Preparation and Characterization of High-purity Cellulose Nanocrystals Films by the Composite Enzymolysis of Cotton Pulp Fibers

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Using cotton pulp fibers as raw material, through composite enzymolysis and purification, two different forms of high-purity cellulose nanocrystals were obtained, namely spherical CNCs (SCNCs) and rod-like CNCs (RCNCs). Vacuum filtration was used to obtain pure SCNCs film and pure RCNCs film. The films were characterized by scanning electron microscopy, ultraviolet–visible spectrometry, and X-ray diffraction and tested for their mechanical properties. The films were shown to have good light transmittance and softness, and the structures were unchanged compared with the cellulose raw materials. This article mainly introduces the preparation process of high-purity cellulose nanocrystals films and provides data support for subsequent research.

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Keywords: Preparation; Composite enzymolysis; Cellulose nanocrystals; CNCs film

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INTRODUCTION

Cellulose, the most abundant biomass on Earth, is a biodegradable homopolymer of a (1,4) connected d-glucose unit, which is a linear-chain polymer d-glucose composed of multiple units connected together in a repetitive, overlapping pattern to produce a polymer with high tensile strength. For industrial use, cellulose is obtained from wood pulp and cotton (Satyamurthy and Vigneshwaran 2013). Nanocellulose is an important cellulose derivative that possesses many of the properties known to be desirable for cellulose, including low density, nontoxicity, and high biodegradability (Hubbe *et al*. 2008). Due to its unique shape, size, surface chemistry, and high degree of crystallinity, it also has unique properties such as high mechanical strength, strengthening ability, *etc*. (Klemm *et al*. 2011; Thomas *et al*. 2018). Among the nanocellulose materials (CNM), the most studied include cellulose nanocrystals (CNCs) and cellulose nanofibers (CNFs). Lu and Hsieh (2010) studied cellulose nanocrystals with rod, sphere, and network morphologies. These were prepared by acid hydrolysis of cotton cellulose, followed by freeze-drying. Nogi and Yano (2009) prepared nanocellulose (CNF) into nanocellulose film for the first time, calling it cellulose nanofiber sheets, and studied its optical properties. Since then, nanocellulose-based films have become a hot topic of broad interest in industry and academia.

Mohamed *et al*. (2017) treated *Ceiba pentandra* with chemical alkali extraction and typical acidified bleaching method to obtain CNCs and examined the physicochemical properties of the nanoporous self-assembled CNC membrane. Sun *et al.* (2018) investigated the tailoring of the properties of thin nanocellulose films by controlling the

weight ratios of CNFs and CNCs in the suspensions. The CNFs and CNCs were prepared using the chemical and acid hydrolysis method, respectively, and the thin nanocellulose films were prepared using vacuum filtration. Hassan *et al*. (2021) studied the physicochemical and electrical properties of nanocellulose films prepared by TEMPO oxidation using coconut residue as raw material. There is also interest in the study of modified nanofiber films, but the authors are not aware of any such studies on high-purity CNC films prepared by composite enzymolysis.

In a previous work (Chen *et al*. 2012), enzymatic hydrolysis of cotton fibers was used to obtain nanocrystalline cellulose, and spherical and rod-like CNCs were prepared by complex enzymatic digestion of eucalyptus pulp fibers. By purification, spherical CNC with a purity of up to 99.99% has been obtained (Xu and Chen 2019). Based on the previous research on obtaining RCNCs and SCNCs (Chen *et al*. 2012, 2019), this paper is the first that discusses the preparation of films by obtaining high-purity spherical and rod-like CNCs by complex enzymatic treatment of cotton fibers. The two forms of nanofiber films were characterized to provide data support for further applications.

EXPERIMENTAL

Materials

Cotton pulpboard was purchased from China Shandong Zhong Paper and Pulp & Paper Co., Ltd. (Shangdong, China). Cellulase (*Aspergillus niger*, enzyme activity: 10000 U/mL) and xylanase (*Trichoderma viride* G, enzyme activity: 20000 U/mL) were purchased from China Ningxia Xiasheng Industrial Group (Ningxia, China). HCl and glycerol were analytical purity and purchased from China Guangzhou Chemical Reagent Factory (Guangzhou, China); 0.1 μm PTFE membrane was purchased from China Shanghai Xingya Purification Company (Shanghai, China).

Preparation High-purity SCNCs Film and RCNCs Film

The preparation of spherical CNCs was basically according to previous work (Xu and Chen 2019). A PFI mill (Mark VI No. 621, Norway) was used to refine 4 g of oven-dry cotton pulpboard to the beating degree of 75 °SR before being put into a 1 L beaker with 200 mL of a 50% (V/V) glycerin solution. To complete the swelling treatment of the fibers, the beaker was shaken at 35 °C, 150 r/min for 4 h in a constant temperature water bath shaker. The mixture was then centrifuged at 5000 revolutions per min for 5 min, discarding the supernatant. 400 mL of distilled water was added to wash the residual glycerol, repeating twice.

The pretreated pulp fibers were mixed with 400 mL of distilled water in a 1 L beaker, and 300 U/mL compound enzymes (xylanase: cellulase = 1:9) were added respectively to obtain CNCs samples at different concentrations of complex enzymes. The reaction mixture was shaken at 50 °C, 150 r/min for 4 h in a constant temperature water bath shaker. The enzymatic hydrolysis solutions were adjusted pH to 2 to 12 with 1 mol/L HCl. The flocculated product solution at pH 2 was centrifuged at 3500 rpm for 10 min to get the precipitate. Then, the precipitate was again dispersed into 400 mL of aqueous solution with pH 2 by sonicating 10 min. This process was repeated twice. The obtained precipitate was sonicated in 400 mL of deionized water and centrifuged at 3500 rpm for 10 min to get the supernatant to obtain the pure spherical CNCs (SCNCs) solution.

Following the above steps, the rod-like CNCs were obtained by changing the reaction time and reaction enzyme concentration. The glycerol-pretreated pulp fibers were mixed with 400 mL of distilled water in 1 L beaker, and 20 U/mL compound enzymes were added respectively to obtain CNCs samples at different concentrations of complex enzymes. The reaction mixture was shaken at 50 °C, 150 r/min for 12 h in a constant temperature water bath shaker and then centrifuged at 3000 rpm for 10 min. The supernatant was vacuum filtered with a 0.1 μm pore PTFE membrane and a circulating water multi-purpose vacuum pump, and 200 mL of distilled water was added to wash twice. The rod-like CNCs were scraped from the filter membrane and redispersed in distilled water to obtain a pure rod-like CNCs (RCNCs) suspension.

CNCs suspensions with a solid content of 0.2 g were vacuum filtered *via* a PTFE membrane with a pore size of 0.1 μm, and then gel-like films were obtained. To create pure CNCs films, the gel-like films were vacuum-dried in a drying box set at 45 °C. The whole process is shown in Fig. 1.

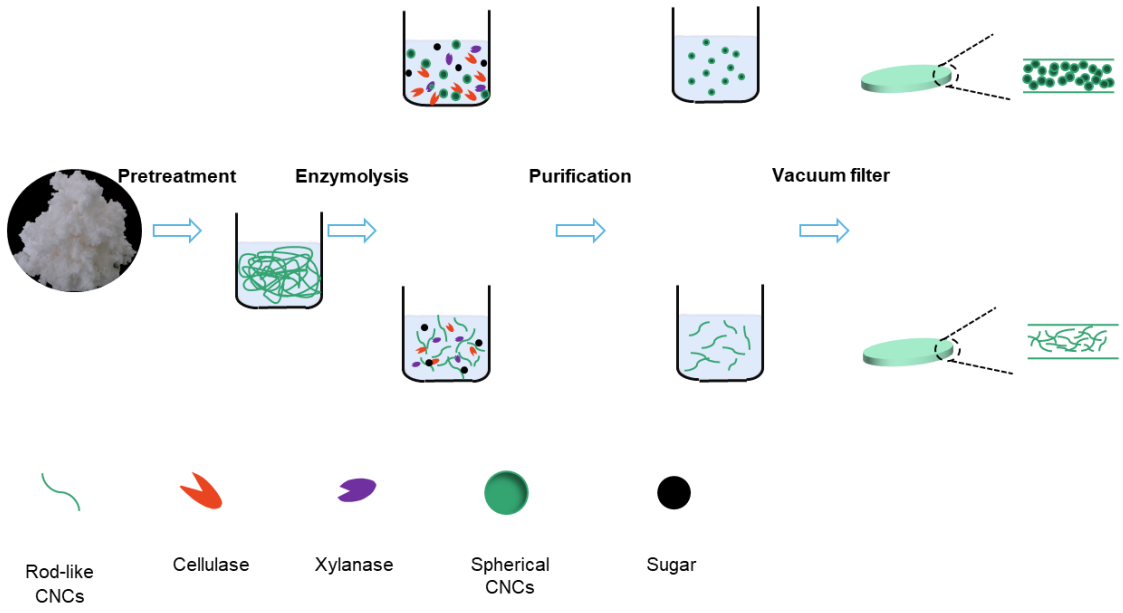

Fig. 1. Preparation of pure SCNCs film and pure RCNCs film (not drawn to scale)

Characterization

Field emission scanning electron microscope (SEM)

One to two drops of 0.05% concentration CNCs samples suspension and CNCs films samples were taken to the mica sheet. After natural airing, the sample was pasted on the conductive sample holder and sprayed with gold. The morphology was observed with a field emission scanning electron microscope (JEOL JSM-7600 F, Tokyo, Japan) at the voltage of 5 kV.

Optical transmittance analysis

The samples were cut into 1 cm × 5 cm long strips and fixed on the UV-Vis spectrophotometer (TU-1810, China) by fixture, and the detection was carried out in the range of 200 to 1000 cm^{-1}.

X-ray diffraction (XRD) analysis

The X-ray diffraction spectra were measured with the Bruker D8 Advance X-ray diffractometer (Karlsruhe, Germany) with an accelerating voltage of 40 kV, current of 40 mA, and scanning range of 5° to 45° (2θ). Pellets were prepared from the dried pure spherical CNCs or eucalyptus pulp fibers. The crystallinity index was calculated by Eq. 1,

$$\mathrm{CrI}=[(I_c-I_a) / I_c]\times 100 \quad (1)$$

where CrI is the crystallinity index (as a percentage), I_c is the peak intensity of crystal plane (002) (maximum intensity at $2\theta = 22.5°$), and I_a is the minimum in intensity corresponding to amorphous content at $2\theta = 18°$.

Mechanical properties analysis

The samples were cut into rectangular strips of 8 mm × 6 mm and placed in a constant temperature and humidity environment (23 °C, 50% relative humidity) for one day for moisture equilibration. The thickness of each strip was measured by a thickness meter (L&W251, Kista, Sweden), and the tensile strength of the composite film was evaluated with an electronic universal testing machine (Instron 5565, Norwood, MA, USA) to obtain the stress-strain curve. Each sample was measured at least three times, and the average value was calculated to show the central tendency.

RESULTS AND DISCUSSION

Characterization of the Pure CNCs Samples

In previous studies, for eucalyptus pulp fibers, the pure CNCs molecules obtained at the enzyme concentration of 300 U/mL were spherical. For the cotton pulp fibers, spherical CNCs were obtained by the same method, and the SEM image is shown in Fig. 2(a). Pure CNCs molecules obtained at the enzyme concentration of 20 U/mL were rod-like, as shown in Fig. 2(b). The surface SEM images of the films made of pure SCNCs and pure RCNCs described above are shown in Figs. 2(c) and 2(d).

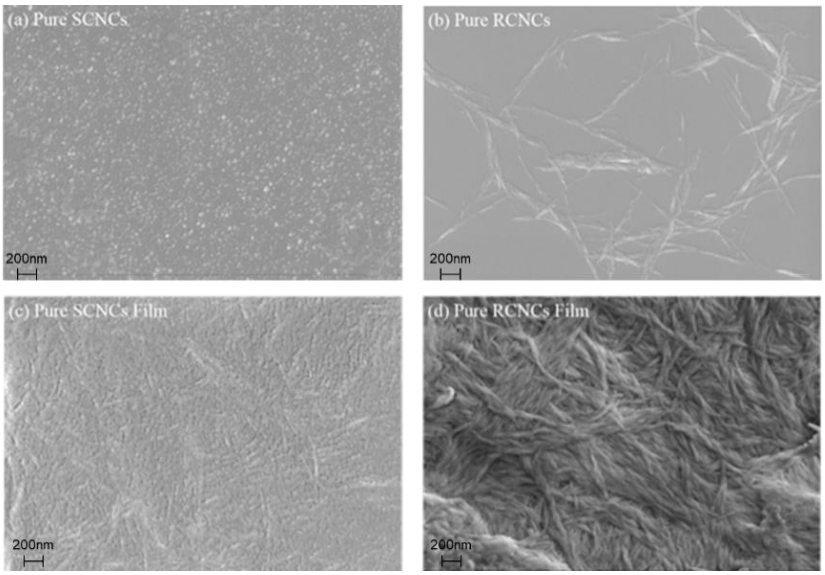

Fig. 2. Morphology of pure SCNCs and pure RCNCs and their films

Figure 2(a) shows spherical CNCs with uniform grain diameter obtained by enzymolysis and purified forms were about 30 nm. In Fig. 2(b), the resulting rod-like CNCs were approximately 20 nm in diameter, mostly 400 to 500 nm in length and a small amount at 300 nm. Figures 2(c) and 2(d) show the patterns of the films prepared by SCNCs and RCNCs. There were spherical cellulose nanocrystals in the SCNCs film, but a small number of cellulose nanocrystals were arranged into lines in a certain order. Given this situation, there were two reasons to consider: method and time. To use vacuum extraction method into film, in the film formation process, filter paper has an impact on pure nanocellulose, leading to the arrangement of nanocellulose in a certain direction. Moreover, with a long extraction time, granular nanocellulose is prone to agglomerate. In Fig. 2(d), long fibers are intertwined and form a dense network structure.

Light Transmittance Performance

Light transmittance is an important indicator of the material application. Figure 3(a) is a physical diagram of pure SCNCs film and pure RCNCs film under natural light. Figure 3(b) is a light transmittance curve of the sample at wavelength within the range of 200 to 1000 nm.

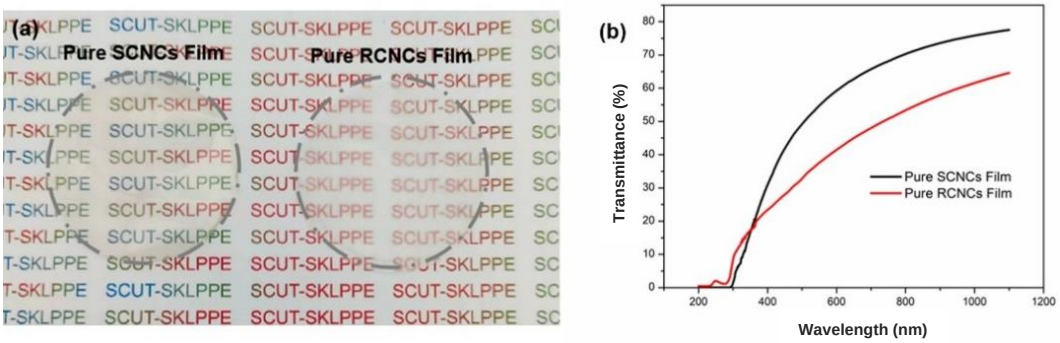

Fig. 3. (a) Pure SCNCs film and pure RCNCs film in natural light; (b) UV-visible spectrum of pure SCNCs film and pure RCNCs film

Compared with pure rod-like CNCs film, the font clarity of passage by pure spherical CNCs films was high, demonstrating that the transmittance of pure spherical CNCs film was higher than rod-like CNCs film. Figure 3(b) shows that the optical transmittance of pure SCNCs film was greater than the optical transmittance of RCNCs film at visible optical wavelength (380 to 760 nm). At 550 nm, the transmissivities of SCNCs film and RCNCs film were 55% and 37%, respectively, because the particle diameter of SCNCs was less than RCNCs. Thus, the transmission rate of pure spherical CNCs film was greater than that of rod-like CNCs film.

Crystal Structure by X-ray Diffraction

Figure 4 and Table 1 are XRD profiles and crystallization of SCNCs film and RCNCs film, respectively. Diffraction maps of pure spherical and rod-like CNCs films showed wide diffraction peaks both near both 2θ =16 º and strong peaks near 2θ =22.5 º. The peak positions of the films were almost unchanged compared with cellulose and spherical cellulose nanocrystals, suggesting that the crystal structure of cellulose was not destroyed during enzymatic hydrolysis and maintained the type I structure of natural cellulose. The crystallization of pure spherical CNCs film was 65.77%, slightly less than rod-like CNCs film 66.43%. Figure 4 shows that the spherical and rod-like CNCs films

decreased first, which was consistent with the crystallization law of the spherical CNCs film. Additionally, the swelling pretreatment of the pulp fiber weakened the hydrogen bonding between the cellulose crystallization areas, which reduced the film crystallization.

The lattice spacing within RCNCs and SCNCs films is shown in Table 1. The θ value at 22.5 º was 0.39 nm, with microcrystal sizes of 2.671 nm.

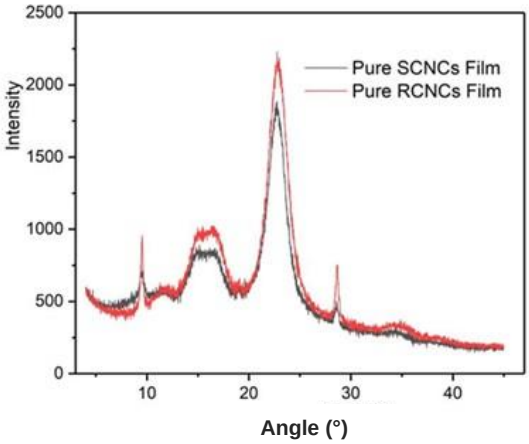

Fig. 4. XRD spectrum of Pure SCNCs Film and Pure RCNCs Film

Table 1. XRD Results of Pure CNCs films

Sample	CrI (%)	*d* (nm)	*L* (nm))
Pure SCNCs Film	65.77	0.39	2.671
Pure RCNCs Film	66.43	0.39	2.671

Tensile Properties

Figure 5 shows stress strain-curves of a pure SCNCs film and a pure RCNCs film. The pure spherical CNCs film had an ultimate tensile strength of 43 MPa, while the pure rod-like CNCs film had an ultimate tensile strength of 148 MPa. The maximum strain was less than 1%, and the film had a certain degree of flexibility. Because the rod-like CNCs were longer than spherical CNCs and can be entangled with each other to form an interwoven network structure, the film was not easy to break. The spherical CNCs were granular and incapable of being interwoven.

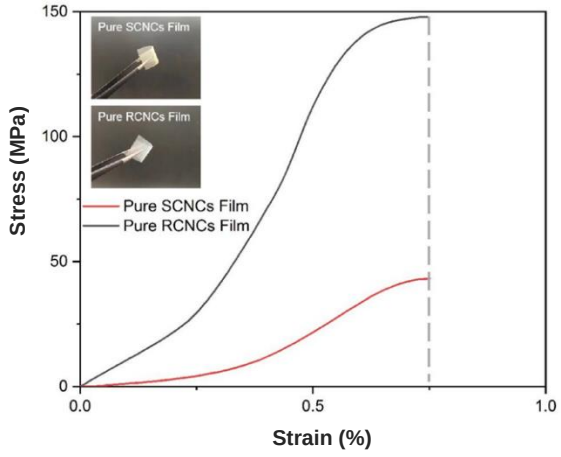

Fig. 5. Stress-strain curves of pure SCNCs films and pure RCNCs films

CONCLUSIONS

1. Using suspensions of high-purity cellulose nanocrystals by the composite enzymolysis of cotton pulp fibers as the raw material, the corresponding films could be obtained by the vacuum filtration method.
2. Under natural light conditions, the transparency of pure SCNCs film was higher than that of pure RCNCs film, and under visible light wavelength of 550 nm, the transmittances of SCNCS and RCNC films were 55% and 37%, respectively. However, through mechanical property analysis, the softness of pure SCNCs film was lower than that of pure RCNCs film.
3. The peak position of the films was almost unchanged compared to cellulose and spherical cellulose nanocrystals, suggesting that the crystal structure of films was not destroyed during the preparation and maintains the type I structure of natural cellulose.
4. In this paper, original work was reported for the preparation of high-purity cellulose nanocrystals films by the composite enzymolysis of cotton pulp fibers, which is a method with outstanding advantages in terms of green and sustainable development.

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