Preparation of convertible cellulose from rice straw using combined organosolv fractionation and alkaline bleaching

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Abstract. Rice straw was employed to prepare microcrystalline cellulose (MCC). First, crude cellulose was isolated from rice straw using organosolv fractionation in the presence of mixed solvent composed of ethanol and H2O. Most hemicellulose and lignin were removed while little silica was not. Second, crude cellulose was bleached in Na2CO3/H2O2 mixed solution to remove residual silica as well as small amount of hemicellulose and lignin. After that, the removal rates of hemicellulose, lignin and silica reached up 91.5, 92.8 and 97.9%, respectively. Cellulose from rice straw was further converted into MCC in the presence of hydrochloric acid aqueous solution, and the cellulose content reached up to 92.4% in MCC. Infrared spectrometer (IR) and X-ray diffractometer (XRD) were used to characterize the structure of rice straw and its derived samples.

1. Introduction
Lignocellulosic biomass has been considered a promising alternative for traditional fossil and used as sustainable feedstock for chemicals as well as energies production because it is available and low-cost. Cereal straws, common and available biomass, are primarily composed of cellulose, hemicellulose, lignin and a small amount of ash (mainly containing silica). Up to now, cereal straws are widely used as an alternative for non-wood pulping raw material to obtain paper pulp. In fact, both sugar and lignin fractions in cereal straw are potential resources for fuels and chemicals production. Cellulose is the major component of cereal straws, which can be isolated and well transferred to value-added products including fuel ethanol, methylcellulose and cellulose acetate after the removal of hemicellulose, lignin and ash [1]. Rice straw is one of the most common and abundant straws, and the conversion of cellulose from rice straw is promising.

Effective fractionation of complex lignocellulosic material into its constituents is a key operation for the isolation and further conversion of cellulose. Organosolv fractionation has been widely investigated since it generally aims to fractionate lignocellulosic biomass into its natural structure as much as possible. Although the transformation of straw fiber resources into high quality industrial products provides a prospective solution to straw disposal and alternative to fossil fuels, α-cellulose isolated from rice straw is difficult to convert efficiently due to its natural structure with high degree of polymerization (DP) value. Microcrystalline cellulose (MCC) is a derivative from cellulose, which is generally synthesized via the partial hydrolysis of wood and cotton cellulose catalyzed by dilute mineral acid. Compared to α-cellulose, MCC is easier to convert to valued chemicals because of moderate DP value [2]. The preparation of MCC from agricultural residues such as soybean husk, wheat and rice straw has become an active research field. This work focused on isolating cellulose
from rice straw and its subsequent conversion to MCC. Cellulose isolation was performed by organosolv fractionation in the mixture of ethanol/H$_2$O, and MCC was prepared by the partial hydrolysis of cellulose in the presence of hydrochloric acid.

2. Experimental

2.1. Isolation of cellulose from rice straw

2.1.1. Removal of soluble impurity. The removal of soluble impurity was carried out according to the reported procedures [3]. Rice straw was cut to 1-2 cm and washed with warm water 3 times to remove dirty and aqueous soluble substances, followed by drying at 50 °C under vacuum. The rice straw was then crushed into powder. Typically, 10 g rice straw powder was Soxhlet extracted using toluene-ethanol (2:1, v/v) mixture. After extracting 24 h, the powder was dried at 50 °C under vacuum, thus giving extracted rice straw.

2.1.2. Organosolv fractionation of rice straw. To a 100 mL stainless steel autoclave, 3 g extracted rice straw, 30 mL ETHANOL/H$_2$O mixture (1:1, v/v) and H$_2$SO$_4$ (0.05 M in the mixture) were added. The mixture was then heated at 150 °C for 6 h under stirring. The solid residue was collected after filtration, washed thoroughly by warm water and dried at 50 °C under vacuum. Crude cellulose was acquired and weighed to determine yield.

2.1.3. Bleaching of crude cellulose. Typically, 2 g crude cellulose from rice straw was added into a 100 mL round-bottom flask, followed by the addition of 20 mL Na$_2$CO$_3$ and H$_2$O$_2$ mixed solution (8 and 2 wt% in the mixture, respectively). The mixture was heated at 60 °C for 10 h before cooling to room temperature. The solid was collected by filtering and dried at 50 °C under vacuum for 10 h, then giving rice straw cellulose.

2.2. Preparation of MCC

Generally, 1 g cellulose isolated from rice straw and 25 mL 6 wt% hydrochloric acid aqueous solution were added into a 100 mL round-bottom flask. The mixture was then heated at 80 °C for 1 h under stirring. The mixture was cooled to room temperature before neutralizing by 5 wt% NaOH aqueous solution until pH value of the mixture increased to 7, followed by standing 10 h to precipitate. The as-precipitated powder was collected by filtration, washed with 95 wt% ethanol solution, dried at 60 °C under vacuum for 10 h, thus giving MCC. Yield of 76.2% and DP value of 231 were acquired.

2.3. Determination and calculation

The contents of cellulose, hemicellulose, lignin and silica were determined according to the reported procedures [4]. The DP value was determined using intrinsic viscosity measurement, in which the samples were dissolved in copper-ammonium hydroxide solution [5]. The yields of crude cellulose, cellulose and MCC from rice straw were calculated according to equation (1), and the removal rates of hemicellulose, lignin and silica were calculated according to equation (2), as shown in the following.

\[
\text{Yield} \, (\%) = \frac{m'}{m_o} \times 100\% \quad (1)
\]

\[
\text{Removal rate} \, (\%) = \frac{mC-m_1C'}{mC} \times 100\% \quad (2)
\]

Where $m'$ and $m_o$ are product mass obtained and raw material employed for each procedure, respectively; $m$ and $C$ are the mass of extracted rice straw employed in section 2.2.2 and component contents in extracted rice straw; $m_1$ and $C'$ are the mass and component content of products including crude cellulose and cellulose, respectively.

2.4. Measurements
Infrared spectrometer (IR) was performed on an EQUINOX 55 spectrometer in the range from 4000 to 400 cm$^{-1}$. X-ray diffractometer (XRD) measurement was performed on a D/MAX-RB diffractometer with Cu Kα radiation at a scanning rate of 5°/min in the range from 10 to 80°.

3. Results and discussion

3.1. Characterization

3.1.1. IR. The IR spectra of extracted rice straw, crude cellulose, cellulose and MCC are presented in Figure 1. The bands around 3412 and 2921 cm$^{-1}$ presented in the spectra of all samples are attributed to the stretching vibration of –OH group and C–H bond in –CH$_2$, respectively. The band at 1159 cm$^{-1}$ is assigned to the stretching vibration of characteristic β-(1–4)–glycosidic bond in glycogen [6]. In addition, the band at 795 cm$^{-1}$ is responsible for the stretching vibration of Si–O–Si in silica. In the spectrum of extracted rice straw (Figure 1a), the small shoulder band at 1736 cm$^{-1}$ is ascribed to the characteristic of aliphatic esters in hemicellulose and/or lignin. The absorption at 1517 cm$^{-1}$ belongs to the aromatic skeleton vibrations of C=C in bound lignin [4]. In the IR spectrum of crude cellulose (Figure 1b), the bands around 1736 and 1517 cm$^{-1}$ almost disappear while the band at 795 cm$^{-1}$ still can be observed. These results indicate nearly complete removal of hemicellulose and lignin but incomplete removal of silica. In the IR spectrum of cellulose derived from rice straw (Figure 1c), no band around 795 cm$^{-1}$ could be observed, displaying nearly complete removal of silica from rice straw after bleaching. All characteristic bands of cellulose are observed but no new bands appear in the spectrum of MCC (Figure 1d), indicating MCC almost has the same chemical composition as that of cellulose.

![Figure 1. IR spectra of (a) extracted rice straw, (b) crude cellulose, (c) cellulose and (d) MCC.](image)

3.1.2. XRD. The XRD patterns of extracted rice straw, crude cellulose, cellulose and MCC are presented in Figure 2. The peak around 22.4° of (200) reflection in all samples is contributed to the typical crystal lattice of cellulose I$_\beta$. Compared to the pattern of extracted rice straw (Figure 1a), new diffraction peaks appear around 16.1°, 34.3° and 44.6°. In the XRD patterns of crude cellulose and cellulose (Figure 3b,3c), the shoulder peak at 16.1° of (101) reflection is assigned to the typical crystal lattice of cellulose I$_\alpha$ [7]. The diffraction peak at 22.4° of (002) reflection is wide and round in the curve of extracted rice straw (Figure 3a) but becomes relatively sharper and narrower after organosolv fractionation and bleaching (Figure 3b, 3c), indicating the removal of amorphous components such as hemicellulose, lignin and silica. The curves in Figure 3 displayed that MCC possesses similar typical
crystal lattice to that of crude cellulose and cellulose except the disappearance of the diffraction peak at 44.6° (Figure 3d), indicating the partial hydrolysis of cellulose mainly occurs in amorphous area in the presence of acid.

Figure 2. XRD patterns of (a) extracted rice straw, (b) crude cellulose, (c) cellulose and (d) MCC.

3.2. Isolation of cellulose from rice straw

3.2.1. Organosolv fractionation of rice straw. It has been reported that cellulose could be extracted from straw by hydrolyzing hemicellulose, fragmenting and dissolving lignin in liquid phase via organosolv fractionation. Generally, the hydrolysis of hemicellulose is improved in acidic condition, in which H₂SO₄ is widely employed. The influence of H₂SO₄ concentration was investigated, as shown in Table 1. The yield of crude cellulose dropped with raising H₂SO₄ concentration in the range from 0.03 to 0.06 M. It can be ascribed to improvement in the removal rates of hemicellulose, lignin and silica resulted from xylan hydrolysis and delignification. As a result, the yields of crude cellulose slightly decreased from 69.7 to 58.2% correspondingly. Further increasing in H₂SO₄ concentration led to slight increase in the removal rates of hemicellulose and lignin, and the yield of crude cellulose also changed insignificantly. These results indicated that enhancement in acid concentration gave insignificant effect on the hydrolysis of cellulose once H₂SO₄ concentration above 0.06 M. So it is reasonable to conclude that the hydrolysis of cellulose hardly occurs in the present pretreatment conditions. The results in Table 1 also revealed that the removal rate of silica changed in a range from 8.1 to 15.5%, indicating that acidic condition is not beneficial for removing silica and further pretreatment for crude cellulose is essential.

Table 1. Organosolv fractionation of rice straw using H₂SO₄ with various concentrations.

| Entry | H₂SO₄ concentration (M) | Removal rate (%) | Yield (%) |
|-------|------------------------|------------------|-----------|
|       |                        | Hemicellulose     | Lignin    | Silica   |               |
| 1     | 0.03                   | 52.0             | 41.2      | 8.1      | 69.7         |
| 2     | 0.04                   | 56.1             | 50.8      | 9.5      | 64.6         |
| 3     | 0.05                   | 81.6             | 63.4      | 11.2     | 61.3         |
| 4     | 0.06                   | 83.1             | 65.7      | 14.2     | 58.2         |
| 5     | 0.07                   | 83.8             | 68.1      | 15.5     | 57.9         |

Fractionation conditions: 2 g treated rice straw, 20 mL solvent (ethanol/H₂O=1:1, v/v), 150 °C for 6 h.

3.2.2. Bleaching of crude cellulose
The results in Table 2 showed that most hemicellulose and lignin were removed via organosolv fractionation. However, the removal of silica was inefficient. Obviously, the remained silica, small amount of hemicellulose and lignin affected the purity and quality of cellulose, which is critical to convert cellulose to chemicals. Therefore, crude cellulose was further treated by H$_2$O$_2$ and/or aqueous Na$_2$CO$_3$ solution, and the results were presented in Table 2. Only small change in the removal rate of hemicellulose was observed in the presence of H$_2$O$_2$ or Na$_2$CO$_3$ alone. However, the removal rates of lignin and silica were obviously enhanced. Surprisingly, the removal rates of all components were also enhanced by treating in H$_2$O$_2$ and Na$_2$CO$_3$ solution subsequently. Removal rates of hemicellulose, lignin and silica at 93.8, 91.7 and 97.5% were obtained, respectively (entry 3). Similar results were obtained by varying the order of Na$_2$CO$_3$ and H$_2$O$_2$ (entry 4). Positive effect of combined H$_2$O$_2$ and Na$_2$CO$_3$ could be attributed to the decomposition of H$_2$O$_2$ to HOO$^-$ in alkaline solution, which not only acts as a bleacher but also an oxidant. The latter leads to the oxidation of lignin and thus gives hydrophilia group, promoting the dissolution of lignin and thus the removal rates were improved. The improvement in delignification also led to a positive impact on the removal rate of hemicellulose since hemicellulose and lignin are structurally linked via covalent bond, which breaks during the dissolution of lignin. In addition, the saponification of ester bond between hemicellulose and lignin generally occurs in alkaline solution [8]. All these factors promoted the removal of hemicellulose and lignin. Although the removal rates are relatively high via the treatment of H$_2$O$_2$ and Na$_2$CO$_3$ subsequently, the time required was too long and the process was too complex. So, crude cellulose was treated simultaneously by H$_2$O$_2$ and Na$_2$CO$_3$. The results in Table 2 revealed that the removal rates of hemicellulose, lignin and silica still reached up 91.5, 92.8 and 97.9% (entry 5), respectively.

Table 2. Influence of beaching condition on cellulose from crude cellulose.

| Entry | H$_2$O$_2$ | Na$_2$CO$_3$ | T (°C) | Removal rate (%) | Yield (%) |
|-------|------------|-------------|--------|-----------------|----------|
|       |            |             |        | Hemicellulose    | Lignin    | Silica   |        |
| 1     | 2          | /           | 60     | 84.5            | 75.2      | 90.1     | 83.5   |
| 2     | /          | 10          | 95     | 85.7            | 91.3      | 91.7     | 67.3   |
| 3a    | 2          | 10          | 60/95  | 93.8            | 91.7      | 97.5     | 69.2   |
| 4b    | 2          | 10          | 95/60  | 92.9            | 93.0      | 97.7     | 66.7   |
| 5     | 2          | 10          | 60     | 91.5            | 92.8      | 97.9     | 67.5   |

Reaction conditions: 1.0 g crude cellulose, 20 mL Na$_2$CO$_3$ and H$_2$O$_2$ solution, 60 °C for 10 h.

a) 1) 60 °C for 10 h in H$_2$O$_2$ solution; 2) 95 °C for 10 h in Na$_2$CO$_3$ solution.

b) 1) 95 °C for 10 h in Na$_2$CO$_3$ solution; 2) 60 °C for 10 h in H$_2$O$_2$ solution.

3.3. Determination of composition and DP value

Table 3 gave the composition and DP value of rice straw, crude cellulose, cellulose and MCC. The contents of cellulose, hemicellulose, lignin and silica are 36.8, 20, 14.3 and 6 wt% in rice straw (entry 1), respectively. The content of these components increased slightly after the removal of waxiness, fat and solubility by extracting in the mixture of toluene/ethanol (entry 2). The contents of hemicellulose and lignin dropped obviously after organosolv fractionation by ethanol/H$_2$O while the silica content is contrary to those, indicating that silica was ineffectively moved (entry 2). It is in accordance with the results given in Table 1. In addition, the silica content dropped significantly after bleaching (entry 3), confirming effective removal of silica in H$_2$O$_2$/Na$_2$CO$_3$ solution. The content of hemicellulose, lignin and silica dropped to 4.9, 2.2 and 0.9%, and cellulose content at 90.1% was obtained after bleaching. Among all the samples, MCC gave the highest cellulose content, which reached up 92.4%. It also can be seen that the DP value increased after toluene/ethanol extraction and ethanol/H$_2$O fractionation since most amorphous and low molecular components were removed. Cellulose isolated from rice straw possessed a close DP value to that of crude cellulose, further confirming that hemicellulose and lignin were almost removed via organosolv fractionation. The results in Table 3 also showed that significant drop in DP value was observed in MCC, and DP value at 231 was determined, indicating partial hydrolysis of cellulose.
Table 3. Composition and DP value of rice straw and its derivatives.

| Sample       | Component (wt%) | DP value |
|--------------|-----------------|----------|
|              | Cellulose       | Hemicellulose | Lignin | Silica |
| Rice straw   | 36.4            | 20.4      | 14.3   | 6.1    | 594   |
| Treated rice straw | 42.2            | 23.5      | 16.1   | 8.0    | 608   |
| Crude cellulose | 67.3            | 8.5       | 3.5    | 9.6    | 682   |
| Cellulose    | 90.1            | 4.9       | 2.2    | 0.9    | 652   |
| MCC          | 92.4            | 4.9       | 1.8    | 1.1    | 231   |

4. Conclusions
In this paper, MCC with high cellulose content and moderate DP value was derived from rice straw. Mixed solvent of ethanol/H₂O with a volume ratio of 1:1 was effective for the removal of most hemicellulose and lignin via organosolv fractionation. Contrarily, silica was mainly removed via bleaching in the mixed solution of Na₂CO₃/H₂O₂. After organosolv fractionation and bleaching, the removal rates of hemicellulose, lignin and silica at 91.5, 92.8 and 97.9% were obtained, respectively. Cellulose isolated from rice straw was further converted to MCC. The cellulose content reached up to 93.2% and moderate DP value at 231 was given.

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