Preparation of nanocellulose from steam exploded poplar wood by enzymolysis assisted sonication

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Abstract
By exploiting cellulose nanofibril’s high aspect ratio and nano-order-unit interconnected web-like structure of poplar wood, a new approach was designed. Steam explosion was used for the pretreatment of the poplar, followed by enzymatic hydrolysis assisted sonication for the preparation of nanocellulose. The effects the cellulase dosage, enzymatic hydrolysis time and temperature on the yield of nanocellulose were studied. Under optimal conditions nanocellulose yield was approximately 13.2%. The chemical composition, crystallinity, and morphology of the composites were characterised using FT-IR, x-ray diffraction and TEM. The results demonstrated that the structure was not destroyed during the preparation process, that the crystal form remained cellulose I, and the crystallinity was 61.98%, 9.15% higher than that of poplar cellulose. The width was between 20 and 50 nm, with high aspect ratio and a web-like entangled structure. Therefore, nanocellulose prepared using this method is an ideal toughening material that could be applied in composite materials.

1. Introduction
Poplar is a fast-growing species of tree and is widely planted and abundantly available. It is an important industrial raw material, and is mainly used for the production of plywood, veneer laminated wood, furniture, fiberboard, etc. A small portion of the processing residue is used in papermaking, but the majority is simply discarded or burned, resulting in tremendous waste and a negative environmental impact. Poplar wood processing residue contains 48.05% cellulose, 25.93% hemicellulose and 19.85% lignin [1]. Due to its high content of cellulose, poplar wood processing residue is also a good source of cellulose, and can be employed in the preparation of nanocellulose. This will promote high value usage of poplar wood and a more efficient use of industrial waste.

Nanocellulose possesses many unique properties compared to natural cellulose, including natural renewability, biodegradability, biocompatibility, high strength coefficient, high specific surface area, high aspect ratio, dimensional stability, and thermal stability (~200 °C). It is widely used in the production of optical transparent materials, reinforced polymer nanocomposites, biomimetic materials, sensors and energy collectors [2, 3]. Jabbour et al. [4] prepared a layered porous nanofibers/graphite-based lithium battery cathode material, which is highly flexible and has good mechanical properties, whilst maintaining high chargeability following many charging-discharging cycles.

Reports have shown that nanocellulose can be prepared using a verity of methods, which are divided into three types [5—6]: (1) Cellulose nanofibers, which are mainly prepared using a chemical-physical method, with an average size of 5–60 nm and a few microns in length. (2) Nanocellulose crystals are mostly prepared by acid
hydrolysis of fibres, with an average size of 5–70 nm and 100–250 nm in length. Bacterial nanocellulose is synthesised using bacteria and has an average size of 2–10 nm in width and around 100 nm in length [7].

During the preparation of nanocellulose, acid hydrolysis is normally employed, and sulphuric acid is used as an inorganic acid [8–9]. The utilization of sulphuric acid evokes certain disadvantages. The introduction of a sulphuric acid group into the hydrolysis process promotes the degradation of cellulose [6], particularly at high temperatures, which influences its thermal stability and alters performance. Tang et al [10] used phosphoric acid to treat cellulose for the preparation of nanocellulose. In general, acid hydrolysis requires the treatment of large amounts of acid, which results in the generation of unwanted pollutes. In the case of enzymatic hydrolysis under mild conditions, such pollutants are not generated and also the process consumes less water, but results in low yield. Therefore, other methods are often used to assist the process [11–12]. Cellulase comprises three major highly specific enzymes namely; the endo-glucanases, the exo-glucanases and β-glucosidases catalyses the conversion of cellulose to glucose in an enzymatic hydrolysis. Zhu et al [13] treated bleached sulphate eucalyptus pulp with cellulase, followed by mechanical homogenisation, giving a highly crystalline nanocellulose, where the crystallinity increased by 24% compared with the original cellulose. A hydrolysate treated cellulase can also be converted into ethanol using yeast fermentation at 92% conversion rate. Deepa et al [14] extracted nanofibers from banana fibers via a steam explosion technique. Assessment of fiber chemical composition before and after treatment showed evidence for the removal of hemicelluloses and lignin that occurred during steam explosion treatments. Percentage yield and aspect ratio of the nanofiber obtained by this technique is found to be very high in comparison with other conventional methods.

In this paper, poplar wood was pretreated using steam explosion, and then enzymatic hydrolysis assisted sonication was used for the preparation of nanocellulose. Steam explosion pretreatment not only removed several hemic-fibres, but also aided the removal of lignin through sodium chlorite and enzymatic hydrolysis of cellulase. The effects of cellulase dosage, enzymatic hydrolysis time and enzymatic hydrolysis temperature on the yield of poplar nanocellulose were studied, and the chemical compositions, crystalline forms, and crystallinity and morphology characteristics were all characterised and analysed.

2. Experiment

2.1. Materials

Poplar wood was collected from Changde City in the Hunan Province in China. Cellulase was purchased from Novozymes. Sodium chlorite was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Phosphoric acid was purchased from Chongqing East Sichuan Chemical Co., Ltd. Glacial acetic acid was purchased from Sinopharm Chemical Reagent Co., Ltd. All chemical reagents were of analytical grade.
2.2. Preparation of nanocellulose

2.2.1. Preparation of poplar cellulose

2.2.1.1. Steam explosion pretreatment

Naturally air-dried poplar wood was crushed into sawdust, which had a diameter of 0.2–0.8 cm. The poplar wood sawdust was pre-impregnated for 1 h in 2 wt% dilute phosphoric acid, and then mixed using a solid-liquid ratio of 1:2. The pre-impregnated wood sawdust was then blasted under 2 MPa pressure for 180 s \[15\]. The explosives were collected, washed it using suction, and dried ready for further use.

2.2.1.2. Delignification

Under acidic conditions (pH 4-5, acetic acid adjusted), 2 g of treated poplar wood powder (based on its dry weight) was dispersed in 60 ml of sodium chlorite solution (9.7 g/l) for 1 h at 75°C. It was then subjected to conventional washing operations: deionized water was filtered several times, then rinsed with absolute ethanol, and finally washed in deionized water and dried giving poplar cellulose.

2.2.2. Enzymatic hydrolysis

(1) 0.5 g poplar cellulose was dispersed in 25 ml of acetic acid buffer solution (pH 4.7) in a 100 ml flask. 50(or 100, 150, 200, 250) U/g of cellulase enzyme (enzyme activity 1400 U g\(^{-1}\)) was added and shaken at 200 rpm for 12 h at 50°C, using a constant temperature shaker (760R, A Plus, USA).

(2) 0.5 g of poplar cellulose was dispersed in 25 ml of acetic acid buffer solution (pH 4.7) in a 100 ml flask. 200 U/g of cellulase enzyme (enzyme activity 1400 U g\(^{-1}\)) was added and shaken at 200 rpm for 8(or 12, 16, 20, 24) h at 50°C, using a constant temperature shaker (760R, A Plus, USA).

(3) 0.5 g of poplar cellulose was dispersed in 25 ml of acetic acid buffer solution (pH 4.7) in a 100 ml flask. 200 U/g of cellulase enzyme (enzyme activity 1400 U g\(^{-1}\)) was added to the flask and shaken at 200 rpm for 12 h at 50(or 40, 40, 60)°C using a constant temperature shaker (760R, A Plus, USA).

2.2.3. Ultrasonic treatment

Following the enzymatic reaction, cellulose was inactivated in a water bath at 95°C over a period of time and then treated using an ultrasonic cell pulveriser (SCIENTZ-IID, Ningbo Xingzhi Biotechnology Co., Ltd., China.) for 20 min at 200 W. The sample was then centrifuged at 11,000 rpm for 10 min and the supernatant was poured out to remove cellulose. The process was repeated several times until pH 7 solution was obtained. The speed was adjusted to 2,000 rpm for 10 min, and the turbid upper liquid was collected, which contained nanocellulose. This process was repeated several times until the turbid upper liquid became clear. Finally, the suspension was dried in an oven at 60°C until the moisture content was reduced to 25–30 ml. It was then freeze-dried (FD-1C, Beijing Detianyou Technology Development Co., Ltd) to constant weight prior to characterization \[16\].

2.3. Analysis and characterisation

2.3.1. Chemical composition analysis

The chemical compositions (cellulose, hemicellulose, lignin) of poplar wood, steam explosion-treated poplar wood and poplar wood cellulose were determined according to the Laboratory Analytical Procedures of National Renewable Energy Laboratory.

2.3.2. Nanocellulose yield calculation

The nanocellulose yield (%) was calculated using the following equation \[17\]:

\[
\text{yield(\%)} = \left( \frac{m_1 - m_2}{m_3V_1} \right) \times 100\%
\]

where \(m_1\) is the total mass of the oven-dried nanocellulose and bottle, \(m_2\) is the weight of the bottle, \(m_3\) is the weight of the poplar cellulose, \(V_1\) is the total volume of nanocellulose suspension, \(V_2\) is the volume of nanocellulose suspension for oven-drying.

2.3.3. Particle size and zeta potential analysis

The dried samples were re-dispersed in distilled water, and sonication using an ultrasonic processor. The measurements of particle size distribution, average particle size and zeta potential of the nanocellulose samples were obtained using Zetasizer Nano ZS 90 (Malvine, UK).
2.3.4. Fourier transform infrared spectroscopy analysis
The chemical structure of poplar, steam explosion treated poplar, poplar cellulose and nanocellulose was analysed using Fourier Transform Infrared Spectroscopy (Nicolet Avatar-330, USA).

2.3.5. X-ray diffraction analysis
The crystalline structures of poplar, steam explosion treated poplar, poplar cellulose and nanocellulose were analysed using x-ray diffraction (Xpert Pro, Netherlands Panaco) in continuous scanning mode with an angular range 2θ from 10° to 50°. The scanning rate was 2 °/min and the step was 0.02°. Based on XRD data, the crystallinity index (CrI, %), which expressed the relative degree of crystallinity, was calculated using a method as described (Segal et al 1959).

2.3.6. TEM observations
The microscopic morphology was characterised using a transmission electron microscope (Jeol 2100F). A uniformly dispersed nanocellulose suspension droplet was placed on a carbon-coated grid. The sample was dried at room temperature prior to analysis. The working voltage was 80 KV [18].

3. Results and discussion

3.1. Chemical composition analysis
The fibre chemical composition components of poplar wood, steam explosion-treated poplar wood and poplar wood cellulose are displayed in table 1. The results demonstrate that most hemicellulose and some lignin in poplar wood are removed following steam explosion treatment. Sodium chlorite treatment removed almost all the remaining lignin and hemicellulose. Lignin removal rate is 83.5%, and cellulose content is 81.8%, which was used in the preparation of nanocellulose.

### Table 1. Chemical composition.

| Component     | Poplar wood | Blast poplar | Poplar cellulose |
|---------------|-------------|--------------|------------------|
| Cellulose (%) | 47.0        | 51.0         | 81.8             |
| Hemicellulose (%) | 26.2        | 12.4         | 10.1             |
| Lignin (%)    | 19.7        | 18.2         | 3.0              |

3.2. Influence of enzymatic hydrolysis conditions on the yield

3.2.1. Influence of enzyme dosage on the yield
As shown in figure 1, the yield of cellulose nanocrystals is dependent on enzyme dosage. At low dosage most of the cellulose remained, resulting in low yield. As enzyme dosage increases, the cellulose nanoparticle yield also increases. When the enzyme dosage exceeds 200 U/g, the yield decreases. Therefore, to ensure optimal yield of nanocellulose, the enzyme dosage was 200 U/g.

3.2.2. Influence of time on enzymatic hydrolysis
As shown in figure 2, the time of enzymatic hydrolysis influences the final yield. After 12 h the yield is optimal, however, as the reaction time is prolonged the nanocellulose hydrolysates to glucose decreasing the yield. Due to limited binding sites, the yield of cellulose nanoparticles tended to stabilise after 16 h of enzymatic hydrolysis.

3.2.3. Influence of temperature on enzymatic hydrolysis yield
Figure 3 shows that temperature has a great influence on the enzymatic hydrolysis yield. The optimum temperature is 50 °C. Below this temperature, enzyme activity is slow, resulting in lower yield. Beyond this temperature the enzyme becomes inactivate, hence, a gradual decrease in yield is observed.

3.3. Nanocellulose size and zeta potential analysis
The average particle sizes of the samples are shown in figure 4. It is apparent that sonication plays an important role in particle size. The average particle size of nanocellulose (figure 4(a)) without sonication is 1600 nm, whereas sonicated nanocellulose’s particle size is significantly reduced. Enzymatic hydrolysis temperature also influences the average particle size. At 40 °C (figure 4(b)), the average particle diameter is 674 nm. When the enzymatic hydrolysis temperature increases to 50 °C (figure 4(c)), the particle diameter reduces to 310 nm.
However, at 60 °C (figure 4(d)), the average particle size increases to 565 nm due to changes in enzyme activity. Optimal enzymatic hydrolysis and particle size is obtained at 50 °C.

Figure 5 proves that the zeta potential of the sample is negative. The surface of the sample is negatively charged in water. The absolute value of cellulose nanoparticles, which are untreated by sonication is 21 mV, which is lower than that of cellulose nanoparticles which were treated by sonication. Enzymatic hydrolysis temperature has little effect on the zeta potential, but its optimal value is obtained at 50 °C.

3.4. FT-IR spectra analysis
The FT-IR spectra of poplar wood, steam explosion treated poplar, poplar cellulose and nanocellulose are displayed in figure 6. Characteristic peaks of cellulose molecules include the stretching vibration of –OH at 3420 cm\(^{-1}\), the bending vibration of –CH\(_2\) and –OCH at 1430 cm\(^{-1}\), the stretching vibration of C–O at 1059 cm\(^{-1}\), and the isotope carbon (C\(_1\)) vibration at 890 cm\(^{-1}\). In the case of the stretching vibration absorption peak of –OH at 3420 cm\(^{-1}\), the absorption band gradually narrows with the enzymolysis treatment of the sample. This is mainly due to the hydrogen in cellulose being destroyed during the preparation process. In the spectrum of steam explosion treated poplar (figure 6(b)), the intensity of the deformation vibration of –CH\(_2\) at 1460 cm\(^{-1}\) decreases, and disappears in the spectrum of poplar cellulose (figure 6(c)). This change indicates that the side chain of lignin is degraded.
Figure 3. Effect of temperature on enzymatic hydrolysis yield.

Figure 4. Nanocellulose average particle size of enzymolsysis at 50°C without sonication (a), at 40°C with sonication (b), at 50°C with sonication (c), and at 60°C with sonication (d).

Figure 5. Zeta Potential of enzymolsysis temperature 50°C without sonication (a), enzymolsysis temperature at 40°C with sonication (b), at 50°C with sonication (c), and at 60°C with sonication (d).
3.5. X-ray diffraction analysis

Figure 7 shows the x-ray diffraction patterns of steam explosion treated poplar, poplar cellulose and nanocellulose. Sample (a) shows three diffraction peaks are observed at $2\theta = 16.5^\circ$, 22.6°, and 34.3°, corresponding to (110) crystal plane, (200) crystal plane, and (004) crystal plane, respectively, which belonged to cellulose I. The diffraction peak positions of sample (b) and (c) are identical with those of sample (a), which belong to cellulose I. This proves that nanocellulose is still the native cellulose, and retains its monoclinic structure. Based on the Segal method, the crystallinities of the steam explosion treated poplar, poplar cellulose and nanocellulose are 44.95%, 52.83%, and 61.98%, respectively. The crystallinity of the samples gradually increases, due to removal of some amorphous parts of lignin, hemicellulose, and cellulose during the preparation process.

3.6. TEM observations

TEM images of nanocellulose after enzymatic hydrolysis (200 U/g, 12 h, 50 °C) / sonication process are shown in figure 8. Figures 8(a) and (b) show that nanocellulose is in filament bundles, but due to their amorphous areas in the internal structure of cellulose, the cellulose still exhibits an agglomeration phenomenon [19]. Figures 8(b) and 8(a) show the width in nanometer-scale using high power electron microscopy, where between 20 and 50 nm, and is incredibly uniform (figure 8(d)). The nanofibrils also entangle the filaments in the network structure, which is caused by interaction between the hydroxyl groups [20] (figures 8(c) and (d)), which greatly increases the interface with the polymer bonding area and plays a toughening role allowing full nano-effect in polymers.
4. Conclusion

Poplar wood was pretreated using the steam explosion method, followed by enzymatic hydrolysis assisted sonication in order to prepare the nanocellulose. The effects of cellulase dosage, enzymatic hydrolysis time and temperature on the yield of nanocellulose were studied. Optimal enzymatic hydrolysis conditions were determined as 200 U/g, 12 h and 50 °C, giving nanocellulose in approximately 13.2% yield. The chemical composition, crystallinity, and morphology of the composites were all characterised using FT-IR, x-ray diffraction and TEM. The results showed that the structure was not destroyed during the preparation process, that the crystal form remained as cellulose I. The crystallinity was 61.98%, which was 9.15% higher than that of poplar cellulose. The width was between 20 and 50 nm, with high aspect ratio, and with a web-like entangled structure. Therefore, nanocellulose prepared using this method is an ideal toughening material, which can be used in the manufacturing of composite materials.

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