International Biodeterioration & Biodegradation 64 (2010) 13–19

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Xylanase production by *Paenibacillus campinasensis* BL11 and its pretreatment of hardwood kraft pulp bleaching

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ARTICLE INFO

Article history:
Received 8 September 2009
Received in revised form
7 October 2009
Accepted 7 October 2009
Available online 2 November 2009

Keywords:
Bleaching pretreatment
Hardwood kraft pulp
Paenibacillus
Xylanase

ABSTRACT

Production of a single component 41 kDa xylanase from *Paenibacillus campinasensis* BL11 was conducted under various pH and temperature at the shake flask level. Alternative carbon and nitrogen sources were also evaluated. Up to 10.5 IU/mL and 29.39 IU/mg specific activity of xylanase in crude extract was obtained at 24 h, 37 °C, pH 8. In addition, same level of xylanase productions was also obtained on rice husk and rice straw at 2 days of incubation. Relative xylanase activities of 56.8% and 51.9% were found after 4 h incubation in pH 7 and pH 9 at 65 °C, respectively. Xylanase pretreatments (2.5 IU per gram of oven-dry pulp) increased brightness (as much as 4.4 and 3.9%) and viscosity (as much as 0.5 and 0.3 cP) of pulp after full chlorine dioxide bleaching for untreated and oxygen delignified hardwood kraft pulp. Increased benefits of pretreatment were found with increasing xylanase dosage and pretreatment time.

1. Introduction

Enzymes are extremely efficient and highly specific biocatalysts. With the advancement in biotechnology, the demand for enzyme application to amend conventional processes is ever increasing in pulp and paper industry (Bajpai, 2004). Xylanases were first used for pre-bleaching kraft pulp in 1994. The potential of xylanase as a pre-bleaching agent in the pulp and paper industry was recently demonstrated on the commercial scale (Viikari et al., 1994; Subramaniyan and Prema, 2002). Since the major advantage to employ xylanase pretreatment was to reduce the usage of chlorine and/or chlorine dioxide in the first stage of bleaching and to derive an environmental-friendly bleaching process. The mechanisms by xylanase-assisted bleaching includes: (i) depolymerization of xylan precipitated on the fiber surface, then opening up the pulp structure to access by bleaching chemicals (Wong et al., 2001); (ii) release of chromophores with smaller residual lignin molecules by cleavage of the glycosidic bonds in carbohydrate portion of lignin-carbohydrate complex (Beg et al., 2001).

Microbial diversity was explored as an invaluable xylanases resource for industrial applications, *e.g. Arthrobacter* sp. MTCC 5214 (Khandeparkar and Bhosle, 2007), *Bacillus coagulans* (Choudhury et al., 2006), *Bacillus pumilus* (Asha and Prema, 2007). The suitability of xylanase for bleaching pretreatments was generally dependent on the enzyme stability at high optimum pH and temperature, and the devoid of cellulase activity (Subramaniyan and Prema, 2002). Hence, xylanases from extremophilic organisms has got most attention recently, due to superior stability at higher pH and temperature (Subramaniyan and Prema, 2002).

Paenibacillus species were capable to hydrolyze plant materials, and frequently isolated and identified from soil and plant related sources (Rivas et al., 2005a,b, 2006). Suihko also isolated *Paenibacillus* species in paper mills and paper products (Suihko et al., 2004). Several members of the genus *Paenibacillus* secrete diverse assortments of extracellular polysaccharide hydrolysing enzymes and their xylanolytic and cellulolytic systems are being gradually identified (Gallardo et al., 2003; Pason et al., 2006; Lee et al., 2007). However, few studies did address utilization aspects of their polysaccharide hydrolases (Garcia et al., 2002). The efficacy of *Paenibacillus* sp. xylanase for bleaching pretreatment was not reported.

One objective of the present study was to investigate xylanase production from recently reported *Paenibacillus campinasensis*

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doi:10.1016/j.ibiod.2009.10.001

BL11, with single component 41 kDa xylanase (Ko et al., 2007). Productions under various pH and temperature nutrients at the shake flask level were evaluated. Cultivation with alternative carbon and nitrogen source was also conducted. Thermostabilities of xylanase were evaluated with conditions as same as the pre-bleaching applications of actual kraft pulp mills. Crude xylanase was employed to treat both oxygen delignified and control mill hardwood kraft pulp prior to full chlorine dioxide (DED) bleaching, sequentially conducted by chlorine dioxide (D_0)-alkaline extraction (E)-chlorine dioxide (D_1) stages. To assess the benefit of chlorine dioxide saving in the first bleaching stage, 5 different chlorine dioxide charges (expressed as well as active chlorine multiples (Reeve, 1996)) were applied on xylanase treated and control pulps. Kappa number represents the amount of residual lignin in pulp. Viscosity represents the molecular weights of pulp cellulose. Selectivity, defined as the ratio of the decrease the kappa number to the change of decreasing viscosity, is commonly used to evaluate the efficiency of oxygen bleaching (Fu and Lucia, 2003) and other enzyme pretreatment processes (Shatalov and Pereira, 2008).

Using different chlorine dioxide charges at the first stage, pulp bleachability and selectivity enhancement by xylanase pretreatment can be properly identified by comparison of brightness and viscosity values after full DED bleaching. This is the first time that these variables are being considered together in a study on *Paenibacillus* xylanase-aided bleaching.

2. Experimental methods

2.1. Microorganism and growth measurements

The thermo-alkaline *Paenibacillus campinanesis* BL11 used in this study was isolated at 90 °C, pH 9 environment in a kraft pulp mill (Ko et al., 2007). Chemicals used were from Sigma (St. Louis, USA) or Merck (Darmstadt, Germany). This strain was routinely plated on Luria–Bertani (LB) plates containing 0.2% (w/v) birchwood xylan, and incubated at 37 °C for 1–2 days. The LB plates were prepared with 10 g Bacto-tryptone, 5 g yeast extract, 5 g NaCl, 18 g agar per liter. Cell growth was monitored by direct cell counts. Additional cell growth experiments were performed by collecting cell suspension, diluting it with alkaline medium without xylan and plating it directly on xylan medium agar plates. After the plates were incubated for 24 h at 37 °C, colonies were counted for determination of colony forming units (CFU). Cell dry weight was also determined by vacuum filtration using a 0.45 μm pore size cellulose acetate membrane.

2.2. Enzyme assays

Crude enzyme solution was obtained as cell-free supernatants collected by centrifugation at 10,000 × g at 4 °C for 20 min. Xylanase activity was measured using birchwood xylan as substrate. The reaction mixture contained 0.5 mL of appropriately diluted enzyme and 0.5 mL of 1% (w/v) water-soluble birchwood xylan in 50 mM Tris–HCl buffer, pH 8. The reaction mixture incubated at 50 °C for 30 min, and the produced reducing sugars were assayed by the dinitrosalicylic acid method (Bailey et al., 1992; Miller, 1959). The D-xylose was used as standard. Soluble xylan was prepared by suspending 1 g of birchwood xylan in 100 mL of 50 mM Tris buffer, pH 8, and autoclaving for 30 min. A unit enzyme activity was defined as the amount of enzyme that produce 1 μmol of reducing sugar per min. The total extracellular protein concentration was measured by the Lowry method, with bovine serum albumin as standard. All the assays were carried out in triplicate.

2.3. Effect of pH and temperature on growth of P. campinanesis and xylanase production

The culture was primarily incubated in trypticase soy broth (TSB) medium (37 °C, pH 7, 100 rpm) for 24 h and inoculated into xylan medium. Xylan medium contained (Nakamura et al., 1993): 0.25 g peptone, 0.25 g yeast extract, 0.05 g K_2HPO_4, 0.01 g $MgSO_4 \cdot 7H_2O$, 0.25 g birchwood xylan, and 50 mL deionized water. Erlenmeyer flasks (250 mL) containing 50 mL of xylan medium were inoculated with a culture of *P. campinanesis* BL11 and incubated with shaking on a rotary shaker at 100 rpm. The culture broth was then processed for cell growth and xylanase measurement. For determination of optimum pH and temperature for xylanase production, the culture was grown in the xylan medium at pH 7–9, 37 °C, and at 25, 37, 50 °C under pH 8.

2.4. Effect of nutrient source on xylanase production

Several carbon sources (rice bran, rice straw, rice husk, eucalypt wood, bagasse and wheat straw) were studied at levels of 0.5–2% (w/v) at pH 8, containing 0.5% yeast extract and 0.5% peptone as nitrogen sources. Two percent solutions of xylose, glucose, or arabinose were also examined. The inoculum was inoculated as previously described. Xylanase activity was measured for 1–3 days cultures at pH 8. Effects on enzyme level of cultures on 1% birchwood xylan were compared to previously obtained enzyme levels with 0.5% xylan. Five nitrogen sources (yeast extract, peptone, casein, soybean meal, and corn steep liquor) were tested at concentrations equivalent to 0.5% yeast extract plus 0.5% peptone. For these studies, 0.5% birchwood xylan was used as the carbon source. The shake flask production of crude xylanase was performed in 50 mL medium with 0.05 g K_2HPO_4 and 0.01 g $MgSO_4 \cdot 7H_2O$. The pre-culture was also inoculated in 2.5 mL TSB medium. All natural biomaterials were grinded to less than 40 meshes, and sterilized prior to use.

2.5. Thermostability of crude xylanase

The thermostability of crude xylanase was determined by incubating the crude enzyme at 55, 60 and 65 °C, at pH 7 and 9 from 1 to 4 h. The crude xylanase was collected at the 48th hour cultivation, 37 °C, pH 8, by using 0.5% birchwood xylan, 0.5% yeast extract, 0.5% peptone in the media specified above. Then the respective enzyme activities were assayed.

2.6. Pulping processes

The hardwood kraft pulp was kindly provided by Taiwan Pulp and Paper Cooperation. Wood chips were consisted as following: *Eucalyptus globulus* Labill. 43.7%, *Eucalyptus* Leizhou No. 1 (*Eucalyptus citriodora* Sm. × *Eucalyptus exserta*) 30.8%, *Rhizophora* sp. 13.3% and mixed northeastern Chinese hardwood (mainly aspen and birch) 12.2%. The kraft pulp was collected from the mill production line before and after oxygen delignification process. The conditions of kraft pulping process were: 23% sulfidity, 18% alkali charge, 1/3 wood/liquid ratio. The cooking temperature was increased from 45 to 165 °C within 2 h, then kept at 165 °C for another 2 h. Oxygen delignification was performed in the following conditions: 10% consistency; 11 kg NaOH per ton of air-dried pulp, 98 °C with 4 kg/cm^2 oxygen pressure for 1 h. Original kappa numbers were 17.5 and 10.7; original viscosities were 16.6 and 15.1 cP (TAPPI) for the hardwood kraft pulp before and after oxygen delignification.