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Enzymatic hydrolysis of maize straw polysaccharides for the production of reducing sugars

Ming Chen, Jing Zhao, Liming Xia *

Department of Chemical Engineering and Bioengineering, Zhejiang University, Hangzhou 310027, China

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Abstract

Enzymatic hydrolysis of maize straw polysaccharides was investigated for the production of reducing sugars. After maize straw was pretreated with 2% sodium hydroxide at 80 °C for 1 h to delignify, the cellulosic residues were hydrolyzed by cellulase from *Trichoderma reesei* ZU-02 and the hydrolysis yield at 48 h was 65.9%. A certain amount of cellobiose was accumulated in the hydrolysate due to low cellobiase activity in *T. reesei* cellulase. Supplementing cellobiase from *Aspergillus niger* ZU-07 greatly reduced the inhibitory effect caused by cellobiose, and the hydrolysis yield at 48 h was improved to 81.2% with cellobiase activity enhanced to 10 CBU/g substrate. The addition of 5 g/l Tween-80 improved the enzymatic hydrolysis by increasing the hydrolysis yield of 7.5%. Fed-batch hydrolysis was started with a batch hydrolysis containing 80 g/l substrate, with cellulosic residues added at 6 and 12 h to get a final substrate concentration of 110 g/l. After 72 h of hydrolysis, the reducing sugars concentration reached 89.5 g/l with a hydrolysis yield of 83.3%. The hydrolysate from fed-batch hydrolysis contained 56.7 g/l glucose, 23.6 g/l xylose, and 5.7 g/l arabinose, which is suitable for subsequent fermentation process.

Keywords: Maize straw; Polysaccharides; Enzymatic hydrolysis; Cellulase; Cellobiase

1. Introduction

Lignocellulosic biomass, which includes agricultural residues, paper wastes, wood chips, etc., is an ideal inexpensive, renewable, abundantly available resource (Ho, Chen, & Brainard, 1998; Sun & Cheng, 2002). 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 production of ethanol, xylitol, organic acid, and other chemicals (Xia & Sheng, 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).

The high cost of cellulase enzymes often restricts the large-scale application of these enzymes in the bioconversion of lignocellulosic biomass. In previous publication, we have reported low-cost cellulase production by *Trichoderma reesei* ZU-02 using solid-state fermentation (Xia & Cen, 1999). However, the most widely used cellulase from *T. reesei* is poor in cellobiase, and thus restricts the conversion of cellobiose to glucose (Shen & Xia, 2004). The accumulation of cellobiose will cause severe feedback inhibition to the cellulase reaction. Therefore, improving the activity of cellobiase in the cellulase system is crucial to raise the enzymatic hydrolysis yield.

Maize straw is one of the most abundant agricultural wastes, and it is estimated that about 250 million tons are produced annually in China. Currently, this straw is predominantly disposed of by direct burning in open field due to lack of effective utilization, which also causes serious environmental pollution. It is an important issue to deal with the agricultural waste both for the comprehensive

* Corresponding author. Tel.: +86 571 8795 1840; fax: +86 571 8795 1358.
E-mail address: xialm@zju.edu.cn (L. Xia).

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utilization of lignocellulosic resources and for the prevention of environmental pollution. In this work, enzymatic hydrolysis of maize straw polysaccharides with cellulase from *T. reesei* ZU-02 and cellobiase from *Aspergillus niger* ZU-07 was studied for the production of reducing sugars, which can then be further converted into value-added products.

2. Materials and methods

2.1. Lignocellulosic material and characterization

Maize straw was kindly provided by Tianguan Group Co. Ltd., China. The sraw was milled to pass through 20-mesh screen. The milled straw was washed thoroughly with tap water to remove some sticky clay, then filtered and air-dried. The sample was stored in a plastic bag and kept at 4 °C for use in all tests. The main composition of the raw material was as follows (dry weight basis): cellulose 38.7%, hemicellulose 21.7%, lignin 19.3%, and others 20.3%.

2.2. Pretreatment of maize straw

Before enzymatic hydrolysis, maize straw was delignified by alkali pretreatment to increase the exposure of polysaccharides to hydrolytic enzymes. Straw sample was treated with 2% NaOH at 80 °C for 1 h. The solid cellulosic residues were collected and washed thoroughly with tap water to neutral pH, then dried at 50 °C. The main composition of the delignified maize straw was as follows (dry weight basis): cellulose 64.1%, hemicellulose 24.6%, lignin 8.6%, and others 2.7%.

2.3. Microorganism

The strain *T. reesei* ZU-02 (a mutant strain originally from ATCC 56764) was used for cellulase production. *A. niger* ZU-07 (obtained from the Laboratory of Renewable Resource Engineering, Purdue University) was used for cellobiase production.

2.4. Enzyme production

Cellulase and cellobiase used for enzymatic hydrolysis were produced according to the methods of Xia (Xia & Cen, 1999) and Shen (Shen & Xia, 2004), respectively. Each gram of dry cellulase koji produced by *T. reesei* ZU-02 contained 146 filter paper activity units (FPU), 12 cellobiase units (CBU), and 1458 U of xylanase activity. Each gram of dry cellobiase koji produced by *A. niger* ZU-07 contained 376 CBU and no detectable filter paper activity.

2.5. Enzymatic hydrolysis

2.5.1. Batch enzymatic hydrolysis

Batch enzymatic hydrolysis was performed in 250 ml Erlenmeyer flasks containing a 100 ml mixture of 0.05 M citrate buffer solution (pH 4.8) and solid substrate. Cellulase from *T. reesei* ZU-02 and cellobiase from *A. niger* ZU-07 were used for enzymatic hydrolysis. After the enzymes were added, flasks were incubated at 50 °C in a rotary shaker at 150 rpm. Samples were taken from the reaction mixture periodically for sugar analysis.

2.5.2. Fed-batch enzymatic hydrolysis

Fed-batch enzymatic hydrolysis was carried out at pH 4.8 and 50 °C in a 2 l reactor. Experiments were started with initial 80 g/l substrate and enzyme loadings of 20 FPU/g substrate and 10 CBU/g substrate. Delignified straw residues were then added twice at 6 and 12 h to get a final substrate concentration of 110 g/l, simultaneously adding certain amount of cellulase (10 FPU/g fed substrate). The total hydrolysis time was set 72 h.

2.6. Analysis methods

Filter paper activity and cellobiase activity were determined according to standard IUPAC (International Union of Pure and Applied Chemistry) procedures (Ghose, 1987). One FPU is defined as the amount of enzyme that releases 1 μmol of glucose equivalents from Whatman No. 1 filter paper per min. One CBU is the amount of enzyme that converts 1 μmol of cellobiose to 2 μmol of glucose per min. Xylanase activity was assayed according to the method of Bailey (Bailey, Biely, & Pountanen, 1992).

The cellulose was determined by HNO_3–ethanol method, lignin by 72% H_2SO_4 method, and hemicellulose by two-brominating method (Liu, 2004). The reducing sugar was determined using the 3,5-dinitrosalicylic acid (DNS) method (Ghose, 1987). Glucose, xylose, cellobiose, and arabinose were determined using a HPLC system (Model 500, Syltech, USA) equipped with an organic acid column (IC Sep ICE-Coregel 87H3, Transgenomic, USA). Deionized water was used as the mobile phase at a flow rate of 0.5 ml/min. The column temperature was fixed at 60 °C. The eluate was detected by a refractive index detector (Model 6040 XR, Spectra-Physics, USA).

The yield of enzymatic hydrolysis was calculated as follows:

Hydrolysis yield (%) = reducing sugar (g) × 0.9 × 100/polysaccharides in substrate (g).

At least three parallel samples were used in all analytical determinations, and data are presented as the mean of three replicates.

3. Results and discussion

3.1. Enzymatic hydrolysis by cellulase from T. reesei ZU-02

Pretreated maize straw sample (80 g/l) was hydrolyzed by cellulase from *T. reesei* (20 FPU/g substrate) at pH 4.8, 50 °C for 60 h. Glucose, xylose, arabinose, and cellobiose produced during hydrolysis were determined by HPLC (Fig. 1). The reducing sugar concentration reached 52.0 g/l

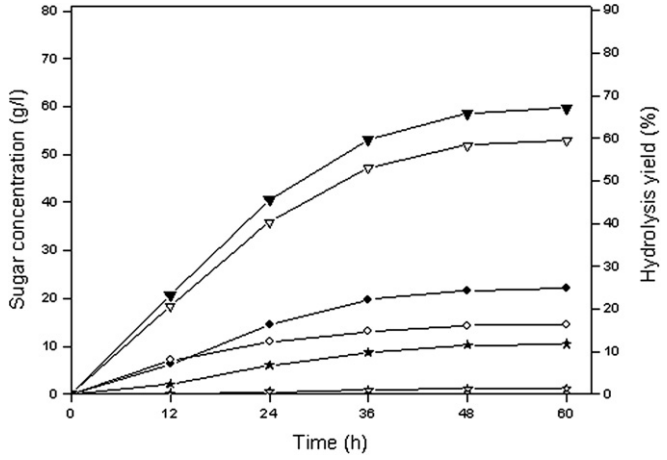

Fig. 1. Time course of enzymatic hydrolysis by *T. reesei* cellulase (20 FPU/g substrate; 1.64 CBU/g substrate). (●) Glucose; (○) xylose; (★) cellobiose; (☆) arabinose; (▽) reducing sugar; (▼) hydrolysis yield.

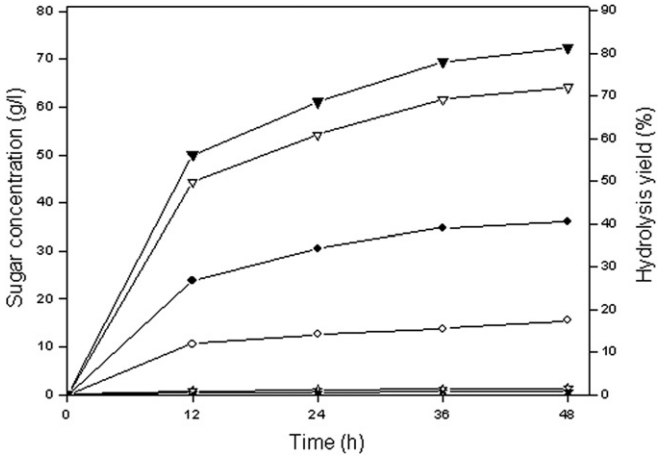

Fig. 2. Time course of synergistic hydrolysis by *T. reesei* cellulase and *A. niger* cellobiase (20 FPU/g substrate; 10 CBU/g substrate). (●) Glucose; (○) xylose; (★) cellobiose; (☆) arabinose; (▽) reducing sugar; (▼) hydrolysis yield.

and the hydrolysis yield was 65.9% after 48 h of hydrolysis, and prolonged hydrolysis time beyond 48 h helped little in increasing the hydrolysis yield. Xylose and arabinose were detected out in the hydrolysate, showing the presence of xylanase in *T. reesei* cellulase, so there is no need to add xylanase to the hydrolysis system.

It was found that a high amount of cellobiose existed in the cellulosic hydrolysate, indicating relatively low cellobiase activity in *T. reesei* cellulase. At the *T. reesei* cellulase dosage of 20 FPU/g substrate, only 1.64 CBU/g substrate of cellobiase was present in the hydrolysis system. The accumulation of cellobiose caused severe feedback inhibition to the cellulase reaction, as the enzyme is more susceptible to end-product-inhibition caused by cellobiose than glucose (Duff & Murray, 1996; Wen, Liao, & Chen, 2004).

3.2. Effects of cellobiase activity on enzymatic hydrolysis

To weaken the feedback inhibition caused by cellobiose accumulation, cellobiase produced by *A. niger* ZU-07 was supplemented to the hydrolysis system to enhance the total activity of cellobiase. For the given cellulase dosage of 20 FPU/g substrate, both reducing sugar concentration and hydrolysis yield increased with increasing the cellobiase activity till 10 CBU/g substrate. Further addition of cellobiase did not improve the hydrolysis (data not shown). Time course of synergistic hydrolysis by *T. reesei* cellulase and *A. niger* cellobiase (20 FPU/g substrate; 10 CBU/g substrate) is shown in Fig. 2. Due to the improvement of cellobiase activity in the hydrolysis system, the cellobiose concentration staged at a very low level during the whole hydrolysis process. Thereby the feedback inhibition caused by the cellobiose accumulation was greatly reduced, which resulted in a higher reducing sugar concentration and hydrolysis yield. At 48 h, the hydrolysis yield was increased to 81.2% and the reducing sugar concentration reached 64.1 g/l. In the following hydrolysis experiments, *T. reesei* cellulase was supplemented with cellobiase from *A. niger* (2 FPU:1 CBU) to avoid product inhibition caused by cellobiose accumulation.

3.3. Effects of enzyme dosage on enzymatic hydrolysis

Enzymatic hydrolysis of pretreated maize straw sample at 80 g/l substrate concentration, using different enzyme dosages (presented as FPU/g substrate), is shown in Fig. 3. In experiments it is found that liquefaction of the substrate usually took 3–4 h, but it took about 12 h to liquefy the substrate at an enzyme dosage of 7 FPU/g substrate, indicating obviously insufficient enzyme activities at this level. For each enzyme dosage, hydrolysis yield increased sharply for the first 12 h, and then more slowly from 12 to 48 h. The optimal enzyme dosage was identified as 20 FPU/g substrate, i.e. an enzyme complex including

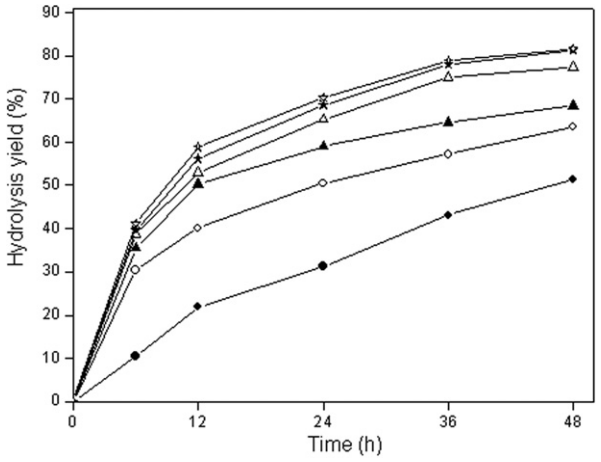

Fig. 3. Effects of enzyme dosage (presented as filter paper activity per gram of substrate, FPU/g substrate) on the enzymatic hydrolysis. (●) 7 FPU/g substrate; (○) 10 FPU/g substrate; (▲) 13 FPU/g substrate; (△)17 FPU/g substrate; (★) 20 FPU/g substrate; (☆) 23 FPU/g substrate.

cellulase of 20 FPU/g substrate and cellobiase of 10 FPU/g substrate, and further increase in enzyme dosage did not produce a corresponding increase in the hydrolysis yield.

3.4. Effects of substrate concentration on enzymatic hydrolysis

The effects of substrate concentration on enzymatic hydrolysis were investigated at fixed enzyme dosage of 20 FPU/g substrate (Fig. 4). Little difference in hydrolysis yield was observed within the substrate concentration ranging from 30 to 80 g/l. For example, the hydrolysis yield at 30 g/l substrate concentration was 84.3% at 48 h, just a little higher than that at 80 g/l substrate concentration at 48 h. Previous investigations have shown that high substrate concentration usually resulted in lower hydrolysis yield due to product inhibition (Ramos, Breuil, & Saddler, 1993), enzyme inactivation (Reese, 1980) and reactivity decrease of the substrate (Sinitsyn, Gusakov, & Valasenko, 1991). In this case, sufficient enzyme dosage and low substrate concentration are possible explanations for why little difference in hydrolysis yield was observed at different substrate concentration. However, it was difficult to get higher substrate concentration than 80 g/l in batch hydrolysis process because of the mixing and heat transfer problems.

3.5. Effects of surfactant on enzymatic hydrolysis

Non-ionic surfactant Tween-80 was evaluated for its ability to improve the enzymatic hydrolysis of pretreated straw samples. Hydrolysis was performed at 80 g/l substrate concentration using enzyme dosage of 20 FPU/g substrate for 48 h. As results shown in Fig. 5, the addition of surfactant Tween-80 improved the enzymatic hydrolysis effectively. Adding 5 g/l Tween-80 increased the hydrolysis yield from 81.2% to 87.3%, i.e. an increase of 7.5%. Eriksson, Borjesson, and Tjerneld (2002) have reported that the improved conversion of lignocellulose with the addition of non-ionic surfactant can be explained by the reduction of the unproductive enzyme adsorption to the lignin part of the substrate. The possible mechanism is that the hydrophobic interaction of surfactant with lignin occurs on the lignocellulose surface, which releases the non-specifically bound enzyme (Eriksson et al., 2002).

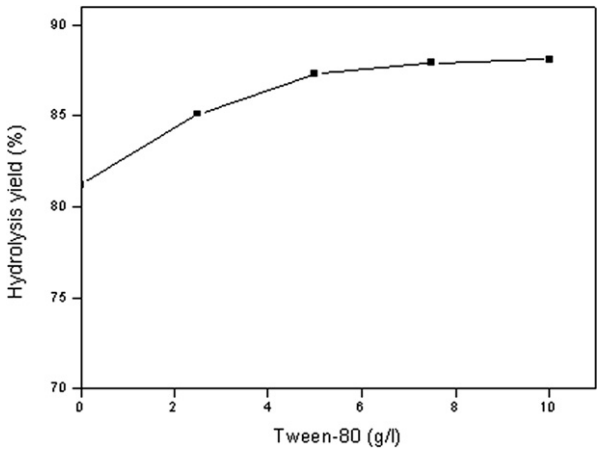

Fig. 5. Effects of surfactant Tween-80 addition on the enzymatic hydrolysis.

3.6. Fed-batch enzymatic hydrolysis

In ethanol production from lignocellulosic materials, the efficient recovery of ethanol seems to require ethanol concentration higher than 40 g/l (Phillips & Humphrey, 1983), which in turn require starting concentrations of fermentable sugars at least higher than 80 g/l. Raising the substrate concentration in batch hydrolysis helps to obtain higher reducing sugar concentration. However, too high a substrate concentration would cause mixing and heat transfer problems due to the rheological properties of a very dense fibrous suspension (Rudolf, Alkasrawi, Zacchi,

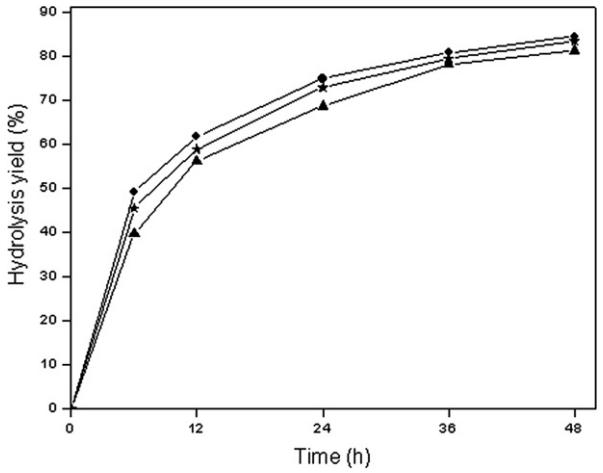

Fig. 4. Effects of substrate concentration on the enzymatic hydrolysis at fixed enzyme dosage (20 FPU/g substrate; 10 CBU/g substrate). (●) 30 g/l substrate concentration; (★) 55 g/l substrate concentration; (▲) 80 g/l substrate concentration.

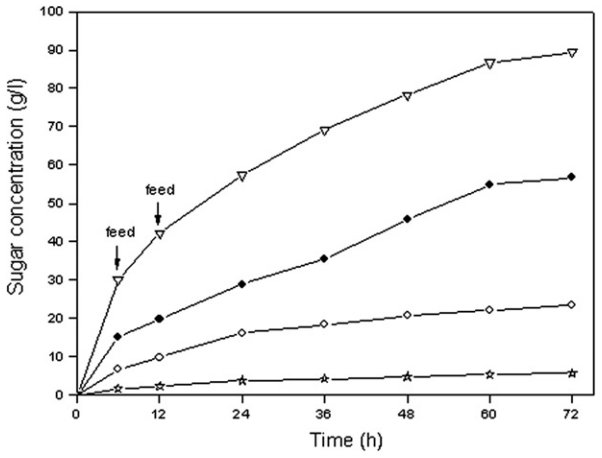

Fig. 6. Time course of fed-batch synergistic hydrolysis by *T. reesei* cellulase and *A. niger* cellobiase. (●) Glucose; (○) xylose; (☆) arabinose; (▽) reducing sugar.

& Liden, 2005). Applying fed-batch enzymatic hydrolysis will be a good solution to these problems.

Fed-batch hydrolysis process was started with a batch hydrolysis containing 80 g/l of substrate concentration, with cellulosic residues added at 6 and 12 h to get a final substrate concentration of 110 g/l. Since added substrate was gradually degraded, the viscosity of reaction mixture was kept at low level. After 72 h of hydrolysis, the reducing sugar concentration reached 89.5 g/l with a hydrolysis yield of 83.3% (Fig. 6). The enzymatic hydrolysate contained 56.7 g/l glucose, 23.6 g/l xylose, and 5.7 g/l arabinose, suitable for subsequent fermentation process for ethanol production.

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