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Comparative Study of Cellulose Hydrogel Films Prepared from Various Biomass Wastes

Cho Cho, Thinzar Aye, Aung Khaing and Takaomi Kobayashi

Abstract

The conversion of biomass waste products to valuable products like cellulose hydrogel films is important in cell regeneration. In this study, the various biomass wastes: thanaka heartwood (TH), sugarcane bagasse (SB) and rice straw (RS) were used as cellulose resources. They were chemically treated using acid and alkali to obtain cellulose fibers. The yield percent of cellulose fibers depends on the nature of biomass materials. Scanning Electron Microscope (SEM), X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) analyses showed that the amount of lignin and hemicellulose from these samples were successfully reduced by chemical treatment. Cellulose fibers were treated using the dimethylacetamide/lithium chloride (DMAc/LiCl) system to obtain cellulose hydrogel solutions. Following this, the cellulose hydrogel films were prepared employing the phase inversion method without cross-linker. These films were transparent and flexible. In the present study, water retainable property and viscoelasticity of cellulose hydrogel films were measured. Antimicrobial activity tests of cellulose solutions have been carried out to be utilized to hydrogel films for biomedical application.

Keywords: cellulose hydrogel films, phase inversion method, biomass waste, antimicrobial activities

1. Introduction

Hydrogels are three-dimensional polymeric networks kept together by cross-linked covalent bonds as well as weak cohesive forces such as hydrogen or ionic bonds [1]. Hydrogels consisting of hydrophilic polymer networks can absorb up to thousands of times their dry weight in water. Natural and synthetic polymer-based hydrogels are the two types of hydrogels, classed based on polymer source. Synthetic polymer-based hydrogels are fabricated from poly ethylene glycol, poly acrylic acid, polyacrylamide, poly vinyl alcohol etc. [2–5]. Natural polymer-based hydrogels are derived from various natural polymers, such as cellulose, gelatin, peptides, chitosan and alginate, etc. [6–8] and can be employed as advanced materials for tissue and organ repair and regeneration [9–11].

Commonly, all plant biomass consists of cellulose, hemicellulose, lignin, pectin and protein with cellulose being the major component making up to 33% of plant biomass. Use of cellulose has several advantages including biocompatibility, biodegradability, renewability, good mechanical strength, being environmentally

friendly, and one of the safest materials on the earth [12]. Consisting of a linear chain of β (1 \rightarrow 4) linked *D*-glucose units, it has properties of tasteless, odorless, insolubility in water and most organic solvents - a characteristic owing to its hydrogen bonds and Van der Waals forces that make it difficult for the dissolution [13].

Abundant in hydrophilic functional groups, including hydroxyl, carboxyl, and aldehyde groups, cellulose and its derivatives are ideal materials to prepare hydrogels films. Biodegradability, biocompatibility, non-toxicity, hydrophilicity, and tissue-mimicking characteristics of cellulose-based hydrogels make them useful in a variety of sectors, including food, agriculture, environmental remediation and medicinal applications, such as drug delivery [14, 15], tissue engineering [16–19], wound dressing [20–22], bio imaging [23–25] and wearable epidermal sensors etc. [26, 27]. The possibility of these numerous biomedical applications draws researchers into exploring renewable plant biomass-based cellulose alternatives to create hydrogel films [28–31].

Myanmar is an agricultural country with paddy crop production being the main agricultural production of the country. After being harvested; rice straw is generated in the field. Sugarcane bagasse is also fibrous by-product remaining after sugar extraction from sugarcane. And, these biomass wastes are always in abundance in many areas. The bark of thanaka is used for the production of cosmetic products such as thanaka powder, liquid, and paste [32]. The heartwood is often unused and becomes waste after the bark has been used up. As a result, as sustainable cellulose, thanaka heartwood, sugarcane bagasse and rice straw are biomass wastes that can potentially be converted into a valuable product like hydrogel films and they will become cost-effective items and can reduce environmental pollution. For this purpose, this study was carried out to convert cellulose solutions from these resources by using DMAc/LiCl systems to prepare cellulose hydrogel films. This research article has also reviewed the comparative study on the properties and nature of cellulosic hydrogels from various plant biomass wastes for biomedical applications.

2. Materials and methods

2.1 Materials

Thanaka samples were obtained from Pakokku, Pakokku Township, Magway Region, Myanmar. Rice straw samples were obtained from Helgu Township, Yangon Region, Myanmar and Sugarcane bagasse collected from Nawaday Sugar Mill, Pyay Township, Bago Region, Myanmar.

2.2 Preparation of treated fiber

First, the collected raw materials were cut, washed and dried at 40°C. For the preparation of acid-treated samples, 10 g of raw samples were immersed in 500 mL, 4 vol% H₂SO₄ solution and stirred at 90°C for 2 h. After cooling down, the samples were washed, filtrated and collected as acid-treated samples. Similarly, they were again immersed in 500 mL, 10 vol % NaOH solution and stirred at 90°C. They were washed, filtrated and collected as base-treated samples. Again, they were immersed in 200 mL, 10 vol % NaOCl and stirred for the color bleaching. Then, cellulose fiber was obtained and dried at 50°C [29–31]. Percent cellulose fibers were found to be 28.50% of TH, 20.31% of SB and 21.63% of RS. Among them, the highest yield percent of cellulose was obtained from thanaka heartwood samples due to the hard portion (stem) of the plant. The yield percent of cellulose depends on the nature of plant materials.

2.3 Preparation of cellulose solution

100 mL of deionized water was added into 1 g of cellulose fiber and stirred at room temperature overnight. They are then filtered and washed with ethanol. After that, 100 mL of ethanol was added to the swollen fibers and the mixture was stirred for 24 h. Then, ethanol was removed and added to 50 mL of DMAc and stirred overnight. Finally, 8 wt % of LiCl and DMAc were added to the swollen cellulose fibers and the solvent system adjusted to obtain a 1% concentration of cellulose solution. The mixture was stirred at room temperature to get the hydrogel solution.

2.4 Preparation of cellulose hydrogel films

For hydrogel film preparation, 10 g of cellulose solution was poured into a Petri dish (10 cm diameter) and kept in a container filled with ethanol for 24 h. After this, transparent hydrogel films were obtained. Then, these films were washed with distilled water several times and immersed in distilled water and kept at room temperature. **Figures 1–3** illustrated the raw samples to cellulose hydrogel films for thanaka heartwood, sugarcane bagasse and rice straw, respectively. It was found that all cellulose hydrogel films were transparent and flexible but a little difference in their strength.

2.5 Characterization techniques

The structural changes of samples were analyzed by FTIR 8400 Shimadzu spectrophotometer by using the KBr pellet method in the MIR radiation with the wavelength from 4000 cm^{-1} to 400 cm^{-1} range with a resolution of 4.0 cm^{-1} . The surface morphology of the samples was investigated by SEM (JEOL 15 kV). The X-ray diffraction (XRD) patterns of raw sample and pretreated cellulose fibers were recorded with $\text{CuK}\alpha$ radiation at 40 kV and 30 mA in the range of $2\theta = 10^\circ\text{--}40^\circ$ by X-ray diffractometer (Smart Lab, Rigaku, Japan). The samples were dried in vacuum at room temperature before measuring them. The crystallinity index (CI) was calculated using the equation: $\text{CI} (\%) = (I_{002} - I_{\text{am}}) / I_{002} \times 100$, where I_{002} is the maximum intensity of the peak (002) lattice diffraction and I_{am} is the intensity of diffraction attributed to amorphous cellulose [33]. Viscoelasticity of the hydrogel film, 2 cm in diameter with 5 mm in thickness, was determined by Auto Paar-Rheoplus equipment (Anton Paar Japan, Tokyo) in wet conditions at 37°C .

2.6 Study on antimicrobial activity

The study on antimicrobial activity of DMAc, DMAc/LiCl and cellulose solutions was performed by the agar well diffusion method [34]. Nutrients agar was

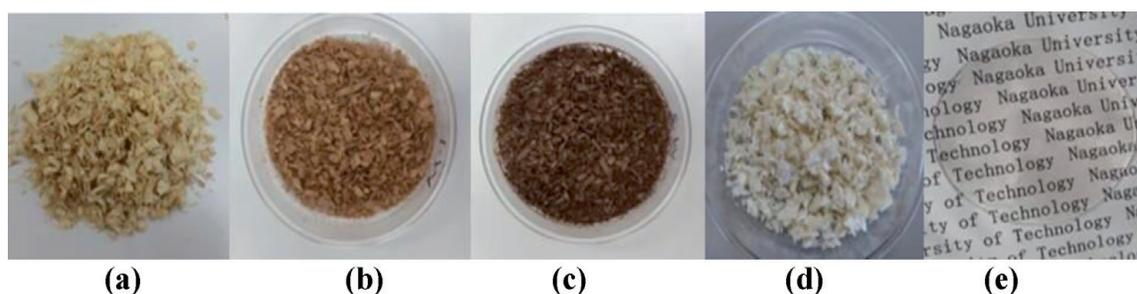


Figure 1. Photos showing (a) thanaka heartwood (b) acid-treated sample (c) base-treated sample (d) cellulose fiber and (e) thanaka heartwood cellulose hydrogel film (THCF).

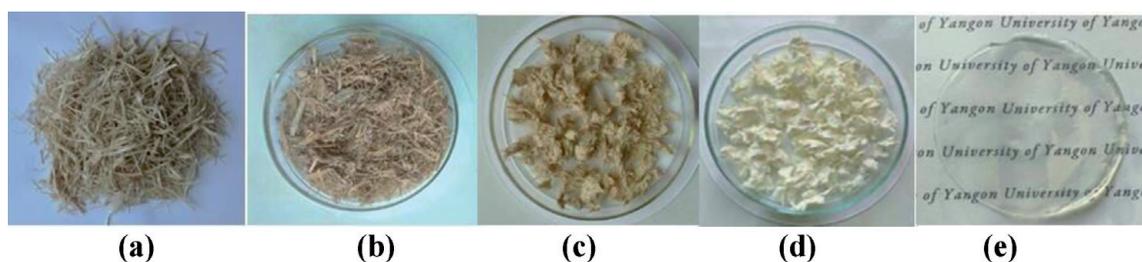


Figure 2.

Photos showing (a) sugarcane bagasse (b) acid-treated sample (c) base-treated sample (d) cellulose fiber and (e) sugarcane bagasse cellulose hydrogel film (SBCF).

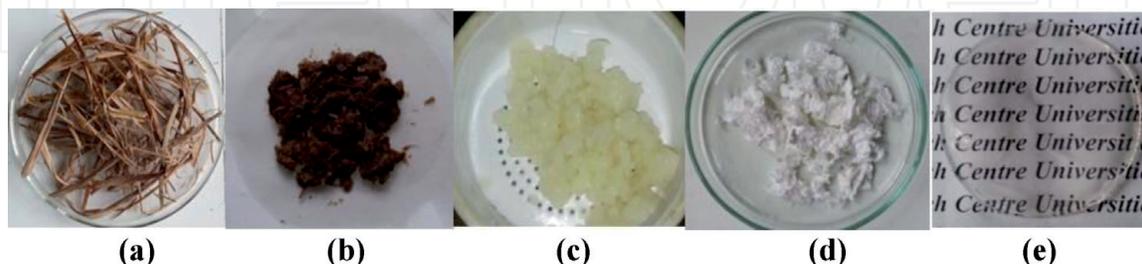


Figure 3.

Photos showing (a) rice straw (b) acid-treated sample (c) base-treated sample (d) cellulose fiber and (e) rice straw cellulose hydrogel film (RSCF).

prepared according to Cruickshank's methods [35]. Firstly, nutrient agar (medium) was boiled and 20–30 mL of the medium poured into test tubes which were plugged with cotton wool. Secondly, the test tubes with the medium were autoclaved at 121°C for 15 minutes and they were cooled down to 30–35°C. Finally, the medium was poured into the sterilized petri dishes and 0.1–0.2 mL of test organisms were added into the dishes. In this study, the tested microorganisms are *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Escherichia coli*. The agar was allowed to set for 2–3 hours. Fourthly, 10 mm agar-well was made with the help of a sterilized agar-well cutter. Finally, about 0.2 mL of samples were introduced into the agar-well and incubated at 37°C for 24 to 36 hours. The inhibition zone that appeared around the agar-well was measured for the indication of the presence of antimicrobial activity.

3. Results and discussion

The structural changes for each biomass sample: thanaka heartwood, sugarcane bagasse and rice straw of (a) raw sample (b) acid-treated sample (c) base-treated sample and (d) cellulose fiber were shown in **Figures 4–6** respectively. The broad peaks at 3400 cm^{-1} in all samples were due to the stretching vibrations of O-H groups of water contents. The peak at 2900 cm^{-1} was due to C-H stretching. From the comparison of the presented FTIR spectra, the peaks around 1510 cm^{-1} and 1520 cm^{-1} showed the presence of lignin and lignocellulose in the initial raw sample. Moreover, the band at around 1200 cm^{-1} disappeared in treated fiber which was attributed to the removal of hemicelluloses. The changes of the bands in the region from 1423 cm^{-1} to 1080 cm^{-1} were due to the removal of lignin and hemicelluloses [36, 37]. This is a clear indication that the amount of lignin from the raw sample was successfully reduced by the chemical treatments.

The surface morphologies of biomass raw samples and their pretreated samples were shown in SEM images of thanaka heartwood in **Figure 7**, sugarcane bagasse in

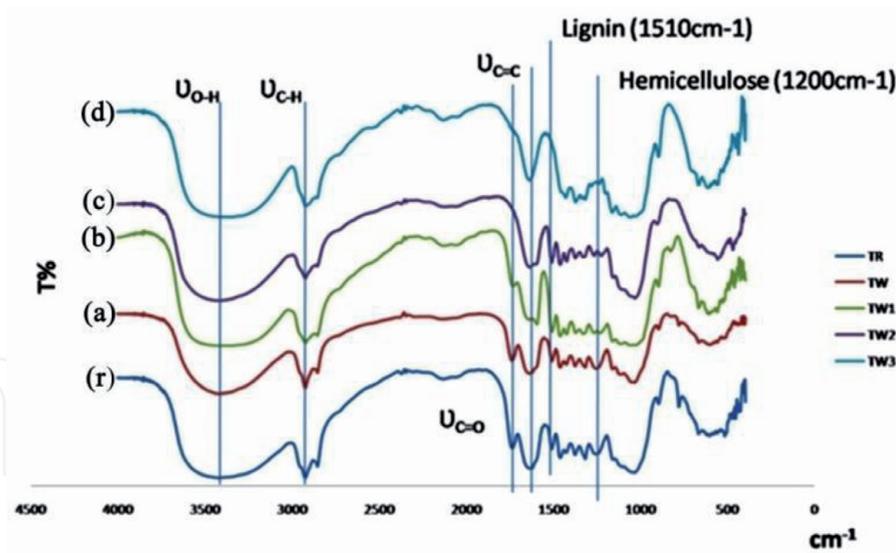


Figure 4.
 FTIR spectra of (a) thanaka heartwood (b) acid-treated (c) base-treated samples and (d) cellulose fiber.

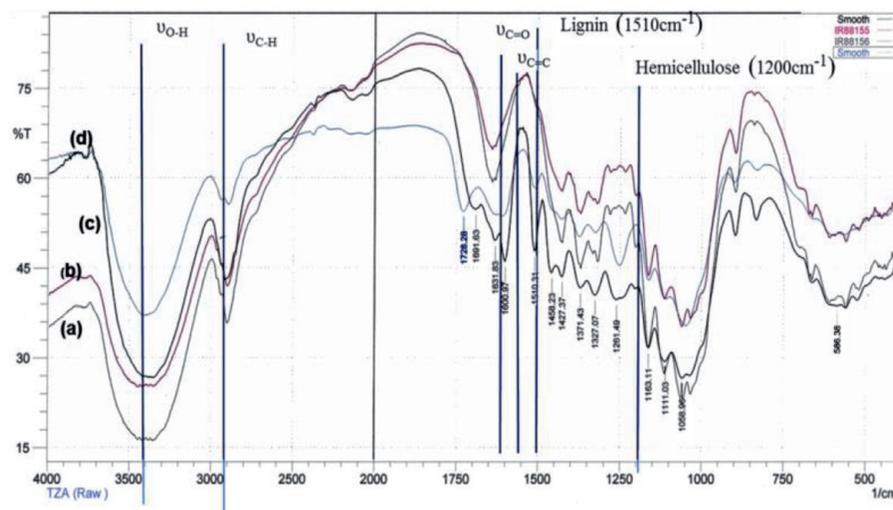


Figure 5.
 FTIR spectra of (a) sugarcane bagasse (b) acid-treated (c) base-treated samples and (d) cellulose fiber.

Figure 8 and rice straw sample in **Figure 9**. Morphological changes in the raw, acid and base pretreated samples were clearly seen in these SEM images. The fibrous patterns of the plant cell wall appeared in the raw samples shown in **Figures 7(a), 8(a), and 9(a)**. **Figures 7(b), 8(b), 9(b)** and **7(c), 8(c), 9(c)** represented the distorted structure of lignin and hemicellulose of plant cell wall structure due to pretreatment of acid and base. Pretreatment of NaOH tended to decrease the lignin and hemicellulose which in turn increased the content of cellulose. It also resulted in an increase in cellulose accessibility shown in **Figures 7(d), 8(d)** and **9(d)**.

Water content of the hydrogel films was determined by weighing dry and wet samples by the following procedure. Disk samples with 5 mm diameter were cut from cast films and dried in a vacuum oven for 24 h and weighed (W_0). Then, samples were immersed in distilled water for 36 h. After that, films were removed from the water and wrapped with filtered paper in order to remove excess water and weighed again (W_1). Finally, the equilibrium water content was calculated from the wet (W_1) and dried (W_0) hydrogel films. For each sample, four independent measurements were done and averaged by the following formula: Water content (%) = $(W_1 - W_0) / W_0 \times 100$. According to the calculation, water content was 165.5%

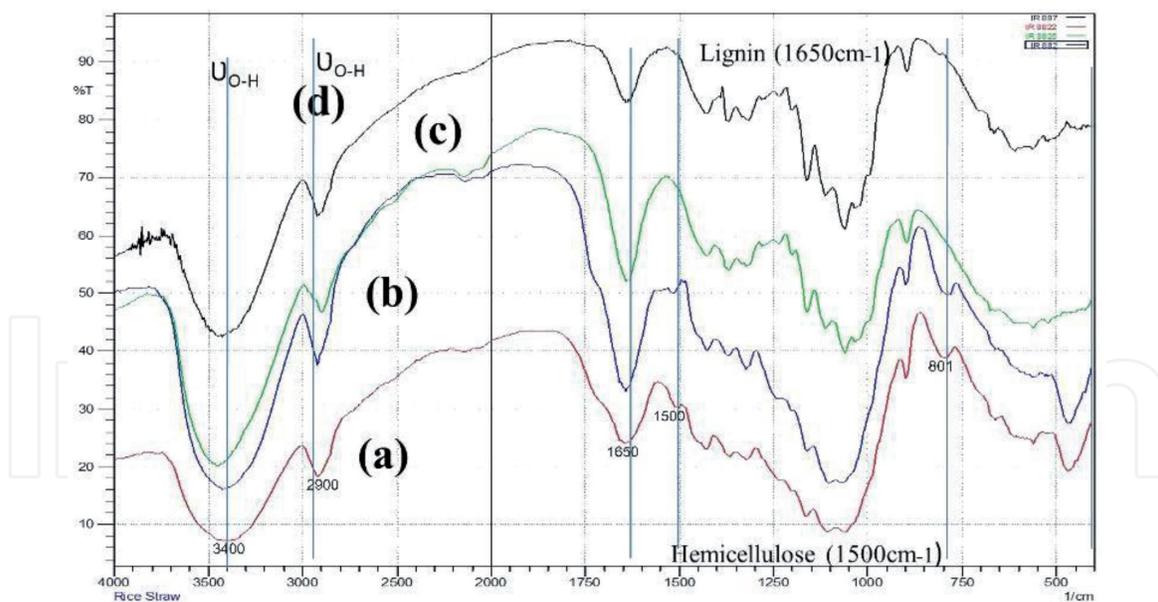


Figure 6. FTIR spectra of (a) rice straw raw (b) acid-treated samples (c) base-treated and (d) cellulose fiber.

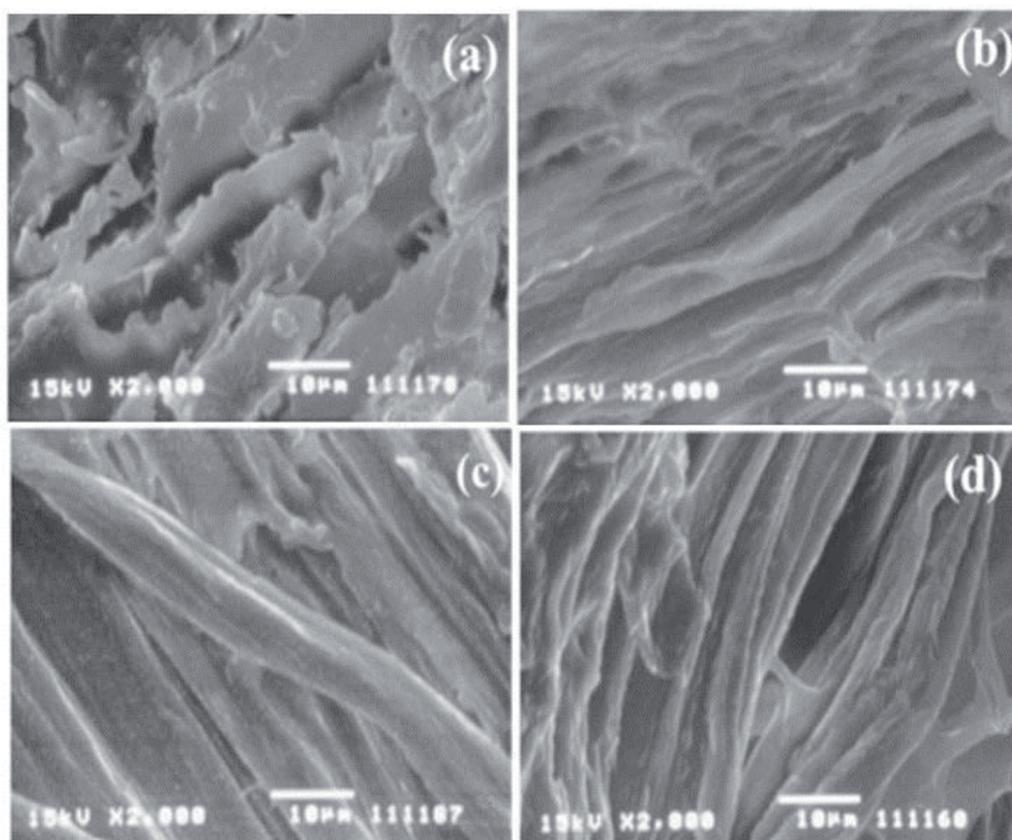


Figure 7. Comparative SEM images of (a) thanaka heartwood (b) acid-treated (c) base-treated samples and (d) cellulose fiber.

for THCF, 188.47% for SBCF film and 168.63% for RSCF film. Thus, the films have water retainable property which will be the formation of the interaction of hydrogen bonding networks of the resultant cellulose in the hydrogel films.

XRD measurement was carried out to evaluate the effect of treatment conditions on the crystalline structure of raw samples, treated fibers and cellulose fibers. The patterns of (a) to (d) exhibited typical crystalline lattice of cellulose with peaks at 22.3° and 16.4° [38, 39]. This cellulose crystalline can be found in natural

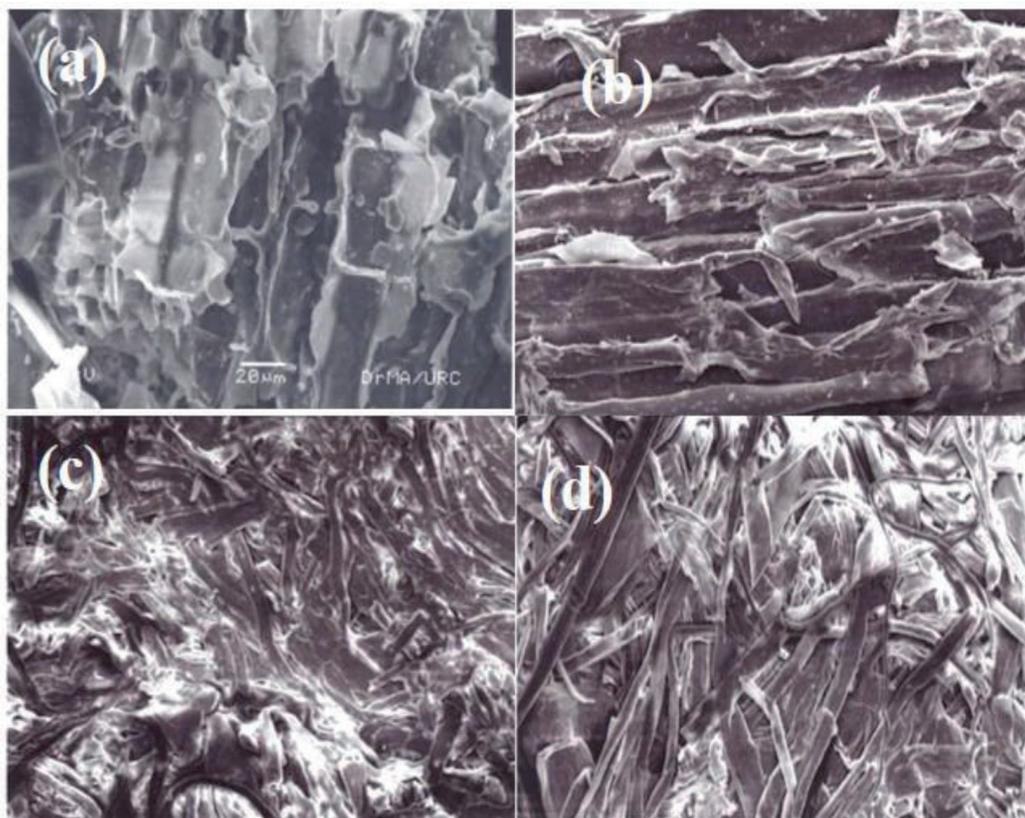


Figure 8.
Comparative SEM images of (a) sugarcane bagasse (b) acid-treated (c) base-treated samples and (d) cellulose fiber.

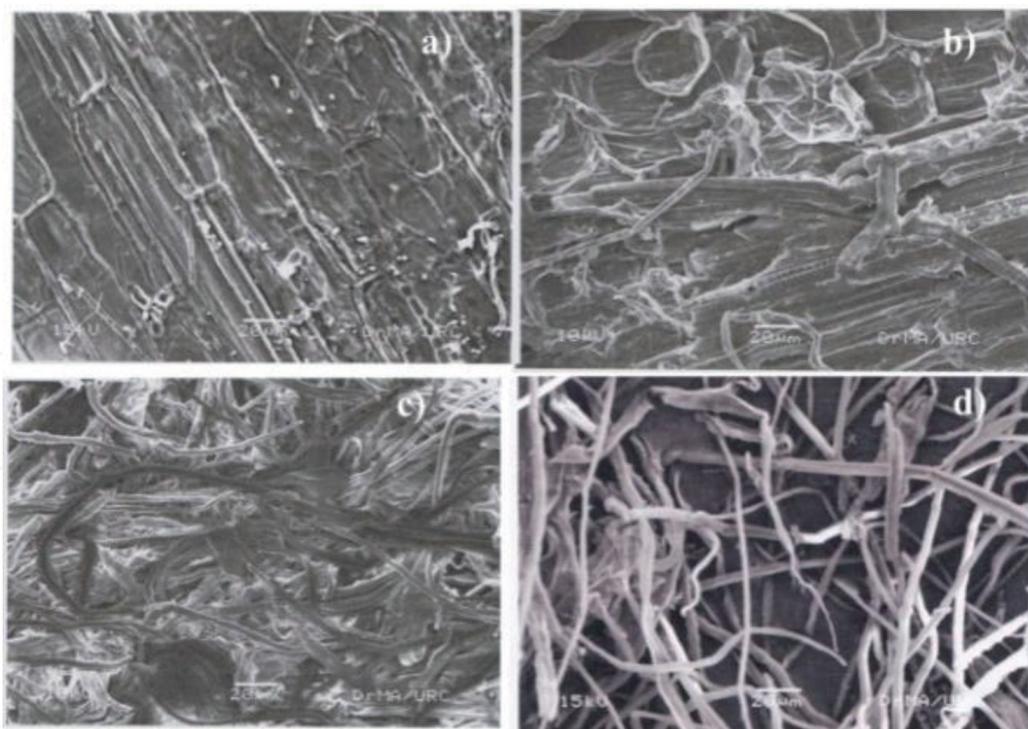


Figure 9.
Comparative SEM images of (a) rice straw (b) acid-treated (c) base-treated samples and (d) cellulose fiber.

plant cellulose. **Figure 10** showed the XRD patterns of thanaka heartwood and the treated fibers. In the crystallinity of thanaka heartwood and cellulose fibers, the peak ratio showed that thanaka sample and pre-treated fibers were at 68.5%, 82.1%, 84.2%, and 85.4%, respectively. **Figure 11** showed the XRD patterns of the bagasse

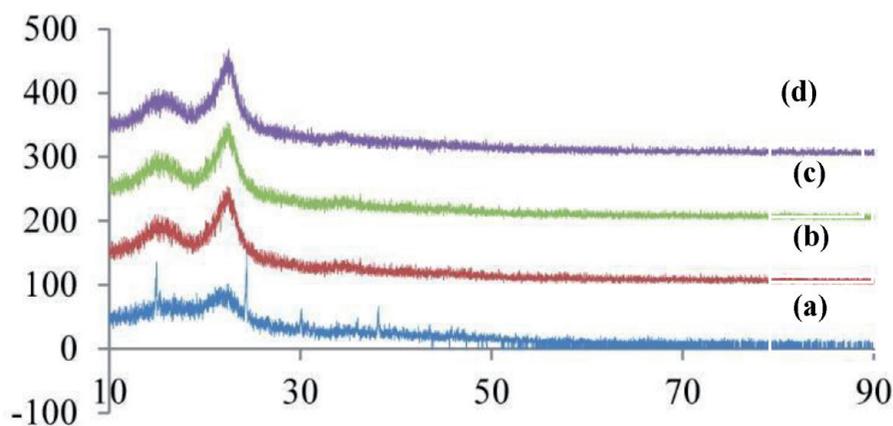


Figure 10. XRD patterns of (a) thanaka heartwood (b) acid-treated (c) base-treated and (d) cellulose fiber.

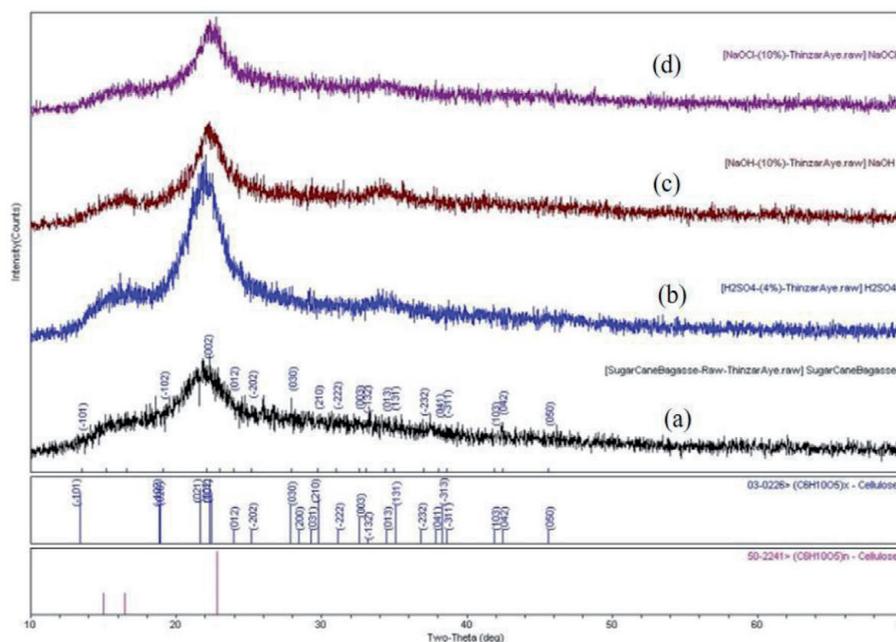


Figure 11. XRD patterns of (a) sugar cane bagasse (b) acid-treated (c) base-treated and (d) cellulose fiber.

and the purified fibers. The crystallinity indexes of sugar cane bagasse raw sample, acid-treated sample, base-treated sample and cellulose fiber were 44.1%, 58.8%, 59.1% and 60.2% respectively. **Figure 12** showed the XRD patterns of rice straw and the purified fibers. The crystallinity indexes of rice straw, acid-treated sample, base-treated sample and cellulose fiber were 71.43%, 75.7%, 76.25% and 78.57%, respectively. The increment of the crystallinity in the pre-treated fibers was due to the removal of hemicelluloses and lignin by sodium hydroxide and sodium hypochloride treatment, indicating especially higher purity of cellulose fibers.

Viscoelasticity measurements indicated the relationship between storage elastic modulus G' , loss elastic modulus G'' and strain for the hydrogel films. **Figure 13** showed the viscoelastic data for (a) THCF films (b) SBCF films and (c) RSCF films. It was noted that the deformation of THCF occurred at $G' = 8 \times 10^4$ and $G'' = 8 \times 10^3$ Pa at 1×10^{-2} to 9% strain, SBCF at $G' = 7 \times 10^4$ and $G'' = 6.5 \times 10^3$ Pa at 1×10^{-2} to 35.71% strain, the RSCF film at $G' = 8 \times 10^4$ and $G'' = 7.5 \times 10^3$ Pa at 1×10^{-2} to 12.45% strain [40]. The loss elastic modulus (G'') of SBCF film was lower than that of the others. This meant that the elastic nature was low and deformation was high in SBCF film. The crossover point of G' and G'' meant fracture of materials or inability to follow deformation due to the rigid polymer network. In the prepared

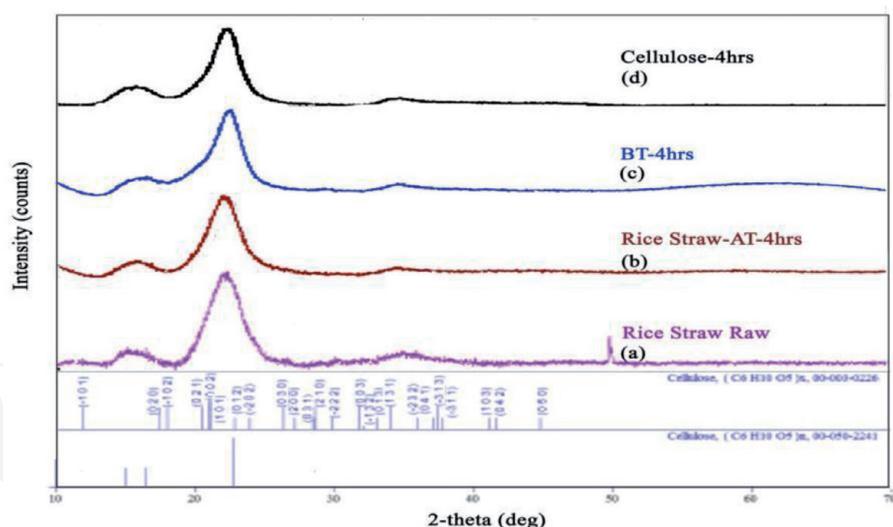


Figure 12.
 XRD patterns of (a) rice straw raw (b) acid-treated (c) base-treated and (d) cellulose fiber.

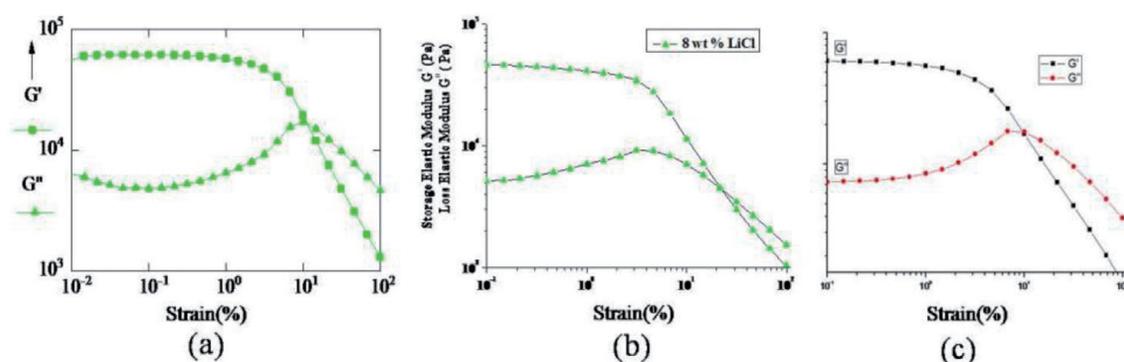


Figure 13.
 Viscoelasticity of (a) thanaka heartwood cellulose hydrogel film (THCF), (b) sugarcane bagasse cellulose hydrogels films (SBCF) and (c) rice straw cellulose hydrogel film (RSCF).

cellulose hydrogel, cross points of G' and G'' shifted towards a low strain range in order of strength of cellulose fiber. The crossover point of THCF film was found to be 9% and that of RSCF was 12.45% strain. In the case of SBCF, the G' and G'' values overlapped at 35.71% strain. It seemed that the SBCF film showed a very soft and less elastic nature than the other. Based on the comparison of viscoelasticity measurement for these three films, THCF film was the strongest and most elastic compared to the other two films as a consequence of its rigid polymer network of hardwood resource.

Table 1 showed the measurements of inhibition zones of cellulose solutions. The inhibition zone appearing around the agar-well was measured; an indication of the presence of antimicrobial activity. The measurable zone diameter, including the well diameter showed the degree of antimicrobial activity. The well diameter was 10 mm in this experiment. The larger the inhibition zone diameter showed stronger antimicrobial activity on the test organisms. According to the results, cellulose solution samples showed an inhibition zone more than the DMAc/LiCl solution, noting the DMAc solvent had no antimicrobial activity. It was found that all cellulose solutions showed antimicrobial activity and TH cellulose solution exhibited high activity against all test microorganisms (diameter of inhibition zone ranging 12–18 mm). On grounds of the antimicrobial activity results and water retaining properties, cellulose hydrogel films prepared from plant biomass waste can be applied as wound dressing, medical plaster and facial mask for biomedical applications.

Samples	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus pumilus</i>	<i>Candida albicans</i>	<i>Escherichia coli</i>
DMAc	—	—	—	—	—	—
DMAc + LiCl	15 mm (+)	15 mm (+)	15 mm (+)	15 mm (+)	12 mm (+)	15 mm (+)
RS cellulose solution	17 mm (++)	15 mm (+)	16 mm (++)	17 mm (++)	15 mm (+)	15 mm (+)
SB cellulose solution	16 mm (++)	15 mm (+)	16 mm (++)	17 mm (++)	13 mm (+)	15 mm (+)
TH cellulose solution	18 mm (++)	18 mm (++)	15 mm (+)	17 mm (++)	12 mm (+)	18 mm (++)

Table 1.
Antimicrobial activity results for test samples.

4. Conclusion

By using DMAc/LiCl system, conversion of biomass waste to valuable products like cellulose hydrogel films was successfully prepared. Comparative studies on the properties and nature of cellulosic hydrogels from various plant biomass wastes were also reviewed. The yield percent of cellulose fibers: 28.50% of thanaka heartwood, 20.31% of sugarcane bagasse and 21.63% of rice straw was found in the study. It was regarded that the amount of the cellulose fibers depends on the nature of biomass materials. Cellulose hydrogel films were transparent and flexible but of slightly variable strengths due to the different kinds of cellulose sources. The water retainable property of SBCF was greater than that of THCF and RSCF. Based on the comparison of viscoelasticity measurement, it was found that THCF and RSCF were stronger, more elastic in nature and of a less deformed nature than the SBCF film. Since the cellulose solutions displayed antimicrobial activities against six test organisms, the cellulose hydrogel films could be utilized in biomedical applications.

Acknowledgements

We are extremely thankful to experts at Open Access Funding Page and especially to Mia Vulovic, Author Service Manager, for granting a free publishing fee allowing us to share our research with a wider audience. The authors: Cho Cho, Thinzar Aye and Aung Khaing, would like to express our sincere thanks to Prof. Dr. Pho Kaung, Rector of the University of Yangon for motivating and heartening us in our research. We are greatly appreciated Prof. Dr. Ye Chan, Head of Universities' Research Centre (URC), for his permission to carry out this research. We are deeply indebted to Prof. Dr. Takaomi Kobayashi, Department of Science of Technology Innovation, Nagaoka University of Technology, Nagaoka, Japan, for his invaluable advice and endless support. Our special thanks go to all of the URC staff.

Conflict of interest

The authors declare no conflict of interest.

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