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Research Article

Investigation of bleaching stage in cellulose isolation from pineapple leaves for cellulose nanocrystals production

Co Dang Pham[1,2], Nhi Trang Vo[1,2], Nga H. N. Do[1,2], Kien A. Le[3], Phung K. Le[1,2,*]

ABSTRACT

Nanocrystalline cellulose has emerged as a substantial nanomaterial in recent years due to its peculiar characteristics such as bio renewability, sustainability, and low toxicity while having high mechanical strengths, optical transparency, and much more. Meanwhile, pineapple leaves (PL) as by-products after fruit harvest exhibit a huge potential in cellulose and nanocellulose extraction due to their high cellulose content (approx. more than 36%). The latest studies have successfully recovered cellulose from pineapple leaves, in which the bleaching stage greatly affects the properties of the produced cellulose. In some cases, this stage can result in a reduction in cellulose content because of the excessive use of chemicals. However, the effects of influential factors such as solid-liquid ratio, reaction time, and reagent concentration in the bleaching stage have not been widely investigated although they are necessary to scale up the cellulose recovery process. In this study, cellulose was extracted from PL using alkali treatment with sodium hydroxide and bleaching with hydrogen peroxide before synthesizing nanocellulose. The characterization of PL, cellulose, and nanocellulose was performed by Thermogravimetry Analysis (TGA), Fourier Transform spectroscopy (FTIR), X-ray diffraction (XRD), Transmission Electron Microscope (TEM) and Dynamic Light Scattering (DLS). The results indicated that bleaching with H_2O_2 at 6% after 60 min at a solid-liquid ratio of 1:20 yielded an impressively high cellulose content of 94.25%. Obtained nanocellulose possessed high crystallinity index of approx. 80% with a diameter in the range of 15-30 nm. Along with further research related to the application of organic nanoparticles, this study has a great impact on the proposing processes with better stability, which is meaningful in terms of green chemistry towards sustainable development by satisfying most principles of this theory.

Key words: Bleaching step, Cellulose recovery, Nanocellulose, Morphology

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INTRODUCTION

Cellulose is considered one of the most abundant polysaccharides on the Earth, which is usually found in plants, waste, or agricultural residues[1]. This linear organic polymer consists of several hundred to many thousands of D-glucose units linked via β-1,4-glycosidic bonds. The intramolecular hydrogen linkage between hydroxyl groups and oxygen of the contiguous ring attributes to not only the unbranched structure of cellulose but also to the stability of these chains[2]. Along with the presence of numerous hydroxyl groups resulting in a strong affinity to many substances, cellulose possesses an attractive mechanical property. In specific, the tensile strength of the cellulose chain is in the range of 4.9-7.5 GPa and approximately 150 GPa for the theoretical modulus, in addition to its low density (nearly 1.6 g/cm^3)[3]. Besides, due to its low toxicity and biodegradability, cellulose exhibits a huge potential for various applications such as food packing and water treatment[4].

Nanocellulose (NC) is a valuable material that is extracted from cellulose and is usually categorized into three types nanocellulose fibers (CNF), nanocellulose crystals (CNC), and bacterial nanocellulose (BNC)[5]. Along with the intrinsic characteristics which are obtained from cellulose, NC is utilized in various applications due to its superior properties such as high surface area and high surface strength[6]. CNC, known as a typical crystalline material, has a diameter in the range of 20-50 nm and dozen to hundreds of nanometers in length. CNC could be used as a mechanical reinforcing agent, thickening agent, rheological modifier, and in drug delivery applications, etc.[7]. Thus, the CNC extraction from pineapple leaves (PL) is not only an effective solution to solve environmental problems of waste products from agricultural production but also creates a foundation for the synthesis of advanced materials.

Extensive studies have been executed to investigate the extraction process of cellulose in general and nanocellulose in particular from many types of biomass such as rice straw, coconut coir, and sugarcane bagasse. Among these, PL with a production of 28.18 Mt in 2020 occupy approx. 1 M ha of

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plantation area in the world and are one of the most enormous cellulose-rich biomass sources[8]. In specific, due to the high cellulose content (more than 36%)[9], PL can be employed for the synthesis of valuable materials and nanocellulose production, in particular[10]. However, as can be seen, despite a lot of recent research on PL utilization, most of this raw material is still processed by burning or landfilling, which leads to a negative impact on human health and global warming[11]. Studies related to the investigation of the effect of influential factors such as solid-liquid ratio, reaction time, and reagent concentration on the cellulose production process, have not been widely investigated so far. Do et al. synthesized cellulose microfibers from PL using alkaline and oxidation method at a bench scale. It also revealed that obtained product had a cellulose content of nearly 79%[12]. To be employed in diverse applications, cellulose production should be explored to increase cellulose recovery efficiency with higher cellulose purity. In another study, Shih et al. treated PL with NaOH and HCl, followed by bleaching 2 times with $NaClO_2$ and 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) to prepare purified cellulose for nanocomposite production[13]. Despite the successful cellulose and nanocellulose extraction, this process required a long time for each stage. Consequently, harmful components, such as dimethyl sulfide and hydrogen sulfide, were generated after the bleaching process[14]. In addition, the mechanism of this step was studied in some previous papers. Particularly, cellulose decolorization occurs mainly due to the oxidation of carbonyl and quinoid structure of the lignin side chain, which destroys its chromophore sites. H_2O_2 may also change the solubility of lignin, hence enhancing lignin oxidative degradation, by reacting with the benzoquinone structure or the side chain carbonyl and C-C double bond of lignin. However, in the presence of alkali, H_2O_2 treatment might lead to carbohydrate degradation. Nevertheless, H_2O_2 bleaching is still considered an effective method for the treatment of lignocellulosic biomass[15]. The investigation of influential factors, therefore, plays an important role in reducing energy consumption, required time, and even the cost of the final product. This also helps further increase the potential of cellulose application in various fields.

In this work, the affecting factors in the bleaching stage in cellulose preparation such as H_2O_2 concentration, time, and solid-liquid ratio, were investigated. The morphology, thermal degradation, functional groups, and cellulose content after each step were characterized using different techniques. Finally, nanocellulose was also extracted from PL to prove the quality of obtained cellulose.

MATERIALS AND METHOD

Materials

PL (*Ananas comosus* L.) were collected in Tien Giang province, Viet Nam after harvesting pineapple fruits. Fresh PL were washed with tap water, dried under the sunlight until their moisture is below 12%, and then pulverized into powder (80 mesh). The chemicals purchased from the commercial were analytical grade, including sodium hydroxide (NaOH, 99%) and hydrogen peroxide (H_2O_2, 30%) without any further purification. All solutions were prepared in distilled water (DW).

Investigation of the bleaching step

PL powder was treated with NaOH 5 wt.% (solid-liquid ratio of 1:15 g/mL) for 120 min at 80 °C. The solid was subsequently washed with distilled water until reaching neutral pH, followed by drying and grinding to obtain the pretreated PL (PP) for further investigation of the bleaching stage. The studied parameters are shown in Table 1. The bleached pulp is denoted as BPL.

Nanocellulose production from BPL

BPL with the highest cellulose content was hydrolyzed with H_2SO_4 64 wt.% (solid-liquid ratio of 1:20 g/mL) at 45 °C for 60 min. Subsequently, DW was added to quench the reaction, followed by centrifuging the mixture at 5000 rpm for 5 min to collect the precipitate. This step was repeated 3 times before dialyzing the obtained NC until reaching neutral pH. After dialysis, the NC suspension was freeze-dried to collect dry CNC.

Characterization

The composition of PL including holocellulose and lignin was determined by employing the National Renewable Energy Laboratory (NREL) method[16]. The residue yield (RY) is defined as the mass percentages of the produced product compared to the initial material in each step, as described in **Eq. 1**:

$$RY_i\,(\%) = \frac{w_i}{w_{i-1}} \times 100 \qquad (1)$$

where: W_i (g) is the product weight of step i.

Fourier Transform spectroscopy (FTIR) results were recorded on a Bruker Tensor 37 spectrometer. Thermogravimetry Analysis (TGA) was executed on a LINSEIS DSC PT 1600 (France) interfaced with a heated rate of 10 °C/min from 30 °C to 700 °C under the air atmosphere. The structural characteristics of PL, APL, and BPL samples were studied using an

Table 1: Experimental parameters to investigate the effects of H_2O_2 concentration, solid-liquid ratio, and time on cellulose content

H2O2 concentration (%)	Solid-liquid ratio (g:mL)	Time (min)
2; 4 ; 6; 8; 10	1:20	60
6	1:10 ; 1:15; 1:20; 1:25; 1:30	60
6	1:20	30; 45; 60; 75; 90

SEM Prisma E with a 10 kV operation voltage. X-ray diffraction (XRD) analysis was performed by Bruker Advance D8 Diffractometer (Germany). The crystallinity index (CrI) is determined using **Eq. 2**:

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (2)$$

The crystalline and amorphous areas are represented by I_{002} ($2\theta = 22.5°$) and I_{am} ($2\theta = 18°$), respectively. The apparent size distribution and zeta potential of the CNC sample were recorded using a Malvern Zetasizer Nano ZS90. Transmission Electron Microscope (TEM) images of CNC were captured on a JEM-1400 at 100 keV at room temperature.

RESULTS AND DISCUSSION

Pretreatment step

As can be seen in Figure 1a, the diameter of PL fibers was reduced to about 5 μm after grinding, with a dark brown color due to the presence of a substantial amount of non-cellulosic content (i.e., lignin and hemicellulose). The dark color got lighter after alkali pretreatment (Figure 1b), which indicated the partial removal of lignin. This was further confirmed through the NREL analysis (Figure 1c). The cellulose content in raw PL was found to be 45.20%; grinding PL into powder only helped reduce the fiber diameter, as the cellulose content remained relatively unchanged (44.88%), while alkali treatment significantly increased this value to 73.05%. Although purified cellulose was yet to be obtained after this step, alkalization was proved to be effective in breaking the lignocellulosic complex in PL structure and partially dissolving lignin [17], serving as crucial preparation for the following bleaching stage.

Investigation of the bleaching stage

From a general perspective, bleaching referred to as the cellulose purification step, is a crucial stage in the process. As mentioned, H_2O_2 in the presence of alkali could lead to a decrease in cellulose content via carbohydrate degradation. The concentration of H_2O_2 in this step, therefore, was explored, with the result shown. The experiments were carried out at a fixed solid-liquid ratio of 1:20 for 60 min, and the results are shown in Figure 2.

The results showed that the holocellulose content was in the range of 86.41% to 95.90%. As can be seen, the lignin content gradually decreased from 13.59% to 4.1%. This can be explained by the increase in H_2O_2 concentration which significantly affects the lignin component deep inside the structure of PL. In addition, the amount of lignin declined marginally from 5.75 to 4.10 with increasing H_2O_2 concentration from 6% to 10%. However, the difference in cellulose and lignin content of BPL did not alter significantly when increasing H_2O_2 concentration over 6%, the 6% point was considered the most suitable for this parameter.

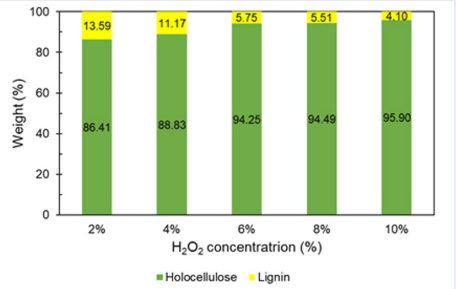


Figure 2: Effect of H_2O_2 concentration on the composition of BPL.

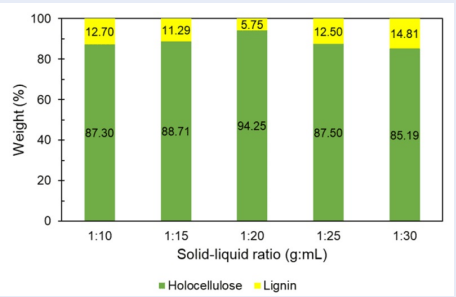
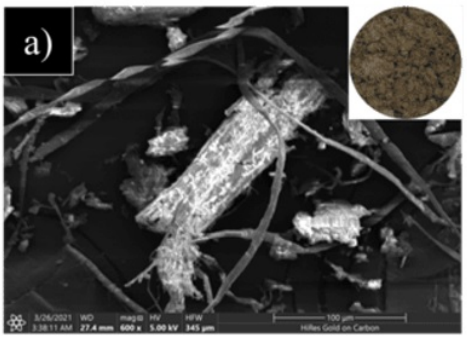


Figure 3: Effect of solid-liquid ratio on the composition of BPL.



c)

Samples	Cellulose content (%)
Raw pineapple leaves (PL)	45.20
Pineapple leaves powder	44.88
Pretreated pineapple leaves (PP)	73.05

Figure 1: SEM images of (a) raw pineapple leaves (PL) fibers and (b) pretreated PL powder (PP); (c) cellulose content of the samples determined by the NREL method

With an H_2O_2 concentration of 6%, the impact of the solid-liquid ratio on cellulose content was studied in 60 min. The results (Figure 3) demonstrate that the obtained holocellulose content was in the range of 85.19% to 94.25% and slightly affected by the solid-liquid ratio. The highest holocellulose content reached the point of 94.25% with the solid-liquid ratio at 1:20 and the lowest was 85.19% at the ratio of 1:30. In addition, the holocellulose content decreased gradually when increasing the ratio from 1:20 (94.25%) to 1:30 (85.19%). This can be explained by the larger amount of H_2O_2 in the reaction. Apart from decolorizing and removing lignin in PL, H_2O_2 also causes cellulose degradation. When the solid-liquid ratio increased to a point that the majority of lignin was removed, the cellulose breakdown started to be more noticeable, which lower the cellulose content and hence, amplified the lignin concentration in the product. Based on the results obtained, the solid-liquid ratio of 1:20 was chosen for the following experiments on bleaching time.

To investigate the effect of bleaching time on cellulose content, PP was bleached using H_2O_2 6% with the solid-liquid ratio at 1:20 at a different time (from 30-90 min). Figure 4 reveals that holocellulose content rose gradually from 30 min (86.52%) to 60 min (94.30%) before a downward trend in longer reaction time. In the previous work, Wu et al. proved that long bleaching time led to a decrease in the cellulose content of the final product [18]. After most of the recalcitrant compounds such as lignin and hemicellulose were removed, the oxidant (H_2O_2) caused carbohydrate degradation. This directly influences the cellulose content and results in an increase in lignin content after 60 min of bleaching. Kenly et al. reported that the bleaching stage using H_2O_2 for nanocellulose production required a shorter reaction time than TEMPO oxidation [19]. Therefore, the 60-min duration was selected as the most appropriate bleaching time.

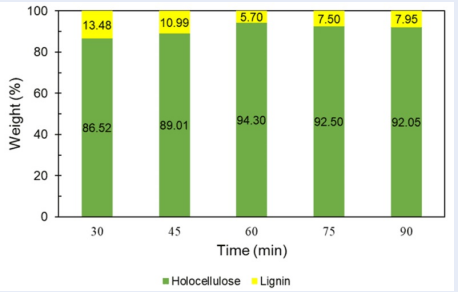


Figure 4: Effect of reaction time on the composition of BPL.

After bleaching, the cellulose obtained a bright appearance (Figure 5a) and a purity of over 94%, with a diameter of around 5 μm (Figure 5a).

The changes in material components and properties, as well as the effectiveness of the bleaching step, were

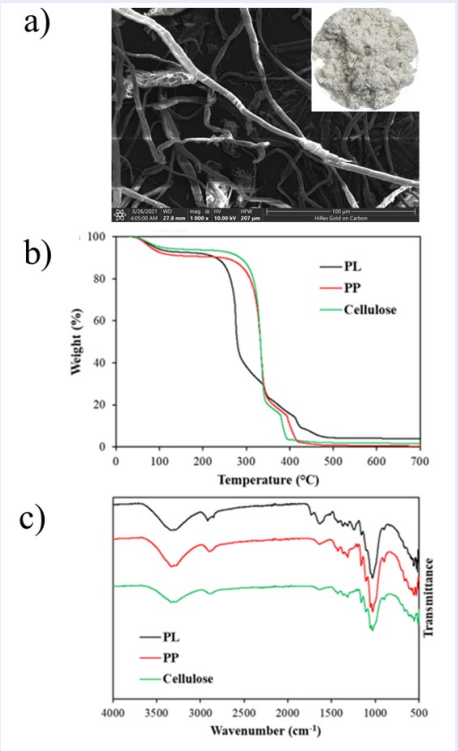


Figure 5: (a) Appearance and SEM image of cellulose from pineapple leaves; (b) TGA results and (c) FTIR spectrum of the samples

confirmed through FTIR and TGA. The removal of the majority of hemicellulose, lignin, and pectin from PL after alkalization and bleaching was attributed to the difference in TGA results (Figure 5b). Specifically, the initial decomposition temperature of about 20% mass was found at approximately 250 °C for PL, while this figure was up to 325 °C for cellulose. The significant improvement in cellulose decomposition temperature could be explained by the absence of components degrading at a lower temperature such as hemicellulose in raw material[20]. The elimination of non-cellulosic components in PL was confirmed by FTIR spectra (Figure 5c). Signals at 1738 cm^{-1}, 1517 cm^{-1}, and 1255 cm^{-1} were assigned to the vibrations stretching of the ether group and the occurrence of C=C in the aromatic ring and C=O bonds, which indicated the existence of lignin and hemicellulose[21]. These bands were visible in all samples but with significantly reduced intensities in the cellulose sample, proving the effectiveness of the pretreatment and bleaching stage on lignin removal.

PL-derived CNC

To assess the quality of cellulose isolated from PL in this study, aside from analysis techniques, the cellulose which was bleached with H_2O_2 6% in 60 min, at a solid-liquid ratio of 1:20, was further employed to produce CNC using hydrolysis. The obtained nanomaterial exhibited an obvious difference in crystallinity from cellulose, as determined by XRD results (Figure 6). It can be observed that both samples show typical diffraction peaks of cellulose I at 2θ = 15.5°, 18°, 22°, and 34.5°, which represent the (110), (101), (200), and (004) planes, respectively[9]. After the bleaching stage, obtained cellulose had a relatively low crystallinity index of nearly 67.97%, which can be explained by the presence of amorphous cellulose and non-cellulosic constitutes, as well as the effect of NaOH on the structure of the material[22]. Meanwhile, the crystallinity index of CNC increased significantly to more than 81%, which was the result of the elimination of these non-crystalline areas through acid hydrolysis.

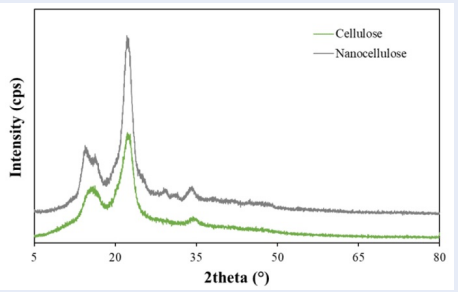


Figure 6: XRD patterns of cellulose and nanocellulose from pineapple leaves

The size distribution of the CNC sample was estimated using the DLS technique due to its facileness and convenience. The result in Figure 7a showed the size distribution of the sample, with an average diameter was 303.9 nm and a zeta potential of -40.6 mV. As the absolute zeta potential was higher than 30, the sample was considered to be a stable suspension[23]. Subsequently, TEM was used to confirm the shape and dimensions of CNC derived from cellulose. The sulfuric acid hydrolysis successfully yielded rod-like crystals 15-30 nm wide and 150-300 nm long (Figure 7b). This result was in agreement with previous studies[24].

CONCLUSIONS

This paper comprehensively studied the effects of various factors in the bleaching step for cellulose recov-

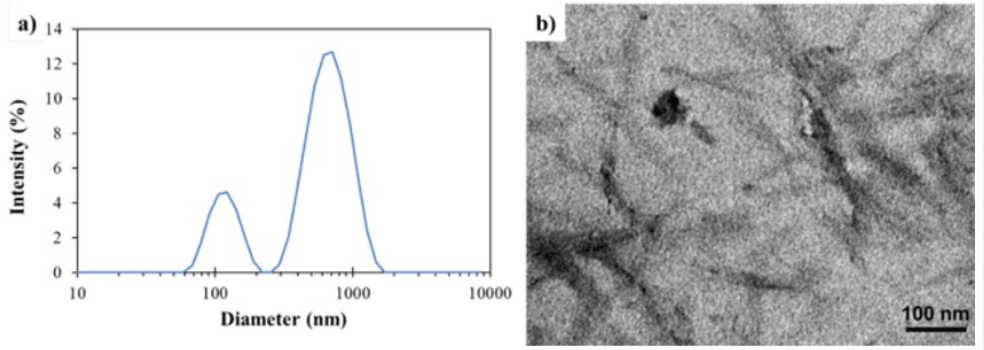


Figure 7: (a) Size distribution and (b) TEM image of nanocellulose from pineapple leaves

ery from PL. By determining the most suitable oxidant concentration, solid-liquid ratio, and reaction time, a facile process to extract high-purity cellulose with minimized chemical consumption was developed. From characterization results, the obtained cellulose showed little to no trace of lignin and a crystallinity index of approximately 67.97%. The cellulose content of over 94% obtained after the facile process exhibited the great potential of the material in nanocellulose production on a larger scale for various applications.

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ABBREVIATION

BNC: Bacterial nanocellulose
BPL: Bleached pulp
CNC: Nanocellulose crystals
CNF: Nanocellulose fibers
DLS: Dynamic light scattering
DW: Distilled water
FTIR: Fourier transform spectroscopy
NC: Nanocellulose
PL: Pineapple leaves
TEM: Transmission electron microscope
TEMPO: (2,2,6,6-tetramethylpiperidine-N-oxyl)
TGA: Thermogravimetry analysis
XRD: X-ray diffraction

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Co Dang Pham: Conceptualization, Visualization, Writing – original draft. **Nhi Trang Vo**: Methodology, Investigation, Formal analysis. **Nga H.N. Do**: Characterization, Data analysis, Writing – review & editing. **Kien A. Le**: Data curation, Resources, Writing – review & editing. **Phung K. Le**: Writing – review & editing, Funding acquisition, Project administration.

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Tạp chí Phát triển Khoa học và Công nghệ – Engineering and Technology 2022, 5(4):1751-1758

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Bài nghiên cứu

Khảo sát ảnh hưởng của quá trình tẩy trắng đến cellulose thu được từ lá dứa ứng dụng trong việc sản xuất nano cellulose

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TÓM TẮT

Trong những năm gần đây, nano-cellulose có cấu trúc tinh thể được xem là một vật liệu nano quan trọng nhờ vào các đặc tính đặc biệt của chúng như khả năng tái tạo sinh học, tính bền vững, độc tính thấp trong khi có độ bền cơ học và độ truyền suốt quang học cao cùng nhiều đặc tính khác nữa. Trong khi đó, phụ phẩm lá dứa sau khi thu hoạch trái rất giàu tiềm năng cho việc sản xuất cellulose cũng như nano-cellulose nhờ vào hàm lượng cellulose cao (khoảng hơn 36%). Nhiều nghiên cứu gần đây liên quan đến việc thu hồi cellulose từ lá dứa đã chỉ ra tầm quan trọng của công đoạn tẩy trắng đến tính chất của cellulose thu được. Trong một số trường hợp, cellulose bị thất thoát trong công đoạn tẩy trắng do sử dụng quá mức các chất hóa học. Tuy nhiên, những tác động của các yếu tố như tỉ lệ rắn lỏng, thời gian phản ứng, nồng độ các chất trong quá trình tẩy trắng vẫn chưa được nghiên cứu rộng rãi, mặc dù đây là điều cần thiết để thu hồi cellulose ở quy mô lớn. Trong nghiên cứu này, cellulose đã được thu hồi từ lá dứa bằng phương pháp kiềm hóa với natri hydroxit và tẩy trắng với hydro peroxit trước khi được sử dụng để sản xuất nanocellulose. Cấu trúc của lá dứa thô, cellulose và nanocellulose được phân tích bằng các phương pháp hiệt trọng lượng (TGA), quang phổ hồng ngoại biến đổi Fourier (FTIR), nhiễu xạ tia X dạng bột (PXRD), tán xạ ánh sáng động (DLS). Kết quả cho thấy sau quá trình tẩy trắng dài 60 phút với H_2O_2 6% ở tỉ lệ rắn-lỏng 1:20, cellulose thu được có độ tinh khiết đạt 94,25%. Nanocellulose thu được sở hữu độ tinh thể cao khoảng 80% và đường kính sợi nằm trong khoảng 15-30 nm. Cùng với những tìm hiểu sâu hơn liên quan đến khả năng ứng dụng của hạt nano hữu cơ, nghiên cứu này có đóng góp to lớn trong việc đề xuất những quy trình với độ ổn định cao hơn, điều có ý nghĩa về mặt hóa học xanh hướng tới sự phát triển bền vững nhờ việc đáp ứng những tiêu chí của lý thuyết này.

Từ khoá: Tẩy trắng, Thu hồi cellulose, Nanocellulose, Hình thái học

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