Preparation of cellulose nanofibrils (CNF) with uniform diameter distribution from okara by ultrasonic and high-speed stirring treatment method using deep eutectic solvent

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Research Article

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Abstract

Cellulose nanofibrils (CNF) is a nanomaterial with excellent performance. It is an important issue to control the CNF diameter during its preparation. In this study, a green and environmentally friendly method for rapid CNF preparation with great cost advantage is reported. Deep eutectic solvent (DES) and different mechanical treatment methods were used to obtain CNF with different diameter distribution. The results showed that ultrasonic treatment resulted in CNF with a uniform diameter distribution of 28 nm and high-speed stirring treatment resulted in CNF with a uniform diameter distribution of 27 nm. The method of using high-speed stirring to prepare CNF has great energy consumption advantages compared with traditional high-pressure homogenization methods. The yield of okara CNF before and after DES treatment is not much different. The recovery rate of DES achieved more than 90% and demonstrated excellent treatment effect indicating DES-pretreatment for okara CNF preparation is economical, convenient and effective.

1. Introduction

With the depletion of the non-renewable petroleum resources, the development of high-value biological sustainable resources is imminent. Natural cellulose contained in various types of plants is the most abundant, sustainable and most widely distributed biological resource in nature. Nanocellulose or cellulose nanoparticles are cellulose elements with at least one dimension less than 100 nm(Isogai et al. 2011). In recent years, cellulose nanofibrils (CNF) or cellulose nanocrystals (CNC) have been widely used in pharmaceutical, biomedicine, electronic devices and polymer nanocomposites reinforcement materials(Thomas et al. 2018) due to its unique characteristics such as large surface area, high modulus, highly porous structures and biodegradability(Klemm et al. 2011).

CNF can be separated from a variety of cellulose sources including bleached kraft pulp, bleached sulfite pulp, bamboo pulp, balloonflower residue, straw, wheat straw, cocoa pods shell, bagasse, beet pulp, corn cob and Japanese cedar(Liu et al. 2016). Previous study showed, though the microfibrillation degree proportionally increased with the number of homogenization steps and gradually decreased after reaching a certain threshold, the performance of the resulted CNF remains constant(Li et al. 2020). In addition, raw banana, pineapple leaf fiber, flax, hemp, jute, and sisal and abaca leaf bast were also used to produce CNF(Chávez-Guerrero et al. 2019). Although wood is the main raw material for CNF production, industrial and agricultural wastes have been attracted great attention in recent years and used as an alternative fiber source for CNF preparation due to their abundance, low cost and renewable characteristics(Nechyporchuk et al. 2016).

Nowadays, China is one of the major soybean processing countries in the world(Li et al. 2018). As the world's major food and oil crops, soybeans processing for protein, soy flour, tofu produces a large amount of by-products called okara(Nsor-Atindana et al. 2019; Vong et al. 2017). Okara is a biomass resource rich in nutrients and its main components include dietary fiber (60% - 70%), protein (13% - 20%), fat (6% - 19%), lignin (≈1%), and ash (3.5% - 5%)(Mateos-Aparicio et al. 2010a; Mateos-Aparicio et al. 2010b).

Furthermore most okara is burned as waste or used as animal feed in Asian countries(Nagano et al. 2020). It also contains a small amount of soybean isoflavones, vitamins, soybean saponins, phytic acid, minerals and other substances(Li et al. 2012; Stanojevic et al. 2013). Okara is derived from the cotyledons of soybeans. The cotyledons are the vegetative tissues of soybeans and are also called parenchyma since they are composed of parenchyma cells. The fine fibers of parenchyma cells existing in the primary wall are loosely arranged and have a small binding force between each other and make it easy to be separated for CNF preparation(Peiyi et al. 2019). The traditional method for CNF preparation is to remove the lignin from the raw materials by acid and alkali process followed by mechanical treatment(Benini et al. 2018; Ilyas et al. 2017). The process is not conducive to lignin and chemical recovery and requires a lot energy, which does not follow the current green manufacturing practice and meet the environmental protection requirements.

DES synthesized by reaction of two or more monomer coordination substances which has a melting point much lower than either of the individual components is considered as green solvent(Paiva et al. 2014). The use of low melting point DES for okara CNF preparation can effectively reduce its impact on the structural properties of okara CNF(Bubalo et al. 2016). The used eutectic liquid can be effectively recycled and reused which can significantly reduce the production cost and the environment burden(Sirviö et al. 2018).

At present, high-pressure homogenization is the most commonly used method for CNF preparation(Wang et al. 2019). However, the method is not conducive to large-scale production because of the high energy requirement(Li et al. 2019). In this context, we investigated the use of DES for okara cellulose pre-treatment and compared the performance of different mechanical dissociation methods for CNF production. We found that the dissociation method using ultrasound and high speed stirring produced CNF with uniform diameter distribution. Compared to high-pressure homogenization method, mechanical stirring showed greater energy consumption advantage and provided a new avenue for CNF preparation. We found that the recovery rate of DES reached more than 90% within three times of recovery and no diminished treatment efficiency was observed. This research provides a green, environmentally friendly and low energy consumption method for CNF preparation from okara by ultrasonic and high-speed stirring treatment method using deep eutectic solvent.

2. Materials And Methods

2.1 Materials

Okara was obtained from a soy milk factory in Heilongjiang Province, China. Choline chloride ($C_5H_{14}ClNO$) was purchased from Adamas Beta (Shanghai) Chemical Reagent Co., Ltd. Oxalic acid ($C_2H_2O_4\ 2H_2O$) was purchased from Tianjin Zhiyuan Chemical Reagent Co., Ltd. All chemicals are analytical grade and used without further purification.

2.2 Pre-treatment of Okara cellulose using DES

The eutectic solvent was prepared by mixing choline chloride and oxalic acid in a 100 ml round bottom flask at a molar ratio of 1:1. The reaction was conducted at a constant temperature of 80°C under continuous magnetic stirring until the solution turned clear and transparent. The resulted DES was then used to treat the okara. Okara and DES were mixed in a conical flask at a ratio of 1:20. The reaction was carried out at 100 ℃ for 30 - 100 min with a stirring speed of 40 r/min. At the end of the experiment, reverse phase solvent - distilled water was used to precipitate the cellulose. The solid substrate was washed by distilled water until the filtrate became neutral. The collected cellulose was made into 2 wt% suspension using distilled water.

2.3 High Pressure Homogenization Treatment

2% cellulose suspension was homogenized for 15 times using a high-pressure homogenizer under a pressure of 60 MPa.

2.4 High Speed Stirring Treatment

2% cellulose suspension was poured into a high-speed mixer and processed for 3 minutes at a speed of 6000 r/min.

2.5 Ultrasonic Treatment

2% cellulose suspension was dissociated for 5 minutes using the cell shredder (JY92-IIDN, Ningbo Xinzhi Biological Technology Co. Ltd., China) at a ultrasonic frequency of 25 KHz and output power of 800 W. All samples were then freeze-dried at -47℃ for 12 - 24 h.

2.6 Recovery of DES from the spent liquor

The recovery of DES was carried out by vacuum distillation. The distillation temperature was set at 60°C and the rotation speed was 80 r/min.

2.7 Characterizations

2.7.1 Scanning Electron Microscopy (SEM) Analysis

SEM (S-4800, Hitachi, Tokyo, Japan) was used to visualize the structure of various CNFs. The SEM images were recorded at a low voltage of 5 kV and a working distance of 5 mm. The CNFs were affixed to metal stubs using double-faced tape and then coated with platinum by ion sputter instrument. The diameter of CNF was analyzed using Nano Measurer software (Department of Chemistry, Fudan University, Shanghai, China).

2.7.2 Recovery rate of DES and yield of cellulose

The recovery rate of DES was defined as ratio of the amount of DES recovered from the spend liquor to its original amount used for okara treatment. The yield (%) of cellulose extract by recycled DES was

determined with Eq(1) :

$$Yield(\%)=\frac{(M_2-M_3)}{M_1}\times 100\% \qquad (1)$$

2.7.3 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) (Vertex, Brock, Germany) was used to identify the structure changes in okara nanocellulose. The dried okara celluloses were mixed with KBr at a ratio of 1:100 and was then thoroughly grounded in an agate grinder. Certain amount of the mixture was tableted at a pressure of 10 MPa for 2 min. The tablets were placed in an infrared spectrometer and scanned for 40 times from 500 cm-1 to 4000 cm-1 at a wave number resolution of 1 cm-1.

3. Results And Discussion

3.1 Effect of high pressure homogenization on CNF morphology and diameter distribution

Figure 1 shows the morphology and diameter distribution of okara CNF prepared by high pressure homogenization. As a conventional mechanical treatment method, high-pressure homogenization is usually used to mechanically dissociate the okara fibers. During the dissociation process, okara fibers are gradually converted to CNF. However, the mechanical energy consumption during high-pressure homogenization is very large and the machine is prone to blockage which would negatively affects the CNF yield. During the high-pressure homogenization process, the pressure is continuously increased to 60 MPa to increase the mechanical shear force on okara fiber to achieve fully okara cellulose nanofibrillation. In the previous research, the morphology of the prepared okara CNF by the times of high-pressure homogenization was discussed in detail. The results showed that 15 times high-pressure homogenization was the optimum homogenization condition. The diameter distribution of CNF prepared from less than 15 times high-pressure homogenization was not uniform and no obvious difference between the CNF obtained from more than 15 times high-pressure homogenization.

It can be seen from Fig. 1 that the diameter distribution of CNF prepared by high-pressure homogenization varies from 5 nm to 45 nm (relatively concentrated in the size range of 25 - 30 nm) with an average diameter of 30 ± 14 nm.

3.2 Effect of ultrasonic treatment time on CNF morphology and diameter distribution

Ultrasonic treatment was used to mechanically dissociate the 2% cellulose to prepare okara CNF. The effect of treatment time on morphology and diameter distribution of CNF was investigated and the result is shown in Fig. 2. Less than 5 minutes ultrasonic treatment was not enough to completely dissociate cellulose. More than 5 minutes ultrasonic treatment consumed more energy but no difference was observed in CNF diameter and morphology. Thus, 5 minutes was determined as the optimum ultrasonic treatment time.

The diameter of CNF obtained by 5 minutes ultrasonic treatment was 6 - 50 nm and had more uniform diameter distribution than those obtained from high-pressure homogenization. Compared to high-pressure homogenization, ultrasonic dissociation process required less mechanical energy and the loss of the sample is small. Moreover, the operation of ultrasonic treatment is simple and the ultrasonic treatment can evenly disperse the suspension. The average diameter of CNF obtained by 5 minutes ultrasonic treatment was 28 ± 9 nm.

3.3 Effect of high-speed stirring speed and mixing time on CNF morphology and diameter distribution

High-pressure homogenization, ultrasonic treatment and high-speed stirring are the three most commonly used mechanical dissociation methods for CNF preparation. High-pressure homogenization requires the most and high-speed stirring requires the least energy input. Previous research showed high-speed stirring treatment can fully dissociated the cellulose sample and results in uniformly dispersed suspension(Li et al. 2020). It is thus necessary to evaluate the high-speed stirring speed and time on its efficiency and effectiveness for CNF preparation.

Table 1 effect of stirring speed and mixing time on CNF diameter prepared by high speed stirring

Stirring speed / r · min-1	Mixing time / min	Average diameter / nm
3000	3	44
	6	27
6000	3	27
	6	26

Single-factor experiment was carried out to determine the effect of stirring speed and mixing time on the average diameter of the produced CNF. Table 1 shows the results. From the observation of the sample's macroscopic morphology during the experiment, different degrees of dispersion was observed at different stirring times. Less than 3 minutes high speed stirring was not enough to fully disintegrate the cellulose and some granular lumps were still observed. As shown in Table 1, when the stirring speed was set at 3000 r/min, the average diameter of the CNF decreased from 44 nm to 27 nm with the increase of the stirring time from 3 minutes to 6 minutes. The increase of the stirring time did not significantly change the average diameter of CNF when the stirring speed was set at 6000 r/min. When the stirring time was extended to 6 minutes, both stirring speed resulted in similar CNF diameter at about 27 nm. Therefore, the CNF preparation through high speed stirring was conducted at 6000 r / min and 6 minutes.

Fig. 3 shows the morphology and diameter distribution of okara CNF prepared by high-speed stirring. The CNF prepared by high-speed stirring had an average diameter of 27 ± 2 nm.

In summary, three different mechanical methods were used for okara cellulose treatment, namely, high-pressure homogenization, ultrasonic treatment and high-speed stirring. Nano Measurer software were used to analyze the above SEM to obtain the corresponding size distribution at different mechanical

dissociation conditions. Then the properties of the CNF can be controlled for different applications by adjusting the mechanical treatment parameters.

Table 2 Control of CNF diameter by adjusting mechanical treatment parameters

Mechanical dissociation	Speed / r · min-1	Pressure / MPa	Frequency/Khz	Time / min	Average diameter / nm	Power consumption/W
High pressure homogenization	-	60		15	30 ± 14	375
Ultrasonic		-	25	5	28±9	75
High speed stirring	6000	-		3	27 ± 2	12.5

Table 2 shows the CNF diameter distribution at different experimental conditions for each mechanical treatment method. As seen from Table 2, high speed stirring consumed much less energy to produce CNF compared to the other two mechanical treatment methods.

Figure 4 shows the size distribution of CNF prepared by different mechanical dissociation methods. CNF can be prepared combining DES pretreatment to extract okara cellulose and different mechanical treatment. The diameter distribution is relatively wide, ranging from 5 nm to 50 nm. Sum up, ultrasonic treatment and high-speed stirring can obtain uniform CNF size distribution which provides new inspiration for the application of CNF.

3.4 FTIR analysis of the recycled DES

Rotary evaporation was used to recover the DES after its treatment of okara cellulose. The recovered DES was characterized by infrared spectroscopy.

The infrared spectrum of DES as shown in Fig. 5 shows the following typical peaks. 3272 cm^{-1} is a nonlinear tensile vibration peak. The two absorption peaks of CN and CH bending vibration are located at the front end of 3018 cm^{-1}, while the tensile vibration of CO and CN is placed at 1637 cm^{-1}. At the front end, 1477 cm^{-1} is the bending vibration peak of CH, 1406 cm^{-1} is the bending vibration peak, 1284 cm^{-1} is the CN tensile vibration peak, and 870 cm^{-1} is the NH bending vibration peak.

According to the spectrum of oxalic acid, the absorption peaks at 1128 cm^{-1} and 1259 cm^{-1} were ascribed to the stretching vibration of CC, the stretching vibration of CO triggers the absorption peak at 1446 cm^{-1}, the stretching vibration of CO triggers the peak at 1685 cm^{-1}, and the characteristic peak of the tensile vibration of hydroxyl is at 3429 cm^{-1}. Compared with oxalic acid, the hydroxyl stretching vibration peak in the ChCl-O spectrum clearly shifts and forms a broad peak, which indicates the formation of

intermolecular hydrogen bonds between choline chloride and oxalic acid. In summary, the results indicate the formation of eutectic solvent from choline chloride and oxalic acid.

It can be seen from Figure 6 that the infrared absorption peak position of the DES recovered by vacuum distillation is basically the same as that of the original DES. The absorption peak at 3411 cm^{-1} corresponds to the stretching vibration of the hydroxyl group in DES, the absorption peak at 1686 cm^{-1} corresponds to the CO stretching vibration in okara CNF, and the absorption peak at 1264 cm^{-1} corresponds to the okara CNF. The tensile vibration of CN in the okara CNF caused the absorption peak at 783 cm^{-1} due to the bending deformation vibration of OH and CH bonds in CNF. The hydroxyl group at 3411 cm^{-1} was caused by the humidity of the air during the experiment. The recovered DES solvent showed the characteristic absorption peak resulted from okara CNF.

3.5 The effect of recycle times on the pretreatment effect

It can be seen from Fig. 7 that the recovery rate of DES solvent reached 93% and the yield of okara cellulose was about 25% for the first three cycles. With the increase of the cycles, the recovery rate of DES dropped from 91% to 84% and the corresponding okara cellulose yield dropped from 21.84% to 19.32%. The decrease in the recovery rate is mainly due to the loss of DES during the rotary evaporation process. Fresh DES should be added to reaction system to compensate for the DES loss after four cycles.

4. Conclusion

Through SEM and diameter distribution analysis, it is concluded that preparation of CNF with uniform diameter distribution from okara can be achieved by high-pressure homogenization, ultrasonic and high-speed stirring treatment using deep eutectic solvents. The effect of different mechanical treatments on CNF morphology and diameter distribution were evaluated. The properties of the CNF can be manipulated through the judicial selection of the mechanical treatment process conditions.

High-speed stirring using less energy was proposed as a new method for preparing CNF with uniform diameter distribution. The average diameter of the prepared CNF was 27 nm. DES solvent could be recycled up to three times and the recovery rate reached more than 90%. The yield of okara cellulose was 20.07% - 24.86%.

Declarations

Acknowledgements

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Figures

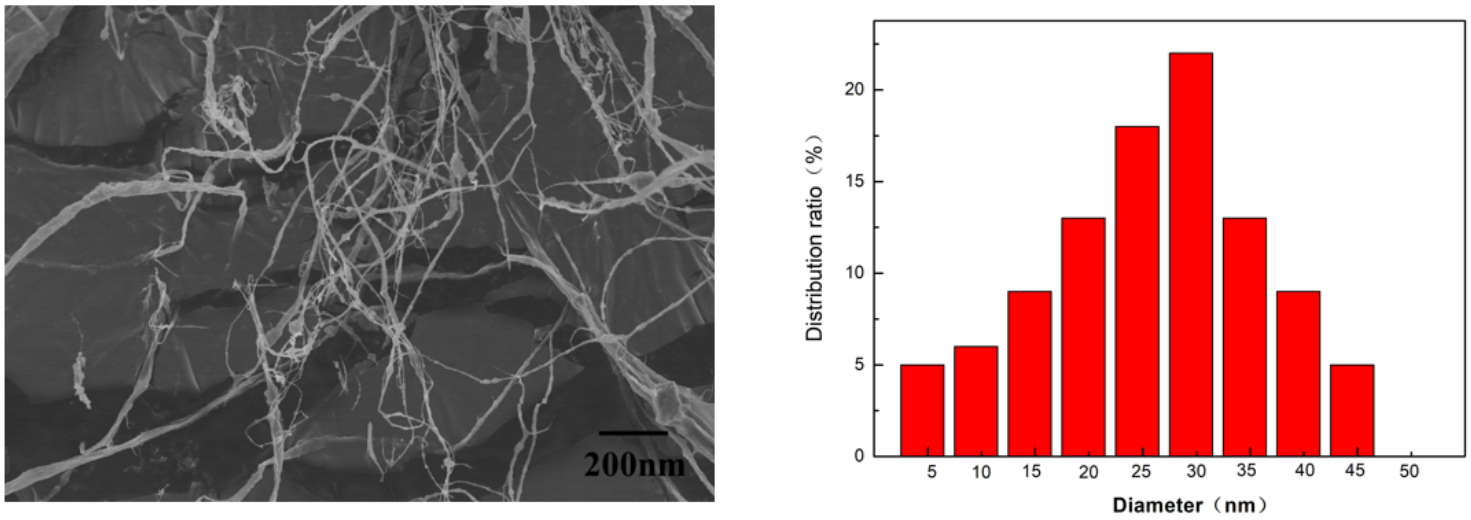

Figure 1

Morphology of CNF prepared by high-pressure homogenization

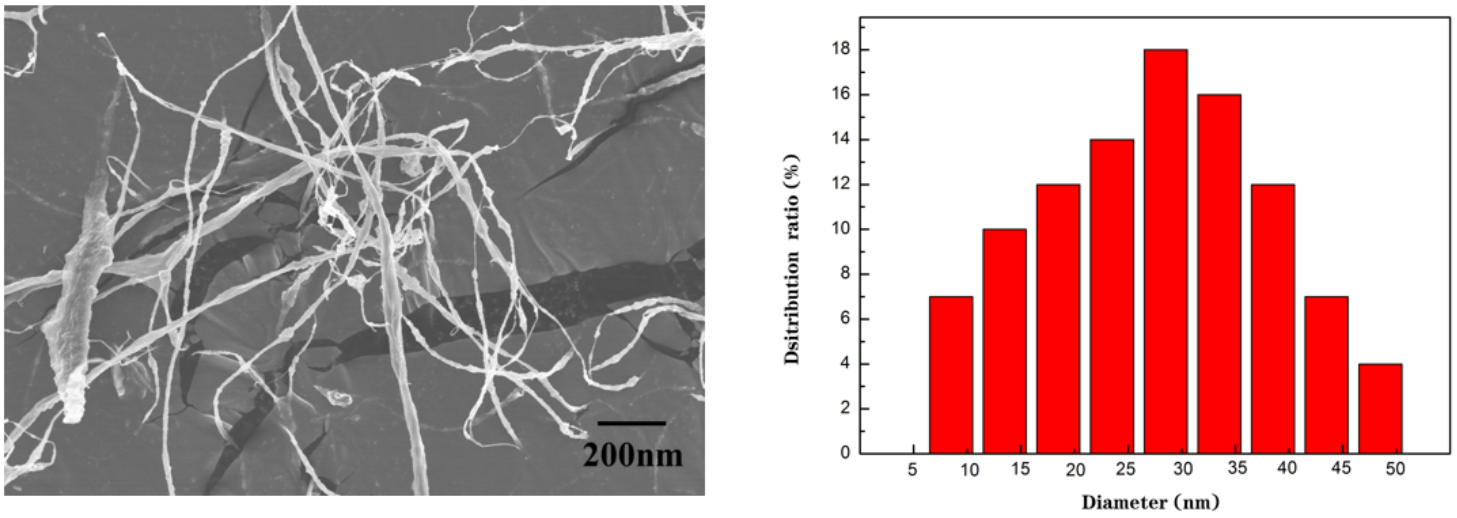

Figure 2

Morphology and diameter distribution of CNF prepared by ultrasonic treatment

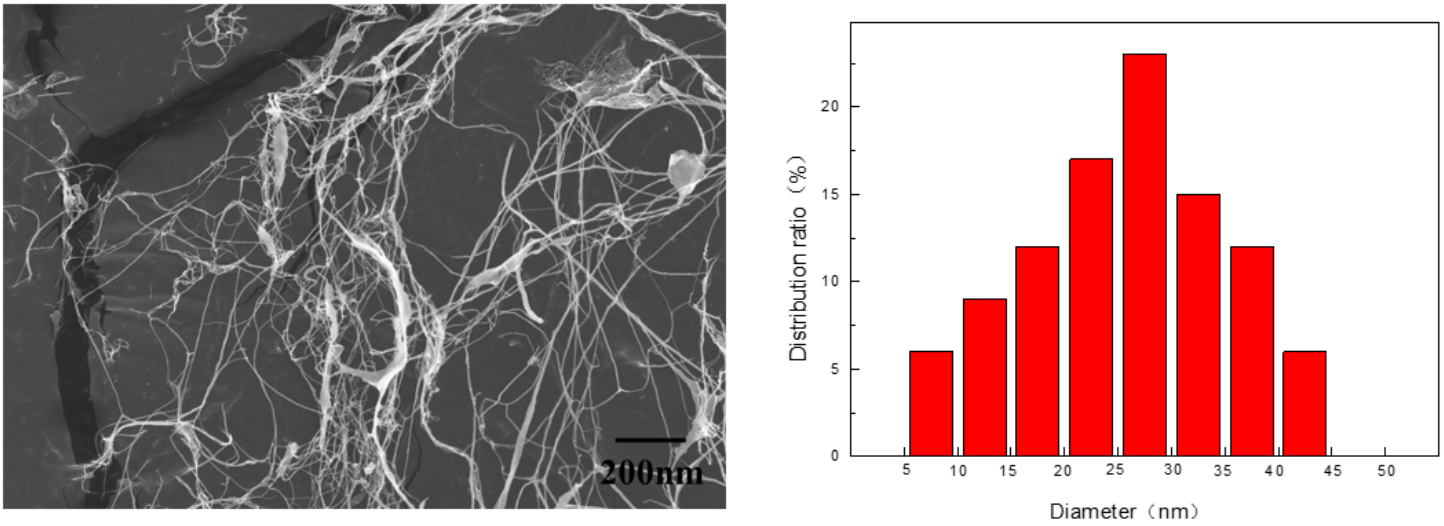

Figure 3

Morphology and diameter of CNF prepared by high-speed stirring

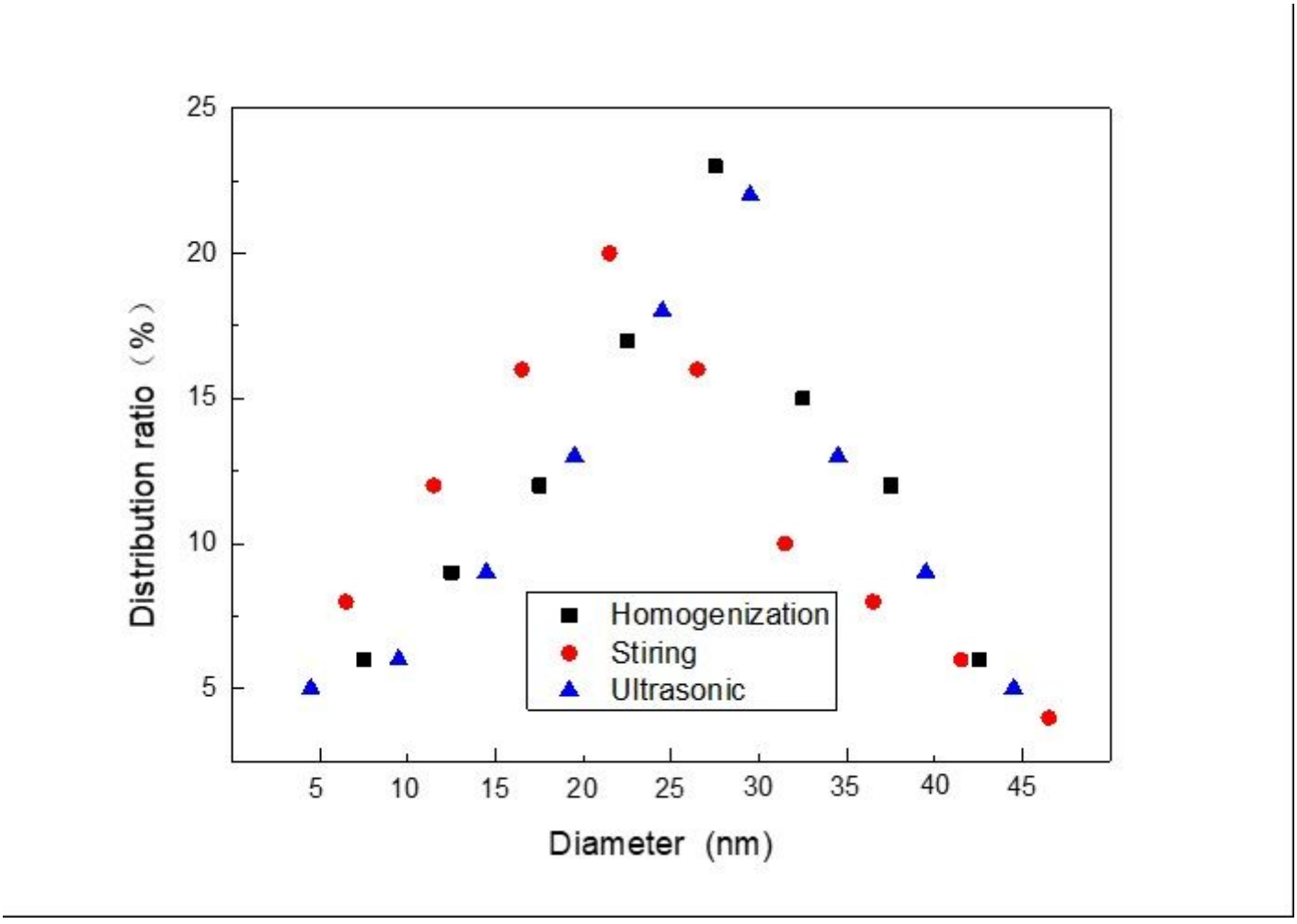

Figure 4

Size distribution of CNF using different mechanical dissociation

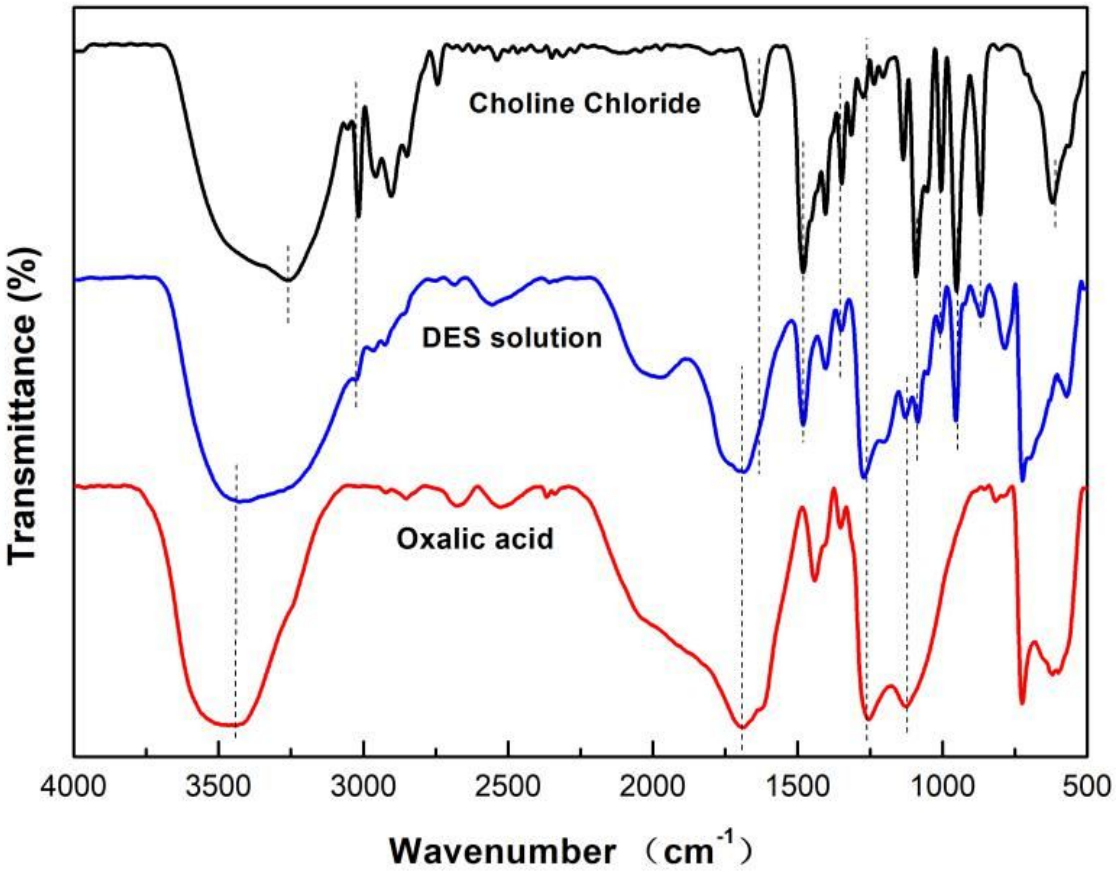

Figure 5

FTIR spectrum of oxalic acid, choline chloride and DES

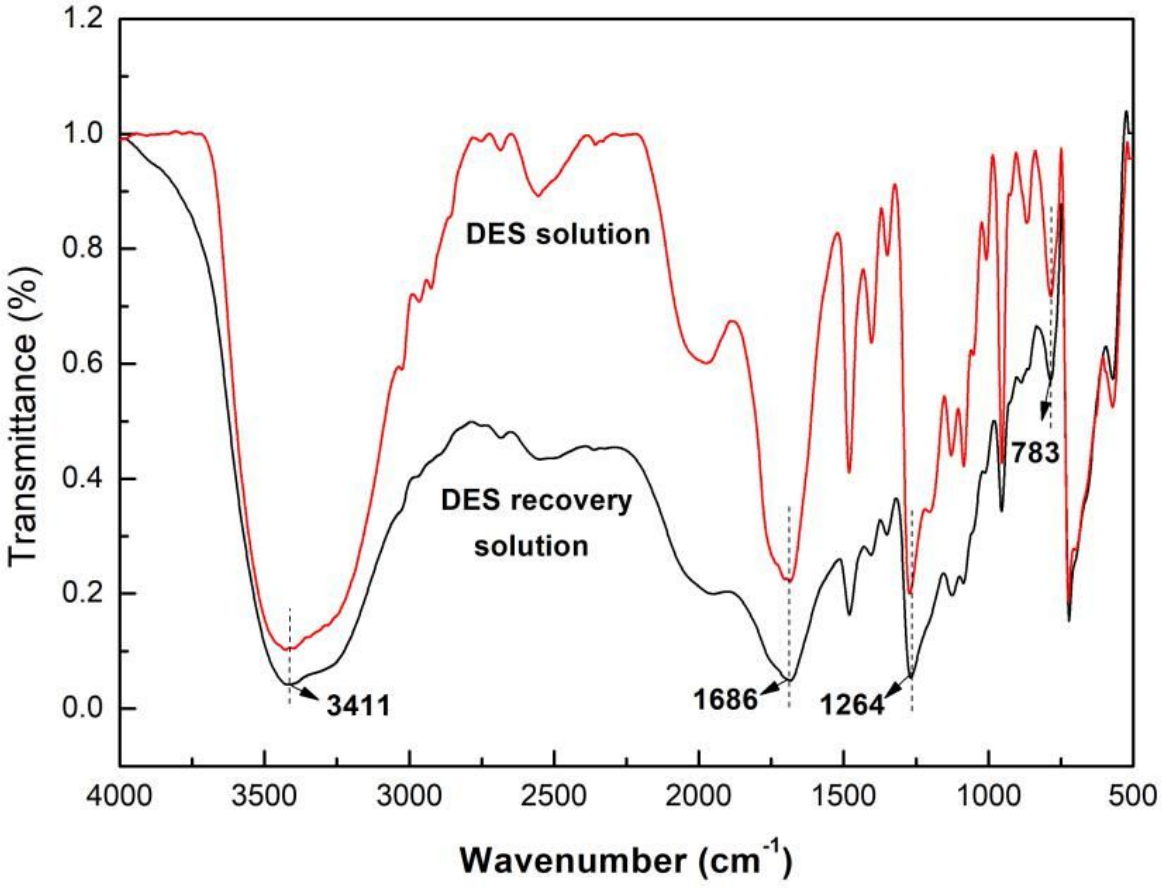

Figure 6

FTIR spectrum of CNF obtained before and after recovery of DES treatment liquid

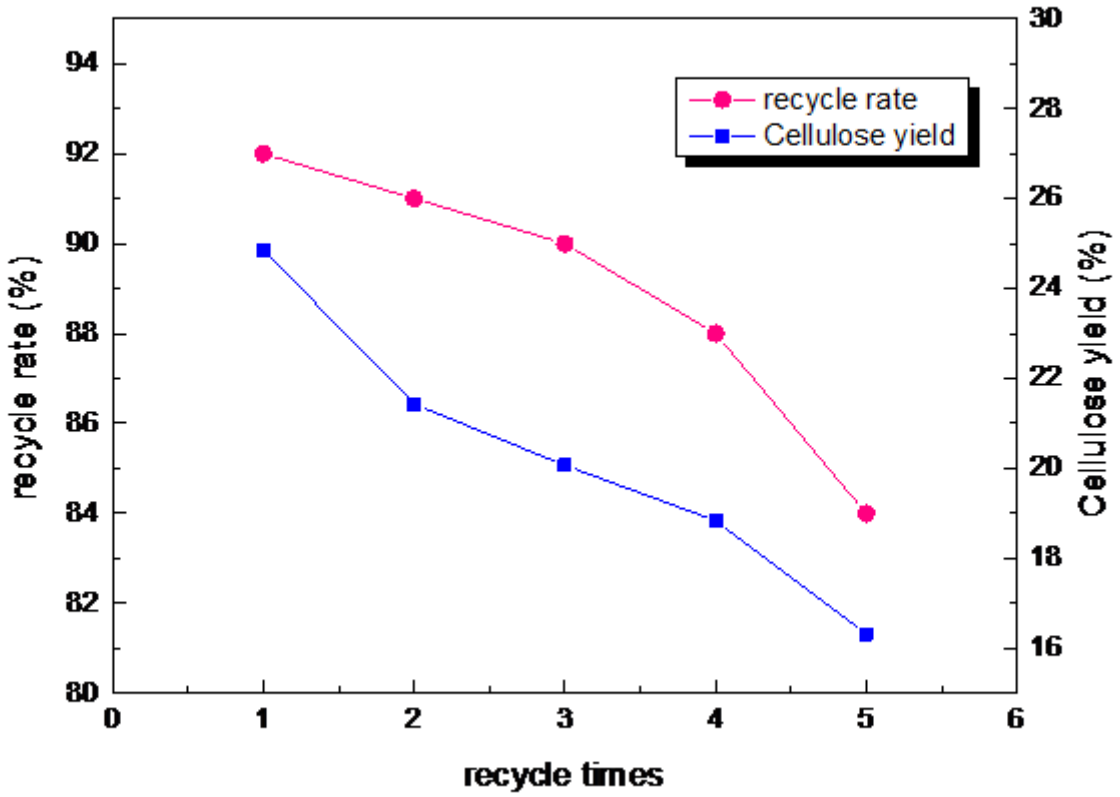

Figure 7

DES recycle rate and cellulose yield

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