

Extraction, separation and refining of microcrystalline cellulose from wheat straw using various pretreatments

Li Jinbao^{1,2}, Dong Huiling¹, Xiu Huijuan¹, Zhang Meiyun¹,
Kamireddy Srinivas Reddy³, Zhang Xuefei³, Ji Yun^{3*}

(1. College of Light Industry & Energy, Shaanxi University of Science & Technology, Xi'an 71002, China;

2. State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China;

3. Department of Chemical Engineering, University of North Dakota, Grand Forks ND 58202, USA)

Abstract: This study focused on the manufacture of microcrystalline cellulose (MCC) from wheat straw using environmentally-friendly solvents. Raw cellulose was separated from wheat straw after thermal decomposition of lignin followed by dissolution of lignin using a recyclable ethanol/acetic acid/water solvent system. Then, pure cellulose was produced using a four-step refining process, including chelating, O₃, H₂O₂, and xylanase treatments. Finally, MCC was obtained through hydrolysis, drying, and mechanical treatments. The effects of acetic acid, O₃, H₂O₂, NaOH, pretreatment time, and temperature on the properties of wheat straw cellulose (including Kappa number, yield, α -cellulose content, crystallinity, KMnO₄ value, degree of polymerization (DP), and brightness) were investigated. The results showed that the addition of acetic acid enhanced lignin removal and hemicellulose degradation, improving the purity of the raw cellulose. The optimized acetic acid dosage in the wheat straw thermal decomposition step was 2% (w/w). The optimized O₃ dosage was 1.2% (w/w). The optimized conditions for H₂O₂ treatment were found to be 3% (w/w) H₂O₂ and 1.8% (w/w) NaOH at 70°C for 120 min. The KMnO₄ value was 2.0, brightness was 84.1% ISO, the viscosity was 934 mL/g, and the DP was 626 for refined cellulose. Xylanase effectively improved the α -cellulose content of wheat straw cellulose. With an optimized xylanase dosage of 1.5 IU/g, the α -cellulose content was 94.7%, the brightness was 85.6% ISO, and the DP was 615 for wheat straw cellulose.

Keywords: wheat straw, cellulose; separation, refining, low pollution process, microcrystalline cellulose (MCC)

DOI: 10.3965/j.ijabe.20160902.1386

Citation: Li J B, Dong H L, Xiu H J, Zhang M Y, Reddy K S, Zhang X F, et al. Extraction, separation and refining of microcrystalline cellulose from wheat straw using various pretreatments. *Int J Agric & Biol Eng*, 2016; 9(2): 137–145.

1 Introduction

The isolation of cellulose from biomass usually

involves pulping, which includes delignification and bleaching^[1,2]. Lignocellulose is recalcitrant to normal treatments due to its complex structure, including three different compounds (cellulose, hemicellulose, and lignin)^[3,4]. Generally, microcrystalline cellulose (MCC) is obtained by the hydrolysis of cellulose using dilute acid pretreatment, followed by drying and crushing. These MCC particles have high fluidity with diameters of 20–80 μm ^[3]. MCC has been widely used in the cosmetics, food, and pharmaceutical industries. The applications of MCC can vary greatly due to its broad spectrum properties; for example, MCC may be used as a suspension stabilizer. MCC also can be used as an excipient because of its chemical inertness, nontoxicity and great hygroscopicity^[5,6]. In addition, MCC can be used in for swelling treatment in synthetic leather

Received date: 2014-08-15 **Accepted date:** 2015-12-16

Biographies: Li Jinbao, Associate Professor, Research interests: lignocellulose conversion and utilization, Email: ljb952@163.com;

Dong Huiling, Master candidate, Research interests: renewable material, Email: 652743567@qq.com; Xiu Huijuan, Lecturer, Research interests: cellulose modification, Email: xiuhuijuan@

sust.edu.cn; Zhang Meiyun, Professor, Research interests: cleaner pulping, Email: myzhang@sust.edu.cn; Kamireddy Srinivas Reddy, Engineer, Research interests: biomass pretreatment, biomedical application, Email: kamireddy.srinu@gmail.com;

Zhang Xuefei, Engineer, Research interests: material science, nanotechnology, Email: xuefei.zhang@gmail.com.

*Corresponding Author: Ji Yun, Associate Professor, Research interests: biomass conversion, lignin, biofuels and renewable chemicals. Department of Chemical Engineering, University of North Dakota, USA. Tel: +1-701-777 4456; Email: yun.ji@

enr.und.edu.

production^[7]. In the pharmaceutical industry, MCC can be used to produce very hard tablets which disintegrate rapidly in water due to swelling of the MCC particles and destruction of the bonding forces holding them together^[8-10].

Currently, the raw materials used for MCC production are cotton pulp and wood pulp^[11]. This study focused on the production of MCC from wheat straw as it is abundantly available and economical. Using conventional methods, MCC is produced from treating pulp with mineral acids, such as hydrochloric acid, phosphoric acid, or sulfuric acid. However, these mineral acids tend to affect the final product quality, as they lead to degradation and are highly corrosive^[8]. In another method, raw cellulose is first separated from wheat straw using ethanol extraction. The cellulose thus obtained is further refined using chelating, O₃, H₂O₂ and xylanase treatment. This method provides a wider product diversity and improved bleach ability of raw cellulose^[8,9]. However, the high Kappa number resulting from this method is unfavorable for cellulose refining.

Our previous study indicated that increasing the acidity of the system is beneficial for hemicellulose hydrolysis^[10,12]. In this study, a solvent comprising ethanol, acetic acid, and water (organosolv) was formulated in order to dissolve lignin and hemicellulose and to separate raw cellulose from wheat straw. Acetic acid assists in hemicellulose hydrolysis through cleaving of acetyl linkages^[13]. In addition, since ethanol and acetic acid have low vapor pressures, they can be easily recycled by distillation. The cellulose products obtained after organosolv extraction still contain lignin; thus, the hardness of the cellulose is high. In addition, wheat straw ash includes metal ions such as Fe³⁺, Mn²⁺, and Cu²⁺^[4]. These metal ions can react with O₃ and H₂O₂; thus, a chelation step was added prior to O₃ and H₂O₂ treatment to eliminate the influence of those heavy metals. The O₃ process can effectively reduce the lignin content in the cellulose and reduce the hardness of the products. The H₂O₂ process can increase the whiteness and stability of the cellulose product. Because the xylan content of wheat straw feedstock is high, xylanase enzymes hydrolyze 5-carbon carbohydrates, thus significantly

improving the purity of the cellulose product^[14-17].

This study investigated the effect of acetic acid on Kappa number, yield, α -cellulose content and crystallinity; the effect of O₃ on KMnO₄ value, yield, α -cellulose content, degree of polymerization (DP), and brightness; and the effects of H₂O₂ and NaOH dosage, treatment time, and temperature on KMnO₄ value, brightness, and viscosity. The effect of xylanase treatment on the quality of refined cellulose was also analyzed.

2 Materials and methods

2.1 Raw materials

Wheat straw was collected from a farm in Xi'an, China, and stored for six months in a sealed container before it was milled to 20-40 mm. The average composition of the wheat straw was 78.3%(wt) holocellulose including pentosan, 17.7%(wt) lignin, and 6.3%(wt) ash. Analytical grade acetic acid, potassium permanganate, potassium iodide, citric acid, trisodium citrate, sodium thiosulfate, sodium oxalate, magnesium sulfate, and sodium hydroxide were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. in China. Analytical grade hydrogen peroxide, ethanol, and hydrochloric acid were purchased from Chengdu Kelong Chemical Reagent Factory in China. Xylanase was purchased from Novozymes in China. Copper ethylenediamine solution was freshly made in the lab.

2.2 Pretreatment procedure

2.2.1 Thermal decomposition treatment and high temperature displacement washing

A 15 L ZQS electro-thermal rotating cooker was purchased from the machinery factory of Shanxi University of Science and Technology. The cooker pot was equipped with high pressure 2 L cans and was heated using an oil bath. Pretreatment was done using a 55% (w/w) ethanol solution and a solid-liquid ratio of 1:8 at 195°C for 60 min. Five different concentrations of acetic acid in the thermal decomposition solution were studied: 0%, 1%, 2%, 4% and 6% (acetic acid: ethanol w/w). Solids that were separated from wheat straw after the thermal decomposition and filtration steps were treated using a thermal displacement washing method in a 2.3 L ZQS electro-thermal rotating cooker manufactured

by the machinery factory of Shaanxi University of Science and Technology. The resultant solids were washed again by DI water in order to remove residual ethanol. Afterwards, crude cellulose was subjected to high temperature thermal displacement washing to remove the residual chemicals in the cellulose product. Three 30-min washes were performed using a 70% v/v ethanol solution, a solid-liquid ratio of 1:8, and a temperature of 145°C.

2.2.2 Refining process from chelating

Chelating was carried out in high-density polyethylene bags which have a melting point between 135°C to 140°C. Chelating treatment of crude cellulose can prevent adverse effects of metal ions during O₃ and H₂O₂ treatment. The treatment conditions for chelation were 6% (w/w) pulp, 0.4% (w/w) EDTA, pH of 6.0, temperature of 90°C, and duration of 80 min. The resultant product was washed by DI water until the wash liquid pH was neutral.

2.2.3 O₃ treatment

O₃ treatment was carried out in graduated cylinders with a rubber cork. Two glass tubes were inserted into the rubber cork for the O₃ gas inlet and outlet. In accordance with literature review of O₃ treatment, the temperature was room temperature, the pH was adjusted to 2.5 by adding HCl, and the pulp concentration was 45% (w/w)^[12]. This treatment involved a gas-solid reaction between O₃ and lignin, which made the crude cellulose fluffy and increased the permeability of O₃. The flow rate of O₃ was determined by titration of KI and Na₂S₂O₃. Treatment time was calculated based on the quantity of O₃.

2.2.4 H₂O₂ treatment

H₂O₂ treatment was carried out in high-density polyethylene bags heated by a water bath. Pulp was vigorously mixed every 10 minutes in order to keep the reaction uniform. The effects of H₂O₂, NaOH, temperature, and time on the refining of cellulose were investigated under the conditions of 12% (w/w) pulp concentration, 1.0% (w/w) tri-sodium citrate, and 0.5% (w/w) MgSO₄.

2.2.5 Xylanase treatment

There is a small amount of hemicellulose still present in the form of xylan after O₃ and H₂O₂ treatment^[13].

Xylanase was used to remove xylan to obtain high-purity cellulose. Xylanase treatment was carried out in an HNY-100D incubation shaker provided by Changzhou Noki Instrument Co., Ltd. of China. The pH of crude cellulose was maintained at 7.0±0.1 by the addition of 10% (w/w) citric acid and 5% (w/w) NaOH. Then xylanase was added and the pulp concentration was adjusted to 3% (w/w) using DI water. The hemicellulose hydrolysis was performed at 50°C for 3 h. Xylanase was de-activated by immersion of mixtures into a 95°C water bath for 15 min. The results of cellulose refining under 0.5-2.5 IU/g on xylanase loading (absolutely dry materials) are further discussed in the results section.

2.3 Analysis and characterization

MCC obtained in each step was washed using DI water. Parameters, such as α -cellulose content, Kappa number, DP and viscosity, were tested according to TAPPI Standards T203 OS-61, T236 OM-2006 and T230 OM-2004. Brightness and pentosan content were analyzed using TAPPI Standards T452 OM-2002 and T223 CM-84. Crystallinity was determined using a Japanese RIGUKU D/MAX-1200 X-ray diffractometer using Cu Ka radiation at a scanning speed of 3 °/min. KMnO₄ values were tested according to Chinese standard GB/T 1547-2004. The morphology of wheat straw was observed by under a microscope (Model PMB5-223IPL-5, Waters, USA). Resultant solids in each step were dried in an electro-thermal drying oven (Model DHG-9053A, Shanghai Chunjia Electronic Equipment Co., Ltd, China). The cellulose yields (*Y*) in each step were calculated using Equation (1).

$$Y = \frac{\text{Absolute weight of cellulose after treatment}}{\text{Absolute weight of cellulose before treatment}} \times 100\% \quad (1)$$

3 Results and discussion

3.1 Thermal decomposition process of wheat straw crude cellulose in ethanol/acetic acid/water ternary solution system

3.1.1 Effects of acetic acid dosage on Kappa number and yield of crude cellulose

The relationship between the acetic acid dosage with a constant ethanol/water ratio and the Kappa number of

crude cellulose was obtained as shown in Figure 1. It is clear that an increase in the acetic acid concentration reduced the Kappa number of crude cellulose, which is an indicator of lignin removal. However, the Kappa number decrease was found to be slower at higher acetic acid dosage, suggesting lignin removal was limited. When the Kappa number dropped to 20.3 (i.e., at high acetic acid dosage), lignin removal rate was low. Therefore, 2% (w/w) acetic acid dosage was chosen to as an optimum condition. The Kappa number rose to 22.8 without acetic acid. An 11% drop of Kappa number showed that the addition of acetic acid assisted lignin removal during the crude cellulose purification process.

The morphologies of crude celluloses treated by different acetic acid dosage were shown in Figure 2a. The dark spots were lignin particles adsorbed on the surface of the crude cellulose. Lignin was primarily observed for the cellulose that was pretreated with no dosage of acetic acid. Figures 2b and 2c show that the

lignin particles adsorbed on the crude cellulose surface disappear gradually with increasing acetic acid dosages. This suggests that lignin dissolved at the beginning of treatment precipitates and decomposes on the cellulose surface that has larger specific surface area and adsorption capacity^[18,19]. This validates the observed results that higher acetic acid dosages resulted in lower residual lignin in the raw cellulose.

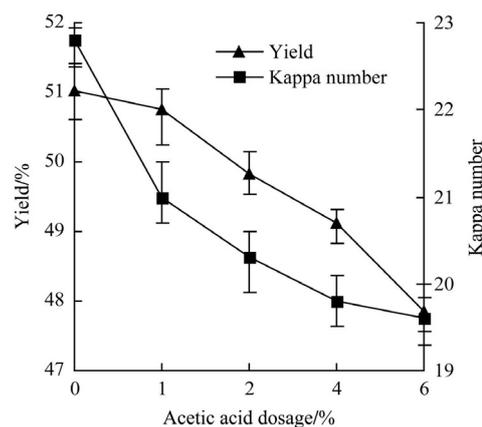


Figure 1 Effect of acetic acid dosage on kappa number and yield of crude cellulose

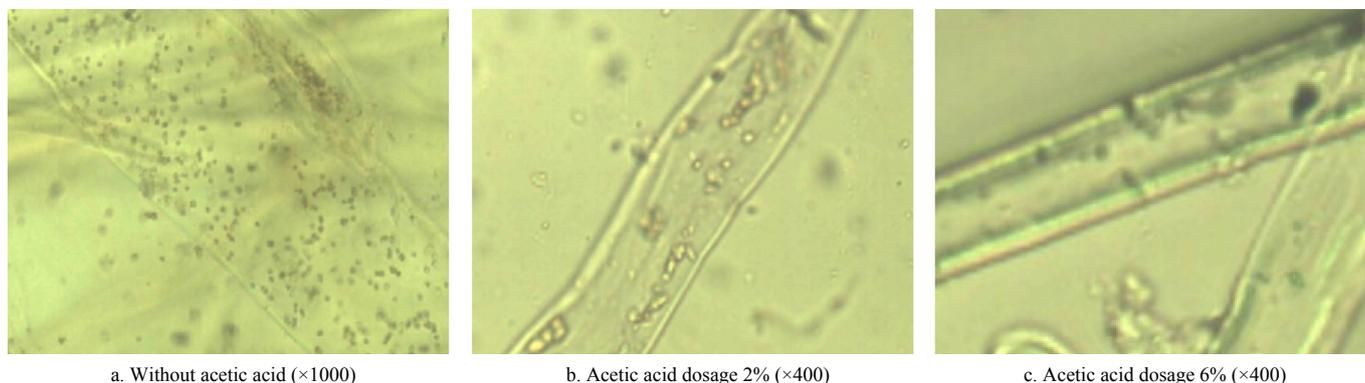


Figure 2 Surface morphology of crude cellulose fiber with different acetic acid dosages

Figure 1 demonstrates that the yield of crude cellulose decreases from 51.02% to 47.85% with the increase of acetic acid dosage from 0% to 6% (w/w). The trend of decrease is intensified with the increase in acetic acid concentration. A comprehensive analysis of the effect of acetic acid dosage on Kappa number shows that lignin removal is obvious accompanied with a slow decrease in yield when the acetic acid dosage is controlled below 2% (w/w). This means the reaction selectivity of acetic acid to lignin is preferred in the mentioned acetic acid dosage, which is favorable for lignin removal. In addition, acetic acid dosage can not only reduce the load of subsequent cellulose refining process but also protect

the strength and yield of cellulose^[20].

3.1.2 Effects of acetic acid dosage on α -cellulose content and crystallinity of the crude cellulose

The α -cellulose content of crude cellulose impacts the yield of cellulose after refining. Therefore, the influence of acetic acid dosage on the α -cellulose content of crude cellulose was studied, as shown in Figure 3. The α -cellulose content rises with the increase of acetic acid dosage; the increase is more predominant when the acetic acid dosage is lower than 2% (w/w). The reason is that the promotion of lignin removal and hemicellulose degradation is more distinguished below this concentration, which increases the purity of crude

cellulose; in other words, the α -cellulose content is raised. However, the α -cellulose content only increased marginally when acetic acid dosage exceeds 2% (w/w). Considering the Kappa number and yield from Figure 1, the promotion of lignin removal and the selectivity of acetic acid to lignin are both debilitated accompanied with intensified acid degradation of cellulose at acetic acid dosages more than 2% (w/w). The loss of yield of approximately 3% (wt) is mainly attributed to the degradation of cellulose. The maximum α -cellulose content was observed to be around 84.3% (wt) at 2% (w/w) acetic acid dosage. This resulted in almost stable α -cellulose content. Therefore, the acetic acid dosage was selected to be 2% (w/w) to maximize the yield and purity of crude cellulose.

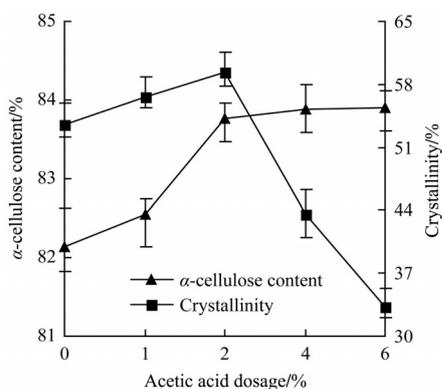


Figure 3 Effects of acetic acid dosage on α -cellulose content and crystallinity of crude cellulose

Cellulose is composed of both crystalline and amorphous regions. MCC is mainly extracted from the crystalline region of cellulose; the larger the crystalline region, the higher the yield of MCC. Therefore, it is necessary to analyze the crystallinity of crude cellulose. From Figure 3, acetic acid dosage has a large influence on the crystallinity of crude cellulose. The crystallinity of crude cellulose increased initially and then decreased when acetic acid dosage was higher than 2% since at lower dosages (<2%) acetic acid degraded mostly hemicellulose and lignin. However, at higher acetic acid dosages, the selectivity of lignin/hemicellulose to cellulose degradation decreased. Therefore, the cellulose crystallinity was decreased. The maximum crystallinity value of 59.4% was observed when the concentration of acetic acid was 2% (w/w). The selectivity of acetic acid is better when its concentration

is lower than 2% (w/w). However, selectivity of acetic acid to lignin becomes trivial when its concentration is more than 2% (w/w). In addition, the removal rate of lignin is slower accompanied with the intensified acid degradation of cellulose. The decline is not favorable for the yield of cellulose hydrolysis or the physical property of microcrystalline cellulose^[21]. Therefore, the optimal acetic acid dosage is confirmed to be 2% (w/w).

3.2 Ozone refining process

3.2.1 Effects of ozone dosage on potassium permanganate value and yield of refined purified cellulose

The crude cellulose extracted after the organosolv process was subjected to ozonolysis in order to remove the residual lignin. In this treatment, the KMnO_4 value was measured to characterize the lignin content in crude cellulose during O_3 treatment. The relationships between the O_3 dosage and KMnO_4 value as well as the yield of crude cellulose after O_3 treatment are presented in Figure 4. O_3 is a strong oxidant which has a very obvious effect on lignin removal, resulting in a rapid decrease in the KMnO_4 value of crude cellulose. The KMnO_4 value drops very quickly, especially when the O_3 dosage is between 0.2% and 0.8%, and then tends to stabilize with increasing O_3 dosage. This indicates the effect of lignin removal using O_3 is significant when O_3 dosage is lower than 0.8%. The KMnO_4 value drops slowly due to the poor selectivity of O_3 to lignin after the O_3 dosage is raised to 0.8%. It can be concluded that the optimal O_3 dosage is 0.8%-1.6%.

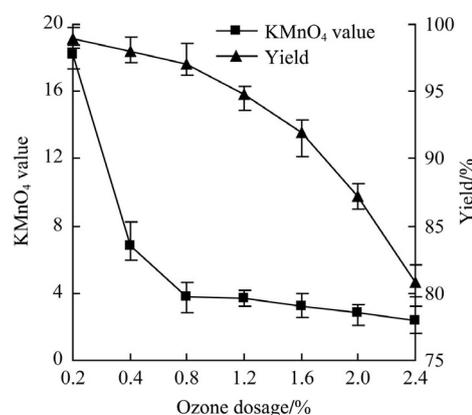


Figure 4 Effects of O_3 dosage on KMnO_4 value and yield of O_3 purified cellulose

It is revealed in Figure 4 that the yield decreases gradually with increasing O_3 dosage. The yield is only

80.9% when O₃ dosage reaches 2.4%, which indicates a serious loss of crude cellulose. The reasons for the decrease include the removal of most of the residual lignin and the oxidative degradation of hemicellulose and cellulose in crude cellulose resulting from large O₃ dosage. It is known that most of the lignin has been removed when O₃ dosage is 0.8%. The KMnO₄ value drops slowly if the O₃ dosage is kept rising. In this situation, cellulose loses its impermeable lignin seal and reacts with O₃ more rapidly, which leads to the intensified degradation of cellulose and drop of yield. It also means the selectivity of O₃ to lignin is poor.

3.2.2 Effects of ozone dosage on degree of polymerization and α -cellulose content of refined crude cellulose

The DP of cellulose was measured because it is a commonly accepted indicator for refined cellulose quality. The DP of crude cellulose is also influenced by O₃ dosage, as shown in Figure 5. The decrease of DP is initially slow but becomes rapid after O₃ dosage is more than 1.6%. This can be explained by the following: firstly, the glucoside bond is cleaved at the center or the end of cellulose molecular chain by O₃. Then, the amount of de-polymerization increases with the increase in O₃ dosage, increasing the possibility of terminating oxidation. Finally, most of the O₃ is consumed by removal of residual lignin and hemicellulose degradation as well as a small amount of amorphous cellulose degradation when the O₃ dosage is lower than 1.6%. However, crystalline cellulose degradation is enhanced when O₃ dosage is more than 1.6%, resulting in the sharp drop of DP. It is revealed from the relationship between O₃ dosage and α -cellulose content that α -cellulose content increases but tends to be stable once O₃ dosage rises to 1.2%. This indicates that an O₃ dose of 1.2% is sufficient for refining^[22].

The brightness is another important parameter for MCC. The brightness continues to increase with increasing O₃ dosage from 26.4% ISO to 61.5% ISO, but is still below the required value of 75%-85%. Nevertheless, brightness cannot be improved by simply increasing O₃ dosage without considering the intensive decrease of yield and DP. Therefore, treatment only by

O₃ cannot meet the required brightness. Alkaline H₂O₂ treatment can be applied with the O₃ treatment in order to increase the brightness.

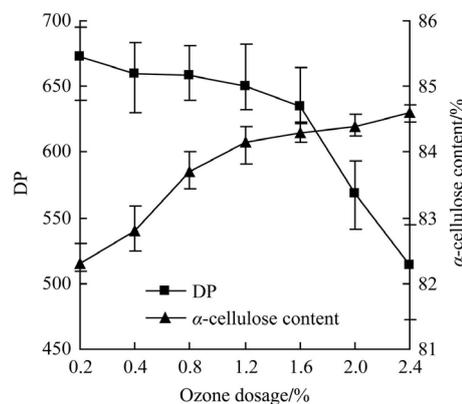
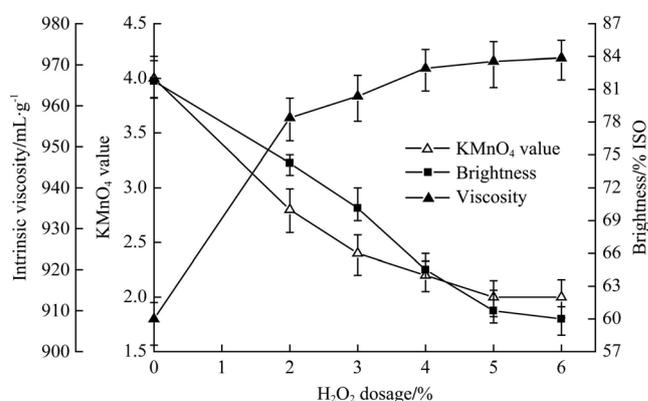


Figure 5 Effects of O₃ dosage on α -cellulose content and DP of O₃ purified cellulose

3.3 Investigation of hydrogen peroxide refining process

3.3.1 Effects of hydrogen peroxide of dosage on crude cellulose refining

The H₂O₂ dosage level is one of the important effects that influences the crude cellulose refining. The brightness of refined cellulose increases rapidly and reaches 83.0% ISO when H₂O₂ dosage is higher than 4%, as evident from Figure 6. There is also a quick drop of the KMnO₄ value. This may be explained by dissolution of the chromophores of residual lignin with the increase of H₂O₂ dosage. At the same time, the celluloses go through degradation in alkaline environment to some extent, leading to the continuous decline of viscosity and DP^[21,22].



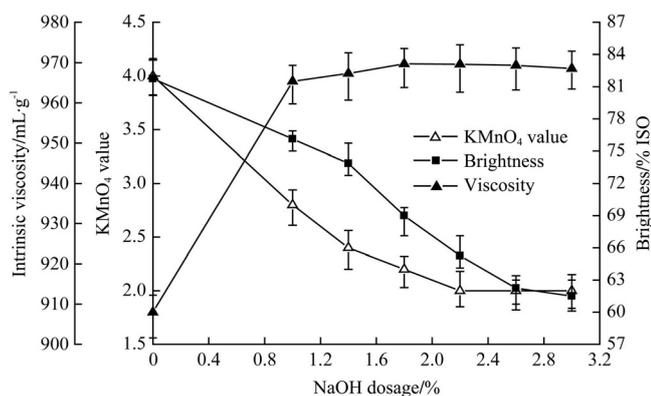
Note: pulp consistency of 12%, H₂O₂ dosage of 3%, temperature of 70°C, time of 120 min, and stabilizer dosage of 1%.

Figure 6 Effects of H₂O₂ dosage on the characteristics of the hydrogen peroxide purified cellulose

The changes of KMnO_4 value and brightness tend to be stable when H_2O_2 dosage is larger than 4%, as presented in Figure 6. However, the rapid drop of viscosity does not become smooth and relatively stable until the H_2O_2 dosage is more than 5%. This is probably because of the lignin removal capacity of H_2O_2 is limited. However, its strong oxidation results in serious degradation of cellulose, leading to the large viscosity drop. Considering the KMnO_4 value and brightness show no significant differences between 3% and 4% H_2O_2 dosage, 3% H_2O_2 dosage is selected in order to maintain higher viscosity.

3.3.2 Effects of NaOH dosage on crude cellulose refining by H_2O_2

The relationship of NaOH dosage to the properties of refined cellulose by H_2O_2 is shown in Figure 7.



Note: pulp consistency of 12%, NaOH dosage of 2%, temperature of 70 °C, time of 120 min, and stabilizer dosage of 1%.

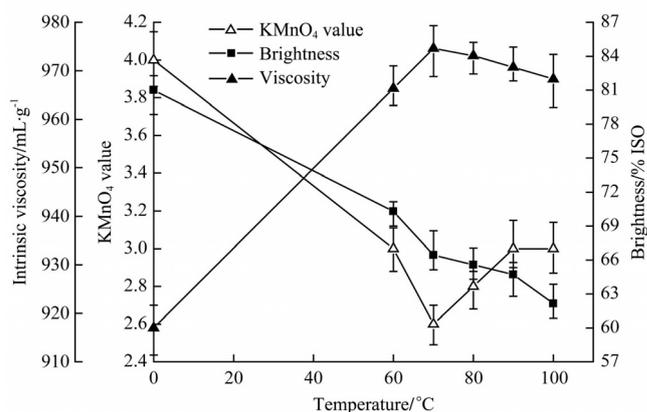
Figure 7 Effects of NaOH dosage on the characteristics of hydrogen peroxide purified cellulose

The KMnO_4 value decreases gradually with the increase of NaOH dosage. The KMnO_4 value tends to be constant when NaOH dosage is more than 2.2%. The brightness increases and then decreases slowly after reaching the peak value of 83.1% ISO at 1.8% of NaOH dosage. The viscosity decreases continuously^[17,19]. The reason for the observed trends is that the increase of OH^- concentration promotes the ionization of H_2O_2 to generate HOO^- , which can attack lignin. This results in the drop of KMnO_4 value and the increase of brightness. Too much NaOH enhances inefficient decomposition of H_2O_2 , which is not favorable for HOO^- generation and inhibits the refining of crude cellulose. The increased alkaline concentration speeds up the degradation of

cellulose, which favors the stabilization of KMnO_4 value and brightness and the continuous decline of viscosity. Therefore, the optimal NaOH dosage is 1.8%^[21].

3.3.3 Effects of temperature on crude cellulose refining by H_2O_2

The influence of temperature on crude cellulose refining by H_2O_2 is shown in Figure 8. KMnO_4 value dropped by 0.4, brightness increased by 3.6% ISO, and intrinsic viscosity decreased by 9 mL/g when the temperature was raised from 60°C to 70°C^[16]. This indicates that an increase in temperature improved the oxidation of H_2O_2 , resulting in better refining. However, the KMnO_4 value began to increase, brightness began to decrease, and the viscosity decreased continuously when temperature continued to increase. This is probably because of more intensive decomposition of H_2O_2 and cellulose degradation at high temperature. Therefore, the optimal temperature for refining is 70°C^[14].



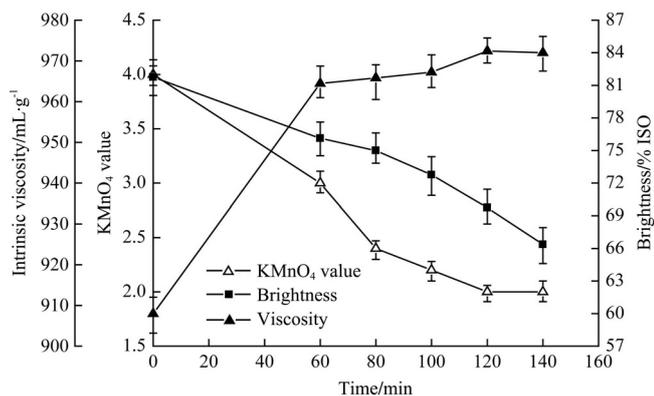
Note: pulp consistency of 12%, H_2O_2 dosage of 3%, NaOH dosage of 1.8%, time of 120 min, and stabilizer dosage of 1%.

Figure 8 Effects of reaction temperature on the properties of the hydrogen peroxide purified cellulose

3.3.4 Effects of treatment time on crude cellulose refining by H_2O_2

Temperature and time are related factors. The increase of temperature results in increasing reaction rates and consequently decreasing reaction times if other factors are kept constant. The effect of time on crude cellulose refining by H_2O_2 is shown in Figure 9. An increase in the refining time from 60 min to 120 min led to a drop in the KMnO_4 value from 3.0 to 2.0 and an increase in the brightness from 80.7% ISO to 84.2% ISO. However, KMnO_4 value was unchanged and the brightness changed slowly if the treatment time was

increased above 120 min. This indicates that the refining effect cannot be improved only by elongation of refining time. It was also observed that the viscosity continuously decreased due to the cellulose decomposition in alkaline environment with the increase of refining time. Therefore, the optimal refining time is 120 min. The parameters of celluloses refined by H₂O₂ are listed in Table 1^[12,13].



Note: pulp consistency of 12%, H₂O₂ dosage of 3%, NaOH dosage of 1.8%, temperature of 70°C, and stabilizer dosage of 1%.

Figure 9 Effects of reaction time on the properties of hydrogen peroxide purified cellulose

Table 1 Parameters of refined cellulose

α -cellulose content/%	Pentosan content/%	DP	Brightness/%ISO
84.28	9.01	626	84.16
94.67	4.29	615	85.60

Note: first row: H₂O₂ dosage of 3%, NaOH dosage of 1.8%, temperature of 70°C, time of 120 min. second row: the optimized condition.

3.4 Effects of xylanase on the crude cellulose refining

The first row of Table 1 shows that the α -cellulose content of wheat straw was higher compared to initial composition. However, the amount of pentosan content was also high. In order to remove remaining pentosan, xylanase enzymes were used for further refining. The effects of xylanase dosage on the α -cellulose content and xylan content were studied as shown in Figure 10.

From Figure 10, α -cellulose content rises as hemicellulose content drops rapidly after treatment using xylanase enzyme. The α -cellulose content increased from 84.3% to 87.0% at a xylanase dose of 0.5 IU/g. The highest α -cellulose content was observed to be 95.4% at a xylanase dose of 2.5 IU/g. The results showed that enzyme treatment can hydrolyze most of the pentosan, which is mainly xylan. Considering the cellulose content and cost of production, the optimal xylanase dose

is 1.5 IU/g. At optimum conditions, the pentosan content dropped from 9.0% to 4.3% (w/w)^[15,16].

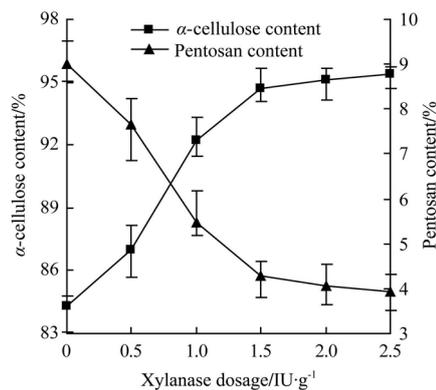


Figure 10 Effects of xylanase dosage on α -cellulose content and pentosan content

The parameters of the resultant α -cellulose obtained under the optimized thermal decomposition, separation and refining processes are shown in Table 1.

4 Conclusions

The organosolv method is an effective way to decompose the recalcitrant nature of wheat straw and to extract and separate crude cellulose. This process is environmentally friendly, and the solution can be recycled. The addition of acetic acid enhances the removal of lignin and degradation of hemicellulose, which improves the purity of crude cellulose. The lignin removed by acetic acid is mainly adsorbed and deposited on the surface of cellulose. Based on the experimental results, acetic acid dosage in the wheat straw was optimized. This was followed by chelation, O₃, and H₂O₂ xylanase treatment. Overall, the production of MCC is more economical since it is produced from wheat straw as compared to cotton or wood. In addition, the organosolv agents have low vapor pressure and they are easily recyclable at low temperatures, making the process more economical and more environmentally friendly. The process can lead improved MCC production from wheat straw and may lead to commercialization.

Acknowledgements

The authors wish to acknowledge specialized research funding of the Doctoral Program of Higher Education Projects (for the priority development areas) by Ministry of Education of China (Grant No. 20126125130001),

State Key Laboratory of Pulp and Paper Engineering open foundation (Grant No. 201505), and the Doctoral Scientific Research Foundation (Grant No. BJ13-02) by Shaanxi University of Science and Technology. We thank Stacy Bjorgaard at the University of North Dakota for proofreading the paper. .

[References]

- [1] Diaz M J, Eugenio M E, Lopez F, Alaejos J. Paper from olive tree residues. *Industrial Crops and Products*, 2005; 21(2): 211–221.
- [2] Kamireddy S R, Li J, Abbina S, Berti M, Tucker M, Ji Y. Converting forage sorghum and sunnhemp into biofuels through dilute acid pretreatment. *Industrial Crops and Products*, 2013; 49(7): 598–609.
- [3] Yan L, Li F W, Yang J L, Zhu Q S. Direct visualization of straw cell walls by AFM. *Macromolecules Bioscience*, 2004; 4(2): 112–118.
- [4] Kamireddy S R, Li J, Degenstein J, Tucker M, Ji Y. Effects and mechanism of metal chlorides salts on pretreatment and enzymatic digestibility of corn stover. *Industrial Engineering and Chemistry Research*, 2013; 52(5): 1775–1782.
- [5] He Y L, Liao X X, Huang K L, Wu R, Wang B, Liu Y H, et al. Study process of microcrystalline cellulose. *Technology & Development of Chemical Industry*, 2010; 39 (1): 12–16.
- [6] Wang D, Shang S B, Song Z Q, Lee M K. Evaluation of microcrystalline cellulose prepared from kenaf fibers. *Journal of Industrial Engineering and Chemistry*, 2010; 16: 152–156.
- [7] Berti M T, Kamireddy S R, Ji Y. Row spacing affects biomass yield and composition of kenaf (*Hibiscus cannabinus L.*) as lignocellulosic feedstock for bioenergy. *Journal of Sustainable Bioenergy Systems*, 2013; 3: 68–73.
- [8] Li J B, Zhang M Y, Liu Y S, Zhang Y. Preparation and characterization of wheat straw MCC for synthetic leather. *Journal of Functional Materials*, 2011; 42(9): 1606–1609.
- [9] Kamireddy S R, Degenstein J, Berti M, Ji Y. Pretreatment and enzymatic hydrolysis of kenaf biomass as a potential source for lignocellulosic biofuel and green chemicals. *Journal of Current Organic Chemistry*, 2013; 17(15): 1–9.
- [10] Degenstein J, Kamireddy S R, Tucker M, Ji Y. Oligomer saccharide reduction during dilute acid pretreatment co-catalyzed with Lewis acids on corn stover biomass. *Int J Agric & Biol Eng*, 2013; 6(2): 54–62.
- [11] Ejikeme P M. Investigation of the physicochemical properties of microcrystalline cellulose from agricultural wastes I: Orange mesocarp. *Cellulose*, 2008; 15: 141–147.
- [12] Kamireddy S R, Schaefer C, Defrese M, Degenstein J, Ji Y. Pretreatment and enzymatic hydrolysis of sunflower hulls for fermentable sugar production. *Int J Agric & Biol Eng*, 2012; 5(1): 62–70.
- [13] Sakhawy M E, Hassan M L. Physical and mechanical properties of microcrystalline cellulose prepared from agricultural residues. *Carbohydrate Polymers*, 2007; 67: 1–10.
- [14] Kamireddy S R, Degenstein J, Berti M, Ji Y. Pretreatment and enzymatic hydrolysis of kenaf biomass as a potential source for lignocellulosic biofuel and green chemicals. *Journal of Current Organic Chemistry*, 2013; 17(15): 1–9.
- [15] Xu Y J, Li K C, Zhang M Y. Lignin precipitation on the fibre surface in the ethanol-based organosolv pulping. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2007; 301(1): 255–263.
- [16] Xu Y J, Zhang M Y. Removal of residual lignin of ethanol-based organosolv pulp by an alkali extraction process. *Journal of applied polymer science*, 2007; 106, 630–636.
- [17] Xu Y, Ren W, Liu S. Auto-catalyzed ethanol-water pulping of wheat straw. *Journal of Northwest Institute of Light Industry*, 1999; 17(2): 40–44.
- [18] Kamireddy S R, Kozliak E, Tucker M, Ji Y. Determining the Rate Constants of sunflower hulls using dilute acid pretreatment in the production of xylose and furfural. *Green Processing and Synthesis*, 2014; 3(1): 69–75.
- [19] Zhang M. Features of non-wood fiber auto-catalyzed ethanol pulping. *Transaction of China Pulp and Paper*, 2004; 19(2): 16–20.
- [20] Zhang M, Xu Y, Pu W. The advance of non-timber fiber self-catalysis ethanol pulping study. *China Paper Industry*, 2007; 28(6): 77–79.
- [21] Shatalov A A, Pereira H. O₃-based TCF bleaching of organosolv pulps. *Bioresource Technology*, 2008; 99: 472–480.
- [22] Xu Y J, Zhang M Y, Li K C. Study on phenomenon and mechanism of Lignin precipitation during ethanol pulping. *Transaction of China Pulp and Paper*, 2008; 23(1): 1–8.