# ENHANCED ENZYMATIC CONVERSION AND GLUCOSE PRODUCTION VIA TWO-STEP ENZYMATIC HYDROLYSIS OF CORNCOB RESIDUE FROM XYLO-OLIGOSACCHARIDES PRODUCER'S WASTE

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A study was conducted to investigate the hydrolysis of cellulose-enriched corncob residue, a cellulosic waste from the xylo-oligosaccharides industry, by two processes. The corncob residue was hydrolyzed by cellulases via direct hydrolysis and two-step hydrolysis. Cellulases were produced by Trichoderma reesei RutC-30 with kraft pulp as the substrate. When the cellulase dosage was above 8 FPU•g-1 of corncob residue and the corncob residue concentration was 3%, over 90% hydrolysis yield and 49.99% glucose yield were obtained at 48 h. To enhance the hydrolysis yield of corncob residue, a new process coupling enzymatic hydrolysis, separation, and acid treatment was investigated. The corncob residue was first hydrolyzed using cellulase for 24 h. Then the remaining solids of corncob residue was separated from the liquid containing soluble oligosaccharides, and allowed to subsequently hydrolyze, using the adsorbed enzyme for 24 h. Using this method, the total hydrolysis yield was up to 97.60%, which represents an increase by 7.5% in comparison to the direct 48 h enzymatic hydrolysis. When the hydrolysates of the two-step enzymatic process were subjected to the concentrated acid hydrolysis at 110 °C for 2 h, the glucose yield could be increased from 43% to 90%.

Keywords: Cellulase; Enzymatic hydrolysis; Corncob residue; Trichoderma reesei

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#### INTRODUCTION

Agricultural residues such as corncob represent large renewable resources of lignocellulosic biomass (Kumar et al. 2008). In the main agricultural countries such as USA, Canada, and China, corncob is an abundant lignocellulosic by-product from farming land. The typical composition of corncob (Demirbas 2005; Mosier et al. 2005) by dry base is cellulose (30–40%, w/w), hemicellulose (35–40%, w/w), and lignin (10–20%, w/w). The unusually high content of hemicellulose in corncob justifies it as an attractive feedstock for pentose-based biotransformation aimed at production of bulk chemicals (Hermann et al. 2007) and bioethanol (Wyman 2007; Chandrakant and Bisaria 1998).

Corncob is treated by diluted alkali in a xylo-oligosaccharides plant. After separation of the alkali extractives containing xylose and xylo-oligosaccharides, the waste residue rich in cellulose is discarded or furnace-burned in many cases. However, this valuable cellulosic residue should be utilized in an economical and environmentally friendly way (Mosier et al. 2005). In the process of xylo-oligosaccharides production, lignin is swelled by alkali (Carrillo et al. 2005), the remaining cellulose is changed in morphology and crystallinity, and thus it is more appropriate for further enzymatic hydrolysis and bioethanol production (Ai-Zuhair 2008).

Ordinary cellulose resists hydrolytic cleavage due to its crystalline structure that retards cellulose biodegradation in the solid residues by cellulytic enzymes. In fact, the hydrolysis of cellulose requires synergistic actions of several cellulase components in a heterogeneous reaction system (Gan et al. 2003), which includes endocellulase, exoglucanase, and  $\beta$ -glucosidase. Among the most prolific producers of cellulose degrading enzymes is the filamentous fungus *Trichoderma reesei*, whose hypercellulolytic mutant strains secrete large amounts of cellulases, up to 40 g·L<sup>-1</sup>. Unfortunately, the activity of the  $\beta$ -glucosidase from this culture system is quite low (Wen et al. 2004, 2005a, 2005b), although this fungus produces a complete set of cellulases. So far the yields have been low by direct employment of this cellulase system. Supplying additional  $\beta$ -glucosidase for the process (Yun et al. 2001) could increase the yields, but the cost of enzymes would increase considerably.

Additionally, during the biomass degradation, the physicochemical heterogeneity of the substrate and enzyme interactions, especially in the case of high substrate coverage, is important. Most fungal cellulases exhibit bifunctional organization of the catalytic domain and cellulose-binding domain (Rabinovich et al. 2002a). The twodomain structure of the cellulases may give rise to non-uniform interaction with the substrate and is strongly influenced by adsorption (Gilkes et al. 1992). As for *Trichoderma reesei*, at least four types of enzymes (Rabinovich et al. 2002b) (CBHI, CBHII, EGI, and EGII) have been confirmed to bind insoluble cellulose tightly and play a central role in the decomposition of crystalline cellulose. Hence, the adsorption capability of the cellulases to cellulose substrate might be an important factor affecting the rate and degree of enzymatic hydrolysis of the biomass.

In order to tap into the potential of the corncob residue cellulose from xylooligosaccharides plant waste for chemicals and energy production, further studies are needed to investigate the efficiency of the cellulose hydrolysis process for the corncob residue and to provide basic information on the commercial utilization of corncob residue. Therefore, the aim of our work was to increase understanding of corncob residue enzymatic hydrolysis and develop a method to improve the hydrolysis yield.

#### EXPERIMENTAL

#### Materials

Corncob residue, a xylo-oligosaccharides plant waste, was obtained from a local xylo-oligosaccharides manufacturer, Jiangsu Kangwei Biotechnology Co., Jiangsu Province, China. The residue was washed extensively with distilled water until neutral pH, filtered, dried in air at ambient temperature, and stored in a sealed plastic bag at 4 °C. The sample was used throughout the experiments and referred to as corncob residue.

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Corn cob residue was brown, and its microscopic view (Fig.1.) showed that it had a more porous, net-like structure in comparison to natural corncob. The water content of corncob residue was 48% (w/w), which was determined by the Chinese Standard of GB/T 2677.2-1993 (Zhao et al. 2008). The cellulose content was 85% (w/w, dry basis), which was determined by the nitric acid-ethanol method (Wang 1995).



Fig. 1. Comparison of microscopic views (300x) of (a) natural corncob and (b) alkali residue from corncob

## Methods

#### Cellulase production

Cellulase enzyme was produced with T. reesei RUT-C30 (ATCC 56765) (Tangnu et al. 1981). The basal medium was as follows: peptone (0.1%, w/v), Tween 80 (0.1%, w/v); and mineral salts were (in  $g \cdot L^{-1}$ ) as follows: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.4, KH<sub>2</sub>PO<sub>4</sub> 2.0, urea 0.3, CaCl<sub>2</sub>•2H<sub>2</sub>O 0.4, MgSO<sub>4</sub>•7H<sub>2</sub>O 0.3; and (in mg•L<sup>-1</sup>) FeSO<sub>4</sub>•7H<sub>2</sub>O 5.0, MnSO4•H<sub>2</sub>O 1.6, ZnSO<sub>4</sub>•7H<sub>2</sub>O 1.4, CoCl<sub>2</sub>•6H<sub>2</sub>O 3.7. The pH of the medium was adjusted to 4.8 using 0.05 M acetate buffer. The medium was autoclaved for 15 min. The strain was maintained on potato dextrose agar slants for 6 days at 28 °C before using it in enzyme production experiments. To prepare the inoculum, the formed spores were seeded into 250 mL Erlenmeyer flasks containing 50 mL of growth medium and incubated on a rotary shaker at 170 rpm and 30 °C. Glucose (1%, w/v) was added to the basal medium to grow cells. After 36 h growth, 5mL of exponential inocula were pipetted into 250 mL Erlenmeyer flasks containing 45 mL of inducing medium. Kraft pulp (1%, w/v) was added to the basal medium to induce cellulase production. The culture was incubated in an orbital shaker at 170 rpm, 29 °C for 4 to 6 days. After incubation, the culture broth was centrifuged at 10,000 g for 30 min. The supernatant was maintained as cellulase and stored at -20 °C. All the experiments were performed in triplicates and the average values were represented.

#### Enzymatic hydrolysis

The hydrolysis of corncob residue was carried out in 50 mL Erlenmeyer flasks. The corncob residue was weighed and transferred to a 50 mL Erlenmeyer flask. Acetate

buffer (0.05 M) was added to keep the pH at 4.8. The flask was put in a shaking water bath set at 50 °C, 100 rpm and allowed to equilibrate for several hours. The appropriate amounts of cellulase from *T. reesei* were added, and the hydrolysis was carried out in 20 mL medium (pH 4.8) at 50 °C and 100 rpm for 48 h. At specific time intervals, the aliquots were withdrawn, placed in boiling water for 15 min to deactivate the enzymes, and then centrifuged for 15 min at 3000 g. The supernatants were stored at -20°C for subsequent sugar analysis. The hydrolysis yield and glucose yield were calculated according to the following equations (Chen et al. 2007):

Hydrolysis yield (%) = Reducing sugar  $\times 0.9 \times 100$  /cellulose content in the substrate

Glucose yield (%) = Glucose  $\times 0.9 \times 100$  / cellulose content in the substrate

#### Two-step enzymatic hydrolysis process

Two-step enzymatic hydrolysis was carried out at pH 4.8 and 50 °C and 100 rpm, as mentioned above. Experiments were started with 3% substrate and enzyme loadings of 8 FPU•g<sup>-1</sup> substrate. At the designated time, the sample was vacuum filtered to separate the liquid and solid residues. The liquid was analyzed regarding its sugar content. The solid fraction was then re-impregnated with acetate buffer (0.05 M, 12 mL) and hydrolyzed again at 100 rpm and 50 °C (second hydrolysis step). After subsequent 24 h auto-hydrolysis, the liquid obtained was analyzed regarding its sugar content. The hydrolysates from the two steps were combined together, and the overall reducing sugar and glucose content were determined to calculate the overall hydrolysis and glucose yields.

Furthermore, a process incorporating the two-step hydrolysis and a subsequent acid treatment is proposed. After two-step enzymatic hydrolysis (24h and 24 h subsequent hydrolysis), 2% H<sub>2</sub>SO<sub>4</sub> was added to the combined hydrolysates for further hydrolysis at 110 °C for 2 h.

#### Analytical methods

Filter paper activity (FPA) was determined according to standard International Union of Pure and Applied Chemistry (IUPAC) procedures (Ghose 1987). One unit of filter paper activity (FPU) is defined as the amount of enzyme that forms 1mmol glucose (reducing sugar as glucose) per minute. Endoglucanase activity (CMCase) was determined using carboxymethyl cellulose sodium as a substrate (Mandels et al. 1976). One unit of endoglucanase activity is the amount of enzyme that forms 1mmol glucose per minute.  $\beta$ -glucosidase activity was assayed by monitoring the release of p-nitrophenol from p-Nitrophenyl- $\beta$ -D-Galactopyranoside (Kaya et al. 2008).

Total reducing sugars were determined by the DNS method, using glucose as the standard (Ghose 1987). Glucose was determined by HPLC (Angilent 1100, Germany) with an Aminex HPX-87H Column (Bio-Rad, USA) (Sluiter et al. 2006). The column temperature was fixed at 50°C. 5 mM  $H_2SO_4$  was used as the mobile phase at a flow rate of 0.6 mL min<sup>-1</sup>. The eluate solution was detected by a refractive index detector (G1362A XR RI). All the samples were diluted and passed through a 0.2µm filter before HPLC analysis.

## **RESULTS AND DISCUSSION**

#### **Cellulases Production**

At an initial pH of 4.8, the cultivation with *T. reesei* grown on 1% kraft pulp was carried out for 140 h. During the cultivation, samples were withdrawn at every 24 h and the enzyme activity was analyzed. The time course of cellulases production by *T. reesei* is shown in Fig. 2.



Fig. 2. The time course of cellulases production by Trichoderma reesei

The  $\beta$ -glucosidase(BG) activity increased throughout the cultivation and reached its maximum value of 0.41 U•mL<sup>-1</sup> at the end. Endoglucanase (CMCase) activity reached 1.01 U•mL<sup>-1</sup> at 96 h, and then decreased sharply. A similar trend was observed for cellulase (refer to cellulolytic complex, FPA); it reached peak levels of 1.00 FPIU•mL<sup>-1</sup> at 96 h. A similar maximum cellulase production was reported for 1% microcrystalline cellulose induction using *T.reesei* QM9414 (Hari Krishna et al. 2000). The pH remained relatively constant during the first 80 h, and after that time it increased in an accelerated manner. Furthermore, after the pH exceeded 5.6 at 96 h, endoglucanase and cellulase activities decreased simultaneously. As a result, we concluded that cellulase activity had reached its peak, and the cellulase was harvested on the 4th day and at a pH of 5.6. When the pH exceeded 5.6, the CMCase and FPA would decrease quickly.

In the cellulase system, the ratio of FPA, CMCase, and  $\beta$ -glucosidase activity was 1:1:0.2. The low level of BG activity suggested that the used cellulase from *T. reesei* is poor in BG, and thus might restrict the conversion of glucose (Xia and Shen 2004).

# **One-Step Enzymatic Hydrolysis**

To characterize the hydrolysis properties of cellulase produced in fungal systems and to investigate its hydrolysis performance of the corncob residue, the enzyme produced by *T. reesei* was used directly to hydrolyze the corncob residue. The enzyme dosage was standard for FPA. Hydrolysis experiments were performed with 3% corncob residue for 48 h, and the enzyme dosages were adjusted from 2 to 10 FPIU•g<sup>-1</sup> substrate, respectively. The influence of the enzyme dosage on the enzymatic hydrolysis of corncob residue is shown in Fig. 3.

When the enzyme dosage was changed from 2 to 5 FPIU•g<sup>-1</sup> substrate, the hydrolysis yield increased sharply from 54.5 to 86.9%. Further increasing enzyme dosage did not produce a corresponding increase in the yield. Based on these results, when the cellulase dosage was above 8 FPIU•g<sup>-1</sup> of substrate and the reaction time was 48 h, the hydrolysis yield reached over 90%. As the cost of cellulase contributes significantly to the total cost of the hydrolysis process, the enzyme dosage should be minimized as much as possible (Chen. 2007). The enzyme dosage ought to be not more than 5-8 FPIU•g<sup>-1</sup> substrate.



Fig. 3. Effect of the enzyme dosage on the enzymatic hydrolysis of corncob residue

For further study, the production of reducing sugars and glucose is shown in Figs. 4 and 5, respectively. The substrate concentration was 3%, and the enzyme dosage was in the range from 2 to 8 FPIU $\cdot$ g<sup>-1</sup> substrate.

When the enzyme dosage increased from 2 to 8 FPIU•g<sup>-1</sup> of substrate, the reducing sugar and glucose increased from 8.12 to 13.30 g•L<sup>-1</sup>, and from 3.08 to 7.38 g•L<sup>-1</sup>, respectively. The obvious difference between the reducing sugar and glucose concentrations showed that the hydrolysate contained a large amount of oligosaccharides. The differences of the reducing sugar concentration at 48 h were very little between two enzyme dosages (5 and 8 FPIU•g<sup>-1</sup> substrate, shown in Fig. 4.). But the level of glucose released at the 8 FPIU•g<sup>-1</sup> substrate dosage was much higher than at the 5 FPIU•g<sup>-1</sup> substrate level (Fig. 5.). In addition, when the enzyme dosage was 8 FPIU•g<sup>-1</sup> of substrate, reducing sugar increased quickly to 8.75 g•L<sup>-1</sup> in the first 4 h, and thereafter the rate of increase slowed down. At the 48 h point, it appeared to reach a saturated status (13.30 g•L<sup>-1</sup>). In contrast, the concentration of glucose increased steadily from 12 h to 48h and showed no trend of decrease at the end time. This result may be explained based

on a supposition that the cellulases from *T. reesei* (Nidetzky et al. 1994) contained two types of enzymes; one was enzyme to dissolve cellulose molecules from supermolecular aggregates (Zhang and Lynd. 2004) in the corncob residue directly, and it could be reflected by FPA and CMCase. The other was  $\beta$ -glucosidase, which was only responsible for degrading the soluble oligosaccharides. In previous studies, the ratio of FPA and BG was generally about 2:1 (Bak et al. 2009; Yang and Wyman 2006), which is regarded as an appropriate enzyme system. In our cellulase system,  $\beta$ -glucosidase was lower in activity compared to FPA (5:1, shown in Fig. 2). The lack of  $\beta$ -glucosidase obviously resulted in the accumulation of soluble oligosaccharides and inhibited the hydrolysis process, and the inhibition effect of glucose was not serious with respect to  $\beta$ -glucosidase. Hence, when the enzyme dosage increased from 5 to 8 FPIU•g<sup>-1</sup> substrate, the yield of reducing sugar did not increase proportionally to that of the glucose. Considering the glucose yield, when using our cellulase system, 8 FPIU•g<sup>-1</sup> substrate was the more appropriate enzyme dosage than 5 FPIU•g<sup>-1</sup> substrate in the hydrolysis system.



Fig. 4. Reducing sugar production during the hydrolysis of corncob residue at different enzyme dosage

The effect of substrate concentrations on enzymatic hydrolysis of corncob residue is shown in Fig. 6. The cellulase dosage was 8 FPIU•g<sup>-1</sup> of substrate. The increase of substrate concentration from 3% to 10% resulted in a decrease in the degree of saccharification, probably due to difficulties in diffusion at higher concentration and endproduct inhibition (Wen et al. 2004). The best yield of hydrolysis was found with 3% (w/v) substrate load, resulting in more than 90% of hydrolysis yield and 49.99% glucose yield.



Fig. 5. Glucose production during the hydrolysis of corncob alkali residue at different enzyme dosage



Fig. 6. Effect of substrate concentration on the enzymatic hydrolysis of corncob alkali residue

#### A Two-step Enzymatic Hydrolysis Process for Corncob Residue

As mentioned in the previous discussions, the hydrolysis of cellulose is a complex enzymatic process composed of saccharification of cellulose and subsequent hydrolysis of the soluble oligosaccharides by two types of enzymes. The heterogeneous hydrolysis process first needs cellulase adsorbed onto the corncob residue. In order to investigate the cellulase adsorption on corncob residue, the cellulases adsorbed on corncob residue were separated, and their effects of the subsequent hydrolysis are shown in Fig. 7. In this experiment, 3% corncob residue and 8 FPU•g<sup>-1</sup> substrate cellulase loadings were mixed and allowed to interact at pH 4.8, 50 °C, and 100 rpm. At a designated time, the sample

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was vacuum-filtered to separate the liquid and solid residues. The solid residues contained remaining corncob residue and the adsorbed cellulase on the residue. Adding 12 ml of 0.05 M acetate buffer in a 50 mL flask, the solid residues began to undergo the subsequent hydrolysis at 50 °C and 100 rpm for 24 h. No additional enzyme was added to the hydrolysis system.



Fig. 7. Effect of the subsequent hydrolysis by the adsorbed cellulases

As shown in Fig. 7, the effect of the subsequent hydrolysis of the corncob residue by the adsorbed cellulases was significant. When the enzymes were adsorbing on the corn cob residue in the first step, the amount of the corn cob residue was decreasing, because of the hydrolysis process. Thus, the adsorption equilibrium on FPA and the amount of the solid were responsible for the fractional recovery of the enzymes in the second step enzymatic hydrolysis. In addition, both the change of amount in adsorbed enzymes and the decrease of the substrate lead to the change in FPIU/g substrate. It also influenced the hydrolysis and glucose yield in the subsequent hydrolysis. When the adsorbed time exceeded 24 h, the hydrolysis and glucose yields did not increase obviously and seemed to reach a plateau at 24 h. It is suggested that the enzyme remaining on the target substrate at 24 h was the most active and could achieve a maximum hydrolysis in this subsequent hydrolysis process. On the other hand, glucose formation was still found (Fig. 7). It was shown that these adsorbed enzymes mainly produced oligosaccharides and also had the ability to convert oligosaccharides to glucose (Karlsson et al. 2001). Furthermore, as observed in Figs. 4 and 5, after direct 24 h enzymatic hydrolysis, the ratio of the release reduce sugar with glucose was 2.33. This was much lower than after the 24 h subsequent enzymatic hydrolysis (3.44) when the adsorbed time was 1 h. This result suggested that  $\beta$ -glucosidase had weaker affinity to the corncob residue and stayed mainly in the liquid.

To avoid the feedback inhibition of oligosaccharides accumulation in the one-step hydrolysis process, a new two-step hydrolysis process coupling enzymatic hydrolysis and separation was proposed. Firstly, the corncob residue was hydrolyzed using enzymes, then the remaining solid of corncob residue was separated from the liquid containing soluble oligosaccharide, and buffer was added to the solid residue and allowed to subsequently hydrolyze the substrate, using the adsorbed enzyme (Garrote et al. 2002) for 24 h. The difference of the one-step process and the two-step hydrolysis process in hydrolysis and glucose yield is shown in Fig. 8.



Fig. 8. Changes in hydrolysis and glucose yield vs. first hydrolysis time using two methods

Compared to the one-step process, the two-step hydrolysis process improved the hydrolysis yield significantly. When the direct hydrolysis time was 48 h, the hydrolysis and glucose yields of the one-step hydrolysis were 90.11% and 49.99%. But the two-step hydrolysis (the 48 h first hydrolysis plus 24h subsequent-hydrolyze) could make the hydrolysis and glucose yields increase to 98.66% and 54.38%, respectively. Additionally, using the two-step hydrolysis process, only 36 h hydrolysis process (12h and 24 h subsequent hydrolysis) was needed to achieve 91.84% hydrolysis yield, which required 48h in one-step enzymatic hydrolysis. The two-step hydrolysis process cut down the reaction time greatly. On the other hand, when taking the same time, the two-step hydrolysis could get the higher hydrolysis yield than the one-step hydrolysis. Using the two-step hydrolysis process, 48h hydrolysis process (24h and 24 h subsequent hydrolysis) gained an overall 97.60% hydrolysis yield, which was increased by 7.5% in comparison with the direct 48 h enzymatic hydrolysis. The two-step process decreased the glucose yield from 49.99% to 42.99%, compared with the one-step hydrolysis. It was confirmed that the separation process removed  $\beta$ -glucosidase with the liquid. The hydrolysis solution of the two-step process resulted more the oligosaccharides than the direct enzymatic method. Supplementing  $\beta$ -glucosidase in this hydrolysis system could take care of the accumulation of the oligosaccharides and increase the glucose yield (Chen et al. 2007). However, adding  $\beta$ -glucosidase will increase the cost of hydrolysis obviously. An alternative measure was to hydrolyze the oligosaccharides in the two-step enzymatic hydrolysate by H<sub>2</sub>SO<sub>4</sub>. 1%-5% concentration H<sub>2</sub>SO<sub>4</sub> was used to hydrolyze cellobiose at 110 °C for 2 h. The result suggested that when the concentration of H<sub>2</sub>SO<sub>4</sub> reached to 2%, at least 90% cellobiose could invert to glucose. In order to avoid degradation of glucose (Taherzadeh and Karimi 2007; Hendriks and Zeeman 2009), a blank experiment with glucose was carried out under the same conditions (2% H<sub>2</sub>SO<sub>4</sub>, 110 °C for 2 h). This result showed that the loss of glucose was within the range of error and can be considered negligible. Therefore, after a 24h-24h two-step hydrolysis, the hydrolysates were combined, and 2% concentration H<sub>2</sub>SO<sub>4</sub> was added to further hydrolysis at 110 °C for 2 h. The total glucose yield reached 90.11 %. This result suggested that acid treatment is an effective way to invert oligosaccharides to glucose. A new process incorporating the two-step hydrolysis and a subsequent acid treatment is schematically illustrated in Fig. 9.



Fig. 9. A new process incorporating the two-step hydrolysis and a subsequent acid hydrolysis

#### CONCLUSIONS

Waste corncob residue from the xylo-oligosaccharidess industry was used as biomass raw material for its potential in saccharification and glucose production, with the aim of manufacturing chemicals and energy. The cellulase was produced with *T. reesei* RUT-C30 (ATCC 56765) and employed in the two enzymatic hydrolysis processes.

When the cellulase dosage was above 8 FPU•g<sup>-1</sup> of corncob residue and the corncob residue concentration was 3%, the direct one-step enzymatic hydrolysis achieved over 90% hydrolysis yield and 49.99% glucose yield at 48 h. In the used cellulase system, the lack of  $\beta$ -glucosidase obviously resulted in the accumulation of soluble oligosaccharides and inhibited the hydrolysis process. To eliminate the inhibition of the oligosaccharides, a two-step hydrolysis was proposed. By separating the liquid and solid residues, the soluble oligosaccharides were removed from the solid residues. Using the adsorbed cellulases on the solid residue, the remaining residue was further hydrolyzed for 24 h. This method not only shortened the hydrolysis time significantly, but also increased the hydrolysis yield. The yield of saccharification was improved by 7.5%, and an overall 97.60% yield of the total sugars was obtained by 48 h hydrolysis. Since the  $\beta$ -glucosidase showed low activity in this two-step process, a third process incorporating the two-step hydrolysis and a subsequent aqueous acid hydrolysis was employed. A remarkable total glucose yield of 90% was achieved.

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