Biosynthesis of Xylobiose: A Strategic Way to Enrich the Value of Oil Palm Empty Fruit Bunch Fiber

Lakshmi, G. Suvarna, B. Uma Rajeswari, and R. S. Prakasham*

Bioengineering and Environmental Centre, Indian Institute of Chemical Technology, Hyderabad - 500 607, India

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Xylooligosaccharides are functional foods mainly produced during the hydrolysis of xylan by physical, chemical, or enzymatic methods. In this study, production of xylobiose was investigated using oil palm empty fruit bunch fiber (OPEFB) as a source material, by chemical and enzymatic methods. Xylanase-specific xylan hydrolysis followed by xylobiose production was observed. Among different xylanases, xylanase from FXY-1 released maximum xylobiose from pretreated OPEFB fiber, and this fungal strain was identified as *Aspergillus terreus* and subsequently deposited under the accession Number MTCC- 8661. The imperative role of lignin on xylooligosaccharides enzymatic synthesis was exemplified with the notice of xylobiose production only with delignified material. A maximum 262 mg of xylobiose was produced from 1.0 g of pretreated OPEFB fiber using FXY-1 xylanase (6,200 U/ml) at pH 6.0 and 45°C. At optimized environment, the yield of xylobiose was improved to 78.67 g/100 g (based on xylan in the pretreated OPEFB fiber).

Keywords: Alkaline pretreatment, oil palm empty fruit bunch fiber, xylanase, xylobiose, xylooligosaccharides

Microbial xylanases (1, 4-β-β-xylanohydrolase, E.C. 3.2.1.8) are most widely used biocatalysts responsible for the breakdown of xylan, which have many industrial applications [2]. One of the recent and exciting applications of endoxylanases is xylooligosaccharides (XO) production, which has great commercial value because XOs have potential applications in different sectors such as chemical, food, and pharmaceutical industries. For food applications, xylobiose is especially considered as this xylooligosaccharide has been used as a prebiotic, promoting the proliferation of bifidobacteria, beneficial microorganisms in the human intestine that reduce the risk of colon cancer [12]. XOs are also known to impart physiological significance in human health mainly in reduction of cholesterol levels, maintaining gastrointestinal health, and improving the nutritional and sensory properties of food [17].

Industrially, production of XOs was performed using different chemical and physical methods where controlling of reaction kinetics plays a significant role in synthesis of specific oligomers, which is one of the limiting factors for effective and economic production. Therefore, production of XOs by enzymatic hydrolysis of xylan-rich lignocellulosic materials offers several advantages, like to minimize undesirable by-products, low quantities of monosaccharides production, controlling of monomeric number in oligosaccharides, and not requiring special equipment [4].

Xylan is the major constituent of hemicellulose and the second most abundant renewable polysaccharide after cellulose, and is considered as the substrate for xylooligomer synthesis [9]. To economize the process, several researchers evaluated for XOs production from different lignocellulosic materials such as cotton stalk [4], sugar cane bagasse [9], almond shells [12], corn cobs [19], etc. Considering the fact that oil palm empty fruit bunch (OPEFB) fiber is one of the xylan-rich lignocellulosic materials containing 24% xylan [13], obtained abundantly as a residual matter from oil palm industry, the OPEFB fiber was selected for XOs production. Moreover, the type of xylooligomer production depends majorly on the xylanase enzyme complex. Thus, a selection of a suitable microbial strain that produces preferable enzyme complex is one of the essential steps for XOs production. Hence, in the present study, xylanases produced by five bacterial and three fungal strains were evaluated for the enzymatic hydrolysis of lignocellulosic materials for XOs production. In addition to the source of enzyme complex, reaction process variables such as incubation time, pH, temperature, and enzyme concentration were optimized to improve the XOs production.

*Corresponding author
Phone: +91-40-27191765; Fax: +91-40-27193159; E-mail: prakasam@iict.res.in
Materials and Methods

Materials

OPEFB fiber used in the present study was collected from the palm oil industry located at Rajahmundry, India. The material was washed with water and dried in an oven at 60°C overnight to get constant weight and stored in a dry place until further use.

Alkaline Pretreatment of the Oil Palm Empty Fruit Bunch Fiber

Fifty grams of OPEFB fiber was placed in a 2 L Erlenmeyer flask and 500 ml of 0.5% NaOH solution added. This mixture was subjected to steam treatment at 121°C and 15 lbs pressure. After steam treatment for 20 min, the fiber was squeezed and washed with distilled water several times for neutralization. The final neutralized pretreated OPEFB fiber was dried in an oven at 60°C overnight and used for XOs production.

Microorganisms

Xylanase-producing bacterial isolates, BXY-5, BXY-6, BXY-7, BXY-8, and BXY-9, were grown on nutrient agar slants for 24 h at 37°C. Inoculum was prepared by adding a loopful of each culture from nutrient agar slants to nutrient broth and incubating in a shaker at 150 rpm at 37°C. After 24 h of incubation, each 1% (v/v) of inoculum was added to the production media for xylanase production. Fungal strains FXY-1, FXY-2, and FXY-3 were grown on potato dextrose agar slants for 72 h at 30°C. Spore suspensions were prepared by adding 10 ml of sterile water containing 1% (v/v) Tween 80 to each fungal agar slant and gently scraping with a sterile inoculation loop. The obtained spore suspensions were used for the production of xylanase enzyme.

Xylanase Production

Xylanase production was carried out under submerged fermentation using the following medium components (g/l): KH₂PO₄ - 0.5, K₂HPO₄ - 0.5, MgSO₄ - 0.5, NaCl - 0.5, Beef extract - 10. For bacterial xylanase production, 2% (w/v) wheat bran was used as substrate and the medium pH adjusted to 7.0, whereas for fungal xylanase production, 2% (w/v) OPEFB fiber was used as substrate with medium at pH 5.0 and kept for sterilization. Then 1% of each bacterial suspension or fungal spore solutions was added to the sterilized medium in separate flasks and incubated under agitation at 150 rpm. Bacterial xylanase production was carried out at 37°C for 24 h of incubation, whereas fungal xylanase production was carried out at 30°C for 72 h of incubation. Xylanase activity in the culture filtrate was determined using 1% (w/v) beech wood xylan as substrate according to the method of Bailey et al. [7]. One unit of xylanase activity is defined as the amount of enzyme liberating 1.0 µmol equivalents of xylose in 1 min per milliliter of enzyme solution.

Enzymatic Saccharification

Alkaline pretreated OPEFB fiber was subjected to enzymatic hydrolysis using the following procedure: One gram of alkaline pretreated OPEFB fiber was suspended in 9.0 ml of 100 mM citrate buffer, pH 5.0, and to this mixture, 1 ml of 10 times diluted different bacterial or fungal xylanase enzymes was added and incubated at 50°C in an orbital shaker at 150 rpm. Periodically, samples were collected up to 30 h and the enzymatic reaction was stopped by boiling the samples for 5 min before they were subjected to analysis for xyloooligosaccharides.

Analysis of the Xyloooligosaccharides by Thin Layer Chromatography

The xyloooligosaccharides produced were qualitatively determined by using thin-layer chromatography. In this method, samples were applied to silica plates and developed with a solvent system containing 2-propanol, ethyl acetate, nitro methane, and water (6:1:1:2). Then the plates were air dried and the spots were visualized with an orcinol spray reagent (10 ml of H₂SO₄, 90 ml of methanol, 0.2 g of orcinol) by heating at 100°C. The oligosaccharides in the samples were identified by comparing their chromatographic behavior with the chromatographic behavior of authentic standards.

Analysis of the Xyloooligosaccharides by High-Performance Liquid Chromatography (HPLC)

The obtained xyloooligosaccharides and monosaccharides were analyzed by HPLC (SHIMADZU 10A) equipped with an Aminex HPX-87H column (Bio-Rad) with a refractive index detector using acidified distilled water (14 µl H₂SO₄ in one liter) as a mobile phase with a flow rate of 0.5 ml/min and at 65°C as the working temperature. Quantitative analysis of the reaction mixture was performed using HPLC. Xylotriose, xylobiose, and xylose revealed retention times of 9.0, 11.5, and 13.0 min, respectively. The concentration of the xyloooligosaccharides was quantitatively estimated by comparing the peak area of the analyzed samples with that of standards (xylobiose, xylotriose, xylotetrose, and xylopentose) and expressed as mg/ml of the hydrolysate.

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\text{XOs yield in the hydrolysate (g/100 g)} = \frac{\text{Total xylobiose/xylotriose in the hydrolysate (mg/g)}}{\text{Xylooligosaccharides in the hydrolysate (mg/g)}} \times 100
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Xylooligosaccharides in the hydrolysate (mg/g)

Results and Discussion

Xylanase Production by Isolated Bacterial and Fungal Strains

In general, xylanolytic complexes produced by different microbial strains differ in the ratio of xylanase enzyme components such as endo -1,4-β-xylanases, 1,4-β-d-xylanidoses, α-l-arabinofuranosidase, α-d-glucuronidase, galactosidase, and acetyl xylan esterase, which play a major role on XOs production [8]. For higher xyloooligosaccharide yield, the preferable xylanase enzyme complex should contain low levels of β-xyllosidase activity [4], which is responsible for conversion of xylobiose to xylose. Hence, initial experimental studies were aimed to select a suitable xylanolytic complex-producing microbial strain. For this, five isolated bacterial strains (BXY-5, BXY-6, BXY-7, BXY-8, and BXY-9) and three fungal strains (FXY-1, FXY-2, and FXY-3) were screened. From analysis of the xylanase yield data, it was evident that the selected microbial strains differ in their xylanase-producing capability. Among the selected fungal strains, FXY-1 produced higher amounts of xylanase enzyme (6,207 U/ml) followed by FXY-3 (5,014 U/ml). The xylanase activities produced by