IMPROVEMENT IN THE PRODUCTIVITY OF XYLOOLIGOSACCHARIDES FROM RICE STRAW BY FEED XYLANASE WITH ULTRAFILTRATION

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Abstract - The effective production of xylooligosaccharides (XOs) from rice straw was investigated. Rice straw contains rich hemicellulose which can be hydrolyzed by enzyme; the XOs were obtained under hydrothermal conditions. To improve the productivity of XOs, ultrafiltration was chosen to eliminate xylan in the XOs. Under optimum hydrolysis conditions (1000 IU enzyme/g, 35 °C, 10% substrate concentration, pH 6.5, 6 h), the DP was the lowest. After ultrafiltration, xylan was eliminated. On the basis of experimental data, an industrial XO production process consisting of pretreatment, enzymatic treatment and purification was designed. Using the designed process, 2.9g dry of purified XO was produced from 50g dry rice straw power.

Key words: Rice straw, xylooligosaccharides, feed xylanase, ultrafiltration

INTRODUCTION

Rice is a major crop in the southern part of China. In terms of total production, rice is the third most important grain crop in the world behind wheat and corn. Associated with rice production is a corresponding annual production of rice straw. Rice straw is also one of the most abundant and renewable energy sources in the world. Its annual production is about 731 million tons, which is distributed in Asia (667.6 million tons), America (37.2 million tons), Africa (20.9 million tons), Europe (3.9 million tons) and Oceania (1.7 million tons) (Kim et al., 2004; Faveri et al., 2004). The straw has traditionally been removed from the field by the practice of open-field burning. This practice clears the field for new plantings and cleans the soil of disease-causing agents. Nowadays, field burning is the major practice for removing rice straw, but it increases air pollution and consequently affects public health (Mussatto et al., 2004).

Lignocellulosic biomass, which includes rice straw, is an ideal inexpensive, renewable, abundantly available resource (Ho et al., 1998). Polysaccharides in lignocellulosic materials including cellulose and hemicellulose can be hydrolyzed to monomeric sugars such as glucose and xylose, which can be further used for the production of ethanol, xylitol, organic acid, and other chemicals (Xia et al., 2004). The hydrolysis of polysaccharides is usually catalyzed by hydrolytic enzymes, because enzymatic hydrolysis produces better yields than acid-catalyzed hydrolysis (Pan et al., 2005). Recently, ultrafiltration, a well-established separation process in biotechnology and fermentation industries (Grandison et al., 2002), was used to separate XOs, and polysaccharides (Freixo et al., 2002; Kim et al., 2003; Czermak et al., 2004; Moerman et al., 2004;).

The objective of this work was to evaluate rice straw as a source of fermentable carbohydrate by measuring the yield of sugars that can be obtained by combining the best available methods of pretreat-
ment and digestion with the best available commercial enzyme preparations. Enzymatic hydrolysis of rice straw polysaccharides with Nutrase feed Xylanase was studied for the production of reducing sugars, which can then minimize the cost.

MATERIALS AND METHODS

All chemicals were of analytical grade. Standards of xylose, xylobiose, and birch wood xylan were from Sigma-Aldrich (USA). A feed xylanase was from Nutrase. Rice straw was obtained from Shanghai surrounds, powdered (60–80 mesh) and stored in reagent glass. CarboSepCoregel-87C (dimension: 300mm×7.8mm) was purchased from Transgenomic.

Material characterization

Reducing sugars were quantified with DNS method (Miller et al., 1959) by using xylose as a standard. Total sugar content in the water soluble fraction obtained from the hydrothermal treatment was measured by the phenol-sulfuric acid method (Dubois et al., 1956). Degree of polymerization (DP) was obtained from the reducing sugar and total sugar (Rastall et al., 2003). XOs were determined by HPLC on CarboSepCoregel-87C column after hydrolysis.

Extraction of xylan

Xylans from the rice straw were extracted according to Zilliox and Guohua Hu (Zilliox et al., 1998; Guohua Hu et al., 2009), with slight modification. Crushed rice straw was swollen at 85°C for 3 h in NaOH solution (4%). The solid-to-liquid ratio was 1:10. The extract was centrifuged at 5000rpm for 15 min followed by neutralization with acetic acid to pH 6, then filtration on filter paper. The xylan was precipitated in 3 volumes of 95% ethanol and centrifuged at 5000rpm for 15 min; the precipitate was dissolved with 4 volumes of water and evaporated with Rotary Evaporators. After dialysis in 3500 Da dialysis bags and lyophilization, it was used to enzymatic hydrolysis.

Xylan hydrolysis with feed xylanase

Commercial enzyme concentrates were assayed for the desired enzyme activities according to endoxylanase activity (Bailey et al., 1992). One unit is defined as the quantity of enzymes which liberates 1.0μmol of xylose equivalent per minute under the described conditions. Substrate concentration, enzyme dosage, temperature, pH value were used as the single factor experiment. The optimized factor was used in different zymohydrolysis time. The results of hydrolysis reaction were monitored by measuring the reducing sugars, the total sugar and DP.

Fractionation by ultrafiltration

Ultrafiltration was carried out using a Labscale TFF System (Millipore, USA). Commercial membrane with a molecular mass cut-off (MMCO) at 5 kDa (Millipore USA) was used. The membrane was cut to size and soaked overnight in deionized water prior to use. Filtration experiments were carried out at a constant pressure of 4 bar. The dead-end ultrafiltration cell was filled with 300 ml of enzymically hydrolyzed concentrate and ultrafiltration was allowed to proceed for 5 to 8 h, depending on the used membrane, at room temperature.

When approximately 200 ml of permeate was collected, filtration was stopped. The permeate solution was analyzed by HPLC, and the interception was zymohydrolysis again.

HPLC analysis of xylooligosaccharides

XOs and neutral sugars were chromatographed on a HPLC system equipped with a refractive index detector (Agilent 1100 Series). Before injection, samples were filtered through a 0.20 μm filter. Aliquots of the filtered sample (20 μL) were injected into the HPLC system. The XOs were eluted using distilled deionized water as the mobile phase. The CarboSepCoregel-87C column (300 mm×7.8 mm) was used at 75°C and a flow rate of 0.6 mL/min. A complete analysis of the XOs was carried out in 30 min. Concentration of an oligosaccharide was quantified us-
ing average peak areas compared with a mixture of standard oligosaccharides (X1–X6) and expressed as mg/mL oligosaccharide.

RESULTS AND DISCUSSION

Characterization of rice straw

The compositions of lignocellulosic biomass vary and the structures of rice straw are very complex. Generally, their major component is cellulose, followed by hemicellulose and lignin. In lignocellulosic materials, cellulose is embedded in a lignin-polysaccharide matrix. Xylan integrates the structure of plant cell wall with covalent and non-covalent interactions (Bailey et al., 1992). Rice straw is a kind of lignocellulosic biomass containing about 32-47% of cellulose, 19-27% of hemicellulose and 5-24% of lignin (Saha et al., 2003; Karimi et al., 2006). Xylan was obtained from rice straw hydrolyzate. After lyophilization, 5.6 g xylan was obtained from 50 g rice straw power.

Optimization of hydrolysis conditions

Optimum hydrolysis conditions for the rice straw were investigated to minimize the expense of hydrolysis. Fig 1 shows the reducing sugars and total sugar content fractions obtained from each single condition.

As shown in the Fig 1. A, the DP changed greatly with the pH. The Nutrase feed enzyme had the high-
Est activity when the pH close to neutral (6-7). The concentration of reducing sugar was increased to 6.5. The DP was lowest at pH 6.5. Enzyme activity was influenced greatly by temperature. Fig 1. B shows that the feed enzyme activity was weaker after 50°C, and the reducing sugar content decreased. Though the DP was lowest at 25°C, the total sugar and reducing sugar were increased after 25°C. For this reason, the temperature of 35°C was selected. Fig 1. C shows the effect of substrate concentration on the hydrolysis conditions. The concentration of total sugar and reducing sugar rose rising within the specific limits (the substrate less than 10%). The optimal concentration was 10%; the DP was the lowest. As shown in

![Graph](image)

Fig. 2. Time effects on the reducing sugars and total sugar for the optimum hydrolysis conditions (enzyme dosage1000IU/g, temperature 35°C, substrate concentration10%, pH 6.5).

In the optimum hydrolysis conditions, the total sugar had changed a little. As shown in Fig. 2, in the first 6 h the concentration of reducing sugar increased, and afterwards the concentration decreased. The DP acquired the lowest value (1.9671) at 6 h.

Fig. 4. The HPLC chromatogram of XOs, produced from rice straw by xylanase from Nutrase feed enzyme. (A without ultrafiltration B with ultrafiltration) X: xylan, X1: xylose, X2: xylobiose, X3: xylotriose, X4: xylotetraose, X5: xylopentaose, X6: xylohexose and >X6: longer chain oligosaccharide.

![Graph](image)

Fig. 3. ultrafiltration effects on xylan and xylooligosaccharides composition. X: xylan, X1: xylose, X2: xylobiose, X3: xylotriose, X4: xylotetraose, X5: xylopentaose, X6: xylohexose and >X6: longer chain oligosaccharide.

![Graph](image)

Fig. 1. D, with the increase of the enzyme, the total sugar content increased. After 1200 IU/g, the reducing sugar content decreased due to mutual inhibition of the enzyme; the DP was the lowest when the enzyme dosage was 1000 IU/g.

**Xylooligosaccharide production**

Fig. 3 compares the xylan and XOs composition with and without ultrafiltration. It was found that without ultrafiltration the xylan composition was more...
than half (54.23%). With ultrafiltration the xylan was separated since its molecular weight is large. XOs produced by feed enzyme were X2 >X1>X3 >X4 >X5 >X6 and small amount of X1 and >X6 during 24 h reaction period (Fig. 3). Considering product composition, xylanase from feed enzyme with ultrafiltration was used in further experiments.

Fig. 4 shows the HPLC chromatogram of XOs from rice straw with xylanase. After HPLC some XOs were larger than X6, such as X7 and X8. These oligosaccharides are shown as >X6 due to the lack of standard. It was found that the HPLC chromatograms of XOs with and without ultrafiltration were similar to each other, while those compositions were different. The enzyme produced mainly X1, X2, X3 and X4 with small amounts of X5 and X6 from rice straw. The relative content of X5 remained at a very low level. These results show that the ultrafiltration took a great role in the production process. After rotary evaporation and lyophilization, the XOs were obtained. 2.9g of XOs was obtained from 5.6g xylan (50g rice straw power).

By-products application

In the production process, some waste that was produced can be used in other ways. The residue centrifuged from the hydrolyzate of NaOH, which contained lots of lignocellulosic was used to produce solid biomass fuel (Chuen-Shii Chou et al., 2009), which reduces the potential pollution of the environment. The alcohol used to precipitate can be recycled, and the hemicellulose (B. Xiao et al., 2001) was obtained. Hemicellulose had been reported to have many biological activities including decreasing blood cholesterol and preventing colon cancer (G.H. Hu et al., 2007).

CONCLUSION

Effective hydrothermal pretreatment of rice straw was investigated. It contributed to improving the yield of sugars in the result and it can remove xylan from the final product. Continuous hydrothermal treatment with Nutrase feed enzyme and the purification obtaining food-grade XOs were demonstrated as a step toward industrial scale production. Following this success, we expect the commercial production of XOs from biomass such as the waste present in the local area.

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REFERENCES

Guohua Hu, Shaohua Huang, Shuwen Cao, and Ma Zhengzhi (2009). Effect of enrichment with hemicellulose from rice


