Nanocellulose Preparation from Cassava Bagasse via Hydrolysis by Sulfuric Acid and Hydrogen Peroxide Medium

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The novel and facile multi-stage method was used for the preparation of nanocellulose from cassava bagasse in Vietnam. Firstly, the cassava bagasse was treated with sodium hydroxide solution followed by chlorine dioxide bleaching for obtaining cellulose pulp with a brightness of 82% ISO. Secondly, the obtained bleached cellulosic pulp was achieved with a dilute sulfuric acid and hydrogen peroxide followed by purification and refining for obtaining nanocellulose. The following optimal conditions of the acidic treatment with adding of hydrogen peroxide for isolation of nanocellulose were proposed: concentration of hydrogen peroxide 0.1 wt.%, the concentration of sulfuric acid 0.25 wt. %, liquor to cellulose ratio 20 to 1, temperature 110°C, time 100 min. The characteristics of nanocellulose were studied by SEM, FTIR, and XRD.

Key Words
Cassava bagasse, Cellulose, Nanocellulose, Nanoparticles, Acid hydrolysis

1. Introduction

It’s well-known that cellulose nanocrystals (CNC), cellulose nanofibers (CNF) and other types of nanocelluloses could be easily obtained from various sources of lignocellulosic wastes such as paper mills sludge, agricultural by-products and residues (rice straw, corn stalks, sugarcane bagasse, etc.)5–8,16,17. The production of nanocellulose is generally carried out in two steps13. The first step is the preparation of cellulosic fibers from raw materials by delignification. Then, it would be used for further processing. There were several methods that could be used for cellulosic fiber production from different lignocellulosic materials13. The most feasible method is alkaline cooking, which is being applied on an industrial scale to produce the cellulose pulp from wood for papermaking and dissolving cellulose. Especially, regarding to the cellulose for nanocellulose fabrication, it is possible to study more eco-friendly methods that employ oxidative delignification agents such as hydrogen peroxide. This method using hydrogen peroxide could alter the cellulose structure at delignification stage, thus facilitating the fabrication of nanocellulose fibers. The second step concerns the fabrication of the nanocellulose by mainly typical processes such as mechanical treatment, acid hydrolysis, enzymatic hydrolysis, TEMPO-oxidation, carboxylation, electro-spinning, etc, which can be used separately or combined10,12,13,15.

Currently, the cassava growing area of Vietnam is stable, about 500,000 ha, with a production of about 100,000 tons per year. There are more than 100 cassava processing factories. The bagasse from the industrial extraction of cassava starch is a solid by-product. It contains 15–50 wt% of cellulose fibers with 3 wt% lignin and 50–80 wt% of residual starch3. Due to these features (low lignin content, availability, etc), it can be considered to be a promising raw material for the extraction of cellulose nanocrystals.

In recent time two-stage (alkali treatment followed concentrated acid hydrolysis) for nanocellulose preparation from cassava bagasse has been investigated, such as using of alkali treatment by KOH solution followed hydrogen peroxide bleaching for cellulose obtaining and concentrated sulfuric acid2, through a combined treatment by KOH and NaClO2 followed homogenization19, sodium hydroxide treatment followed concentrated sulfuric acid18, or sodium hydroxide treatment with sodium chloride and acetic acid bleaching followed homogenization9.

Generally, the use of concentrated acid is devoid of
large acid consumption and a low yield of nanocellulose. Different isolation methods such as acid hydrolysis, mechanical, and TEMPO-oxidation methods with prior post-treatments have been used for the preparation of nanocellulose with various properties related to dimensions, morphology, thermal stability, crystallinity, and mechanical properties. The use of dilute acid such as sulfuric acid combined with oxidation agents such as hydrogen peroxide may be alternative methods for nanocellulose fabrication. Therefore, it is important to study the process using sulfuric acid as a hydrolysis agent for shortening the cellulose chain, and hydrogen peroxide as an oxidizing agent for separating nanofibrils.

Nanocellulose is believed to be a replacement for synthetic materials in more environmentally friendly materials and is an addition to completely new types of biomaterials, i.e., cellulose nanocomposites. Nowadays, cellulose nanocomposites are being used in medical, automotive, electronics, packaging, construction, and wastewater treatment applications.

2. Materials and methods

2.1 Cassava bagasse and chemicals

The cassava bagasse was collected from a local industrial cassava chip in Son Son cassava Company, Phu Tho province, Vietnam.

Commercial enzymes, StargenTM002 (DuPont (USA), cellulase NS 28206 (Novozymes), and Polygalacturonase NS 28212 (Novozymes) were used. Other chemicals were also supplied by Sigma Aldrich including H$_2$SO$_4$ (aqueous solution, concentration of 95 wt%), H$_2$O$_2$ (aqueous solutions, concentration of 34.5 wt%), NaOH (solid, purity of 97%).

2.2 Cassava treatment for obtaining cellulose pulp

Firstly, the cassava bagasse was treated with an enzyme mixture of StargenTM002 (DuPont (USA), cellulase NS 28206 (Novozymes), and Polygalacturonase NS 28212 (Novozymes) with the dosages of 10 GAU/g, 15 CMCcase/g, and 9 U/g over dry bagasse respectively. The 0.1M sodium acetate was used as a buffer solution with a liquor to solid ratio of 12:1 (ml/g) at 45 °C for 3 hours then raised to 60 °C and kept up to 48 hours. Cassava bagasse then washed, dewatered, and used for cellulose preparation.

After the enzymatic treatment, the cassava bagasse pulp was cooked in 50-liter autoclave at 120 °C for 3 hours in sodium hydroxide solution with a dosage of 4% w/w, liquor to solid ratio of 10:1 (ml/g). Cellulose pulp than was washed and submerged tree-stage chlorine dioxide bleaching (Do-EP-D$_1$-EP-D$_2$) to achieve cellulose pulp with a brightness of 82% ISO as follows: Do stage (3.0 % w/w ClO$_2$, 55 °C, 1.0 h), D$_1$&D$_2$ (1.5 % w/w ClO$_2$, 55 °C, 1.5 h), alkaline extraction stage (1% w/w NaOH, 1% H$_2$O$_2$, 70 °C, 1.0 h). These conditions were optimized by separate studies.

2.3. Nanocellulose preparation

For nanocellulose fabrication, the bleached cellulose was submerged in modified hydrolysis by a mixture of hydrogen peroxide and sulfuric acid, with a concentration of 0.1% wt. and 0.25% wt. respectively, at a temperature from 90 °C to 140 °C for 30 - 180 min in an autoclave. The liquor ratio was 20:1. After that, nanocellulose was cleaned by treatment on a dilute solution of hydrogen peroxide and sodium hydroxide followed by washing and then refined mildly for 2 min by a laboratory OSAKA® multifunctional cutter model DH-807 (power 350W, fixed rotation 2500 RPM) until an obtaining of nanocellulose homogeneous suspension, which no precipitated at longtime storage (more than 48 h).

After nanofabrication steps, nanocellulose was centrifuged to determine the yield. The nanocellulose fabrication process is shown in Fig. 1.

2.4 Nanocellulose characterization

The morphological characteristics of the nanocellulose samples were investigated using the JEOL JSM-7600F FESEM microscope at the Advanced Institute of Science and Technology. The samples of nanocellulose in ethanol with a concentration of about 0.05% were sonicated, dropped onto a sample glass holder, dried at room temperature, and then used for SEM analysis. The structural changes of freeze-dried cellulose and nanocellulose samples were determined by SHIMADZU FITR 1S.

The CI of cellulose and nanocellulose was calculated from the XRD spectra according to the amorphous subtraction method described elsewhere using Equation 4 as follows:

$$CI (%) = \frac{(Ic - Iam)}{Ic} \times 100$$

$Ic$: minimum intensity of the peak at 18.7 (amorphous band); Iam: the maximum intensity of the peak at 22.6 (crystal portions).

3. Results and discussion

As known, in acid medium hydrogen peroxide forms anion OH$, which can attack the hydroxyl groups of cellulose, thereby the hydrogen bonds between cellulose macromolecules may be broken down and the fibers can be separated more easily. We applied this idea for nanocellulose preparation in sulfuric acid solution. It can be seen as a modified method of nanocellulose preparation using high concentration sulfuric acid (40-60% wt.). Limited
hydrolysis of cellulose by dilute sulfuric acid was combined with limited fiber separation by hydrogen peroxide at high temperatures, thereby cellulose was modified and nanofibrillation was improved. This is a new approach to the problem, which aims to introduce a novel method of nanocellulose fabrication.

3.1 Influences of hydrogen peroxide

The cellulose treatment at 140 °C for 3 hours, with each agent (hydrogen peroxide and sulfuric acid) separately was conducted to evaluate the effect of each agent on the efficiency of cellulose conversion into nanoforms.

The SEM analysis (Fig. 2) showed that there was almost no difference in fiber morphology between the water-treated samples without hydrogen peroxide and sulfuric acid (Fig. 2a) compared to hydrogen peroxide treated samples (Fig. 2b). Certain, without hydrogen peroxide, the dilute sulfuric acid hydrolyzed the cellulose to nanocellulose, but in this case, the nanofibers remain relatively tightly bound (Fig. 2c), whereas with adding of hydrogen peroxide (Fig. 2d), the nanofibers separate more clearly, formed more separated fibers. This demonstrated the effect of hydrogen peroxide to create a greater repulsion and space between the cellulose macromolecules, whereby the fibers are separated in hydrolysis by sulfuric acid.
### 3.2 Influence of treatment temperature

The series of experiments were carried for the determination of appropriate treatment temperature at 90-140 °C for 180 mins. Hydrogen peroxide and sulfuric acid concentration was 0.5% wt., liquor to solid ratio was 1:10. The SEM analysis of the nanocellulose samples (Fig. 3) obtained from treatment at different temperature levels, showed that from the temperature of 90 °C, the cellulose was hydrolyzed with changes in fiber morphology. However, the fibers were bent, even at higher temperatures (100-110 °C). When the temperature was been taken up to 140 °C, the formation of nanofibers was more expressed, the fibers were relatively well separated. The process of nanocellulose formation was, therefore, better at higher processing temperatures. The obtained nanocellulose had an average diameter of less than 100 nm.

The nanocellulose samples were obtained in a suspension form, without precipitation, when they are stored on open flacons for prolonged periods. All of the nanocellulose samples were obtained in a gel form after refining but easily filtered and dewatered.

The determining yield of the obtained nanocellulose samples (Fig. 4) indicated that when the treatment temperature was higher than 120 °C, the yield of nanocellulose decreased sharply, due to the strong hydrolysis of cellulose, and the nanocelluloses with small sizes were difficult to recover by the centrifugal method. Thus, to obtain nanocellulose with an acceptable yield, it is possible to apply a treatment temperature of about 110 °C.

### 3.3 Optimization of treatment time

Several experiments were carried to investigate the effects of treatment time on the formation and properties of nanocellulose. Treatment temperature was chosen at 110 °C. The SEM analysis of the nanocellulose samples at the same temperature with a treatment of 30 mins to 100 mins...
Fig. 3 SEM of nanocellulose obtained for 180 min with the treatment of cellulose in 0.1% wt. H$_2$O$_2$ and 0.25% wt. H$_2$SO$_4$ solution at different temperature. a: 90 °C, b: 100 °C, c: 110 °C, d: 120 °C, e: 130 °C, f: 140 °C. Zoom images of 100,000x
Figure 4 Influences of temperature to yield of nanocellulose

The results of the FTIR spectra analysis of bleached cellulose (Fig. 6a) and nanocellulose samples obtained at temperatures of 110 °C (Fig. 6b) showed that there have not been any new peaks in the FTIR spectra of the nanocellulose samples, rather it’s being converted to other cellulosic derivatives. It is evidence of a successful nanocellulose fabrication. FTIR of cassava bagasse cellulose showed peaks at 3331 cm⁻¹ (O–H stretching vibrations), 2900 cm⁻¹ (C–H) 1). After the multistep treatment, the peaks the shift of peak at 1641 cm⁻¹ (O–H vibration) was unchanged. Some changes were fixed at 428 cm⁻¹ to about 800 cm⁻¹. The FTIR of nanocellulose showed the peaks as well as the cellulose FTIR. This suggests that the hydrogen peroxide only prepared cellulose fibers, not oxidized.

Figure 5 SEM of nanocellulose samples obtained at 110 °C with different treatment times. a: 30 min (1,000x); b: 60 min (30,000x), c,d: 100 min (30,000x and 100,000x)
cellulose.

Thus, the optimal treatment time should be about 100 mins for the successful conversion of the cellulose into nanofibers with the appropriate size fraction.

XRD diffraction patterns of the cassava bagasse bleached cellulose and nanocellulose are shown in Fig. 7. The intensity of XRD peaks of the RS fiber indicated the increase in the crystallinity due to the removal of non-cellulosic materials and amorphous regions by the treatment 1). The CI of cellulose was calculated using Equation 4) increased from 58.2% to 71.6% after the multistep treatments with H\textsubscript{2}SO\textsubscript{4} and H\textsubscript{2}O\textsubscript{2}. The crystallinity of the prepared nanocellulose was lower than cellulose nanocrystals which were obtained by hydrolysis using concentrated sulfuric acid.

4. Conclusion

Hydrogen peroxide in the dilute sulfuric acid medium is a new agent to separate cassava bagasse derived cellulose into nanocellulose. By multistep appropriate treatment conditions of bleached cellulose in solution with 0.1% wt. H\textsubscript{2}O\textsubscript{2} and 0.25% wt. H\textsubscript{2}SO\textsubscript{4} at a temperature of 110 °C for 100 min were fabricated cellulose nanofibers with fiber diameter < 100 nm. The yield of nanocellulose was about 70% of bleached cellulose or about 22% of oven-dry cassava bagasse.

The nanocellulose gel was filtered and refined easily, as in this study where we used a conventional mixer.

The novelty of this work is the utilization of hydrogen peroxide as a preparing agent of cellulose fibers. It has not oxidized the cellulose. This new method for nanocellulose preparation may be called modifying hydrolysis. The above-mentioned results suggest the prospect of the studied
method of nanocellulose fabrication, which can be applied to different types of cellulose, such as wood cellulose, and on an industrial scale.

In addition, the use of the waste from cassava starch production for the fabrication of biomass-based nanomaterials brings economic benefits and help reduce environmental pollution.

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