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Single-stage method for isolation of cellulose nanocrystals from biomass in one pot

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Abstract

This study aimed to improve the production process of cellulose nanocrystals (CNCs) from plant biomass. For this purpose, a single-stage method for isolating cellulose nanocrystals from biomass was proposed, where the processes of biomass delignification and acid hydrolysis were combined in one pot. The initial biomass was delignified with a 1.5 % sodium chlorite solution at 90oC for 1 h, while the subsequent hydrolysis was carried out with 45% sulfuric acid at 80oC for 1 h. It was shown that the single-stage method is highly productive and allows for a significant increase in the yield of CNCs. In addition, such a method provides wastewater recycling and the return of purified water to the production process, resulting in zero discharge of liquid waste. Applying the single-stage method to flax fibers showed that the isolated CNCs have an average DP of 80, a crystallinity of 74%, a length of 200 nm, and a width of 15 nm.

Keywords: Cellulose nanocrystals; Single-stage production method; Productivity; Yield; Characteristics of nanocrystals

1. Introduction

Cellulose nanocrystals (CNCs) are a special type of cellulose materials made by hydrolysis of initial cellulose fibers with concentrated mineral acids and following mechanical disintegration of particle aggregates by ultrasound or high-pressure dispersers [1-3]. As a result of such treatments, rod-shaped crystalline nanoparticles 100-500 nm in length and 10-40 nm in width are formed. The CNCs have the following important features: high aspect ratio, decreased density, developed specific surface, presence of reactive hydroxyl groups facilitating the functionalization of the surface and improving dispersion within a polymer matrix, biodegradability, stability to increased temperatures, proteolytic enzymes, and some organic solvents, dilute solutions of alkalis and acids, etc. In addition, CNCs exhibit high mechanical properties with an axial Young's modulus of 150-200 GPa, a transverse modulus of up to 57 GPa, and an axial tensile strength of 10-15 GPa [4-10]. Due to these features, CNCs can be used as a high-quality filler for composite materials to reinforce the weak polymer matrix [10]. Other potential applications of CNCs are papermaking, biodegradable materials, thickeners, coatings, pharmaceutics, cosmetics, etc. [11, 12].

Cellulose nanocrystals can be produced from diverse biomass sources such as soft- and hardwoods, bast fibers (e.g., flax, ramie, etc.), herbaceous plants (e.g., miscanthus, switchgrass, Bermuda grass, etc.), annual plants (e.g., palms, bamboo, sisal, etc.), forest residues (e.g., sawdust, twigs, shrubs, etc.), residues of agricultural plants (e.g. stalks, husks, cobs, etc.), residues of textile, pulp, and paper industries and some others [13, 14].

To obtain CNCs, three-stage methods typically are used. In the first stage, the biomass is subjected to kraft or sulfite cooking or treated with alkali solutions at elevated temperatures [15-17]. In the second stage, the lignocellulose is bleached [17, 18], and only in the last third stage, the resulting cellulose is subjected to acid hydrolysis to convert it into CNCs [18]. To isolate free CNCs, mechanical or ultrasonic treatments are applied.

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The hydrolysis stage is performed using various strong acids, phosphoric [19], hydrochloric [20], hydrobromic [21], nitric [22], sulfuric [23], etc. Most often, for this purpose, the cheapest and most stable sulfuric acid is used. The hydrolysis process can be carried out with 60-65 wt. % sulfuric acid at 20-70°C for 30-120 min. [1-3, 23]. However, high-temperature hydrolysis with concentrated acid leads to carbonization and a decrease in the yield of CNCs. Therefore, the temperature of cellulose hydrolysis with 60-65 wt. % sulfuric acid is adjusted to 40-45°C [3, 23].

It should be noted that the conventional three-stage method of CNCs isolation from biomass has a significant disadvantage, namely the low yield of CNCs, estimated at 10-20%. This lack is caused by large losses during cellulose extraction from biomass and the low yield of CNCs during cellulose hydrolysis and disintegration of nanocrystalline aggregates [23, 24]. For example, Kraft delignification of pine woods with subsequent bleaching yields no more than 45% of cellulose [25], after acid hydrolysis of which only about 14% of CNCs can be obtained from this biomass. An additional problem of the conventional method is the formation of large volumes of harmful wastewater that must be neutralized and recycled. Besides, the conventional method is quite prolonged.

This study aimed to improve the process of CNCs production from plant biomass. For this purpose, a single-stage method for isolating cellulose nanocrystals from biomass was proposed, combining biomass delignification and hydrolysis stages in one pot.

2. Material and methods

2.1. Materials

The initial material was natural flax fibers supplied by the Flax Company. These fibers contain 72% cellulose, 19% hemicelluloses, 5% lignin, 3% extractives, and 1% ash. Pure chemicals such as sulfuric acid, peracetic acid, ammonium persulfate, sodium chlorite, and some other chemicals were purchased from Sigma-Aldrich Co.

2.2. Methods of CNCs Isolation from Biomass

2.2.1. Conventional method

In the first stage, about 5 g of chopped flax fibers were treated with 100 ml of boiling 2% NaOH solution for 1 h while stirring and washed to a pH=11 [26]. In the second stage, the resulting lignocellulose was bleached with 100 ml of 5% H_2O_2 at a temperature of 70 °C for 2 h with stirring, after which it was washed and dried at 105°C to constant weight. In the third stage, the bleached cellulose was hydrolyzed with 63.5% sulfuric acid at LSR=10 and temperature 44 °C for 2 h with stirring followed by washing to a neutral pH value [23]. Finally, the CNCs slurry was sonicated at cooling in the ice bath using an ultrasound disperser at 20 kHz and power of 400 W for 30 min. In each stage, the slurry of cellulosic material was separated from the excess liquid phase by centrifugation.

An additional sample was chemically treated to determine the yield of CNCs.

2.2.2. Single-stage method

This method combines biomass delignification and acid hydrolysis in one pot. The biomass is delignified with oxidant solutions to extract holocellulose, after which the obtained holocellulose is hydrolyzed with sulfuric acid to produce CNCs. The oxidizing agents for the single-stage process must meet some requirements, and above all, they must be compatible with the acid. For this reason, three main oxidants were chosen, namely, ammonium persulfate ($(NH_4)_2S_2O_8$), peracetic acid (CH_3CO_3H), and sodium chlorite ($NaClO_2$).

The process can be briefly described as follows. In a chemical flask, about 5 g of chopped fibers were placed, and 50 ml of oxidant solution of various concentrations was added. The required pH values were adjusted using a dilute solution of sulfuric acid. The contents of the flask were heated in a water bath to 90°C and kept at this temperature for 1-2 h while stirring. After that, the temperature of the water bath was lowered, and concentrated sulfuric acid was slowly added to the flask by stirring to achieve the desired final acid concentration. The acid hydrolysis was carried out for 1 h while stirring, and then the obtained acidic CNCs slurry was washed to a neutral pH value. The washed CNCs slurry separated from the water excess by centrifugation at an acceleration of 5000 g. To release free CNCs, the slurry was diluted with water to 1% consistency and sonicated using an ultrasound disperser at 20 kHz and power of 400 W for 10 min while cooling in the ice bath.

An additional sample was chemically treated to determine the yield of CNCs.

2.3. Methods of analysis

The chemical composition of the initial and delignified biomass samples was analyzed using standard NREL analytical methods [27]. The degree of crystallinity of the cellulose samples was determined by the method of wide-angle X-ray scattering (WAXS) [12]. The size and shape of the nanoparticles were studied by the method of transmission electron microscopy (TEM) [28]. The average degree of polymerization (DP) was measured by the viscosity method using diluted solutions of cellulose and nanocellulose in Cadoxen [29]. The delignification degree (DD) of biomass was calculated using the following equation:

$$DD\ (\%) = 100 \cdot ((1 - (L_d/L_o)) \quad \text{.............} \quad (1)$$

where L_o and L_d are the content of lignin in initial and delignified biomass.

3. Results and discussion

A study of the conventional method of CNCs isolation from initial biomass (natural flax fibers) revealed that due to losses of cellulosic material during chemical processing and centrifugation, the final yield of CNCs was low, only 19.2% (Table 1). Thus, using this method, from 1 kg of the initial biomass, only 192 g of CNCs can be obtained in terms of dry matter. The total production time of CNCs for the conventional method is at least 6 hours, while productivity is 0.032 kg CNCs/kg biomass per h. Since this method does not provide for the disposal of wastewater, the total volume of wastewater after the production of 1 kg of CNCs in terms of dry matter can reach 450 liters.

Table 1 Comparison of conventional and single-stage methods of CNCs isolation

Features	Conventional method	Single-stage method
Yield of CNCs, %	19.2	44. 3
Wastewater volume, L/kg CNCs	450	100
Production time, h	6	2.5
Productivity, kg/(kg h)	0.032	0.177

When studying a single-stage method of CNCs isolation, the conditions of biomass delignification were determined. As follows from the results, for complete delignification of initial biomass such as flax fibers with ammonium persulfate (APS) and peracetic acid (PAA), quite high concentrations (10-30 wt. %) of these expensive oxidants are required (Figure 1, Table 2). Unlike these reagents, complete delignification of biomass can be achieved using diluted and inexpensive 1-2 wt. % solutions sodium chlorite (SCL).

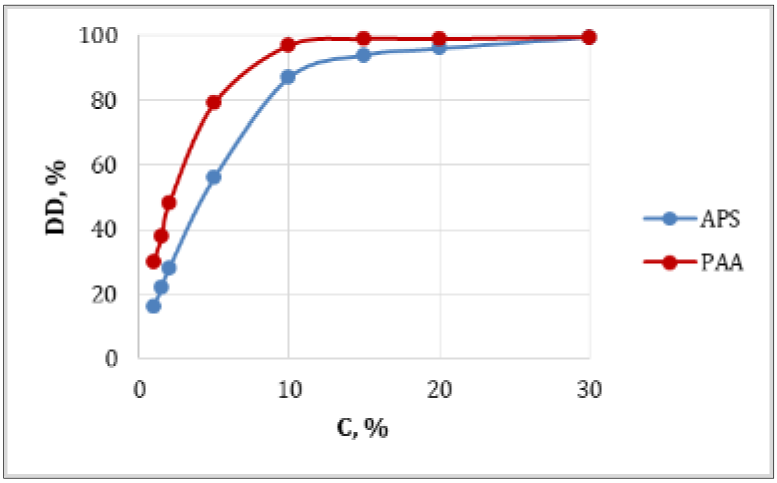


Figure 1 Dependence of delignification degree of initial biomass on oxidant concentration

Table 2 Conditions and results of oxidative delignification of biomass

Oxidant	pH	T, °C	Time, h	Oxidant concentration, wt. %					
				1	2	5	10	20	30
				Delignification degree, %					
APS	1	90	2	14	28	56	87	96	100
PAA	2	90	1	27	48	79	97	100	100
SCL	3	90	1	98	100	100	nd	nd	nd

Note: nd denotes "not determined"

To optimize the delignification process of initial biomass and obtain a cellulose and hemicellulose complex, i.e., holocellulose, a 1.5 wt. % solution of SCL must be used (Figure 2).

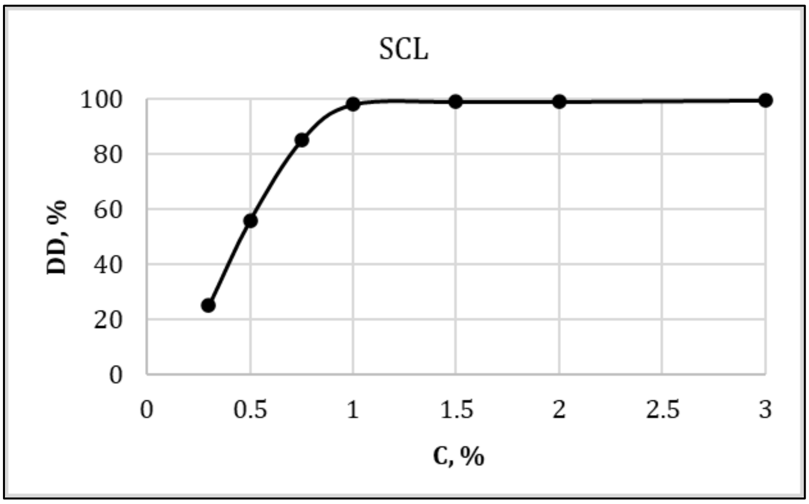


Figure 2 Dependence of delignification degree of initial biomass on SCL-oxidant concentration

Previous research on the hydrolysis process has shown that there is a superposition between the concentration of sulfuric acid and the hydrolysis temperature [30]. As a result, the acid concentration can be reduced from 60-65 wt. % to 40-45 wt. % by increasing the hydrolysis temperature from 40-45˚C to 70-80°C. The economic calculations showed that lowering the acid concentration is more beneficial than raising the hydrolysis temperature [31]. In addition, reducing the acid concentration increases the yield of CNCs.

After acid hydrolysis with 40-45 wt. % acid, hemicelluloses of holocellulose are transformed into oligo- and monosaccharides and dissolved. On the other hand, the remaining cellulose microfibrils are shortened due to local hydrolysis of non-crystalline domains and converted into nanocrystallites.

Thus, in this research, the following procedure of the single-stage method of CNCs production was used. In a chemical flask, about 5 g of chopped flax fibers were placed, and 50 ml of 1.5% $NaClO_2$ solution was added. The pH value was adjusted to 3 using highly diluted sulfuric acid. The contents of the flask were heated to 90°C in a water bath and kept at this temperature for 1 h while stirring. After that, the temperature of the water bath was lowered to 80°C, and 80 wt. % sulfuric acid was slowly added to the flask by stirring to the final acid concentration of 45 wt. %. The acid hydrolysis was carried out at 80°C for 1 h while stirring, and after that, the obtained acidic CNCs slurry was washed to a neutral pH value. The washed CNCs slurry separated from the water excess by centrifugation at an acceleration of 5000 g. To release free CNCs, the slurry was diluted with water to 1% consistency and sonicated using an ultrasound disperser at 20 kHz and power of 400 W for 10 min while cooling in the ice bath.

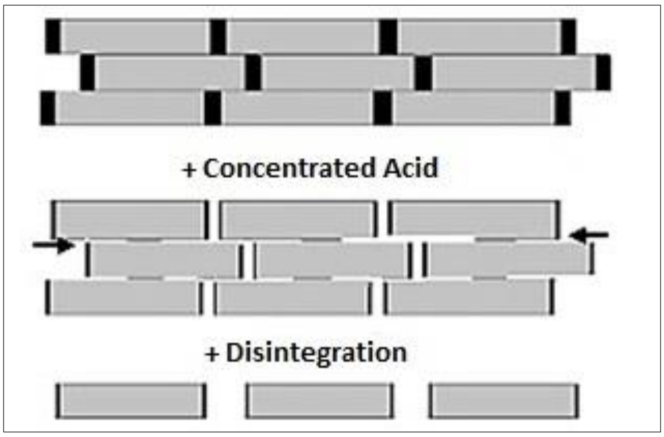
It was found that reducing the number of stages and using a lower concentration of sulfuric acid at the hydrolysis stage makes it possible to decrease losses of cellulosic material and increase the yield of CNCs to 44.0 %, i.e., 2.3 times higher than in the conventional method (Table 1). The total production time of CNCs for the single-stage method is about 2.5 hours, while productivity is 0.177 kg CNCs/kg biomass per h. These features are significantly better than those of the conventional method. Moreover, the single-stage method produces about 4.5 times less wastewater than the conventional method.

In addition, the proposed single-stage method provides for the recycling of wastewater. For this purpose, acidic wastewater is neutralized with calcium oxide or hydroxide:

$$H_2SO_4 + Ca(OH)_2 \rightarrow CaSO_4 + 2H_2O$$

The purified water can be used for the preparation of reagent solutions and washing of CNCs. Calcium sulfate ($CaSO_4$) obtained after the neutralization of acidic wastewater is a valuable by-product that is widely used for the decorative finishing of walls and ceilings, for the production of plasterboard, gypsum concrete, plasters, self-leveling floors, adhesives, and other purposes. The co-production of CNCs and $CaSO_4$ can reduce production costs. Moreover, the recycling of wastewater reduces its volume to zero.

It is known that hydrolysis of cellulose with diluted 10-20 wt.% acid, even at boiling temperature, leads to local cleavage of non-crystalline domains (NCDs), depolymerization of cellulose, and the formation of micron-scale aggregates of crystalline particles called microcrystalline cellulose (MCC) [32]. However, to produce CNCs, increased acid concentrations exceeding 40 wt. % are required. Unlike diluted acid, sufficiently concentrated acid hydrolyzes not only NCDs in cellulose microfibrils but also erodes strong contacts between nanocrystallites. In addition, CNCs obtained by treatment with sulfuric acid of increased concentration contain negatively charged sulfate groups, which impart them a negative Zeta-potential and mutual electrostatic repulsion [2. 3]. The acidic erosion of inter-crystallite contacts together with negative Zeta-potential facilitates the splitting of crystalline aggregates in an aqueous medium with subsequent ultrasonic disintegration with the release of free CNCs (Figure 3). Moreover, forces of electrostatic repulsion and the Brownian motion of nano-scale particles overcome gravity and promote the formation of stable aqueous dispersions of CNCs.



Arrows denote the erosion places between nanocrystallites

Figure 3 Scheme of hydrolysis of NCDs (black areas) and erosion inter-crystallite contacts under the action of concentrated acid solution to isolate CNCs

From the TEM image (Figure 4), it was found that CNCs produced by the single-stage method have a rod shape with a length of 100-300 nm and a width of 10-20 nm.

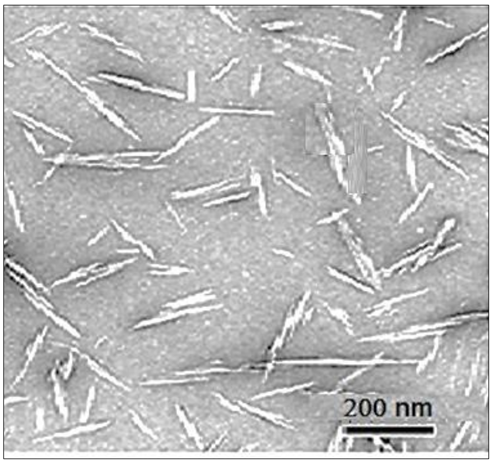


Figure 4 TEM image of CNCs

X-ray studies showed that the crystallinity degree (X) of obtained CNCs is 74 %, which is higher than that of the initial biomass sample with X=68 % (Figure 5, Table 3).

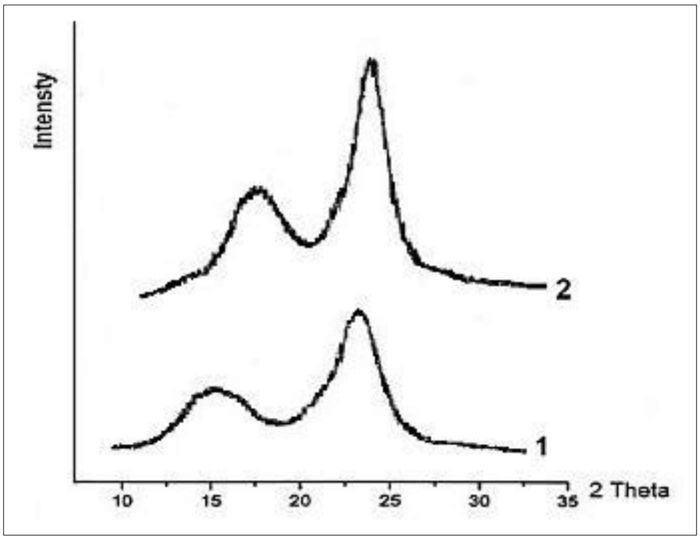


Figure 5 X-ray patterns of initial flax fibers (1) and flax CNCs (2)

Table 3 Average features of CNCs

Features	Value
Crystallinity degree, %	74
Average length, nm	200
Average width, nm	15
Average DP	80

The average features of CNCs produced by the single-stage method were within the range typical for conventional CNCs [1-3, 23].

4. Conclusion

To improve the production process of CNCs from biomass, a single-stage method was developed in which the biomass delignification to extract holocellulose and acid hydrolysis were combined in one pot. The initial biomass was delignified with a 1.5 % solution of sodium chlorite at 90°C for 1 h, while hydrolysis was carried out with 45 wt. % sulfuric acid at 80°C for 1 h.

It was found that compared to the conventional method, the proposed single-stage method is 5.5 times more productive. Moreover, this method increases the yield of CNCs by 2.3 times and reduces the volume of wastewater by 4.5 times. This method also ensures the recycling of the wastewater, the co-production of calcium sulfate simultaneously with CNCs, and the return of purified water to the production process. Thus, wastewater recycling reduces its volume to zero.

The application of the single-stage method was demonstrated using the example of CNCs preparation from natural flax fibers. It was found that the isolated CNCs have an average DP of 80, a crystallinity degree of 74%, a length of 200 nm, and a width of 15 nm.

Compliance with ethical standards

Disclosure of conflict of interest

The author of this paper declares that there is no conflict of interest.

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