

## RESEARCH ARTICLE



# Production of Cellulose Hydrogel from Nipa (*Nypa fruticans* Wurmb) Frond

Alvin G Domingo<sup>1\*</sup>, Jayson F Cariaga<sup>2</sup>, Bjorn S Santos<sup>3</sup>, Shirley C Agrupis<sup>3</sup>

<sup>1</sup> Researcher, Graduate School, Mariano Marcos State University, Mariano Marcos State University

<sup>2</sup> Researcher, National Bioenergy Research and Innovation Center, Mariano Marcos State University

<sup>3</sup> Professor, Graduate School, Mariano Marcos State University

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\* Corresponding author.

[alvindomingo30@gmail.com](mailto:alvindomingo30@gmail.com)

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## Abstract

**Objectives:** The study is aimed to assess the potential of nipa frond biomass to produce cellulose hydrogel by analyzing the elemental composition, morphology, solubility, and effectivity of the isolated nipa  $\alpha$ -cellulose (iN $\alpha$ C).

**Methods:** The iN $\alpha$ C was produced through physico-chemical treatments and examined using a scanning electron microscope. After dissolving the cellulose, it was mixed with various chemical cross-linkers to produce cellulose hydrogels.

**Findings:** Results showed that the iN $\alpha$ C had well-distributed microfibrils of cellulose arranged in parallel orthogonal layers. Solubility (%) reveals treatment 2, involving 10% NaOH pre-treatment of iN $\alpha$ C, yielded the ideal cellulose for eco-friendly and cost-effective cellulose solution production with high solubility percentage. Moreover, cellulose-based hydrogels were more viscous and more turbid than water-based hydrogels, increasing the integrity of the produced hydrogels. **Novelty:** The study demonstrates that iN $\alpha$ C can be transformed into a cellulose-based hydrogel through a one-step chemical crosslinking process, which can streamline the production process and reduce toxic pollution. This has important environmental benefits and potential applications in medical, agricultural, and wastewater treatment.

**Keywords:** Cellulose Hydrogel; Nipa Frond; Biomass; Chemical Crosslinking

## 1 Introduction

Cellulose-based hydrogels have attracted great heed to their numerous advantages such as biodegradability, biocompatibility, non-toxicity, and low cost. With the emergence of this material, cellulose becomes an attractive pathway for fabricating sustainable structures. Because of these numerous advantages, cellulose-based hydrogels have been utilized widely in several applications such as the production of wound dressings, contact lenses, hygiene products, commercial uses in drug delivery, agriculture, wastewater treatment, and also in tissue engineering.

Cellulose is the most sustainable and almost inexhaustible biopolymer material. It has the potential to meet the increasing opportunities in making eco-friendly products. To widen its applications, cellulose needs to undergo dissolution. Different solvent

systems in dissolving cellulose have been studied, such as NaOH/urea<sup>(1)</sup>, ionic liquids<sup>(2)</sup>, LiOH/urea<sup>(3)</sup>, and NaOH/thiourea<sup>(4)</sup>. Different solvent systems give different levels of cellulose homogeneity. In addition, a mixture of alkali/urea in cellulose dissolution is more effective than alkali only. Consequently, to ensure the cellulose is homogenous throughout the process, cellulose dissolution using different solvent systems is important.

NaOH/urea is one of the solvent systems that gained attention due to its simplicity, being environmentally friendly, low toxicity, and low cost<sup>(5)</sup>. Under a low range of concentration and low-temperature conditions, cellulose can be dissolved in NaOH/urea. The interaction occurs when cellulose forms a hydrogen-bond-induced inclusion complex with alkali and urea in the form of a shell. Recent researches prove that cellulose dissolution depends on solvent temperature, stirring rate, stirring time, molecular weight, and crystallinity.

The preparation of cellulose hydrogels from derived celluloses has been the main focus of many recent studies. There have been countless studies that have been tested to prepare derived cellulose-hydrogels, such as pre-esterifying<sup>(3)</sup> irradiation grafting, graft polymerization, and radical polymerization. A series of techniques, such as chemical cross-linking, chemical initiation, microwave, UV-curing technique, glow discharge electrolysis plasma, and  $\gamma$ -ray irradiation, have been introduced for the production of cellulose-based hydrogels<sup>(6)</sup>. Polymerization and initiation techniques enable the preparation of poly (N-isopropyl acrylamide)-cellulose composite hydrogels, which exhibit lower extents of phase temperature over a wider temperature range than the free PNIPAAm hydrogels<sup>(7)</sup>. Cellulose-based hydrogels were also obtained in NaOH/urea system using acrylic acid and cellulose as the raw materials with the help of N, N'-methylenebisacrylamide (MBA) as a cross-linker via glow discharge electrolysis plasma technique<sup>(8)</sup>.

Chemical crosslinking in hydrogel usually focuses on the bonds between the crosslinking agent and polymer. A specific functional group from the crosslinking agent leads to the hydrogel's properties, especially the mechanical strength. Polymers with hydroxyl groups can be crosslinked with glutaraldehyde under extreme conditions: low pH, high temperature, and methanol added as a quencher<sup>(9)</sup>. To form a hydrogel, polysaccharides can be crosslinked with 1, 6-hexamethylene diisocyanate, divinyl sulfone, and many other reagents. The purpose of these reagents is to ensure that a specific polymer or functional group builds a network between bonds to form hydrogels.

However, the techniques mentioned have certain disadvantages which oppose the sense of having an efficient, cheaper, and "green" technology to produce cellulose hydrogels. The tedious and complex preparation process, high cost, less efficiency, and other toxic secondary pollution are some of the drawbacks of these techniques. Also, it requires a lot of chemical reagents which makes the post-treatment very complex.

This study presents a very simple, cheap, and one-step approach to producing cellulose hydrogels from matured nipa frond biomass. Succinctly, the dissolved isolated nipa  $\alpha$ -cellulose (iN $\alpha$ C) was mixed with N, N'-methylene bisacrylamide (MBA), Sodium alginate (SA), and Carbomer polymer (CP). Its solubility, chemical, and physical properties were also determined.

## 2 Methodology

### 2.1 Characterization of the Isolated Nipa $\alpha$ -Cellulose (iN $\alpha$ C)

Briefly, iN $\alpha$ C was produced using acid-base hydrolysis in different concentrations (7%, 10%, 12%, and 15% NaOH) from nipa fronds collected in Pasuquin, Ilocos Norte, Philippines. A commercial cellulose (carboxymethyl cellulose) was bought for comparison. Elemental analysis was done through XRF analysis. Lastly, morphological properties of the celluloses were observed through SEM. Images were obtained up to 2000 magnification times.

Elemental analyses were measured with an Olympus Delta Handheld X-Ray Fluorescence Analyzer (ASTM E1476-16, ASTM E3057-16, and ASTM E3077-17). The X-ray radiation used was a Ni-filtered Cu-K $\alpha$  line (1.54 Å). The current was set at 30 mA and the voltage was set at 40 kV. The cellulose samples were mounted on a solid circular holder.

SEM observed the morphology properties of cellulose samples (Model FlexSEM1000). Prior to taking pictures, the samples were sputtered with gold. Images were obtained at magnifications up to 2000 times.

### 2.2 Cellulose Dissolution through NaOH/Urea

$\alpha$ -Cellulose obtained from the last protocol of the nipa frond underwent drying for 48 hours.

#### 2.2.1 Cellulose Dissolution

7 wt% NaOH/12 wt% urea aqueous solution was prepared by mixing NaOH, urea, and distilled water (7:12:81 by weight) and was pre-cooled to -12.6°C. Cellulose (1 g) was dispersed in the solvent (50 mL) and stirred for 5 min at 5000 rpm. The suspension turned into a clear solution.

### 2.2.2 Solubility test

The cellulose solution was prepared using the method described above. The cellulose solution was centrifuged at 8000 rpm for 20 min to obtain a supernatant solution. NaOH/urea solution containing the dissolved cellulose fraction was neutralized with 10 wt% H<sub>2</sub>SO<sub>4</sub>; the cellulose precipitate (regenerated cellulose) was isolated using filtration methods, washed with water, and dried at 60 °C to a constant weight. The solubility of cellulose in NaOH/urea aqueous solution was calculated by:

$$\text{Solubility (\%)} = W_2/W_1 \times 100$$

Where W<sub>1</sub> is the weight of the original cellulose and W<sub>2</sub> is the weight of dissolved fractions.

### 2.2.3 Preparation of cross-linked cellulose hydrogels

The obtained cellulose solution from the cellulose preparation mentioned in the last protocol was kept at 0-4 °C to obtain a transparent cellulose solution. It was centrifuged at a speed of 750 rotations per minute to remove any insoluble components and bubbles. Next, the desired amount of MBA (N, N'-methylenebisacrylamide) powder, SA (Sodium alginate), or CP (Carbomer polymer) was added to the cellulose solution and was kept stirring for 2 hours until it is homogenous. Then, the mixture was transferred to a container and kept for 12 hours at room temperature. Finally, a transparent cross-linked cellulose gel was obtained. The concentration of cellulose solution was 2 wt% in all of the experiments and the temperature of the hydrogels as they transformed was 25 °C. After that, the viscosity and turbidity were recorded as well.

The weight ratios of the crosslinkers to cellulose in the gels were 0.4:1 and 1:1. The treatments of this experiment are as follows:

- WMBA**- water crosslinked with MBA;
- α MBA**- iNαC crosslinked with MBA;
- CMBA**- carboxymethyl cellulose crosslinked with MBA;
- WSA**- water crosslinked with sodium alginate;
- αSA**- iNαC crosslinked with sodium alginate;
- CSA**- carboxymethyl cellulose with sodium alginate;
- WCP**- water crosslinked with carbomer polymer;
- αCP**- iNαC crosslinked with carbomer polymer; and;
- CCP**- carboxymethyl cellulose crosslinked with carbomer polymer.

### 2.2.4 Viscosity

BDV series digital viscometer (BDV-1S-5S-8S-9S) was used to determine the relative viscosity of the different hydrogels. The instrument features quick and easy operation, high precision measurement, steady rotating speed, excellent anti-interference ability, and a wide range of working voltages. All of the rotors of the equipment were used with varying speeds of rotations per minute. A total time of three minutes was recorded for the gathering of data.

### 2.2.5 Turbidity

Turbidity is a measure of the cloudiness or haziness of a fluid caused by the scattering of light by particles or other material in the solution. In the case of hydrogels, turbidity can provide information about the degree of swelling and particle dispersion in the hydrogel matrix, which can affect its mechanical strength and drug release properties. A UV-Visible spectrophotometer (Thermo Scientific: Evolution 220) was used to determine the different hydrogels' turbidity. The wavelength used was 360 nm at a bandwidth of a fiber. Formazine was bought for the standardization of the turbidity for comparison to the produced hydrogels.

## 2.3 Statistical Treatment of Data

The experiment was laid out in a Complete Randomized Design (CRD). The data were analyzed using the factorial one-way Analysis of Variance (ANOVA).

## 3 Results and Discussion

### 3.1 Elemental Analysis of Isolated Nipa α-Cellulose (iNαC using XRF)

Shows the elemental analysis of isolated nipa α-cellulose (iNαC) in comparison with the standard cellulose using an X-ray fluorescence analyzer. The control cellulose used in this protocol was Carboxymethyl Cellulose (CMC). The qualitative analysis shows the same elements present in the two samples. Meanwhile, quantitative analysis suggests a significant difference in terms of light elements with 99.76% for CMC and 94.66% for the iNαC. This was also observed by Saengkrajang, et al (2021)<sup>(10)</sup>. In

their study, nipa palm revealed the presence of inorganic elements such as Ca, K, Cl, and Si. Interestingly, other elements were detected in both samples such as Strontium, Thallium, Niobium, and Yttrium.

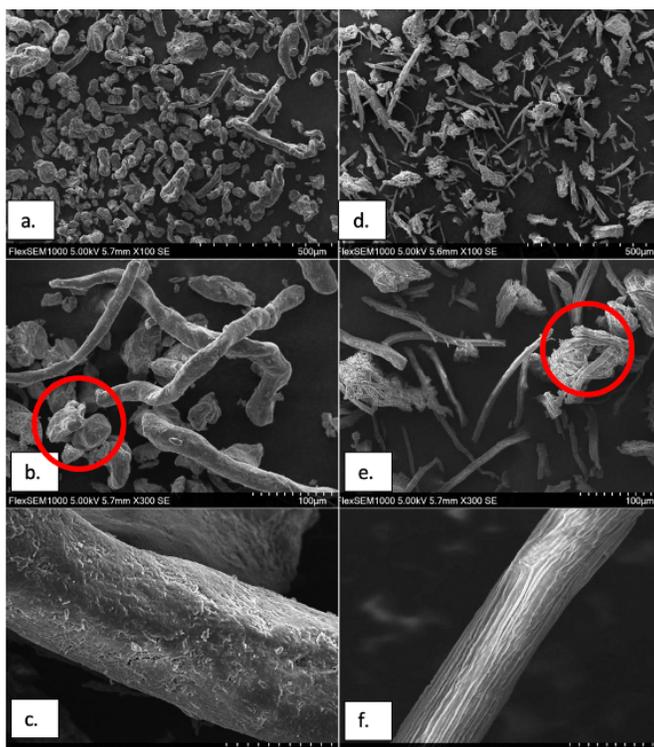
**Table 1.** Qualitative and Quantitative Elemental Analysis through X-Ray Fluorescence Analyzer

Treatment	Light Elements (LE%)	Si(g/L)	Ca(g/L,%)	Th(g/L)	Nb(g/L)	Y(g/L)	Sr(g/L)	Cl(g/L)
CMC	99.67a	1.61	1.48	0.05	0.01	0.01	0.01b	0.10b
iN $\alpha$ C	94.66b	1.41	3.57	0.03	0.02	0.01	0.06a	1.43a
CV, %	0.0383	23.01	n/a	2.95	1.14	1.14	11.13	11.35
Level of Significance	**	ns	n/a	ns	ns	ns	**	**

\*Means with the same letter are not significantly different

### 3.2 Morphological Properties of iN $\alpha$ C through SEM Analysis

Morphologically, cellulose consists of repeating  $\beta$ -D-glucopyranose units that are covalently linked together by acetal functions between the C1 carbon atom and the equatorial -OH group of C4 ( $\beta$ -1,4-glycosidic bonds).



**Fig 1.** Photomicrograph of different Celluloses from CMC (Control) at different magnifications: a.) X100 SE, b.) X300 SE, and c.) X2000 SE; and; iN $\alpha$ C at different magnifications: d.) X100 SE e.) X300 SE f.) X2000 SE using SEM

These morphological properties of cellulose are due to its strong aggregation of highly ordered structural entities from their chemical constitution and spatial conformation as observed in the photomicrograph (Figure 1). To date, morphological studies looked at natural and model cellulose from derived celluloses, and in contrast, this part of the study focuses on the different morphological properties of the CMC (control) and the iN $\alpha$ C through SEM analysis at various magnifications. Figure 1 a and d show the macro fibrils' shape and size distribution in CMC and the iN $\alpha$ C. SEM images (Figure 1 b and e) of both celluloses show a great amount of cellulose agglomeration (encircled) due to the high hydrophilic properties and high reactivity of hydroxyl groups, and as a result, it increases the interactions between cellulose particles. Moreover, CMC (Figure 1 c) shows

many trenches and cracks at higher magnification. While the  $iN\alpha C$  (Figure 1 f) shows well-distributed microfibrils of cellulose arranged in orthogonal layers that are parallel to each other which was also noticed in a study conducted by Nguyen, V. et al in 2021 wherein mechanical and chemical treatment were also used<sup>(11)</sup>. Furthermore, the  $iN\alpha C$  SEM images taken are almost free of trenches, and cracks.

### 3.3 Solubility of the $iN\alpha C$ through NaOH/Urea solution

The solubility characteristics of the  $iN\alpha C$  are given in Table 2. The analysis of variance showed a significant difference between the control cellulose (CMC) and the  $iN\alpha C$  (Treatment 1-Treatment 4). A solubility of 98% was obtained after subjecting the CMC to the NaOH/urea solution while the  $iN\alpha C$  (Treatment 4, 15% NaOH) gave the highest solubility of about 78.34% among the other treatments. Although Treatment 4 (15% NaOH) exhibited the highest solubility, analysis of variance suggests that all treatments are not significantly different. This means that the data encourages the use of 10% NaOH solution (Treatment 2) to produce  $iN\alpha C$  as an ideal concentration in the pretreatment process for a lower cost and environment-friendly production of  $iN\alpha C$ .

Table 2. Effect of NaOH/Urea on the dissolution of the  $iN\alpha C$

Treatment	Initial weight (g)	Insoluble Cellulose (g)	Solubility (%)
CMC (Control)	0.5	0.01	98.00 a
Treatment 1 (7% NaOH)	0.5	0.11	77.32 b
Treatment 2 (10% NaOH)	0.5	0.11	77.53 b
Treatment 3 (12% NaOH)	0.5	0.11	78.13 b
Treatment 4 (15% NaOH)	0.5	0.11	78.34 b
CV, %			11.34
Level of Significance			**

\*Means with the same letter are not significantly different

Compared to the study of Bagheri S., et al, up to 56% was dissolved after subjecting the celluloses to the same solution with varying amounts of urea concentration<sup>(12)</sup>. This is due to its natural recalcitrance to conventional solvents. Pure cellulose has intra- and intermolecular hydrogen bonds, hence, the study recommends derivatizing the cellulose since derived celluloses are easily dissolved into water or acid after modification. That is why, the data suggest that CMC— a cellulose derivative is expected to have the highest solubility since it has been used for its industrial application.

### 3.4 Comparative Relative Viscosity of Cellulose Hydrogels

The comparative relative viscosity of different hydrogels from  $iN\alpha c$ , CMC, and water is presented in Table 3. The analysis of variance shows significant differences between treatments. As expected, the control (CCP100) shows the highest relative viscosity which yields 20000 mPa-s, and then is followed by the  $\alpha CP100$  (19623.22 mPa-s).

Table 3. The viscosity of the chemically crosslinked hydrogels

Treatment	MBA40	MBA100	SA40	SA100	CP40	CP100
Water	15.79 m	14.95 m	127.56 k	190.72 j	782.00 f	490.94 i
CMC	23.00 l	22.28 l	640.87 g	881.28 e	9815.33 c	20000.00 a
$iN\alpha C$	15.06 m	16.56 m	497.34 i	629.50 h	8458.00 d	19623.22 b
CV, %	0.0639	0.0639	0.0639	0.0639	0.0639	0.0639
Level of Significance	**	**	**	**	**	**

\*Means with the same letter are not significantly different

In addition, results reveal that the cellulose hydrogels crosslinked with carbomer polymer exhibited the highest viscosity than the hydrogels chemically crosslinked with sodium alginate while the hydrogels with MBA powder gave the lowest. Hydrogels that are chemically crosslinked with carbomer polymer were the most viscous among the other hydrogels because of their high polymerization with the cellulose solution<sup>(13)</sup>. On the other hand, hydrogels with low viscosity flow easily because of their molecular makeup which results in very little friction when it is in motion.

The data shows that cellulose based-hydrogels were more viscous than water-based hydrogels, therefore increasing the integrity of the produced hydrogels. This is because of the presence of abundant hydroxyl groups in the cellulose molecule, thus cellulose can be used to prepare hydrogels for many functions<sup>(14)</sup>.

Moreover, the MBA hydrogel that was produced gave an almost watery consistency as seen in Figure 2. Meanwhile, the hydrogels from sodium alginate gave smooth viscous solutions. While the hydrogels from the carbomer polymer showed thick viscous solutions that don't easily flow.

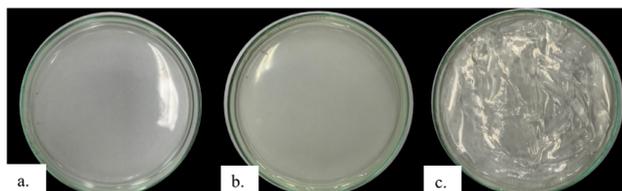


Fig 2. Photographs of the different  $\alpha$  hydrogels chemically crosslinked at 1:1 concentration: a.)  $\alpha$ MBA100, b.)  $\alpha$ SA100, and c.)  $\alpha$ CP100

### 3.5 Turbidity of the Chemically Crosslinked Hydrogels

Figure 3 presents the quantitative analysis of the turbidity of the chemically crosslinked hydrogels from  $\alpha$ CMC, CMC, and water. Analysis of variance shows significant differences between treatments. In MBA-linked hydrogels, the control cellulose (CMBA100) obtained the highest turbidity (4.824 NTU) followed by the hydrogels from  $\alpha$ CMC ( $\alpha$ MBA100, 4.569 NTU), then the least turbid was the hydrogel from water (WMBA100 and WMBA40) amounting to 4.453 NTU.

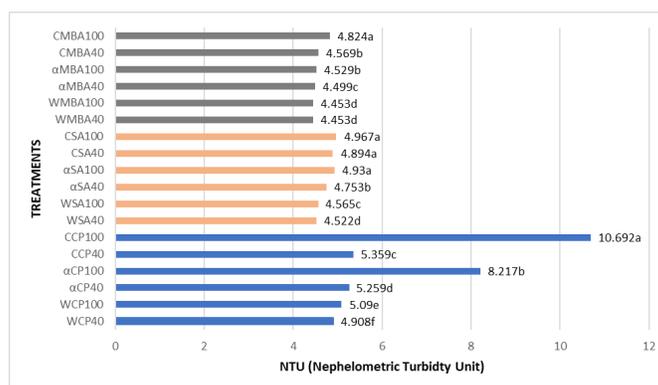


Fig 3. Quantitative Analysis of the Turbidity of the Chemically Cross linked Hydrogels

The same trend can be observed in sodium alginate hydrogels. The control cellulose (CSA100) leads the turbidity (4.967 NTU) among the other SA-linked hydrogels while the hydrogels from water (WSA40, 4.522) gave the least. Moreover, hydrogels chemically crosslinked with carbomer polymer exhibited the highest turbidity among the other chemical crosslinkers. As expected, CCP100 showed the highest turbidity amounting to 10.692 NTU and it was statistically incomparable to the turbidity of  $\alpha$ CP100 amounting to 8.217 NTU, and to the other hydrogels from carbomer polymer. Furthermore, it can be observed that from the trends of the results, turbidity was increased with the increasing polymer and/or crosslinker concentration.

High turbidity can indicate poor particle dispersion in the hydrogel, which can affect its mechanical strength. In drug delivery applications, for example, a high turbidity hydrogel may not release drugs as effectively due to poor particle dispersion, resulting in reduced efficacy. In addition, high turbidity may also limit the visibility of the hydrogel during imaging or surgery, making it more difficult to accurately locate and apply the material.

Low turbidity, on the other hand, generally indicates good particle dispersion in the hydrogel. This can lead to improved drug delivery properties and better mechanical strength of the hydrogel. However, very low turbidity may also indicate that the hydrogel has a low concentration of particles, which may result in reduced drug delivery efficacy or mechanical strength.

The implications of high or low turbidity in a hydrogel depend on the specific application and intended use, and researchers should carefully consider turbidity measurements along with other properties when studying and developing hydrogel

materials.

### 3.6 Potentials of iN $\alpha$ C Hydrogels

The potential of iN $\alpha$ C in manufacturing products has been widely studied and evaluated in reinforced hydrogels with different crosslinkers. This research has opened up a range of possible applications in a variety of fields, including medical, agricultural, and wastewater treatment.

One of the most promising areas of application for iN $\alpha$ C-based hydrogels is in the medical field. Medical hydrogels have the potential to improve wound healing by increasing the permeability and absorbency of bioactive compounds. By incorporating iN $\alpha$ C into hydrogels, the mechanical properties of these materials can be enhanced, leading to more effective wound healing.

In the case of nipa hydrogels, yeast phenols can be added as a bioactive compound for wound healing. Yeast phenols have been shown to have anti-inflammatory and antioxidant properties, which can help to reduce inflammation and promote healing. By incorporating yeast phenols into nipa hydrogels, the wound healing process can be accelerated and improved.

In agricultural applications, hydrogels have been shown to increase water holding capacity and improve water use efficiency. By incorporating iN $\alpha$ C into hydrogels, the mechanical properties of these materials can be enhanced, leading to more effective water retention in soil. This can help crops to resist abiotic factors such as drought, leading to higher yields and more sustainable agricultural practices.

Lastly, hydrogels have potential applications in wastewater treatment. By improving the physical and chemical absorption process, hydrogels can help to remove pollutants and contaminants from wastewater, improving its quality and making it safe for use in agricultural irrigation. By incorporating iN $\alpha$ C into hydrogels, the mechanical properties of these materials can be enhanced, leading to more effective wastewater treatment and safer use of wastewater in agriculture.

Overall, the potential applications of iN $\alpha$ C-based hydrogels are vast and varied, and further research in this area is likely to uncover even more promising applications in the future. The versatility of these materials makes them highly attractive for a wide range of applications, and they have the potential to revolutionize a variety of fields, from medicine to agriculture to environmental science.

## 4 Conclusion

Cellulose hydrogel is one of the sought materials that stirs research and development because of its potential in many fields. Numerous techniques have been studied. However, these techniques have very tedious preparations and create toxic secondary pollution. Therefore, this research generated a one-step simple process in producing cellulose-based hydrogels from nipa frond biomass. Firstly, the iN $\alpha$ C was evaluated chemically and morphologically. Photomicrographs of CMC show many trenches and cracks at higher magnification while the iN $\alpha$ C shows well-distributed microfibrils of cellulose arranged in orthogonal layers that are parallel to each other because of the acid hydrolysis treatment in the isolation and purification methods. To widen the applications of iN $\alpha$ C, it was subjected to dissolution. Solubility (%) reveals that treatment 2 is an ideal pretreatment process for a lower cost and environment-friendly production of cellulose solution. Lastly, the physical properties of the produced nipa hydrogels were determined. The control (CCP100) shows the highest relative viscosity which yields 20000 mPa·s, and then followed by the  $\alpha$ CP100 (19623.22 mPa·s). In terms of turbidity, CCP100 was the most turbid hydrogel amounting to 10.692 NTU. It was statistically incomparable to the turbidity of  $\alpha$ CP100 and the other hydrogels from different crosslinkers. Furthermore, it can be concluded that the approach that was used to obtain the nipa hydrogel is effective and it can be used as a good blank template for many applications.

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