inhabit the cuticle and primary cell wall. Concentrated alkaline solutions of scouring not only cause threat to the environment but also attack non-specifically on cellulose of the fiber leading to weight and strength loss. Effluent wastes of textile industry cause rapid increase in the BOD (biological oxygen demand) and COD (chemical oxygen demand) values of the water bodies.

Research is gaining momentum to replace these harsh chemicals with commercial enzymes which can specifically target the non-cellulosic impurities while maintaining the standard industrial properties of the fiber as it was achieved through conventional strategies [9–18]. Enzymatic treatment accelerates the degradation of complex impurities situated in the primary cell wall thus enhancing its water absorbing properties. A number of reports are available on the use of cellulases and pectinases for bioprocessing of fabric but there are very few reports on xylanases for the purpose of desizing and scouring [19,20]. The greatest hurdle in the commercialization of enzymatic method is offered by the presence of seed

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doi:10.1016/j.enzmictec.2008.03.016

coat fragments attached with the fibers and linters [21]. Enzymatic pretreatment with xylanase leads to the partial hydrolysis of these seed coat fragments thereby making them more accessible to the chemicals during the later stages of bleaching and finishing [20].

In the present investigation, high level production of alkalothermophilic cellulase-free xylanase using agro-residues under solid-state fermentation was achieved from newly isolated strain of *Bacillus stearothermophilus* and was tested for its application in processing of fabrics.

2. Materials and methods

2.1. Microbial strain and its maintenance

Microorganism was isolated from a compost sample and was identified by Institute of Microbial Technology (IMTECH), Chandigarh, India as *B. stearothermophilus* SDX on the basis of its morphological, physiological and biochemical characteristics. The strain has been deposited at MTCC, IMTECH and has been given an accession number 8508. The isolated bacterial strain is Gram positive, spore forming (oval, subterminal), highly thermophilic with minimum, optimum and maximum temperature for growth as 37, 45 and 65 °C, respectively. The strain is an alkalophile and grew optimally at pH 8.0 (range 6.8–12.5). The culture was maintained on Nutrient Agar (NAM) (g/l: peptone, 10.0; yeast extract, 3.0; sodium chloride, 5.0; agar, 2%) slants at 4 °C and as glycerated frozen culture at −70 °C.

2.2. Xylanase production and enzyme extraction

Erlenmeyer flasks (250 ml) containing 10 g of wheat bran was moistened with mineral salt solution (g/l: K_2HPO_4, 2.0; $MgSO_4.7H_2O$, 0.4; pH 8.0) in the ratio of 1:2.5 (w/v), autoclaved at 1.05 kg cm^{-2} for 45 min, cooled, inoculated with 15% (v/w) inoculum (18-h old, ~3.6×10^6 counts/ml) and incubated in a humidified (relative humidity 60–65%) incubator at 37 °C for 72 h. The flasks were gently tapped intermittently to mix the contents. The bacterial bran was extracted from each flask with phosphate buffer (10 mM, pH 8.0, 1:10 (w/v)) by agitation at 150 rpm for 20 min and squeezed through a wet muslin cloth. The crude enzyme was harvested by centrifuging the SSF extract at 12,000 × *g* for 20 min at 4 °C and the clear supernatant was used as the enzyme source.

2.3. Xylanase, cellulase assay

The enzyme activity was determined by measuring the release of reducing sugars during the enzyme-substrate reaction using 3, 5-dinitrosalicylic acid (DNS) reagent [22]. The reaction mixture containing 490 μl of 1% Birchwood xylan prepared in 0.1 M glycine–NaOH buffer (pH 9.5) as the substrate and 10 μl of appropriately diluted enzyme was incubated at 70 °C for 10 min and the reaction was terminated by adding 1.5 ml DNS reagent.

One unit of xylanase activity was defined as the amount of enzyme that catalyzes the release of 1 μmole of reducing sugar equivalent to xylose per min under the specified assay conditions. Xylanase production was expressed as units (U) per gram of dry bacterial bran. Similarly assay for cellulose was carried out with CM-cellulose as substrate. All the experiments were carried out independently in triplicates and results presented are the mean of the three values.

2.4. Chemicals and fabric samples

Birchwood xylan, carboxymethyl cellulose, 3,5-dinitrosalicylic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of the highest purity available commercially. Sized samples of cotton and micropoly fabrics were obtained from Nahar Group of Industries, Punjab, India. Agro residues were procured locally.

2.5. Parametric optimization of xylanase production in SSF

1. Incubation period: SSF was carried out for 120 h and the enzyme was extracted and assayed at regular intervals of 24 h.
2. Inoculum concentration: An 18-h-old seed culture was used as inoculum in the range of 2.5–20% (v/w).
3. Temperature: Xylanase production at different temperatures ranging from 37 to 65 °C was studied.
4. Moistening solutions and moisture level: Various mineral salt solutions as demonstrated in Table 1 were used as moistening agents and different substrate to moistening solution ratio (w/v) in the range of 1:0.5–1:5.0 were tested.
5. pH: pH of the moistening solution giving maximum xylanase yield was varied from 6 to 12 for highest xylanase production.
6. Effect of carbon and nitrogen sources: Different agricultural wastes and byproducts were used as carbon source and organic/inorganic sources of nitrogen were used to study their effects on xylanase production.
7. Effect of various additives: Different additives like peptone, beef extract, yeast extract, etc., at a concentration of 0.2% (w/v) and Tween 80, Tween 20, glycerol, Triton X, olive oil and oleic acid at a concentration of 0.2% (v/v) were supplemented in the medium and the effect of various concentrations of the selected additive was studied.

After optimization of these parameters, xylanase production was carried out under optimized nutritional and fermentation conditions for maximum yield of the enzyme to be applied in textile industry.

2.6. Optimization of enzyme dose for bioprocessing

The optimization of enzyme dose and retention time for desizing and bioscouring was carried out by treating sized fabrics with varying doses of xylanase, ranging between 5 and 10 g/l for variable time intervals starting from 30 min to 3 h.

2.7. Desizing of fabric

Greige cotton i.e., untreated or raw cotton (0701DR007) and greige micropoly (5012117) fabrics (122 gm^{-2}) used for investigation were kindly provided by the Nahar Group of Industries, Nahar, Punjab, India. Before use, fabrics were washed with distilled water at room temperature to extract water-soluble impurities and then air

Table 1
Optimization of enzyme dose for desizing of fabrics

ED[a]	pH	Cotton fabric (0701DR001)		DE[d] (%)	VSR[e]	Micropoly fabric (5012117)		DE[d] (%)	VSR[e]
		Ab[b]	W'ness[c]			Ab[b]	W'ness[c]		
05	7.0	1.4711	17.38	0.35	3–4	0.6286	27.45	0.35	3–4
05	8.0	1.2146	18.84	0.35	3–4	1.6391	28.61	0.35	3–4
05	9.0	1.2176	19.32	0.35	3–4	1.4366	32.84	0.35	3–4
05	9.5	1.2189	19.88	0.2	4–5	1.4347	35.71	0.2	4–5
05	10.0	1.1986	19.39	0.2	4–5	1.3347	33.62	0.35	3–4
7.5	7.0	1.3427	17.26	0.35	3–4	0.5942	26.66	0.35	3–4
7.5	8.0	1.2110	17.83	0.35	3–4	1.4337	27.52	0.35	3–4
7.5	9.0	1.2056	18.22	0.35	3–4	1.4871	28.57	0.35	3–4
7.5	9.5	1.2234	18.59	0.35	3–4	1.6213	29.33	0.2	4–5
7.5	10.0	1.1992	18.93	0.35	3–4	1.5822	30.71	0.2	4–5
10.0	7.0	1.3322	16.11	0.35	3–4	0.5735	25.11	0.35	3–4
10.0	8.0	1.1973	16.68	0.35	3–4	1.3922	25.97	0.35	3–4
10.0	9.0	1.1988	17.33	0.6	2–3	1.3619	26.55	0.6	2–3
10.0	9.5	1.2037	17.67	0.6	2–3	1.3358	27.34	0.6	2–3
10.0	10.0	1.2983	18.03	0.6	2–3	1.3216	28.29	0.6	2–3

[a] Enzyme dose of xylanase enzyme in gram per liter (g/l).
[b] Absorbance at 540 nm.
[c] Whiteness index.
[d] Desizing efficiency.
[e] Violet scale reading.