INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Isolation of α - Cellulose from Nipa (*Nypa fruticans* Wurmb) Frond using Physico-Chemical Treatment

Jayson F Cariaga[1]*, Alvin G Domingo[2], Bjorn S Santos[3], Shirley C Agrupis[4]

1 Researcher, National Bioenergy Research and Innovation Center, Mariano Marcos State University
2 Student Researcher, Graduate School, Mariano Marcos State University, Mariano Marcos State University
3 Researcher, Graduate School, Mariano Marcos State University
4 Researcher, National Bioenergy Research and Innovation Center, Mariano Marcos State University

OPEN ACCESS

Received: 05-12-2022
Accepted: 08-04-2023
Published: 17-06-2023

Citation: Cariaga JF, Domingo AG, Santos BS, Agrupis SC (2023) Isolation of α - Cellulose from Nipa (*Nypa fruticans* Wurmb) Frond using Physico-Chemical Treatment. Indian Journal of Science and Technology 16(23): 1754-1759. https://doi.org/10.17485/IJST/v16i23.2258

* **Corresponding author**.

jfcariaga@mmsu.edu.ph

Funding: National Bioenergy Research and Innovation Center and Research Directorate Funds

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objective: The aim of this research is to create a versatile approach to produce cellulose from nipa fronds, which are a sustainable resource. **Methods:** The study focuses on determining the amount of cellulose in nipa fronds, the effectiveness of a simplified physicochemical pretreatment, and the purity of the isolated nipa α-cellulose. The nipa biomass was pretreated using physical and chemical methods and analyzed through gravimetric analysis to determine its composition, delignification efficiency at varying concentrations of Sodium hydroxide (NaOH), and the purity of the isolated nipa α-cellulose (iNαC) using the National Renewable Energy Laboratory (NREL) protocol, "NREL/TP-510-42618". The data was statistically analyzed using One-way Analysis of Variance. **Findings:** The results showed that increasing the concentration of NaOH led to positive responses in delignification, decreased hemicellulose, and increased recovery of cellulose. The study found that as the concentration of NaOH increased, the amount of α-cellulose recovered also increased. Treatment 4 (15% NaOH) and Treatment 2 (10% NaOH) produced 79% w/w and 77.45% w/w of α-cellulose, respectively. While Treatment 4 was highly effective in breaking down nipa biomass, a statistical analysis revealed that Treatment 2 produced comparable results in terms of producing pulp from nipa frond biomass without requiring higher concentrations of NaOH. This led to a reduction in chemical waste, making Treatment 2 a more sustainable option.

Keywords: α-cellulose; Nipa Frond; Biomass; Soda Treatment; Lignocellulosic; NREL protocol

1 Introduction

Nipa is an economically important palm species in many coastal communities in the Northern Philippines. It is considered the only palm species that thrives well in river estuaries and brackish water[(1)]. The palm is used for various products such as

sugar syrup, local alcoholic beverages, vinegar production, thatching materials, cigarette paper, construction materials, and as a food source[2]. Interestingly, the National Bioenergy Research and Innovation Center (NBERIC) of the Mariano Marcos State University (MMSU) utilizes sap from nipa palm as the primary feedstock for bio-ethanol production. The project aims to vertically integrate country-side fisherfolks and farmers in the mainstream bio-ethanol industry. This initiative of the NBERIC focuses on two things, 1. Country-side development through developing simple and adaptable science-based technologies that can be adapted by simple farmers, and 2. Inclusive economic growth through capacity building and product development of established technologies[3].

The production of bioethanol encompasses a strict consolidated process that requires one hectare of natural nipa plantation to operate one unit of bioethanol distilling facility. One of the processes in bioethanol production is the preparation of the nipa plantation which requires a thinning process or the removal of nipa fronds and extra nipa stands along the path of collection. The arduous process involved in handling Nipa fronds generates a substantial amount of waste residues. The environmental implications of this waste include the obstruction of waterways and river dams, the formation of breeding habitats for pests, and contamination of both water and land ecosystems. The accumulation of Nipa fronds in waterways and river dams can obstruct the natural flow of water and cause flooding, while the decomposition of the fronds can contribute to eutrophication and water pollution. The accumulation of Nipa fronds can also create breeding grounds for mosquitoes and other insects, leading to the spread of diseases. If not disposed of properly, Nipa fronds can accumulate in landfills and contribute to the production of greenhouse gases, which is a potent contributor to climate change. The nipa frond is considered as an agricultural residue and can serve as a biomass source for fuel or in the production of materials. In a study conducted by Evelyn et al. (2020), nipa palm frond residues can be valued as a source of raw materials for the production of medium-density fiberboards, heavy metal adsorbents, pulping materials, fuels, and cellulose derivatives[4]. The problem arising from the accumulation of biomass residue in the production of bioethanol can be addressed through the utilization of the biomass residue and treating it to produce a product. This approach can provide an alternative source of income for nipa communities. One of the promising products that have an increasing demand in the global market is cellulose and its derivatives. As per the latest industry analysis, the global market for cellulose and its derivatives is expected to witness a Compound Annual Growth Rate (CAGR) of 5.5% during the forecast period of 2023-2025. The growth of the market can be attributed to the increasing demand for cellulose and its derivatives in various industries, such as food and beverages, pharmaceuticals, textiles, and cosmetics. Moreover, the eco-friendly nature of cellulose and its derivatives has led to a surge in their demand in recent years, particularly for applications such as packaging and paper production. The growth of the market is also supported by technological advancements in cellulose production and processing methods, which have improved the quality and performance of cellulose derivatives, making them more suitable for a wider range of applications[5].

Presently, the production of cellulose pulp and its derivatives has been increasing interest in non-wood plant fibers as a source of raw materials[6]. Non-wood fibers such as the nipa frond can be used to meet the increasing demand for pulp and paper and the rapid growth of its utilization can be a vital source of renewable materials. Production of pulp and cellulose derivates relies on efficient pulping techniques and cost-effective production[7]. Meanwhile, chemical pretreatments such as the use of white liquor (sodium hydroxide), anthraquinone, and the sulfur-based process are the most efficient way to produce cellulose. However, it imposes an environmental problem with the use of sulfur-based chemicals and the expensive cost of anthraquinone. A new approach has been proposed as a total sulfur-free and chlorine-free pretreatment process, however, anthraquinone is not an over-the-counter chemical and is considered to be very expensive. Furthermore, there are a lot of established methods in the purification of cellulose present as references for chemicals pretreatments such as γ-Valerolactone as a co-solvent[8] acid hydrotropes such as maleic acid, p-toluene sulfonic acid, benzenesulfonic acid and 4-phenol sulfonic acid[9], Dialkylimidazolium-based, and choline-based, and protic ionic liquids[10]. However, its highly technical nature cannot be easily adapted by simple farmers. Therefore, it is important to look for suitable resources, efficient methods, and the most environmentally friendly and cost-effective production technique for cellulose production is a must. Thus, this study established an adaptable method in the production of cellulose using nipa frond as a renewable resource. It specifically evaluates the amount of cellulose present in the nipa frond, evaluates the efficiency of a simplified physicochemical pretreatment, and evaluates the purity of isolated nipa α-cellulose.

2 Methodology

2.1 Simplified Soda Treatment Protocol

2.1.1 Collection of nipa frond

Samples of Nipa fronds were collected from Pasuquin, Ilocos Norte. Only the nipa frond rachis was used in the study. Matured Nipa fronds with lengths between two and three meters and brownish in color were selected. All samples were transferred to the

National Bioenergy Research and Innovation Center (NBERIC), Biomass Characterization Laboratory for further processing. A total of 59 kg of biomass were used in the experiment.

2.1.2 Physical pre-treatment of nipa frond

Samples were washed with distilled water and dried at room temperature (25 °C). After drying, the samples were powdered and sieved in 80 mesh, then weighed to calculate the fresh weight of the biomass. A portion of the sample was taken for moisture content analysis as the basis for the computation of its dry weight.

2.1.3 Soda treatment

Soda treatment trials were carried out in an autoclave. The conditions of this experiment were as follows: liquor-to-material ratio of 6:1, time to maximum cooking temperature of 30 minutes, time of cooking of 60 minutes at 15 psi, with variations in the liquor (NaOH) composition from 7%, 10%, 12% and 15% and time of cool down for 30 minutes. After cooking, the black liquor that remained in the pulp was washed off and properly disposed of and the biomass was screened. Rejects and screened yield was determined on an oven-dry weight basis.

2.1.4 Biomass Composition

The biomass composition of the sample was taken before and after soda treatment following an adapted a modified and combined TAPPI methods from T249 and T222, and modified NREL/TP-510-42618 procedure.

Lignin. Twenty (20) mL of water, 10% acetic acid, and five mL of 5% of sodium hypochlorite were added to the samples. Furthermore, the samples were heated at 75°C for one hour. More acid (2 mL) was added until the samples were thoroughly delignified. After which, samples were washed with water (five times), acetone (twice), and ethanol (once), then dried up at 105°C (90 minutes) and weighed (W_1). The differences between the initial weight and W_1 are the lignin content of the sample.

Hemicellulose. Twenty (20) mL of 24% KOH was added to the samples and air dry for two hours at 20°C the samples were washed with water (5 minutes), 5 % acetic acid (once), acetone (twice), and ethanol (once), then dry at 105°C (90 minutes) and weigh. The differences between W_1 and W_2 are the hemicellulose content of the samples.

Cellulose. The weight of the residue from the preceding procedure is the crude cellulose contents of the samples.

The delignification efficiency and % hemicellulose change of the process was determined using the following equation:

$$Delignification\ efficiency\ (\%) = \frac{A-B}{\frac{(A+B)}{2}} \times 100$$

Where: A = Lignin content(g)of the original sample

B = Lignin content(g)of the original sample after treatment

$$Hemicellulose\ change\ (\%) = \frac{A-B}{\frac{(A+B)}{2}} \times 100$$

Where: A = Hemicellulose content(g)of the original sample

B = Hemicellulose content(g)of the original sample after treatment

2.2 Isolation and Purification of α-Cellulose

Crude cellulose obtained from the soda treatment was used to isolate α-cellulose.

2.2.1 A*-Cellulose Isolation and Purification Protocol*

Five grams of the crude cellulose were weighed into an appropriate container, which was then placed in a constant-temperature (20 °C) water bath. After the sample obtained the temperature of the bath, 17.4% NaOH solution was pipetted into the beaker and the sample was macerated with a stirring glass rod. The NaOH treatment step was repeated and the sample was filtered after 30 minutes using a funnel. The residue on the filter paper was oven-dried at 105 °C for 1 hour, or until no more change in weight was observed. This was followed by cooling. After which, a 400-ml of distilled water was added to the sample and the mixture was filtered. The filtration was repeated several times to ensure that all the residues were retained by the filter paper. The residues were then soaked in 100 mL of 20% acetic acid for 10 minutes, after which the acetic acid was washed off by suction and 1 liter of boiling water was added in small portions at a time. The sample was then kept in an oven at 105 °C to remove moisture. The increase in weight of the filter paper expressed as a percentage of the biomass sample was considered as

the amount of α-cellulose in the sample. The samples were then subjected to bleaching with a NaClO solution using a 1:0.5 weight ratio of α-Cellulose to NaClO. Furthermore, residual acid-insoluble lignin (AIL) and acid-soluble lignin (ASL) content of the α-cellulose were determined using modified National Renewable Energy Laboratory NREL/TP-510-48087 method.

2.3 Statistical Treatment of Data

The study utilized a Complete Randomized Design (CRD) with four NaOH treatments (7%, 10%, 12%, and 15%) administered in triplicate. Factorial one-way Analysis of Variance (ANOVA) was used to analyze the data and evaluate the impact of NaOH concentrations on the degradation of nipa frond biomass.

3 Results and Discussion

3.1 Initial Biomass Analysis of Extractive-free Nipa Frond Biomass

The moisture content of the fresh nipa frond were ascertained to be 81% using the ASTM-D4442 or the Standard Test Methods for Direct Moisture Content Measurement of Wood and Wood-Based Materials. This resulted in the residual quantity of 11 kg of bone-dried biomass. Additionally, the nipa frond biomass is comprised of the organic compounds lignin, hemicellulose, and cellulose. Nevertheless, regulating the influence of environmental variables on the biomass composition poses an arduous challenge due to their susceptibility to the fluctuations in weather patterns, water availability, temporal and spatial temperature differentials, and soil properties [11].

The chemical composition of nipa frond biomass collected from Pasuquin, Ilocos Norte. Like any agricultural non-food biomass, it contains the three major constituents in considerable quantities. It is largely composed of cellulose (43%) followed by lignin (38%), and hemicellulose (19%).

3.2 Effect of Soda Treatment on the Degradation of Extractive-free Nipa Frond Biomass

Table 1 represents the results of an experiment that investigated the impact of varying concentrations of white liquor (NaOH) - specifically, 7%, 10%, 12%, and 15% - on the mass of extractive-free nipa frond biomass. The experiment was conducted using a liquor-to-material ratio of 6:1.

The results of the analysis of variance revealed significant variations among all the treatments. The data demonstrated a positive trend of delignification, reduction in hemicellulose content, and an increase in the cellulose amount as the concentration of NaOH increased. Among all the treatments, Treatment 4 exhibited the lowest amount of lignin content (14.01% w/w) and delignification efficiency of approximately 21.00% w/w compared to the other treatments. Additionally, the same trend was observed in terms of hemicellulose content, which decreased by approximately 37% w/w during the treatment. The TAPPI protocol indicated that after soda treatment, the amount of cellulose was around 83.02% w/w for the same treatment.

Table 1. Effect of soda treatment on extractive-free nipa frond biomass degradation

Treatment	Lignin (%)	Delignification Efficiency (%)	Hemicellulose	Hemicellulose Change (%)	Cellulose
Control	37.58c		18.96b		43.46c
T1 (7% NaOH)	22.44b	12.30b	15.18b	5.88c	62.38b
T2 (10% NaOH)	15.04a	21.30a	5.88a	26.00b	80.72a
T3 (12% NaOH)	14.71a	21.70a	3.27a	36.36a	82.02a
T4 (15% NaOH)	14.01a	21.00a	2.97a	37.00a	83.02a
CV, %	7.90	7.90	25.22	25.22	4.20
Level of Significance, 0.05	**	**	**	**	**

*Means with the same letter are not significantly different

It can be observed that treatment 4 has the best concentration in deconstructing the nipa frond biomass. It achieved the goal of soda treatment, in delignifying the biomass which can be observed in Table 1, showing significant difference against the control and treatment 1. Meanwhile, T2, T3, and T4 shows no significant difference which suggest that there is no need to increase the concentration of NaOH to obtain a delignified biomass. This can be observed almost in all components of the

biomass. which is to degrade lignin and hemicelluloses into small water-soluble molecules which can be washed away from the cellulose without depolymerizing it. Hence, a decrease in chemical waste (black liquor). Consequently, this was also observed in a findings of Evelyn and Sunarno., et al. (2022), in determining the biomass characterization of nipa which yielded 62.1 % w/w of holocellulose which is a combination of the total mass of hemicellulose and cellulose and 18.3% w/w of lignin [4].

3.3 Amount of α-Cellulose after Isolation and Purification Protocol

Cellulose is a sustainable and almost inexhaustible polymeric raw material, with the potential to meet the increasing demand for environmentally friendly products. α-Cellulose is used to define the content of "true cellulose" from plant material in the form of solubility in alkali [12].

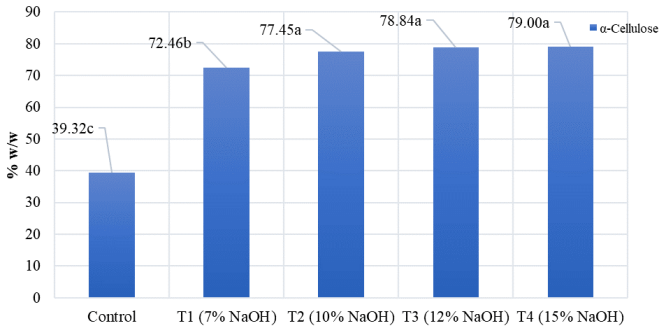

Fig 1. Amount of α-Cellulose after Isolation and Purification Protocol

Figure 1 shows the amount of α-cellulose retrieved after isolation and purification methods. Briefly, an initial weight of about 5 grams of each of the samples was subjected to acid (acetic acid) and base (NaOH) hydrolyses. An increasing amount of α-cellulose can be observed as the treatment concentration increases. Treatment 4 (15% NaOH) afforded the highest amount of α-cellulose among all of the treatments. However, the analysis of variance suggests that treatment 4 is comparable to treatment 2 which gave 79.00% w/w and 77.45% w/w, respectively. α-Cellulose is not a chemical structure but rather the cellulose portion that cannot be dissolved in the 17.5% NaOH at 20°C (16). This means that any undesirable components of the biomass after the initial soda treatment can be further purified to produce α-cellulose in the end process. This also supports the objective of the study to produce α-cellulose since it has the highest degree of polymerization. In comparison to the study conducted by Shaikh, H. M., et al. (2021), date palm trunk mesh (DPTM) afforded 65.91($\pm$2.50)% of α-cellulose after $ScCO_2$ and 20% wt NaOH alkali treatment [13] while nipa palm afforded 79.00% of α-cellulose after subjecting it to 15% NaOH and further purification. Thus, the nipa palm is a potential source of α-cellulose and can be treated with a lower concentration of NaOH in the initial process. Moreover, biomass variability results from many factors such as component analysis methods, harvesting practices, storage conditions, and preprocessing techniques.

The NREL protocol was done to ensure the purity of the isolated nipa α-cellulose (i$N\alpha$C). Specifically, the protocol aimed to determine the total acid-soluble lignin and acid-insoluble lignin on the i$N\alpha$C after acid (acetic acid) and base (NaOH) hydrolyses. Lignin is an organic compound that is chemically bonded to cellulose and hemicellulose. The structural integrity of the plant cells can be attributed to lignin. Therefore, a higher amount of lignin requires more deconstructive force, chemicals, and pretreatments to be applied.

Table 2 shows the effect of acid (acetic acid) and base (NaOH) hydrolyses on the removal of total acid-soluble lignin and acid-insoluble lignin on the i$N\alpha$C. The analysis of variance showed a significant difference in the treatments on the determination of AIL compared to the control. A decreasing amount of lignin can be observed as the concentration increases. Furthermore, a positive trend can also be observed in the removal of ASL. The analysis of variance showed a significant difference among all of the treatments. The removal of the ASL from the biomass by treatment 4 (15% NaOH) gave the least amount of lignin (0.49%) followed by treatment 3 (12% NaOH) which gave 0.52%, then treatment 2 which gave 0.53%, and lastly, treatment 1 (7% NaOH) amounting to 0.66%.

The findings indicate that samples subjected to treatment 4 generally have the lowest amount of total lignin (AIL+ASL) amounting to 0.49% followed by treatment 3 which gave 0.52%, treatment 2 amounting to 0.53%, and lastly, treatment 1 amounting to 0.66% of total lignin. Based on the trends of the results, it can be deduced that the higher the concentration of NaOH, the lower the amount of lignin present in the sample. Moreover, the results of the experiment indicate that the purity of the retrieved α-cellulose from acid-base hydrolyses is 94.58%-99.48%.

Table 2. Effect of isolation and purification methods on the removal of total lignin (AIL and ASL) content (%) of iNαC

Treatment	Acid Insoluble Lignin (w/w%)	Acid Soluble Lignin(%)	Total Lignin (%)
Control	4.44 b	0.98 c	5.42 b
T1 (7% NaOH)	0.11 a	0.55 b	0.66 a
T2 (10% NaOH)	0.00 a	0.53 a	0.53 a
T3 (12% NaOH)	0.00 a	0.52 a	0.52 a
T4 (15% NaOH)	0.00 a	0.49 a	0.49 a
CV, %	26.45	1.56	24.68
Level of Significance	**	**	**

*Means with the same letter are not significantly different

4 Conclusion

The availability of accumulated biomass residue from the operation of the NBERIC for bioethanol production opened an opportunity for the product development of biomass residue to cellulose production using a simplified method. This research showed how feasible the nipa frond biomass is as a source of α-cellulose. Results suggest that biomass treated with 10% NaOH can be used in the isolation and purification process and thus, can be treated with a lower concentration of NaOH in the initial soda treatment. Moreover, the results of the experiment indicate that the purity of the iNαC from acid-base hydrolysis is 94.58%-99.48%. Furthermore, this study shows that the physicochemical treatments and purification methods were effective in producing a high amount of α-cellulose from nipa frond biomass which can be utilized for environment-friendly products. Simplifying the method and removing extra chemicals that cannot be sourced out easily by simple farmers in the production of iNαC believes to be adaptable at the community level.

Acknowledgment

The authors would like to thank the National Bioenergy Research and Innovation Center for facilitating the conduct of the research. Furthermore, the authors would like to extend their gratitude to the Research Directorate of the Mariano Marcos State University and the Department of Science and Technology for the technical and financial support.

References

1) Cheablam O, Chanklap B. Sustainable Nipa Palm (Nypa fruticans Wurmb.) Product Utilization in Thailand. *Scientifica*. 2020;2020:1–10. Available from: https://doi.org/10.1155/2020/3856203.
2) Estira K. Factors Affecting Purchase Intention for Nipa products. *Southern Asian Journal of Science and Technology*. 2022;7:2672–2984. Available from: https://sajst.org/online/index.php/sajst/article/view/258.
3) Madigal J. Development of Nipa (Nypa fruticans) Sap Closed Collection Vessel. *Agro Bali: Agricultural Journal*. 2020;3(2):108–117. Available from: https://doi.org/10.37637/1b.v3i2.611.
4) Evelyn, Sunarno, Andrio D, Aman A, Ohi H. Nypa fruticans Frond Waste for Pure Cellulose Utilizing Sulphur-Free and Totally Chlorine-Free Processes. *Molecules*. 2020;27(17):5662. Available from: https://doi.org/10.3390/molecules27175662.
5) Global Cellulose Fibre Market: By Type: Natural, Man Made; By Application: Textile, Hygiene, Industrial, Others; Regional Analysis; Historical Market and Forecast (2018-2028); Market Dynamics; Value Chain Analysis; Competitive Landscape; Industry Events and Developments. 2022. Available from: https://www.expertmarketresearch.com/reports/cellulose-fibre-market.
6) El-Sayed ESA, El-Sakhawy MS, El-Sakhawy MAMS. Non-wood fibers as raw material for pulp and paper industry. *Nordic Pulp & Paper Research Journal*. 2020;35(2):215–230. Available from: https://doi.org/10.1515/npprj-2019-0064.
7) Liu Z, Wang H, Hui L. Pulping and Papermaking of non-wood fibers, pulp and paper processing. In: Kazi, N S, Ed, editors. Pulp and Paper Processing. 2020. Available from: https://doi.org/10.5772/intechopen.79017.
8) Raj T, Chandrasekhar K, Banu R, Yoon JJ, Kumar G, Kim SH. Synthesis of γ-valerolactone (GVL) and their applications for lignocellulosic deconstruction for sustainable green biorefineries. *Fuel*. 2021;303:121333. Available from: https://doi.org/10.1016/j.fuel.2021.121333.
9) Zhou X, Liu J, Huang T, Bian H, Wang R, Sha J, et al. Near-complete enzymatic hydrolysis efficiency of Miscanthus using hydrotropic fractionation at atmospheric pressure. *Industrial Crops and Products*. 2020;149:112365. Available from: https://doi.org/10.1016/j.indcrop.2020.112365.
10) Zhang J, Zou D, Singh S, Cheng G. Recent developments in ionic liquid pretreatment of lignocellulosic biomass for enhanced bioconversion. *Sustainable Energy & Fuels*. 2021;5(6):1655–1667. Available from: https://doi.org/10.1039/d0se01802c.
11) Jadhav B, Roy R, Rahman MS, Raynie DE. Extraction and Depolymerization of Lignin from Pine Sawdust and Pistachio Shells. *Biomass*. 2022;2(4):348–357. Available from: https://doi.org/10.3390/biomass2040023.
12) Havstad MR. Biodegradable plastics. *Plastic Waste and Recycling*. 2020;p. 97–129. Available from: https://doi.org/10.1016/b978-0-12-817880-5.00005-0.
13) Shaikh HM, Anis A, Poulose AM, Al-Zahrani SM, Madhar NA, Alhamidi A, et al. Isolation and Characterization of Alpha and Nanocrystalline Cellulose from Date Palm (Phoenix dactylifera L.) Trunk Mesh. *Polymers*. 2021;13(11):1893. Available from: https://doi.org/10.3390/polym13111893.