Napier Grass Derived Cellulose Acetate Membrane for Water Purification

Grisha Shrestha[1], Krishna Kandel[2], Girja Aryal[3], Prakash Gautam[3], and

Bhanu Bhakta Neupane[1],*

[1]Central Department of Chemistry, Tribhuvan University, 44613, Kathmandu, Nepal

[2]Department of Chemistry, Birendra Multiple Campus, Tribhuvan University, 44207, Chitwan, Nepal

[3] Research Centre for Applied Science and Technology, Tribhuvan University, 44613, Kathmandu, Nepal

Corresponding E-mail: bhanu.neupane@cdc.tu.edu.np/newbhanu@gmail.com

Abstract

Napier grass is one of the widely cultivated forage plants. The stem region of the plant, an agro waste, is being explored in fabrication of paper, biogas production, and as composite material. The purpose of this study was to extract cellulose from Napier grass, modification of the cellulose to cellulose acetate, and use the acetate in fabrication of membrane for filtration purpose. Cellulose was extracted from lignocellulosic biomass by drying, pulping followed by alkali treatment and bleaching to remove hemicellulose and lignin content from the biomass with the yield of 47%. The obtained cellulose was acetylated through reaction of acetic anhydride in the presence of iodine as catalyst and systematically compared with commercially available cellulose acetate. The yield of cellulose acetate was found to be 41.67%. The cellulose acetate membrane was fabricated using cellulose acetate using calcium carbonate nanoparticles as pore templates. The membrane was able to decrease turbidity from simulated water sample by ~ 96%. This study suggested that the waste Napier grass stem could be used for efficient synthesis of cellulose acetate and fabrication of membrane for water purification.

Introduction

Lignocellulosic biomass is one of the most abundant renewable biomass annual global supply reaching nearly 200 billion tones[1–3]. Due to the renewable and biodegradable nature, the biomass is being used to get wide range of chemicals and materials[1–3]. The biomass consist of cellulose, hemicellulose and lignin as major components. Cellulose is one the key component of the lignocellulose biomass and can be extracted from wide range of plants and bacteria [4–6]. The physical properties of cellulose can be modified considerably by derivatization. The cellulose derivatives is being in wide range of applications   and has attracted significant research attention[7–9].

Cellulose acetate (CA) is being extracted from multiple sources such as bamboo pulp[10], rice husk[8], sugarcane straw[11], and recycled newspapers[12]. CA is being extensively used plastic industry, textile, coatings, consumer products, pharmaceuticals, film, in water purification[13].   The pure cellulose has extremely low solubility in in common solvent. Cellulose acetate is mostly used cellulose derivatives in the synthesis of membranes due

to its versatile solubility. The solubility of cellulose acetate depends on the degree of substitution (DS). The cellulose acetate with degree of substitution of 2-2.5 is soluble in wide range of solvents such as dichloromethane, acetone, dioxane, and methyl acetate [10–12].

The versatile solubility of cellulose acetate allows the synthesis of membranes. The membrane can be mostly fabricated by solvent evaporation and by phase separation. Cellulose can be considered as a polymer with the highest potential in the field of filtration membranes since the membrane is prepared from the natural sources[14]. The phase inversion process is one of the most popular methods used to produce porous polymeric membranes[15,16]. A porous microfiltration membrane of tunable pore size can be also be produced by removing a nanoparticle pore template[17,18].

Napier grass or elephant grass (*Cenchrus purpureus*, synonym *Pennisetum purpureum*) is a perennial, monocotyledonous flowering plant belonging to the family Poaceae and the genus *Pennisetum*[19]. In Nepal, it is also used for feeding grass carp and tilapia[20]. It possess high biomass yield[21], helps in the control of soil erosion[22] and is suitable for cellulosic biofuel production[23]. However, the use of Napier grass as an alternative forage crop has not been much successful due to its limited amount of research.

In this work, several properties of Napier grass fiber were measured and compared with other fiber types. Cellulose was then extracted from Napier grass stem following chemo-mechanical process of fiber processing. The cellulose was modified to get cellulose acetate. The cellulose acetate was characterized and compared with commercially available cellulose acetate. The cellulose acetate membrane was fabricated using calcium carbonate

nanoparticles as pore template followed by dissolving the particles in hydrochloric acid bath. The membrane was then finally tested for turbidity removal.

Materials and Methods

Fiber extraction

The Napier grass stem were collected from Napier grass plant (aged ~1 year) from Kavrepalanchok district of Nepal. The stems were sun dried and retted for a week in a water trough containing tap water (pH 7.2±2). The fibrous mass was washed several time and oven dried for 24 hour at 100±1 ºC.

Chemical analysis of the fiber

The extractive, moisture content, cellulose, hemicellulose, lignin, and ash content in the biomass was determined following literature reported gravimetric methods[24–27]. To determine extractive, 5 g of dried fiber was loaded into cellulose thimble and 300 mL of acetone was used as solvent for extraction using Soxhlet extractor. Temperature was adjusted to 70 °C for 2 h run period. After extraction, sample was dried at room temperature then heated in a hot air oven at 100±1 ºC until constant weight was obtained. The difference in weight between before and after the extraction process gave the extractives content. The process was repeated for triplicate measurements.

To determine moisture content, 1 g of dried fiber was taken in each of three petri dishes and kept in hot air oven for 2 h at 100±1 ºC. The fiber was then kept in a desiccator for 20 min and was weighed. The process was repeated until constant weight was obtained.

Cellulose content was determined by taking 1 g of extractive free fiber. The biomass was initially delignified with 0.7% sodium chlorite (NaClO$_2$) at 100 ºC for 2 h in an acidic solution (pH 4) using a fiber/liquor ratio 1:50. After filtering, the fibers were treated with 5% w/v

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sodium bisulphate and washed with distilled water. The delignified fibers were then treated with 17.5% NaOH for 1 h using fiber/liquor ratio 1:30. The residue was filtered, washed with 10% acetic acid and then with distilled water until neutralization. The fibers were then dried at 60 °C in a hot air oven until constant weight was obtained.

Hemicellulose was determined taking 1 g of extractive free dried fiber in 10 mL of 0.5 N NaOH and heating the content at 80 °C for 3.5 h. It was filtered and washed with distilled water until neutralization. The residue was dried at 105 °C in a hot air oven until constant weight was obtained. The difference between sample weight before and after this treatment was the hemicellulose content of dry biomass.

Extractive content was determined taking 0.3 g of the biomass in 3 mL of 72% H_2SO_4. The sample was treated at room temperature for 2 h with careful shaking at 30 min intervals to allow complete hydrolysis. The content was diluted and washed with distilled water, residue filtered, and dried. The acid insoluble lignin was determined by drying the residue at 105 °C.

Ash content was measured taking 0.5 g biomass in a porcelain crucible and incinerating in an electric muffle furnace at 525 ± 25 °C for 3 h. Ash residue in the crucible was calculated after cooling the crucible at room temperature in a desiccator.

Fiber morphology and strength

The images of raw Napier grass fiber and treated fibers were taken using AmScope brightfield microscope and digital microscopes at different magnifications. The images were analyzed in ImageJ software to get width of fiber. The tenacity of the fibers was calculated using the breaking force using fiber bundle strength tester (TTS India Instruments) and the known weight of fiber measured to nearest accuracy of 1.000 g [28].

FTIR and XRD measurements

Fourier transform infrared (FTIR) data were measured in ATR mode using in the range of 4000-400 cm⁻¹ (IRTracer-100, Shimadzu, Japan). XRD data in the range of 20° to 80° were measured in an X-ray diffractometer (Rigaku D/MAX-2500/pc). The Cu Kα radiation of wavelength 1.54060 Å was used as X-ray source.

Extraction of cellulose

The extractive free dry powdered Napier grass fiber (3 g) was soaked in 90 mL of 5% (w/v) NaOH (Merck Life Science, India) with a fiber/liquor ratio of 1:30 and heated to 80 °C for 5 h. After alkali treatment, slurry was allowed to cool, neutralized with dilute H_2SO_4, and washed several times with distil water. The biomass was bleached by treating with 2% H_2O_2 solution (Fischer Scientific, India) prepared in $NaHCO_3$-Na_2CO_3 solution of pH value 9 while keeping fiber/liquor ratio of 1:30 and stirring it for 5 h at room temperature. After bleaching, the material was washed with distil water, dried in hot air oven until constant weight was obtained[29].

Acetylation of cellulose

The acetylation of the cellulose performed following literature reported method with slight modification[8]. Firstly, 0.6 g of Napier grass cellulose was taken into a 100 mL conical flask. In the flask around 30 mL of acetic anhydride and 0.9 g of iodine were added. The mixture was heated to 80 °C, stirred for 5 h, and allowed to cool to room temperature. Around 30 mL saturated solution of sodium thiosulfate was added in the mixture with continuous stirring. The mixture was then transferred to a beaker washed thoroughly with 75% (v/v) ethanol so as to remove unreacted acetic acid and other byproducts. The solid residue was oven dried at

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60 °C, cooled, dissolved in dichloromethane, and filtered. The filtrate was evaporated to get pure cellulose acetate.

Synthesis of cellulose acetate membrane

Calcium carbonate particles were prepared by following literature reported method[30]. In the first step around 10 g of cellulose acetate was mixed with 130 mL of acetone. In the cellulose acetate polymer solution 10 g of calcium carbonate and 3.5 mL of glycerol were added in a kitchen grinder and homogenized [31]. In next step, two parallel edges of a glass were plastered with four successive layers of transparent sellotape. The surface of the glass was cleaned with small amount of ethanol. The cellulose acetate polymer dispersion was then poured onto the glass surface and labeled using the edge of a stainless-steel ruler. The final membrane sheet was dried in ambient air for 5 min. to remove the calcium carbonate particles, the glass plate containing the membrane sheet was submerged in basin containing 0.1 M HCl. After 20 min, the membrane sheet was washed for around 5 min in a basin containing water. Finally, the membrane sheet was taken out from water and placed on a paper towel.

Application of the membrane in turbidity removal

The filtering efficiency of membrane was determined by checking the turbidity of filtrate. First, turbid solution of calcium carbonate was prepared by dissolving a pinch of $CaCO_3$ in distilled water. 40 mL of $CaCO_3$ solution was filtered using Whatman 1 filter paper. Similarly, each of 40 mL of $CaCO_3$ solution was filtered through CA membrane incorporated with bulk $CaCO_3$ and $CaCO_3$ nanoparticle. Then, the turbidity of each filtrate filtered through filter paper and CA membranes were measured using turbidity meter (TB200, Bante Instruments, China). The time required for each filtration was

also recorded. Triplicate data were collected for both filters. The schematic outline of this work is provided in **Fig. 1.**

Fig. 1: Schematic outline of the research work.

Results and Discussion
Chemical analysis

The moisture content in Napier grass fiber was found to be 8.1 ± 0.1 % (w/w). The average value of extractives, hemicellulose, lignin, ash and cellulose were obtained to be 7.3 ± 0.2%, 27.7 ± 0.6 %, 13.9 ± 0.98 %, 4.5 ± 0.6 %, 48.3 ± 0.3%; respectively. The comparison of chemical composition of Napier grass fibers with some other perennial grasses is shown in **Table 1.** The cellulose content of Napier grass was found to be 48.3% which is significantly higher than in other grasses. The high cellulose content of Napier suggest that Napier grass more useful or profitable for producing different products of cellulose.

Table 1. Comparison with other fiber types

Fiber type	Extractives	Cellulose	Hemicellulose	Lignin	Ash	Reference
Bamboo	2.3	38-54	17-28	14-24	1.7-4.8	[32]
Giant reed	4.55-6.34	29.18-32.03	28.48-32.03	20.92-21.31	4.77-6.14	[33]
Reed canary	1.1	28.0	22.0	14	8	[34]
Napier grass (this work)	7.3 ± 0.2	48.3 ± 0.3	27.7 ± 0.6	13.9 ± 0.98	4.5 ± 0.6	

Fiber morphology and tenacity

The width of raw fiber, water retted fiber, 6% NaOH treated fiber, and extracted cellulose (n=50) was found to be 314.9±36.66 µm, 198.5 ± 32.45 µm, 102±20.5, and 48.4 ± 27.53 µm, respectively. The width of Napier grass fibers keeps on decreasing as it undergoes various

chemical treatment with the removal of various components attached to the fibers. As the fibers are treated in various alkaline and acidic solutions during the extraction process and the components like lignin, hemicellulose and extractives are removed in extraction process so the width of prepared cellulose is decreased[24,27,35,36]. The tenacity of water treated Napier grass (n=20) was found to be 15.02 ± 4.02 g/tex.
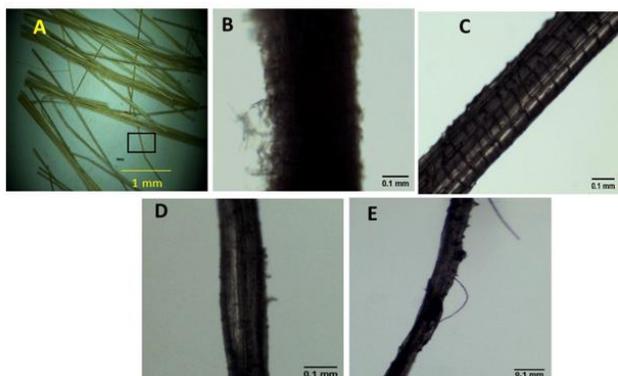

Fig. 2. Fiber Morphology. (A) Images of raw fiber taken measured with digital microscope at magnification of 10X in reflectance mode. (B), (C), (D) and (E) are the images of individual fiber width of raw, water retted, 6% alkali treated, and cellulose fiber measured with a bright microscope in transmittance mode at 100X.

FTIR and XRD studies of extracted cellulose

It is known than alkali treatment followed by bleaching results in removal of hemicellulose and lignin. This results in overall increase in cellulose content. To confirm the hemicellulose and lignin removal, FTIR spectra of raw fiber, extracted cellulose fiber and commercially available cellulose were measured (**Fig. 3A, curves a-c)**. In all FTIR spectra a broad peak around 3400 cm^{-1} is due to O–H stretch frequency of cellulose, hemicellulose and lignin[24,37,38]. A peak in ~2900 cm^{-1} correspond to the asymmetric and symmetric C–H stretching vibrations in lignin, cellulose and hemicellulose[39]. These peaks are more

pronounced or sharper in cellulose samples (Figure 3A, curves b and c) most likely due to removal of the lignin and hemicellulose contents[40]. A peak ~1750 cm^{-1} is attributed to the ester C=O or acetyl groups of non-conjugated not conjugated lignin and hemicellulose. The peak ~1620 cm^{-1} is assigned to C=O stretching of conjugated lignin[41,42]. The later peaks become sharper in cellulose samples indicated removal of hemicellulose and lignin. Absence of peak ~1500 cm^{-1}, which is assigned to aromatic skeletal vibration of lignin and extractives, is absent in cellulose samples indicated lignin removal.
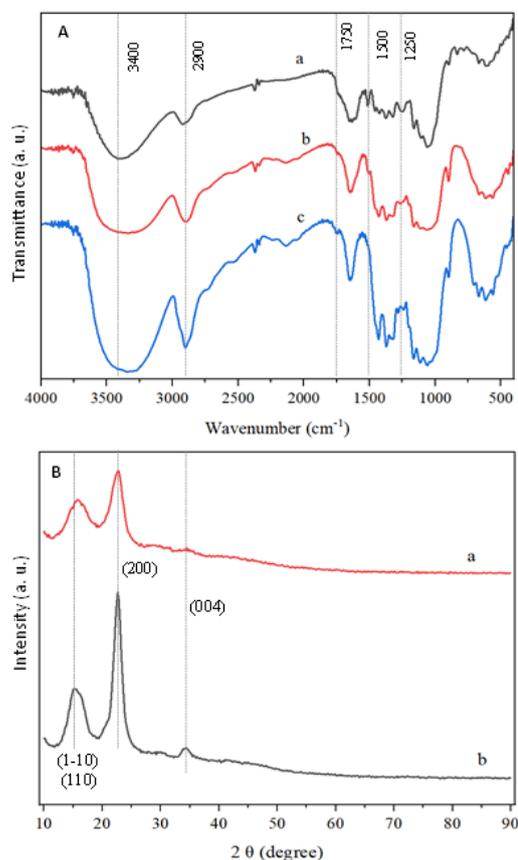

Fig. 3: FTIR and XRD spectra. (A) FTIR spectra raw fiber (curve a), Napier grass cellulose (curve b), and commercial cellulose (curve c). (B) XRD data of Napier grass cellulose (curve a) and commercial cellulose (curve b). The numbers in parentheses indicate the crystalline planes. In both frames, the vertical dotted lines are to indicate the major peaks/features.

Additionally, the ~1250 cm^{-1} assigned to C-O stretching of acetyl groups of hemicellulose and cellulose[43] is weaker in cellulose samples weekend on alkali treatment. This indicates hemicellulose removal. The close resemblance of extracted and commercial cellulose indicate that cellulose of high purity can be extracted form Napier grass. Literature studies suggest that XRD data of cellulosic samples reveal peaks at 2θ of ~15.5°, ~21.5°, and ~35°. These peaks originate from reflection of (1-10) and (110), (200), and (004) planes of crystalline cellulose phase I; respectively[24,44,45]. The first three peaks perfectly match in both extracted and commercial cellulose samples (Figure 3B). However, relatively sharper peaks in commercial cellulose could indicate that the crystallinity of extracted cellulose is lower than that of commercial cellulose.
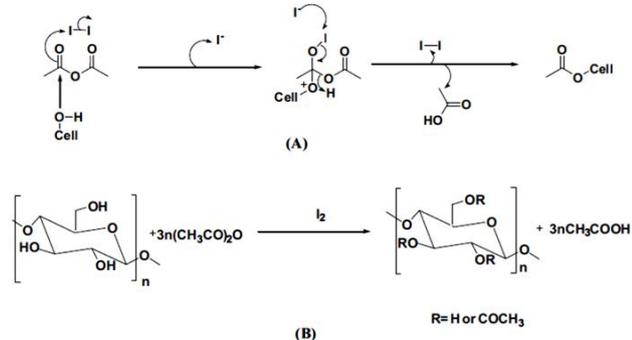
Acetylation of cellulose

Fig. 4. Cellulose acetylation. (A) The proposed mechanism for cellulose (cell-OH) acetylation using iodine as a catalyst. (B) The same reaction shown with cellulose monomeric unit [used with permission from[8]; copyright © 2014 Elsevier Ltd.].

In the acetylation of cellulose, iodine helps to activate the carbonyl carbon of acetic anhydride. The cellulose-OH attacks the carbonyl carbon resulting in sp^3 hybridization (Figure 4). The acetic anhydride then reacts with hydroxyl groups involving a nucleophilic attack on the acyl carbon center of the acetic anhydride. This results in the acetic acid to generate the

ester[46,47]. The addition of saturated aqueous solution of sodium thiosulphate is to make sure that all free iodine is converted to iodide [48]. The mechanism of acetylation cellulose in presence acetic anhydride and iodine catalyst is provided in **Fig. 4**[8].
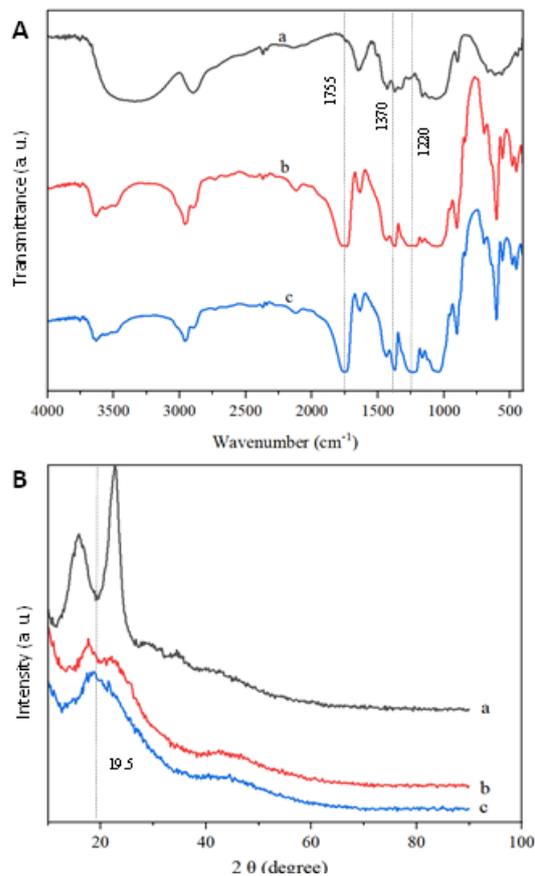

Fig. 5: FTIR and XRD data. (A) FTIR spectra of obtained cellulose (curve a), synthesized Napier grass cellulose acetate (curve b) and commercial cellulose acetate (curve c). (B) The corresponding XRD data. In both frames, the vertical dotted lines are to indicate the major peaks/features.

The FTIR spectra provide evidence for the acetylation. A close inspection of cellulose and acetylated sample (Figure 5, curves a and b) reveals that three prominent peaks viz. around 1755 cm^{-1} (C=O ester), 1370 cm^{-1} (C–H bond in –OCOCH$_3$ group) and –CO stretching band of acetyl group at around 1220 cm^{-1} in acetylated sample. Additionally, a strong band at around 1035 cm^{-1} in acetylated sample is due to the C–

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O–C pyranose ring skeletal vibration[8]. A significantly different band O-H band shape also indicated acetylation. The commercial cellulose acetate sample (Figure 5, curve c) also shows these characteristics peaks further indicating acetylation of cellulose. The further evidence for acetylation of cellulose can be obtained from XRD data. The diffraction pattern of prepared cellulose is changed after acetylation as reduction of sharp peak at $2\theta = 22.9°$ (Figure 5 curve b) compared to cellulose (Figure 5 curve a). The XRD data of both synthesized and commercial cellulose acetate showed a diffraction peak ~19.9° is due to less ordered or amorphous region of cellulose acetate[49] The cellulose acetate has low degree of crystallinity as compared to cellulose because the inter- and intra- molecular hydrogen bonding of cellulose are almost completely broken at the time of substitution of hydroxyl group by acetyl groups[50].

Fabrication of cellulose acetate membrane

If the calcium carbonate particles imbedded in cellulose acetate matrix are solubilized with dilute acid, porous structures as determined by size of particle, will be formed. It can expected that calcium carbonate nanoparticle impregnation result in membrane having smaller pores. Indeed, the optical microscopic image of nanoparticle incorporated membrane showed smaller pores in the optical images (Figure 6). The scanning electron microscopic images of the membrane would provide more information on the pore morphology. The thickness of membrane can be determined by the transparent tape used in the chamber used in casting the membrane and was found to be 95.4 ± 0.4 μm.

Application in turbidity removal

Cellulose acetate membrane is being explored for water purification including turbidity removal [8,31]. The filtering performance of the membranes was tested for turbidity removal. Using solution of 260.2 ± 5.9 NTU, the nanoparticle incorporated membrane removed turbidity by ~96.4% and bulk $CaCO_3$ incorporated by around 49%. The whatman #1 filter resulted in turbidity removal by around 89% (table 2). The turbidity removal by the nanoparticle incorporated membrane is due to small pores. These data suggested that the cellulose acetate membrane can very effectively remove turbidity form simulated turbid solution.
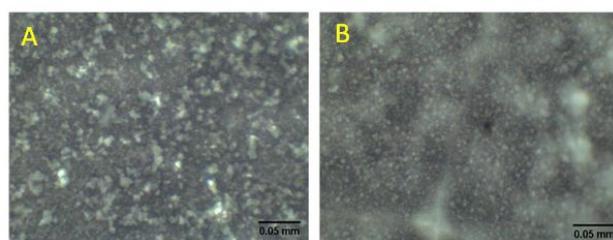

Fig. 6: Optical images. Optical microscopic images of cellulose acetate membrane prepared using bulk calcium carbonate (A) and carbonate nanoparticle (B). Both images were measured at magnification of 100X.

Table 2. Turbidity of solution after using different filters.

Membrane type	Turbidity (NTU) (n=3)	Decrease in turbidity (%)	Filtration time (min)
Whatman #1 filter	28.23 ± 0.16	89.15	2.95 ± 0.06
Bulk $CaCO_3$ incorporated CA membrane	132.90 ± 1.32	48.92	13.88 ± 0.54
$CaCO_3$ nanoparticle incorporated CA membrane	9.18 ± 0.27	96.47	29.95 ± 0.94

Conclusions

To conclude, cellulose was successfully extracted from raw Napier grass lignocellulose biomass. Cellulose acetate was also successfully synthesized through acetylation of cellulose in presence of iodine under solvent free conditions. The cellulose and cellulose acetate yield was found to be ~47% and 41.67%. The FTIR and XRD data of cellulose and cellulose acetate resembled to that of commercial

material suggesting Napier grass biomass can be used for the extraction and synthesis of high quality cellulose and cellulose acetate, respectively. The cellulose acetate was further used to fabricate acetate membrane of controlled porosity using calcium carbonate as pore forming template. The calcium carbonate nanoparticle template resulted in a membrane that can remove turbidity of simulated water by more than 90%. Focus of the current work was to explore the applicability of Napier grass biomass in extraction of cellulose and fabrication of cellulose acetate membrane. In future, the membrane of tunable pore size can be synthesized using calcium carbonate nanoparticle of variable size. The mechanical strength of the membrane and application in filtering environmental water sample could be explored.

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Author's Contribution Statement

Grisha Shrestha: Investigation, Writing: original draft, Methodology. **Krishna Kandel:** Methodology, Writing: original draft. **Girja Mani Aryal:** Investigation and Methodology. **Prakash Gautam:** Methodology, Writing: review and editing. **Bhanu Bhakta Neupane:** Writing: review & editing, Visualization, Validation and data analysis, Resources, Supervision

Conflict of Interest

The authors do not have any conflict of interest throughout this research work.

Data Availability Statement

The data supporting this study's findings are available from the corresponding authors upon reasonable request.

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