

Products of Cellulose Hydrolysis Made by Treatment of Feedstock with Concentrated Solutions of Sulfuric Acid.**Michael Ioelovich***

Department of Chemistry, Designer Energy Ltd., 2 Bergman Street, Rehovot 76100, Israel.

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***For Correspondence**

Department of Chemistry, Designer Energy Ltd., 2 Bergman Street, Rehovot 76100, Israel.

Tel: 972-89366612; Fax: 972-89366614

Keywords: Cellulose, Concentrated sulfuric acid, Hydrolysis, Structural changes, Amorphized cellulose, Nanocellulose**ABSTRACT**

The effect of the concentration of sulfuric acid (SA) and temperature on structure and properties of cellulose had been studied. Investigations showed that after cellulose treatment with concentrated solutions of SA the appreciable depolymerization was observed. Solubility of the initial sample at the room temperature increased gradually in the range of the acid concentration from 50 to 60wt.% SA. When SA concentration reached 65wt.%, then cellulose sample dissolved completely. Amorphized cellulose made by regeneration from 65wt.% SA, was characterized by high enzymatic digestibility. At elevated temperature of hydrolysis, 45°C, content of sulfonic groups and cellulose solubility in SA increase, while yield and DP decrease. After hydrolysis of cellulose with hot 50–60wt.% SA, the crystallinity degree of the obtained cellulose samples changed slightly, and these samples retained mainly the CI crystalline polymorph. However, when SA concentration reached 65wt.%, then cellulose dissolved, and the regenerated cellulose had CII polymorph, reduced crystallinity and low DP. Using optimal conditions of the acidic treatment (C=57–60wt.%, T= 45°C; t=1h) in combination with the high-power disintegration permitted obtaining the CI nanocellulose with the increased yield; this nano-product consists of crystalline cellulose particles having sizes 150–200 × 10–20nm.

INTRODUCTION

The first investigations of cellulose interaction with concentrated sulfuric acid (SA) began in the end of the 19th century and obtained the wide spread in the 20th century with the purpose to develop the methods for hydrolysis and dissolution of cellulose materials. These early investigations showed that at room or lower temperatures, the sulfuric acid with concentrations greater than 63 wt.% caused swelling and dissolving of cellulose samples that was accompanied by hydrolysis process. Increased temperatures of the acidic treatment promoted both to hydrolysis and dissolving of cellulose [1, 2]. At increased concentrations >63wt.% molecules of sulfuric acid are capable to break the hydrogen bonds and penetrate into noncrystalline and crystalline domains of the polymer with forming of the complexes [1]. Moreover, the partial esterification of hydroxyl groups of cellulose and their substitution by sulfonic groups was observed [3,4,5]. Breaking of hydrogen bonds as a result of the forming of the complexes and sulfate esters and the depolymerization of macromolecular chains, are the main factors causing the cellulose dissolution in the enough-concentrated sulfuric acid solutions.

In the last decade of the 20th century and the first decade of the 21th century, interest to the deep study of interaction between cellulose and sulfuric acid was raised due to intensive study of nano-scale cellulose particles (NCP) that can be used as high-quality reinforcing filler for polymers and biodegradable materials, strengthening additive for papers, thickener for dispersions, drug's carrier, implant, and so forth [6,7,8,9,10,11].

The NCP can be made by treatment of celluloses of various origins with concentrated sulfuric acid solutions at various temperatures with the following high-power mechanical or ultrasound disintegration of the acid-treated cellulose in water. The

concentration of sulfuric acid can vary from 45 to 70 wt.%, the temperature can range from 25 to 70°C, and the treatment time can be from 30 min to overnight depending on the temperature [7,8,9,10, 12].

The concentrations of sulfuric acid from 45 to 65 wt.%, were recommended to obtain the high-crystalline nanoparticles having CI crystalline polymorph [9, 13,14,15,16,17,18,19]. However, in the other papers [1, 2, 9, 20,21] it was found that, the sulfuric acid at concentrations greater than 63–64 wt.% causes swelling and dissolving of cellulose samples. When the acidic solution of cellulose was diluted with water the soluble cellulose was regenerated and precipitated in a form of low molecular amorphized flocs having crystalline polymorph of CII-type [9, 20, 21, 22]. So, contrary to statements about the high-crystalline structure of the nanoparticles obtained by treatment of initial cellulose materials with 64–65 wt.% SA [17, 18, 19], the other experiments adduce evidences about amorphized structural state of these particles [9, 20,21,22].

Since the existing results about interaction between cellulose and sulfuric acid were ambiguous, the detailed investigations are required in order to clarify the dependence of the cellulose structure on the conditions of acidic treatment.

EXPERIMENTAL

Materials

Initial cellulose material was cotton cellulose extracted from middle-length cotton fibers “Acala” [36]. This cellulose had 98.5% α-cellulose and average DP about 2300.

Chemical pure 95wt.%-sulfuric acid was supplied from Sigma-Aldrich Co. The concentrated SA was diluted dropwise with water at cooling in an ice bath up to 80 wt.% exactly having density 1.727 g/cm³.

Treatment of Cellulose with Sulfuric acid

The initial cellulose sample was mixed with water in a lab glass, and then 80 wt.% acid (SA) was slowly added at cooling to obtain the required final concentration of SA from 50 to 70 wt.% and acid/cellulose ratio (ACR) 10. The glass was placed into water bath having temperature 25 or 45°C and heated at stirring for 1 h. After the acidic treatment, contents of the glass were poured out into tenfold volume of cold water at stirring. Cellulose sediment was separated from the liquid phase by centrifugation at the acceleration of 4000 g for 10 min, and then the acid-treated sample was washed with water, 5% sodium bicarbonate and finally with distilled water to pH about 6, using centrifugation to remove the liquid phase. To obtain the dry sample, the wet sediment was additionally washed with ethanol and acetone and dried at 60°C overnight and then at 105°C up to constant weight.

Total yield (*Y*) of the acid-treated cellulose including yields of the insoluble and regenerated parts of cellulose was calculated as follows:

$$Y, \% = 100 \left(\frac{W}{W_0} \right), \quad (1)$$

Where *W*₀ is initial dry weight of the sample, *W* is weight of dried sediment.

Determination of Cellulose Solubility in sulfuric acid

The initial sample was treated in a lab glass with 50–70 wt.% SA at ACR = 10, temperature 25 or 45°C for 1h. The insoluble residual part of the sample was separated from the acid by centrifugation at the acceleration of 4000 g for 10 min and then rinsed twofold with the SA solution of the same concentration, washed with water, 5 wt.% sodium bicarbonate and distilled water up to neutral pH value. Finally, the cellulose residue was washed with absolute ethanol and acetone and dried at 60°C overnight and then at 105°C up to constant weight.

Solubility (*S*) of cellulose in solutions of SA was calculated as follows:

$$S, \% = 100 \left[1 - \left(\frac{W}{W_0} \right) \right], \quad (2)$$

where *W*₀ is initial dry weight of the sample, *W* is dry weight of insoluble part of the sample.

Enzymatic Hydrolysis of Cellulose Samples

The samples were hydrolyzed with a mixture of commercial cellulolytic enzyme (cellulase) NS50013 and β-glucosidase NS50010 (Novozymes A/S, Bagsvaerd, Denmark). The loading of cellulase was 5 FPU per 1 g of solid sample and of β-glucosidase was 7 CBU per 1 g of solid sample. Hydrolysis of the samples was carried out in 50-mL polypropylene tubes. The sample containing 1 g of

the solid matter and 10 mL of 50 mM/L acetate buffer (pH = 4.8) were put into the tubes. Then cellulase and β -glucosidase were added. An additional amount of the buffer was supplemented to obtain total volume of the liquid phase 20 mL and concentration of the cellulose sample 50 g/L. The tubes closed with covers were placed in a shaker incubator at 50°C and shaken at 180 rpm for 48 h. The tubes were centrifuged at the acceleration of 4000 g for 10 min in order to separate the cellulose residue from the liquid phase. The sediment was washed with distilled water, with removing the liquid phase by centrifugation. Finally, the cellulose residue was washed with absolute ethanol and acetone and dried at 60°C overnight and then at 105°C up to constant weight. Conversion degree of cellulose (CD) at the enzymatic hydrolysis was calculated as follows:

$$CD = 100 [1 - (\frac{W}{W_0})], \quad (3)$$

Where W_0 is dry weight of the initial sample, W is dry weight of the enzymatic-hydrolyzed sample.

Preparation of Nano Crystalline Particles (NCP)

The NCP were prepared according to the following procedure [9]. The initial cellulose sample was mixed with water in a lab glass, and then 80 wt.% sulfuric acid (SA) was slowly added with cooling to obtain the required final acid concentration from 57 to 60 wt.% and acid/cellulose ratio 10. The glass was placed into water bath having temperature 45°C and heated at stirring for 1 h. After hydrolysis, contents of the glass were poured out into tenfold volume of cold water at stirring. Cellulose sediment was separated from liquid phase by centrifugation at the acceleration of 4000 g for 10 min; washed with water, 5 wt.% sodium bicarbonate, and finally with distilled water to pH about 6–6.5. Then the washed cellulose sediment was diluted with distilled water up to solid concentration 2–3 wt. % and disintegrated by high-pressure homogenizer APV-2000 at pressure 100 MPa during 15 min.

To obtain the dry nanopowder, the following procedure was carried out. The water dispersion of nanoparticles was evaporated in vacuum at 80°C to about 10–15% solid content, washed with absolute ethanol, acetone, and hexane, and finally dried at 50°C up to constant weight.

Determination of Sulfonic Groups

Content of sulfonic groups ($-\text{SO}_3\text{H}$) in hydrolyzed samples was calculated from results of sulfur determination that was performed by means of elemental analyzer FLASH-2000.

Determination of Average degree of Polymerization

The average degree of polymerization (DP) was measured by the viscosity method using diluted solutions of cellulose in Cadoxen [6].

Determination of Structural Characteristics of Cellulose Samples by XRD

A Rigaku-Ultima Plus diffractometer ($\text{CuK}\alpha$ -radiation, $\lambda=0.15418$ nm) was used for X-ray investigations. Diffractograms of dry samples were recorded in the $\phi = 2\theta$ angle range from 5 to 80°. After recording of the diffractograms, the background was separated, and selected X-ray patterns were corrected and normalized. Then, diffractions from crystalline and noncrystalline regions were separated by a computerized method. Crystallinity degree (X) of the cellulose samples was calculated according to the following [24]:

$$X, \% = 100 \frac{\int I_c d\phi}{\int I_o d\phi}, \quad (4)$$

Where I_c and I_o are the corrected and normalized intensities of diffraction from the crystalline part and whole sample, respectively.

Content of CII-crystalline polymorph was determined using the XRD- calibration method of the inner standards [25]:

$$CII, \% = 200 \left(\frac{I_{12}}{I_{15} + I_{16}} \right), \quad (5)$$

Where I_{12} , I_{15} and I_{16} are the corrected and normalized intensities of diffraction from the crystalline domains at the 2θ angle range 12, 15 and 16 degree, respectively.

Shape and size of particles were investigated by SEM “Hitachi S-4700.” The diluted dispersion of cellulose nanoparticles was subjected to ultrasonic treatment for 5 min. A drop of the diluted dispersion was applied onto a substrate and dried and the dry sample was coated with a thin layer of gold. Then the sample was placed in the microscope, evacuated, and electronic image of particles was obtained.

RESULTS AND DISCUSSION

Hydrolysis of Cellulose with SA at Room Temperature to make Amorphized Cellulose

The effect of sulfuric acid concentration on structure and properties of the cellulose were studied at 25°C, for 1 h. The study showed that after cellulose treatment with concentrated solutions of SA the appreciable depolymerization was observed (Fig.1). Solubility of the initial sample increased gradually from 2 to 24 % in the range of the acid concentration from 50 to 60 wt.% SA (Fig. 2), and the obtained non-soluble cellulose residues had relatively low content of sulfonic groups, 0.51–0.57% (Fig 3). However, after cellulose hydrolysis at the acid concentration >60wt. % the considerable rise in solubility of the cellulose was observed.

When SA concentration reached 65 wt.%, then cellulose sample dissolved completely. Cellulose regenerated from 65 wt.% SA contained about 1.21 % of sulfonic groups; it had yield about of 66–68 % and DP of 60–70. Increasing of SA concentration more than 65 wt.% led to diminution in yield of the regenerated cellulose. After treatment of the initial cellulose sample with 70 wt.% SA, the dissolved cellulose cannot be regenerated from the acidic solution by dilution with water due to fast acidic depolymerization of cellulose and forming the water-soluble oligomers [2]. In this connection, the proposal regarding isolation of nanocellulose particles by means of treatment of initial cellulose with 70 wt.% SA at room temperature [26] seems unsuccessful.

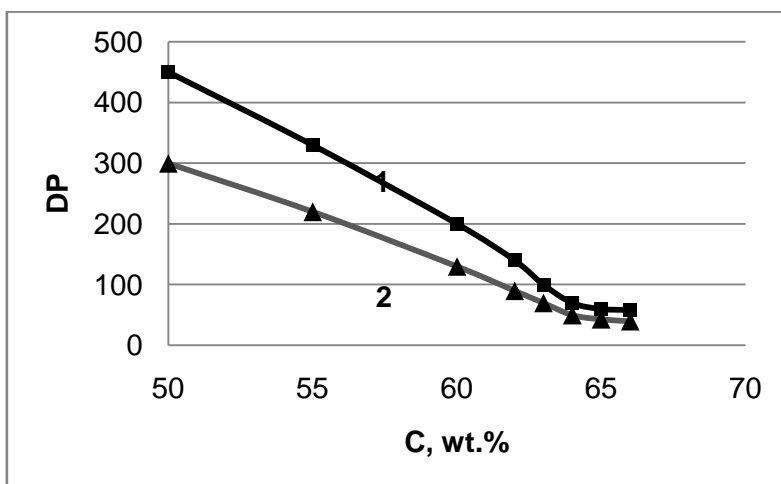


Figure 1: Change of DP of cellulose during hydrolysis at 25°C (1) and 45°C (2) with concentrated acid solutions

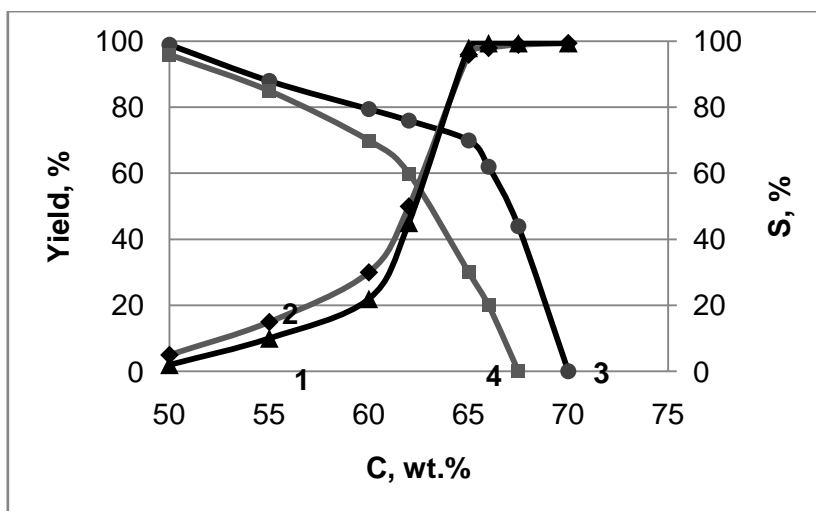


Figure 2: Solubility of cellulose (1, 2) and yield of regenerated cellulose (3, 4) at 25°C (1, 3) and 45°C (2, 4) as a function of sulfuric acid concentration

According to XRD investigations, in the range of SA concentration from 50 to 60 wt.% the crystallinity degree and CI crystalline state of cellulose changed slightly (Figure 4). However at acid concentration higher than 64–65 wt.%, the hydrolysis led to forming of the regenerated cellulose having CII crystalline polymorph and low crystallinity. These results permitted to find the following optimal conditions for production of amorphized cellulose:

- Concentration of SA: 65 wt.%
- Temperature: $\leq 25^{\circ}\text{C}$
- Hydrolysis time: 1 h

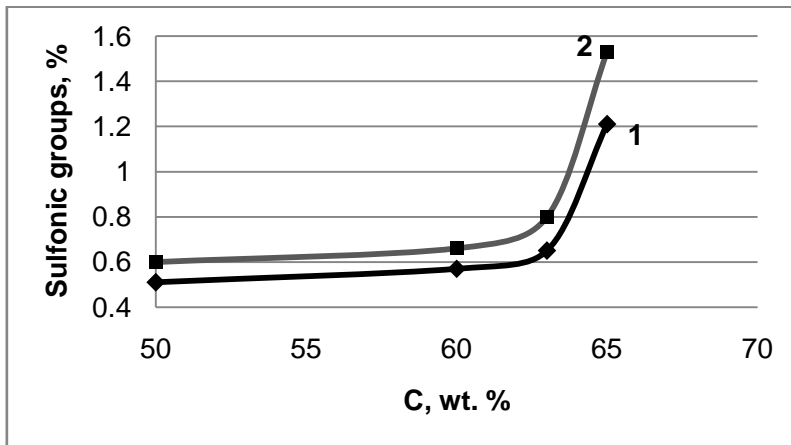


Figure 3: Content of sulfuric groups in hydrolyzed cellulose after hydrolysis at 25°C (1) and 45°C (2) with concentrated acid solutions

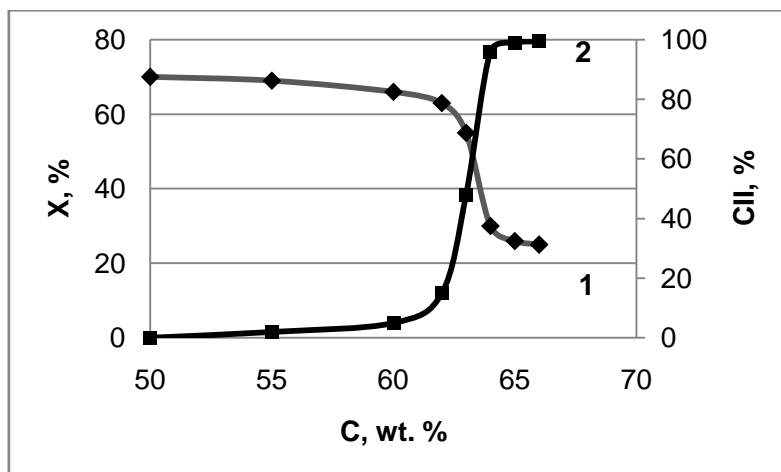


Figure 4: Crystallinity degree (1) and content of CII (2) as a function of sulfuric acid concentration at treatment temperature 25°C

The amorphized cellulose was isolated with increased yield (66–68%) and had a low crystallinity ($X=25\text{--}30\%$), where its crystalline part consisted of crystalline polymorph CII only. Due highly amorphous structure, this product can be used as promising substrate for biotechnology, e.g. for enzymatic conversion of cellulose to glucose (Figure 5).

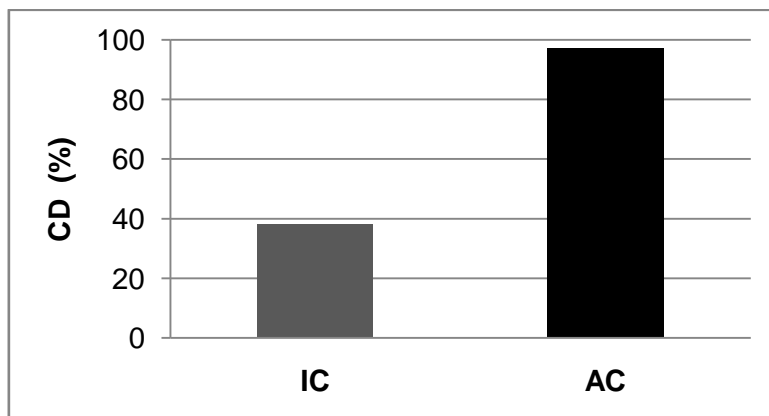


Figure 5: Conversion of the initial cellulose (IC) and amorphized cellulose (AC) to glucose by enzymatic hydrolysis of the samples during 24 h

Temperature 45°C for cellulose hydrolysis with SA was chosen because it regarded as optimal for preparation of nanocellulose [10, 12, 17]. Solubility of cellulose in SA at 45°C was higher, and yield after regeneration was lower than these features at 25°C (Fig. 2). Besides, much more of the sulfonic groups arise at the increased temperature of the acid hydrolysis (Fig. 3). Cellulose can turn into soluble non-regenerable oligomers already after hydrolysis at the elevated temperature with 67 wt.% SA.

After hydrolysis of cellulose at 45°C with 50–60 wt.% SA, the crystallinity degree of the obtained cellulose samples changed low, and these samples retained mainly the CI crystalline polymorph (Fig. 6). When concentration of SA reached 65 wt.%, main part of the initial cellulose dissolved. The cellulose regenerated from 65 wt.% SA had CII crystalline polymorph, decreased crystallinity (25–30%) and low DP (40–50).

To obtain the high-crystalline cellulose nanoparticles having CI polymorph, hydrolysis of initial cellulose materials with 63.5–65 wt.% SA at 45°C was proposed [12,17–19]. However, the experimental results showed (Fig.6) that after treatment with 63.5 wt.% SA crystallinity of cellulose was decreased, while the obtained sample consisted of mixture CI and CII polymorphs.

If the initial cellulose was treated with 65 wt.% SA, then amorphized and depolymerized sample having CII polymorph was formed. Moreover, yield of the isolated cellulose was low, about 30% only (Fig.2).

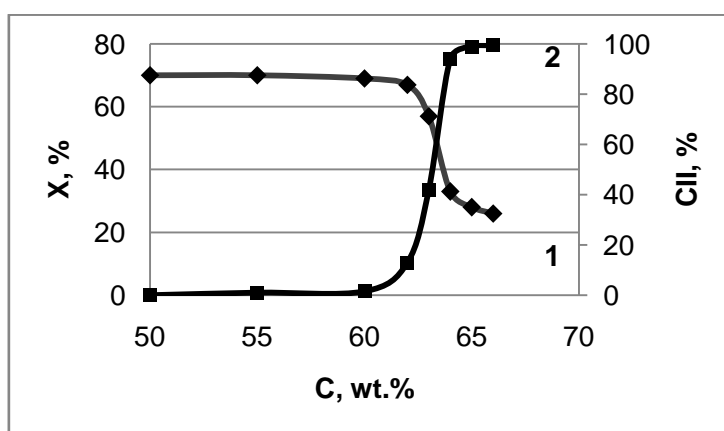


Figure 6: Crystallinity degree (1) and content of CII (2) as a function of sulfuric acid concentration at treatment temperature 45°C

Thus, the known conditions for making of nanocrystalline cellulose particles (NCP) are far from optimal. To prepare really the high-crystalline cellulose nanoparticles, the optimal concentration of SA at the acidic treatment should be in the range 57–60 wt.%.

After treatment of the initial sample with the optimal concentration of SA at 45°C for 1 h and the following high-power disintegration of the hydrolyzed cellulose in water medium, the rod-like NCP (150–200 × 10–20 nm) can be isolated (Figure 7).

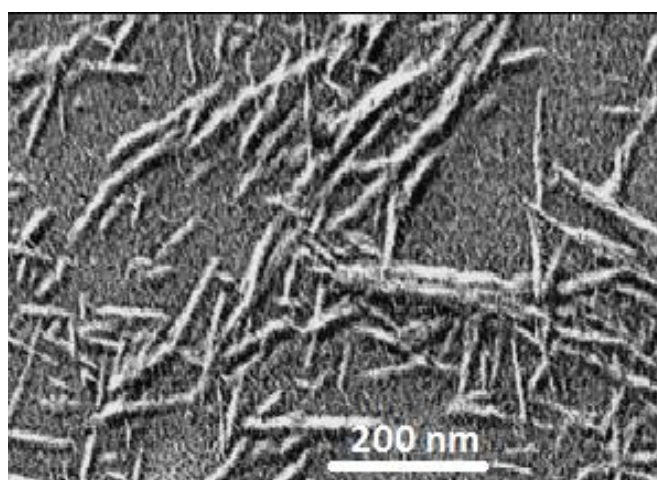


Figure 7: SEM image of rod-like nanocrystalline cellulose particles

Table 1: Main features of the nanocrystalline cellulose particles

Feature	Value
Type of crystalline polymorph	CI
Crystallinity degree, %	70–72
Average particle length, nm	150–200
Average particle width, nm	10–20
Aspect ratio	10–20
Degree of polymerization	130–150

As known, the initial cellulose sample contains nanofibrils that consist of statistically alternated nanocrystallites and non-crystalline domains [8]. During acid hydrolysis at elevated temperature, breaking of the glycoside bonds of non-crystalline domains is taken place that leads to DP-reducing and co-crystallization of nanocrystallites with forming of lateral aggregates of crystallites. The nanocrystallites in these aggregates join together by local lateral crystalline contacts. To isolate the free nanocrystalline particles, it is required to break the crystalline contacts between adjacent nanocrystallites in the aggregates [9]. Treatment of cellulose with 57–60 wt.% SA causes etching of the lateral contacts and sulfonation of surface of individual nanocrystallites. As a result, the crystalline contacts are replaced by weaker amorphous contacts. However, hydrolysis of cellulose with concentrated 57–60% SA only is not enough for releasing of the individual nanocrystallites because even the amorphous contacts join together by hydrogen bonds with average energy 20 kJ/mole and by additional Van der Waals bonds [27]. To breakdown these contacts and isolate the free NCP, it is required the high-power ultrasonic or mechanical disintegration of the hydrolyzed cellulose in the water medium during 10–20 min. Ultrasonic dispersers (20–40 kHz) and high-pressure homogenizers (50–100 MPa) were used for this purpose. To prevent the reaggregation of nanoparticles, solid content of the acid-treated cellulose in water medium at the disintegration should not exceed 2–3%. The determined optimal conditions permit to isolate the NCP (nano-whiskers) with increased yield. As can see from Table 1, the obtained NCP had CI crystalline polymorph and were characterized by nano-sizes (150–200×10–20 nm), decreased DP (130–150) and other features.

CONCLUSION

The effect of concentration of sulfuric acid (SA) and temperature on structure and properties of cellulose had been studied. Investigations of the hydrolysis process of cellulose at room temperature showed that after cellulose treatment with concentrated solutions of SA the appreciable depolymerization was observed. Solubility of the initial sample increased gradually in the range of the acid concentration from 50 to 60 wt.% SA, and the obtained non-soluble cellulose residues had relatively low content of sulfonic groups. When SA concentration reached 65 wt.%, then cellulose sample dissolved completely. Cellulose regenerated from 65 wt.% SA had CII crystalline polymorph, decreased crystallinity degree and low DP. Increasing of SA concentration more than 65 wt.% led to decrease in yield of the regenerated cellulose, and after treatment of the initial sample with 70 wt.% SA the dissolved cellulose cannot be regenerated from the acidic solution by dilution with water due to forming of low-molecular oligomers. The method for production of amorphized cellulose by means of regeneration its solution in 65wt.% SA was proposed. The obtained amorphized cellulose can be used as a promising substrate for biotechnology, e.g. for enzymatic hydrolysis and production of the fermentable sugar – glucose.

At elevated temperature of the acid hydrolysis, 45°C, content of sulfonic groups and solubility of cellulose in SA increase, while yield and DP decrease. Moreover, the cellulose hydrolyzed at the elevated temperature turns to non-regenerable oligomers already at SA concentration of 67 wt.%. After cellulose hydrolysis with hot 50–60 wt.% SA the crystallinity degree of the obtained cellulose samples changed slightly, and these samples retained mainly the CI crystalline polymorph. However, when concentration of SA reached 65 wt.%, then cellulose dissolved and after diluting with water the regenerated cellulose was precipitated. This regenerated cellulose had CII crystalline polymorph, reduced crystallinity (25–30%), and low DP (40–50). Combination of the optimal acidic treatment with the high-power disintegration permits obtaining the NCP (150–200×10–20 nm) with the heightened yield (about 70%). These nanoparticles can be used, for example, as a reinforcing nano-filler for various composites.

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