**Preparation of Nanocellulose by a Combined Method Using Celluloses from Perennial Grasses and Wood:   
Structural Characterization**

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**Abstract.** In the experiment, a combined method was used, including chemical and mechanical treatment of cellulose to obtain nanocellulose. To obtain nanocellulose, cellulose from perennial grasses and wood was used to assess the influence of the nature of these celluloses. At the first stage of the experiment, the selected celluloses were treated with solutions of sodium hydroxide, urea and thiourea. This process allowed us to destroy amorphous regions and weaken intermolecular bonds in celluloses. At the next stage, centrifugation and ultrasonic treatment were carried out to remove impurities and further reduce the particle size. All obtained suspensions were analyzed by the nanoparticle dispersion method. It was found that the structure of nanocellulose contains ordered regions less than 10 nm in size, and the morphology of the particles depends on the type of the source material. Thus, the proposed method allows you to obtain nanocellulose with controlled characteristics, while the choice of raw materials plays a key role in determining its structural properties.

**Keywords:** cellulose, nanocellulose, alkaline treatment, urea, thiourea, centrifugation, ultrasound, structural analysis

**INTRODUCTION**

Over the past decades, research in the field of nanotechnology and nanoparticles has become one of the most dynamically developing areas of science. Nanoparticles are objects in which at least one of the dimensions does not exceed 100 nm, and nanotechnology is a set of methods for working with materials at this level. These technologies open up broad opportunities for creating innovative products and are used in a variety of industries - from energy, electronics and medicine (for example, in drug delivery systems) to the production of cosmetics, lubricants, paints, food packaging and other high-tech products, which clearly demonstrates the diversity of already used and promising nanomaterials.

Among the variety of already used and promising nanomaterials, nanocellulose is of particular interest - a biopolymer of natural origin that combines unique physical and chemical properties and environmental safety, which makes it attractive for many high-tech and environmentally resistant solutions.

There are three main types of nanocellulose: nanocrystalline, nanofibrillated, and bacterial. These varieties have the same chemical composition but differ in morphology, particle size, and degree of crystallinity. In addition, their properties may vary depending on the raw materials used and the production technology [1, 2].

Nanocrystalline cellulose (also called cellulose nanocrystals or cellulose nanowhiskers) is a type of high-strength nanocellulose typically produced by acid hydrolysis of cellulose fibrils. Particles of nanocrystalline cellulose are in the form of short rods or thread-like crystals, so-called whiskers. Their diameter is 2–20 nm and length 100–500 nm. In chemical composition, it consists of 100% cellulose. This implies a high degree of crystallinity - from 54 to 88%, which is due to the predominance of crystalline regions [1].

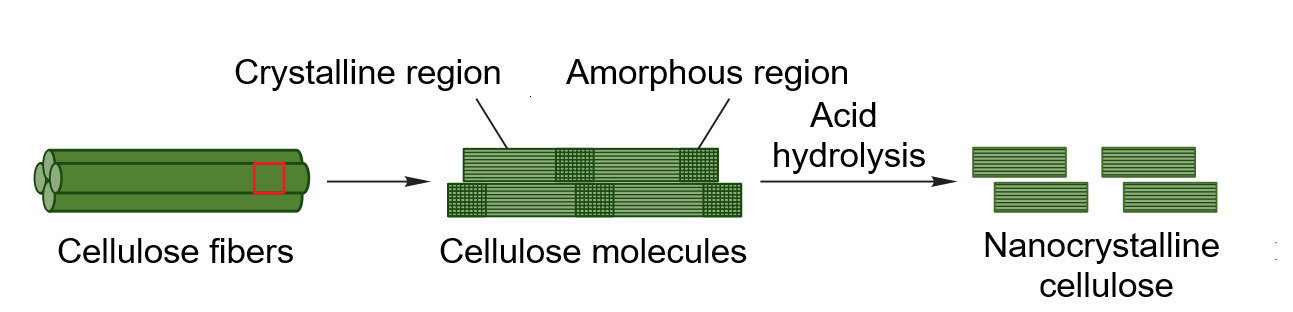
One of the long-known methods for obtaining cellulose nanocrystals is the treatment of cellulose material with acid, followed by the release of cellulose nanocrystals from microfibrils [3, 4]. In this method, acid treatment leads to selective hydrolysis of amorphous areas of cellulose, resulting in the release of rod-shaped nanocrystalline particles [1, 2]. The particle size of the resulting nanocellulose depends on the acid treatment conditions and the type of the original cellulose raw material, and as a result, the particle size varies from 50 to 1160 nm in length and from 3 to 50 nm in diameter [5]. During hydrolysis using sulfuric acid, the surface hydroxyl groups of cellulose are replaced by negatively charged sulfo groups, which provides electrostatic repulsion between the particles and promotes the stability of their aqueous dispersions [6].

The process of obtaining nanocellulose under the action of acidic reagents is schematically shown in Fig. 1.

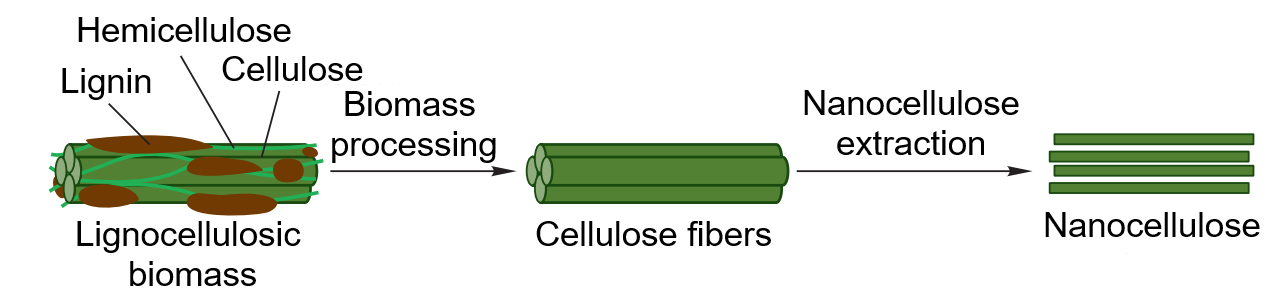
The use of sulfuric acid in a sufficiently high concentration leads to selective hydrolysis of the amorphous regions of the cellulose structure. As a result of this process, the less ordered sections of the macromolecules are destroyed and nanocellulose crystallites with a high degree of crystallinity are released.

Bacterial nanocellulose, unlike the nanocrystalline and nanofibrillated forms that are obtained from lignocellulosic biomass through chemical treatment, is synthesized in the course of a biotechnological process. Its formation occurs during the fermentation of low-molecular polysaccharides by microorganisms, primarily *Gluconacetobacter xylinus*, and is characterized by a significant duration - up to two weeks. After the completion of fermentation, cellulose structures are isolated from the biomass using extraction with organic solvents, which is illustrated in Fig. 2.

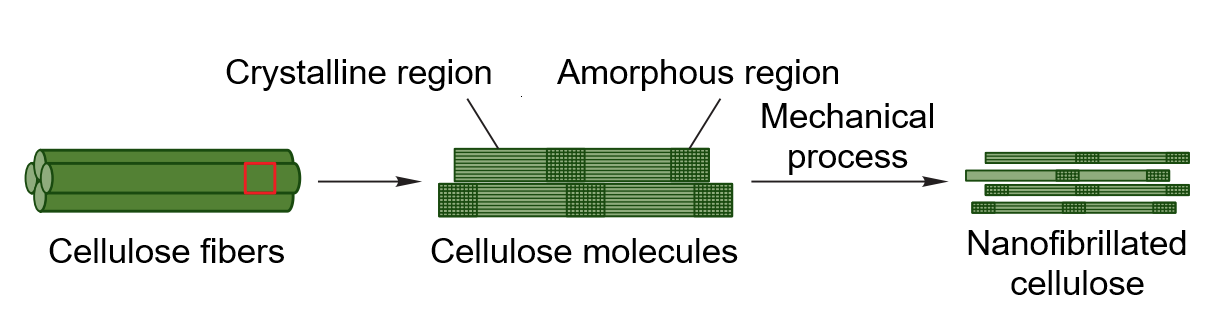
A significant increase in scientific and applied interest in nanosized cellulose is associated with the identification of a complex of its unique physical and chemical properties. These properties open up broad prospects for the practical use of the material and have contributed to the intensification of scientific research aimed at developing various methods for obtaining both nanocellulose and other functional nanomaterials [6, 2, 7].



**FIGURE 1.** The formation of nanocrystalline cellulose in the presence of sulfuric acid



**FIGURE 2.** Scheme for the production of nanocellulose from biomass



**FIGURE 3.** The effect of machining on the process of obtaining nanofibrillated cellulose from wood fiber

In recent years, special attention has been paid to the development of methods for obtaining nanocellulose materials using various types of mechanical action. Such methods include cavitation-hydrodynamic processing, vibration technologies, shock wave action, ultrasonic grinding, and detonation synthesis (Fig. 3). The use of cavitation-hydrodynamic action allows one to obtain suspensions of nanopowders in various dispersion media. In this case, the process is accompanied by the formation and subsequent destruction of gas microbubbles, which within 3-10 seconds at a pressure of 100-1000 MPa causes local heating of the processed material and its destruction. Such an effect, including impact loads, microvacuum effects, and a local increase in temperature, leads to effective grinding of solid matter. A similar destructive effect of cavitation waves is also used in ultrasonic grinding technology, where the effect of acoustic vibrations contributes to the dispersion of the material to a nanoscale state [7, 8, 9].

In addition to the chemical and mechanical methods, research is being conducted based on a combined method for obtaining nanoparticles. The combined method is a combination of chemical and mechanical methods for obtaining nanoparticles.

The combined method of obtaining nanocellulose involves the sequential or parallel use of chemical and mechanical effects, which allows for increased efficiency of the process and targeted management of the morphological and structural characteristics of the final product. At the first stage, as a rule, preliminary chemical treatment of the cellulose raw material is carried out - this can be acid hydrolysis (for example, using sulfuric or hydrochloric acid) or alkaline or enzymatic modification aimed at partially removing amorphous regions and reducing the degree of polymerization of macromolecules. Such preliminary treatment ensures weakening of interfibrillar bonds and increases the susceptibility of the material to further destruction.

The next stage involves mechanical action, including cavitation-hydrodynamic treatment, ultrasonic dispersion, shock wave action, high-shear treatment, or a combination of these. These methods facilitate the efficient grinding of pre-modified fibers and the extraction of nanostructured cellulose elements. In this case, the cavitation effects that occur during ultrasonic or hydrodynamic action provide local heating, micro-vacuuming, and impact destruction of the material, which further intensifies the process of nanoparticle formation.

In preparing the study, various methods for obtaining nanocellulose were considered, including chemical and mechanical methods for processing cellulose raw materials. These methods have both advantages and disadvantages relative to each other. For example, the chemical method of destroying amorphous sections of fibers is more effective than the mechanical method. But in terms of environmental friendliness, the chemical method is inferior to the mechanical method. However, the mechanical method has high energy consumption when achieving the desired particle size.

Taking these features into account, a combined method was chosen that allows combining chemical and mechanical processing and using the best aspects of both methods, minimizing the disadvantages of each of the individual approaches.

The combined method differs from individual physical or chemical methods in that it allows combining their strengths. As a result, it is possible to obtain a material with a more uniform size distribution and improved properties, which makes this approach promising for practical application [10].

For the study on obtaining nanocellulose, the choice of method was obvious based on the above facts. The method we used, combining chemical and mechanical effects on cellulose raw materials, allowed us to weaken the chemical bonds of cellulose, facilitating subsequent mechanical dispersion and reducing energy costs for grinding. The products obtained from the combined method were analyzed in the analysis of the dispersion distribution of nanoparticles.

**MATERIALS AND METHODS**

Several cellulose species were selected for obtaining nanocellulose: Miscanthus “Flamingo”, Miscanthus “Goldfeder”, Miscanthus “Gracillimus” and Broussonetia papyrefera. The analysis of the results presented in Table 1 shows that the moisture content of all three studied Miscanthus samples does not exceed 8.85%, which is consistent with literature data. Miscanthus was found to be rich in cellulose. The maximum amount of cellulose (62.0%) was found in Miscanthus “Gracillimus”, the minimum in Miscanthus “Flamingo” (55.0%). Miscanthus “Gracillimus” contains a record amount of lignin (18.0%), while the Chinese Miscanthus “Goldfeder” contains 1.8 times less lignin (10%) [10]. Initially, all cellulose samples underwent the same combined treatment. This method included preliminary chemical treatment with solutions of caustic soda (NaOH), urea and thiourea. The purpose of this stage was to weaken the hydrogen bonds within the polysaccharide chains of cellulose.

**TABLE 1.** Results of the study of the chemical composition of miscanthus

|  |  |  |  |
| --- | --- | --- | --- |
| **Indicators** | **Components of three species of Miscanthus plant** | | |
| **Miscanthus “Flamingo”** | **Miscanthus “Goldfeder”** | **Miscanthus “Gracillimus”** |
| Indicator humidity | 8.00 ± 0.55 | 8.55 ± 0.52 | 8.85 ± 0.50 |
| Mass share ash, % | 4.20±0.25 | 3.21±0.19 | 4.68±0.28 |
| Mass share lignin, % | 12.00±0.72 | 10.00±0.6 5 | 18.00±1.08 |
| Mass share cellulose, % | 55.00±3.45 | 60.00±3.75 | 62.00±3.87 |

After chemical treatment and soaking in the solution, the material was centrifuged and then subjected to ultrasonic treatment using a probe ultrasonic device. This process was carried out for a certain time at a strictly controlled temperature (Fig. 4).

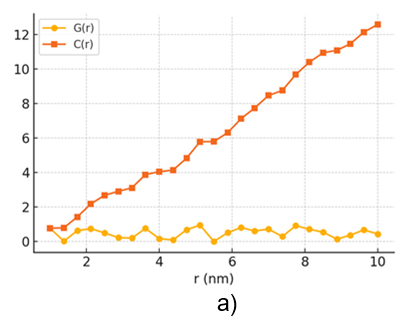
  

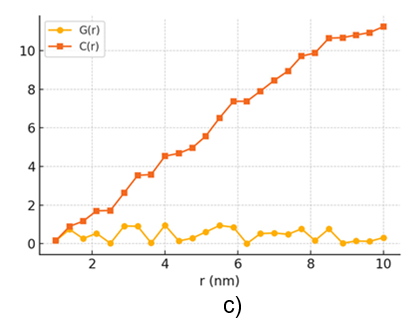
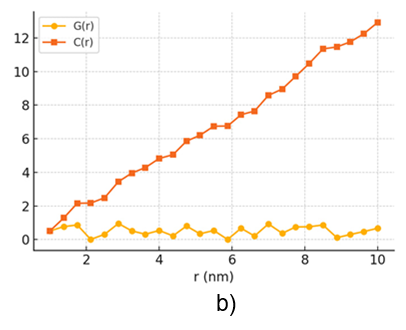
**FIGURE 4.** Stages of the combined method for obtaining nanocellulose

The final products obtained from Miscanthus were designated as samples № 1 and № 2, and from B. papyrefera designated sample № 3. To evaluate the structural characteristics of the obtained samples, the method of dispersive propagation of nanoparticles was used.

**RESULTS AND DISCUSSION**

The graphs corresponding to Fig. 5 (a, b, c) show the graphs of the dispersion distribution functions G(r) and concentration C(r) for different types of obtained nanocellulose. The abscissa axis shows the size of nanoparticles r (nm), and the ordinate axis shows the values of G(r) and C(r), which characterize the degree of dispersion and concentration distribution of particles, respectively.





**FIGURE 5.** Graphs of the dispersion distribution of nanoparticles in the studied samples  
a – sample No. 1, b – sample No. 2, c – sample No. 3

Analysis of the obtained data shows that the C(r) function in all three samples demonstrates a stable growth with increasing nanoparticle size. This indicates a regular accumulation of particles within the nanorange. However, quantitative differences in the growth rate of C(r) between the samples are observed, which reflects the features of the morphology and distribution of particle sizes depending on the initial type of cellulose. For example, in sample № 1, the maximum concentration reaches about 13 units, while in sample 3 3 it is about 11, and in sample № 2 it is also about 13, but with a different nature of increase.

The G(r) function, which reflects the dispersion distribution, has an oscillatory nature with insignificant amplitudes (within 0–1.2), which indicates the presence of certain predominant sizes of nanoparticles and a good degree of dispersion. At the same time, the nature of the oscillations of the G(r) function differs for each type of cellulose, which may be due to differences in the structure, packing density, and degree of fibrillation of the original material.

Comparison of the three graphs allows us to conclude that the structure and behavior of nanosized particles depend on *the nature of the original cellulose.* Despite the general tendency for the concentration of particles to increase with their size, the intensity of this process, as well as the nature of the dispersion, differ. This emphasizes the need for an individual approach to the selection of processing conditions depending on the type of raw material in order to ensure the target morphology and distribution of particles.

Table 2 clearly shows the graphs of the samples in Fig. 5 in which you can identify the average indicator of each sample. The indicator of the dispersion distribution of nanoparticles in all samples looks promising, but among them, sample № 3 has a more stable position to the condition of the reaction. In particular, it can be noted that the optimal conditions for obtaining sample № 3 have already been identified in the experiment, which is proven by this table.

**TABLE 2.** Sample performance in nanoparticle dispersion analysis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| № | **Names** | **r (nm)** | **G (r)** | **C (r)** |
| 1 | sample №1 | 1 – 10 | 0.772 – 0.445 | 0.772 – 12.576 |
| 2 | sample №2 | 1 – 10 | 0.516 – 0.675 | 0.516 – 12.915 |
| 3 | sample №3 | 1 – 10 | 0.158 – 0.313 | 0.158 – 11.245 |

Thus, the conducted analysis confirms that the selected method for obtaining nanocellulose allows the formation of stably dispersed nanostructures, and the differences in the G(r) and C(r) graphs reflect the influence of the type of cellulose on the process of nanoparticle formation.

**CONCLUSIONS**

As a result of the conducted research, the high efficiency of the combined method of obtaining nanocellulose was confirmed, combining chemical preliminary treatment using solutions of caustic soda, urea and thiourea, subsequent centrifugation, as well as ultrasonic dispersion with a probe apparatus. This approach made it possible to obtain samples of nanostructured cellulose from various types of plant raw materials - Miscanthus “Flamingo”, “Goldfeder”, “Gracillimus” and Broussonetia papyrefera - with a good degree of dispersion and stability of nanoparticles in an aqueous environment.

The obtained results of the analysis of the dispersion functions G(r) and C(r) showed that in all samples there is a regular accumulation of particles within the nanorange, as well as a pronounced dependence of the characteristics of nanoparticles on the type of original cellulose. It is especially important that the functions C(r) demonstrate progressive growth, which indicates a high concentration of cellulose nanoparticles in the range from 5 to 100 nm. Differences in the maximum values and the nature of the growth of the function C(r) between the samples indicate the influence of the morphological and structural features of each type of raw material on the yield and size composition of nanocellulose.

The nature of the oscillations of the G(r) function confirmed the presence of predominant nanoparticle sizes in each sample, as well as a good degree of dispersion, which is especially important for the subsequent use of nanocellulose in functional materials. Low oscillation amplitudes and the absence of pronounced anomalies in the graphs confirm the stability and homogeneity of the obtained dispersions. Comparative data analysis showed that despite the general processing technology, the final characteristics of the nanoparticles differ significantly, which requires a flexible approach to the process parameters depending on the specific type of cellulose.

Thus, it can be concluded that the proposed combined method is a universal and adaptable tool for obtaining high-quality nanocellulose from various lignocellulosic materials. It provides the possibility of targeted regulation of the morphological parameters of nanoparticles by varying the processing conditions. The results of this study can be used to develop new biocompatible, environmentally friendly and mechanically strong materials based on cellulose nanostructures, which are used in such areas as packaging, medicine, electronics, filtration systems and composite materials.

The prospects for further research lie in expanding the range of raw materials, optimizing the parameters of ultrasonic dispersion, and studying the functional properties of the obtained nanocellulose for the purpose of targeted application in specific areas of industry and science.

**FUTURE SCOPE**

Nanocellulose still holds potential, but we need to widen the feedstock sources that we use to make it. There is a dire need to review the alternative materials like straw, corn stalks, bamboo, sugarcane bagasse, and other lignocellulosic biomass which will aid in increasing sustainability and making the production financially viable in the various regions.

As suggested by the current literature, optimization of the variables of chemical and mechanical processing (acid concentration, ultrasound frequency, treatment duration, and temperature control) can increase the yield of nanocellulose, reduce the amount of energy necessary and ensure uniformity of particles size. These parameters need to be continuously optimized.

Another opportunity comes in the form of chemical modification or surface functionalization of nanocellulose to increase its range of applications. The material can be functionalized to be used in biomedical gadgets, electronics, intelligent packaging and water-purification systems by introducing functional groups or nanoparticles.

Massive production of laboratory techniques to cost-effective and economically viable is of enormous concern. Processes will be essential to altering nanocellulose into mainstream, continuous, cost-effective, and environmentally-friendly process.

Although nanocellulose is biodegradable and reasonably secure, there is a need to carry out strong environmental-fate and biological-interaction studies. Toxicity, biodegradability in long-term testing at different ecosystems will be a must, not only prior to the regulatory approval, but also at the consumer usage as well.

Other combinations, such as with polymers, resins, or metals result in composites that have the appeal to automotive, aerospace, and construction markets. Future studies should focus their attention on the improvement of the interfacial bonding and compatibility to the extent that these combinations are designed to obtain the highest mechanical strength and functionality.

In addition, the biocompatibility and structural flexibility of nanocellulose has made it an attractive prospect in the regulation of release drug molecules. Research into its pharmaceutical-carrying and -releasing properties at least as far as they can be applied to targeted cancer treatment, wound healing, and long-acting drug release is justified.

Nanocellulose could play a critical role in the development of flexible, lightweight, and biodegradable components for energy storage devices such as supercapacitors and batteries. Additionally, its use in contuctive inks, sensors, and elektronic displays is an emerging area of interest.

Given increasing environmental concerns over plastic waste, nanocellulose holds promise as a biodegradable and functional material for food packaging and other single-use products. Research should be directed toward improving moisture resistance, oxygen barrier properties, and shelf-life enhencement of nanocellulose-based packaging.

The integration of artificial intelligence (AI) and machine learning (ML) in nanocellulose research could significantly accelerate process optimization. AI can help predict optimal processing parameters, control quality, and design new nanocellulose-based materials with desired properties for spesific applications.

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