Pretreatment of wheat straw leads to structural changes and improved enzymatic hydrolysis

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Wheat straw (WS) is a potential biomass for production of monomeric sugars. However, the enzymatic hydrolysis ratio of cellulose in WS is relatively low due to the presence of lignin and hemicellulose. To enhance the enzymatic conversion of WS, we tested the impact of three different pretreatments, e.g. sulfuric acid (H2SO4), sodium hydroxide (NaOH), and hot water pretreatments to the enzymatic digestions. Among the three pretreatments, the highest cellulose conversion rate was obtained with the 4% NaOH pretreatment at 121 °C (87.2%). In addition, NaOH pretreatment was mainly effective in removing lignin, whereas the H2SO4 pretreatment efficiently removed hemicellulose. To investigate results of pretreated process for enhancement of enzyme-hydolysis to the WS, we used scanning electron microscopy, X-ray diffraction, and Fourier transform infrared spectroscopy to analyze structural changes of raw and treated materials. The structural analysis indicated that after H2SO4 and NaOH pretreatments, most of the amorphous cellulose and partial crystalline cellulose were hydrolyzed during enzymatic hydrolysis. The findings of the present study indicate that WS could be ideal materials for production of monomeric sugars with proper pretreatments and effective enzymatic base hydrolysis.

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alkaline solutions efficiently remove lignin by breaking ester bonds, thereby increasing the porosity of biomass.

The contents of lignin after alkali pretreatment were particularly low (121 °C, 4% NaOH, 2.2%), indicating that the porosity of biomass was increased by pretreatment.

Different concentrations of H2SO4, NaOH, and hot water pretreatments to the structural changes of WS, followed by treat with enzymes to hydrolyze the cellulose component. Our results suggested that WS could be a potential raw material for producing monomeric sugars. However, it is less efficient in removing lignin. Previous studies have revealed that after hot water and acid pretreatment, lignin is deposited as droplets on the surface of solid residues, which inhibited the enzymatic hydrolysis of cellulose in materials25–29. Other researchers found that re-localization of lignin during pretreatment improved the accessibility of enzymes to cellulose microfibrils26–32.

Methods used for cellulose conversion from WS were investigated previously. For instance, Jaisamut et al. (2016) pretreated WS with the combination of 180 °C, 30 min, 1% H2SO4, and 2.4% Na2SO4, which resulted in 80% of the cellulose conversion into glucose2. Yin and Wang (2017) demonstrated that after irradiation at 100 kGy, 2% NaOH treatment of WS for 1 h, monomeric sugar yield could reach 72.67%33. These results indicated that different pretreatment methods could affect composition of WS and might significantly affect yield of sugar production. However, comparison of different concentrations of H2SO4, NaOH and hot water pretreatment on enzymatic hydrolysis of WS was not been well investigated yet. Furthermore, variations of compositions like cellulose, hemicellulose and lignin contents of WS from different locations were observed34–36. Hence, more work is needed to develop better pretreatment approaches for enzymatic hydrolysis of WS. Development of optimized means for conversion of WS in production of sugar may potentially have enormous economic benefits and environmental impact. The main objective of present study was to enhance the enzymatic hydrolysis of WS. We tested different concentrations of H2SO4, NaOH and hot water pretreatments to the structural changes of WS, followed by treat with enzymes to hydrolyze the cellulose component. Our results suggested that WS could be a potential raw material for producing monomeric sugars.

Results and Discussion

Compositions of WS. To investigate the potential resource in WS that could convert to monomeric sugars, the compositions of WS were measured. The composition of WS used in this study, as well as comparison to other WSs, is listed in Table 1. The three types of WSs displayed different compositions, and the contents of cellulose and hemicellulose in WS reported by Constant et al. (2016) and Merali (2016) were higher than those of WS in this study. The differences in WS composition may be attributable to variations in geographical location, local temperature, and heterogeneity of feedstock of the WS samples37. The cellulose content of raw WS was 33.7% in present study (Table 1), indicating that it is a significant source of carbohydrates that could be further converted to monomeric sugars. However, the high contents of hemicellulose and lignin in WS (19.1% and 19.8%, respectively), which were inhibit the conversion rate of cellulose, leading to the pretreatments of biomass inevitable.

Effect of pretreatments. In order to investigate the effect of different pretreatments, the compositions of WS after pretreatments were determined. Table 1 shows that the residual contents of hemicellulose in WS after 2% and 4% H2SO4 pretreatments at 121 °C were found to be only 4.1% and 2.4%, respectively, compared to that of 19.1% in the raw WS sample. This finding indicates that acid pretreatment could efficiently remove hemicellulose, which was consistent with the results of a previous study38. NaOH pretreatment results in slight degradation of hemicellulose compared to that using H2SO4 pretreatment (121 °C, 13.1%, and 11.1%, respectively), whereas the contents of lignin after alkali pretreatment were particularly low (121 °C, 4% NaOH, 2.2%), indicating that alkaline solutions efficiently remove lignin by breaking ester bonds, thereby increasing the porosity of biomass39. However, cellulose in WS was also degraded during pretreatment. There was only 29.9% and 19.3% remaining cellulose after 2% and 4% H2SO4 pretreatment at 121 °C. The content of remaining cellulose was higher at 27.1% and 24.9% after 2% and 4% NaOH pretreatment at 121 °C.

| Item | Moisture (%) | Ash (%) | Cellulose (%) | Hemicellulose (%) | Lignin (%) |
|------|--------------|---------|---------------|-------------------|------------|
| Raw WS | 10.3±0.12 | 9.7±0.09 | 33.7±1.62 | 19.1±1.25 | 19.8±1.50 |
| 2% H2SO4-pretreated WS | 23.9±2.98 | 4.1±0.22 | 17.7±1.11 | |
| 4% H2SO4-pretreated WS | 19.3±0.73 | 2.4±0.14 | 15.2±1.17 | |
| 2% NaOH-pretreated WS | 27.1±1.34 | 13.1±0.89 | 3.1±0.52 | |
| 4% NaOH-pretreated WS | 24.9±1.09 | 11.1±1.04 | 2.2±0.18 | |
| Water-pretreated WS | 30.2±1.30 | 17.6±0.89 | 17.8±0.86 | |
| Water-pretreated WS | 23.2±0.14 | 12.3±1.03 | 16.9±1.17 | |
| WS (%) | 3.4 | 44.3 | 24.5 | |
| WS (%) | 8.0 | 37.1 | 23.5 | |

Table 1. Composition of raw WS and pretreated WS using various methods: *All of the percentage compositions of raw and pretreated solid samples are calculated based on the dry mass of raw DG; †Conducted at 121 °C for 1 h; ‡Performed at 175 °C for 1 h; ††Data from Constant et al. (2016)35; †‡Data from Merali et al. (2016)36.
To measure the effect of different pretreatments on cellulose conversion rate, enzymatic hydrolysis was employed. Table 2 shows the conversion rate of cellulose using different pretreatments for enzymatic hydrolysis. The cellulose conversion rate increased with higher temperature. Compared to the cellulose in raw WS, the cellulose conversion rate reached to 100% at 4% NaOH pretreatment. However, after NaOH pretreatment, the conversion rate of cellulose was 86.6%. These high conversion rates could be attributed to the degradation of hemicellulose and lignin after pretreatment (Table 1). The removal of lignin and hemicellulose before enzymatic hydrolysis is highly necessary because it increases the digestibility of cellulose40,41. The low removal efficiency of hemicellulose and lignin by hot water pretreatment at 121 °C indicates that it is not an optimal method for enhancing enzymatic digestibility. Compared to hot water pretreatment at 121 °C, the removal of hemicellulose and lignin was more efficient at 175 °C (12.3% and 16.9%, respectively). However, higher temperatures result in more extensive cellulose degradation.

### Table 2. Wheat straw pretreatment conditions and the cellulose conversion rates: statistical significance

| Sample no.         | Temp. (°C) | Conversion rate of cellulose (%) | Conversion rate of cellulose (%) |
|--------------------|------------|----------------------------------|----------------------------------|
| 0.5% H_2SO_4-pretreated | 121        | 36.3 ± 1.84*                     |                                  |
| 1% H_2SO_4-pretreated | 121        | 39.8 ± 1.55**                    |                                  |
| 2% H_2SO_4-pretreated | 121        | 45.9 ± 1.33**                    | 65.6 ± 1.90****                 |
| 4% H_2SO_4-pretreated | 121        | 49.2 ± 0.42**                    | 86.6 ± 0.73**                   |
| 4% H_2SO_4-pretreated | 50         | 19.2 ± 0.49**                    |                                  |
| 4% H_2SO_4-pretreated | 100        | 23.1 ± 0.75**                    |                                  |
| 0.5% NaOH-pretreated  | 121        | 38.1 ± 1.66**                    |                                  |
| 1% NaOH-pretreated   | 121        | 56.4 ± 3.47**                    |                                  |
| 2% NaOH-pretreated   | 121        | 59.7 ± 0.96**                    | 75.0 ± 2.12****                 |
| 4% NaOH-pretreated   | 121        | 65.8 ± 0.17                      | 87.2 ± 3.42                     |
| 4% NaOH-pretreated   | 50         | 55.6 ± 0.44**                    |                                  |
| 4% NaOH-pretreated   | 100        | 59.3 ± 2.30                      |                                  |
| Water-pretreated     | 121        | 28.3 ± 1.05**                    | 28.7 ± 1.07****                 |
| Water-pretreated     | 100        | 29.7 ± 1.56                      |                                  |
| Water-pretreated     | 175        | 32.9 ± 1.41**                    | 48.7 ± 1.78****                 |

Although hot water pretreatment has advantages such as being inexpensive and does not result in corrosion, the degradation of hemicellulose, lignin after hot water pretreatment at 121 °C was not comparable to that using acid and alkali pretreatment (Table 1). The removal of lignin and hemicellulose before enzymatic hydrolysis of lignocellulose is highly necessary because it increases the digestibility of cellulose40,41. The low removal efficiency of hemicellulose and lignin by hot water pretreatment at 121 °C indicates that it is not an optimal method for enhancing enzymatic digestibility. Compared to hot water pretreatment at 121 °C, the removal of hemicellulose and lignin was more efficient at 175 °C (12.3% and 16.9%, respectively). However, higher temperatures result in more extensive cellulose degradation.

### Enzymatic hydrolysis

To observe the structure changes of the WS, SEM analysis was conducted. The morphological features of raw, pretreated, and enzyme-hydrolyzed WS are shown in Figure 1. Raw WS showed a regular and compact surface structure with fibers arranged in bundles, which impede cellulose access by cellulase. The surface of the WS samples, which is mainly composed of lignin and hemicellulose, was destroyed after acid and alkali pretreatment (Figure 1). The lignin and hemicellulose of the pretreated WS samples were partially removed and broken or became loose, thereby resulting in the exposure of internal structures. Especially after NaOH pretreatment, the WS became loose and scattered, and exhibited fiber porosity on its surface compared to the raw WS. These findings demonstrate that pretreatment could destroy the cellulose-hemicellulose-lignin network, thereby removing some of the external fibers42. The exposure of internal structures of the WS samples increases...
the accessibility of cellulase to the inner cellulose, thereby accelerating the biodegradation process. However, hot water pretreatment only caused minimal changes on the surface of the WS samples (Figure 1), which is consistent with the low efficiency removal of hemicellulose and lignin (Table 1). The structures of the enzymatically hydrolyzed WS samples after acid- and alkali-pretreatments were completely destroyed, indicating that most of the cellulose was degraded by the enzyme. The surface of the enzymatically hydrolyzed WS samples after hot water pretreatment was relatively smooth and showed minimal destruction compared to that using acid and alkali pretreatment, which is indicative of the inefficient removal of hemicellulose/lignin and low enzymatic digestibility of cellulose in WS.

Figure 1. SEM images: all samples were exposed to 121 °C for 1 h except for raw WS.
XRD analysis. Due to crystalline feature of cellulose, pretreatment could potentially enhance the diffusion of cellulase through amorphous cellulose and markedly affect the enzymatic accessibility of lignocellulosic biomass\(^\text{44,45}\). The crystallinity index (CrI) is an important indicator closely related to enzymatic digestibility because it could reflect biomass crystallinity. It could also indirectly indicate the amorphous phase signal of the biomass such as lignin, hemicellulose, and cellulose domains\(^\text{38,46,47}\). The CrI of raw, pretreated, and enzyme-hydrolyzed WS is shown in Figure 2. Compared to the CrI of raw WS (48.27%), the CrI of acid-pretreated and alkali-pretreated WS was considerably higher (61.51% for acid-pretreated WS and 61.84% for alkali-pretreated WS, respectively). The conversion rates of these two pretreatments were as high as 86.6% and 87.2%, which were calculate on cellulose content after pretreatment. This could be attributed to the fact that hemicellulose and lignin were partially degraded during the pretreatment (Table 1). Thus, the crystallinity of the biomass increased, thereby leading to a high cellulose conversation rate. Similar result was reported by previous studies. Haque et al.\(^\text{48}\) reported that compared to raw WS (45.0%), the CrI of WS samples increased to 52.3%, 57.9%, 65.3%, and 71.5% after pretreatment with 0.5%, 1.0%, 1.5%, and 2.0% NaOH at 105 °C, and the reducing sugar yield increased from 45% to 78% with higher NaOH concentrations (1%, 1.5%, and 2.0%) and CrIs. The CrI of hot water-pretreated WS (50.4%) slightly increased compared to that of raw WS, and the conversion rate of cellulose was only to be 28.3%. This could be explained by the inefficient removal of hemicellulose and lignin. The CrIs of enzyme-hydrolyzed WS samples decreased compared to those of pretreated WS samples, which may be attributable to the partial degradation of crystalline cellulose.

FTIR characterization. The FTIR spectra of raw WS, pretreated WS, and enzyme-hydrolyzed WS are shown in Figure 3. The broad band ranging from 3,000–3,500 cm\(^{-1}\) was assigned to the O-H stretching vibration.\(^\text{9}\) The intensity of these absorption peaks indicate the cellulose content in the WS samples.\(^\text{38}\) The intensities of these bands in the FTIR spectra of 4% H\(_2\)SO\(_4\), 4% NaOH, and hot water pretreatments were significantly higher than those of enzyme-hydrolyzed WS. These results suggest that the cellulose content of pretreated WS was higher than those of enzyme-hydrolyzed WS. The bands around 2,850–2,922 cm\(^{-1}\) were attributed to the C-H stretching vibration of the aliphatic chain structure of lignin.\(^\text{49}\) The bands of 4% H\(_2\)SO\(_4\) pretreatment, hot water pretreatment, and enzyme-hydrolyzed WS after 4% hot water pretreatment were similar to that of raw WS, indicating weak lignin removal. Compared to the raw WS, the intensities of these bands in the spectra after NaOH pretreatment and enzyme-hydrolyzed WS after NaOH pretreatment significantly decreased, which indicates that the aliphatic compounds in lignin were efficiently removed. These results are related to the fact that alkali pretreatment...
was more efficient in removing lignin. Furthermore, compared to the raw WS, the bands ranging from 1,635–1,655 cm\(^{-1}\) disappeared, which were attributed to the removal of hemicellulose\(^6\). The range of 500–1,770 cm\(^{-1}\) is considered as the lignin fingerprint region.\(^1\) The intensities of the absorption peaks at 1,735, 1,620, 1,460, 1,257, and 1,070 cm\(^{-1}\) decreased or disappeared compared those of raw WS, indicating that lignin and hemicellulose in the pretreated WS samples were partially removed.\(^9\) The bands within the range of 1,450–1,630 cm\(^{-1}\) were attributed to the aromatic skeleton stretching vibration, and the intensities of these absorption peaks were stronger than those of raw WS\(^1^8\).

In conclusion, the effects of \(\text{H}_2\text{SO}_4\), \(\text{NaOH}\), and hot water pretreatment on cellulose digestibility of WS were investigated. Our findings indicate that \(\text{H}_2\text{SO}_4\) pretreatment could efficiently remove hemicellulose in WS. However, the removal of lignin was poor and only partial cellulose degradation was observed with \(\text{H}_2\text{SO}_4\) pre-treatment compared with previous study\(^1^5\). The cellulose conversion rate was as high as 86.6% with 4% \(\text{H}_2\text{SO}_4\) pretreatment (121 °C). \(\text{NaOH}\) pretreatment could efficiently remove lignin and partially degrade cellulose in WS. Furthermore, among all the pretreatments, the highest conversion rate was obtained by 4% \(\text{NaOH}\) pretreatment at 121 °C (87.2%), which was pretreated at a mild alkali concentration and temperature. Although slight cellulose degradation was observed with hot water pretreatment, the removal of hemicellulose and lignin were not comparable to that using acid and alkali pretreatment, and the cellulose conversion rate by enzymatic hydrolysis was particularly low (28.3%). SEM analysis revealed that lignin and hemicellulose in WS were destroyed after acid and alkali pretreatment, whereas hot water pretreatment only caused minimal changes on the surface of the WS samples. The CrIs of cellulose increased after pretreatments, particularly that using \(\text{H}_2\text{SO}_4\) and \(\text{NaOH}\) pretreatment, which resulted in an enhancement of enzymatic hydrolysis. Thus, \(\text{NaOH}\) pretreatment at a mild concentration and temperature (4% \(\text{v/v}\), 121 °C for 1 h) was determined to be the optimal method for enhancing the enzymatic digestibility of WS.

Materials and Methods

**Materials.** The wheat straw was collected from a farm located in Deyang, Sichuan, China in June 2016. After drying at 45 °C in an oven for two weeks, the WS was milled with a Wiley laboratory mill (Model No. 4, Thomas Scientific, Philadelphia, PA, USA), followed by passing through a 40-mesh screen. The obtained samples were stored in airtight plastic bags for the following experiments. The moisture content and ash content of the samples were determined according to the National Standard Procedures of People's Republic of China.\(^5^1,5^2\). To determine moisture content, 1–2 g of the WS was placed in a preweighed weighing bottle and dried at 105 °C for 4 h. The weighing bottle was then transferred to a desiccator, cooled for 0.5 h, and then weighed. The weighing bottle was again moved, dried for 1 h, and cooled for weighing until constant weight. The moisture content was calculated based on the National Standard Procedures of People's Republic of China (Eq. 1).\(^5^3\) For ash content, 2–3 g of WS was placed in a preweighed porcelain crucible and carbonized in a muffle furnace at 225 °C, followed by burning to ash at 575 °C. The crucible was air cooled for 1 min and then cooled in a desiccator for 0.5 h and weighed. The porcelain crucible was dried at 105 °C and cooled for weighing until constant weight. Ash content was calculated based on the National Standard Procedures of People's Republic of China (Eq. 2).\(^5^4\) Cellulose and hemicellulose of the samples was determined by the method of the National Renewable Energy Laboratory (NREL).\(^5^5\) Briefly, 0.5 g of WS was placed in the qualitative filter paper that was soaked in ethanol for 1–2 h and extraction was performed for 16–24 h in a Soxhlet extractor. After drying, 3 mL of 72% \(\text{H}_2\text{SO}_4\) was added and incubated for 1 h at 30 °C. Approximately 84 mL of deionized water was added to dilute the acid to 4%, followed by heating at 121 °C for 45 min. The solution was filtered through a Buchner funnel, and the filtrate was collected into a new Erlenmeyer flask. The liquid was neutralized using \(\text{CaCO}_3\) and stored at −20 °C for the determination of cellulose and hemicellulose content using HPLC. The lignin content was determined using the method described by Chung et al.\(^5^6\). 5 mg of sample was suspended in 2.5 mL acetyl bromide (25% \(\text{AcBr}\) in acetic acid). 0.1 mL of perchloric acid was added and mixed. The mixture was dried three times in an oven for 10 min each at 70 °C and cooled on ice water for 30 min, followed by transferred to volumetric flask containing 10 mL of 2 M \(\text{NaOH}\) and 12 mL of acetic acid. Lignin content was determined by a UV-VIS at 280 nm using 20.09 g⋅L\(^{-1}\)⋅cm\(^{-1}\). All composition percentages of the raw and pretreated solid samples were calculated on dry mass of WS.

\[
\text{Moisture content (\%)} = \left(1 - \frac{m_1}{m} \right) \times 100; \quad (1)
\]
where \(m\) is the weight of WS before being dried, g; and \(m_1\) is the oven dry weight of WS, g.

\[
\text{Ash content (\%)} = \left(1 - \frac{m_2}{m} \right) \times 100; \quad (2)
\]
where \(m_1\) is the weight of crucible, g; \(m_2\) is the weight of crucible with ash, g; and \(m\) is the oven dry weight of WS, g.

**Pretreatments of WS.** Three different pretreatments were tested in this study, including sulfuric acid pretreatment (0.5–4% \(\text{v/v}\), 50–121 °C, 1 h), sodium hydroxide pretreatment (0.5–4% \(\text{v/v}\), 50–121 °C, 1 h), and hot water pretreatment (50–175 °C, 1 h). The pretreatment was performed according to a previous study.\(^5^6\) Briefly, 5 g of WS was placed in 50 mL of pretreatment liquid and sealed in an Erlenmeyer flask in an autoclave and then heated at different temperatures for 1 h (Table 2). For hot water pretreatment at 175 °C, 5 g of WS was placed in 50 mL deionized water and sealed in a stainless steel pot, followed by heating in an oil bath digester (YYQ-10-1.25, Nanjing Jiezhen Science & Technology Development Co. Ltd., Nanjing City, China) for 1 h. Each sample was immediately cooled and neutralized using \(\text{HCl}\) or \(\text{NaOH}\) heat treatment. The neutralized sample was centrifuged (7,000 g) at 4 °C for 3 min. The residue was washed four times with 100 mL of deionized water and centrifuged previously described, followed by dried at 45 °C for enzymatic hydrolysis.
Enzymatic hydrolysis of pretreated WS. Enzymatic hydrolysis of the pretreated WS as described in a previous study\textsuperscript{65}. Briefly, 1 g of the pretreated WS was immersed in 30 mL of a sodium citrate buffer (pH 4.8, 50 mM). To hydrolyze the lignocellulose biomass, 35 FPU/g cellulase from \textit{Trichoderma reesei} (Celluclast 1.5 L) and 61.5 CBUs/g cellobiose from \textit{Aspergillus niger} (Novozym 188) were added. In addition, sodium azide (0.3% w/v) was also added to the solution to prevent microbial contamination. The lignocellulose biomass was dynamically (150 rpm) incubated at 55 °C for 72 h and then rapidly cooled on ice to stop the reaction. The hydrolysate was then centrifuged to collect the supernatant for the determination the yield of monomeric sugar using HPLC.

SEM observation. The morphologies of raw, pretreated, and enzyme-hydrolyzed WS were characterized by SEM (SU8010, Hitachi High-technologies Co., Tokyo, Japan) as described elsewhere\textsuperscript{56}. All samples were conducted at a 4% concentration and 121 °C for 1 h except for raw WS. Before SEM observation, a thin layer of gold was sputter-coated on the samples to prevent degradation and to render fiber conductivity.

XRD analysis. XRD (Ultima UVX XRD, Rigaku Co., Tokyo, Japan) was used to characterize the crystalline phases of raw, pretreated, and enzyme-hydrolyzed WS, which was operating at 30 kV and 10 mA with Cu radiation (1.54 Å) as previously described\textsuperscript{57}. Except for raw WS, all samples were conducted at a 4% concentration and 121 °C for 1 h. The X-ray diffractograms were recorded from 10° to 40° with a step size of 0.02°. The CrI was calculated using the following Eq. (3)\textsuperscript{56}.

\[
\text{CrI (\%)} = \left( \frac{I_{002} - I_{\text{am}}}{I_{002}} \right) \times 100\%;
\]

where \( I_{002} \) is the maximum peak intensity at lattice diffraction; and \( I_{\text{am}} \) is the intensity of diffraction at \( 2\theta = 18\).°

FTIR analysis. FTIR spectra of different samples were obtained with a FTIR spectrometer (Nicolet 8700, Bruker AXS, Karlsruhe, Germany). The data was recorded within a range of 400–4,000 cm\(^{-1}\) with a detector at 4 cm\(^{-1}\) resolution and 128 scans per sample.

Analytical methods. The yields of monomeric sugars were measured using HPLC. Precolumn derivatization with PMP was performed with an Agilent Zorbax SB-C18 column\textsuperscript{58}. The experiments were conducted in triplicate. All data were expressed as the mean ± standard deviation and analyzed with GraphPad Prism 6 via the Turkey–Kramer test. The cellulose conversion rate was calculated as Eq (4).

\[
\text{Cellulose conversion rate (\%)} = \frac{C_{\text{Glucose}} \times 0.9}{C_{\text{Cellulose}}} \times 100\%;
\]

where \( C_{\text{Glucose}} \) is the concentration of glucose in enzymatic hydrolysat, g/L; and \( C_{\text{Cellulose}} \) is the concentration of cellulose in samples, g/L.

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Acknowledgements
This study was jointly funded by grants from the Foundation for Studies on Pit Mud of Vinasse for Liquor by DGGE and PLFA (project number: ychx00018), the National Key Technology R&D Program (project number: 2013BAD01B05), the Science and Technology Innovation Platform Development Program of Fujian Agriculture and Forestry University (project numbers: PTJH13001, PTJH12015), the United Fujian Provincial Health and Education Project for Tackling Key Research (project number: WKJ-FJ-25) and the Funds for Researches by Leading Personnel in Higher Education Institutions (project number: k8012012a).

Author Contributions
J.Z. and X.G. conceived and supervised the project. Q.Z. and X.G. designed the experiments and wrote the manuscript; Q.Z., T.Z., Y.W., X.C., S.W. and M.Z. carried out the experiments; H.W., X.M. and B.Z. improved the manuscript. All authors reviewed and approved the manuscript.

Additional Information
Competing Interests: The authors declare that they have no competing interests.

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