Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 7 (4): 580-584, 2007
ISSN 1812-5654

Structure and Physical Properties of Natural Gellous Materials

[1]Rike Yudianti, [1]Lucia Indrarti and [2]Jun-ichi Azuma
[1]Division of New Material, Research Center for Physics, Indonesian Institute of Sciences,
Jalan Cisitu 21/154D, Bandung 40135, Indonesia
[2]Laboratory of Forest Biochemistry, Division of Environmental Science,
Graduate School of Agriculture, Kyoto University, Japan

Abstract: This study presents two types of natural gellous materials as cellulose resources including gellous material synthesized by *Acetobacter xylinum* in fermentation process of coconut water with common name Bacterial Cellulose (BC) and gellous material isolated from seed of *Ocimum americanum* called hydrogel. Morphological surface of BC and hydrogel was observed by Scanning Electron Microscope (SEM). These images show randomly arrangement of fibres in three dimensional network having length of 1-5 μm and 3-12μm, respectively in forming a dense reticulated structure. Hydrated fibres were observed evidently by Atomic Force Microscope (AFM) showing that BC and hydrogel have fibres in nanometer scale diameter, 7-10 and 2-3 nm, respectively. At glance, X-Ray diffraction profile of hydrogel shows broadening peaks at 2θ, 16° and 22°. While BC has peaks at 2θ, 14.7, 16.7, 20.5 and 22.5°, attributed to lattice diffractions (100), (010), $(\bar{1}\bar{1}2)$ and (110), respectively. The sharp profile present in BC lead to ordered structure, confirmed by higher crystallinity degree of BC (75%) compared to that's of hydrogel (35%). Water Holding Capacity (WHC) of BC and hydrogel has values about 5.5 and 39.2 mL g^{-1}, respectively while swelling ability of BC and hydrogel in water is 6.2 and 102.2%, respectively. Neutral sugar compositions of BC resulted in less 0.1% arabinose and rhamnose, 1.1% galactose, 98.5% glucose, 0.2 xylose and 0.2 mannose indicating high cellulose content. Meanwhile, hydrogel contains 11.9% (arabinose), 4.5% (rhamnose), 18.6% (galactose), 50.5% (glucose), 13.2% (xylose), 1.3% (mannose) indicating high hemicellulose contents leading to branching of arabinogalactan attached to cellulose.

Key words: Gellous material, Acetobacter xylinum, bacterial cellulose, hydrogel, water holding capacity

INTRODUCTION

Indonesia is rich in cellulose resources as major biopolymer and tremendous economic importance globally. Cellulose materials are very important natural resources because of biodegradability and renewability. Two different types of cellulosic material are known to be present on the earth as hard crystalline cellulose (Bacterial cellulose) and soft amorphous cellulose (hydrogels). Fundamental understanding of the structure of native cellulose has altered in recent years been by discovery that native cellulose microfibril consist of two different co-existing crystalline allomorph, named Iα (triclinic) and Iβ (monoclinic). The relative proportion of these two different crystalline structure varies depending on the source of the cellulose.

Bacterial cellulose is produced by fermentation of coconut water forming white gel-like on the surface coconut water for few day. Few country utilize bacterial cellulose as food product 'nata de coco' containing dietary cellulose fibre, consumed as desert and low calorie additive, thickener, stabilizer and texture modifier. One of the most important features of bacterial cellulose is its chemical purity, which distinguishes this cellulose from plants, usually associated with hemicelluloses and lignin, removal of which is inherently difficult. In addition, high mechanical strength cause bacterial cellulose on widely application possibility, particularly in acoustic diaphragma and electronic paper (Nishino *et al*., 1995; Shah and Brown, 2005; Tajima *et al*., 1995). Another cellulose resources are harvested from plant as most important renewable natural resources. They are known to be present in mistletoe (*Viscum album* L.) (Azuma and Sakamoto, 2003), white mustard (*Brassica alba*), sage (*Salvia* sp.) (Yudianti *et al*., 2004, 2005) and shell bush (*Orthosiphon* sp.). One of cellulose resources is produced by *Ocimum* sp. (Indrarti *et al*., 2004, 2005) having seeds for popular beverage to remedy cough, stomachache and headache. *Ocimum* sp. is one of plants producing hydrogel which proven in utilization for removal of

Corresponding Author: Rike Yudianti, Division of New Material, Research Center for Physics, Indonesian Institute of Sciences, Jalan Cisitu 21/154D, Bandung 40135, Indonesia Tel: 62-22-2503052 Fax: 62-22-2503050

chromium gaining considerable importance as alternative technology for treatment of heavy metal waste (Melo and D'Souza, 2004).

As noted above, Bacterial Cellulose (BC) and hydrogel constitute inexpensive gellous materials commercially available in Indonesia. Presence of cellulose in the structure attracted more attention leading to economically low price and biodegradable. Corresponding in utilization of gellous material as biofunctional application, gellous materials structure and physical properties are being studied. Structure analysis was analyzed by X-ray diffraction and High Performance Anionic Exchange Chromatography (HPAEC), X-ray diffraction analysis conducted to determine order-diorder structure while HPAEC analysis intended to know neutral sugar contributed in gel forming. Physical properties were focused on Water Holding Capacity (WHC) and swelling ability of gels. Presence of nanofibres within gel structure evaluated by Scanning Electron Microscope (SEM) and Atomic Force Microscope (AFM) add a potentially an outstanding gels in numerous application.

MATERIALS AND METHODS

Materials: Bacterial cellulose (BC) is syntesized by *Acetobacter xylinum* in fermentation process of coconut water under static condition and incubated at room temperature for 7-15 days. After incubation, the gel bacterial cellulose produced on the surface of media were harvested and washed with water, 2% sodium hydroxide (NaOH), and throughly washed by distillated water. Drying of BC was conducted by freeze dryer.

Meanwhile, *Ocimum americanum* commercially available at West Java., Indonesia constitutes one of plants utilized as hydrogel resources. Isolation of hydrogel from seed coat of *Ocimum americanum* was conducted after wetting of seed and expanding out hydrogel from seed coat completely. Isolation of hydrogels from seeds coat was conducted by blender for 1 min. Subsequently hydrogel and crushed seed were separated by filtration and obtained hydrogel was dried by freeze dryer.

Methods

X-ray diffraction: X-ray diffraction profiles of BC and hydrogel were measured by X-ray diffraction Rigaku RINT 2500, using Ni filtered Cu-Kα radiation (λ = 0.1542 nm). The operating voltage and current were 35 kV and 40 mA, respectively. The scanning of 2θ (5-40°) was employed with speed of 2°/min. Crystallinity was calculated from diffracted intensity data using the method of Segal *et al.* (1959), where the crystallinity index $I_{(200)/(110)}$–$I_{am}/I_{(200)/(110)}$·; $I_{(200)}$ is the maximum intensity of lattice diffraction for bacterial cellulose and $I_{(110)}$ is the maximum intensity of lattice diffraction for hydrogel, I_{am} is amorphous region at 2θ = 18°.

Neutral sugar composition analysis: Dried samples were hydrolized with 72% (w/w) sulfuric acid followed by diluted sulfuric acid (4%) for 1 h at 120°C. After hydrolysis, neutral sugar compositions were analysed by High Performance Anion Exchange Chromatography (HPAEC) Dionex DX-500 equipped with ED 40 Carbopac PA1 (4.0 mm × 25.0 cm) which well-suited to analysis of monosaccharides and separation of linear homopolymers. HPAEC is coupled with Pulsed Amperometric Detection (PAD) permits direct quantification at low picomole levels with minimal sample.

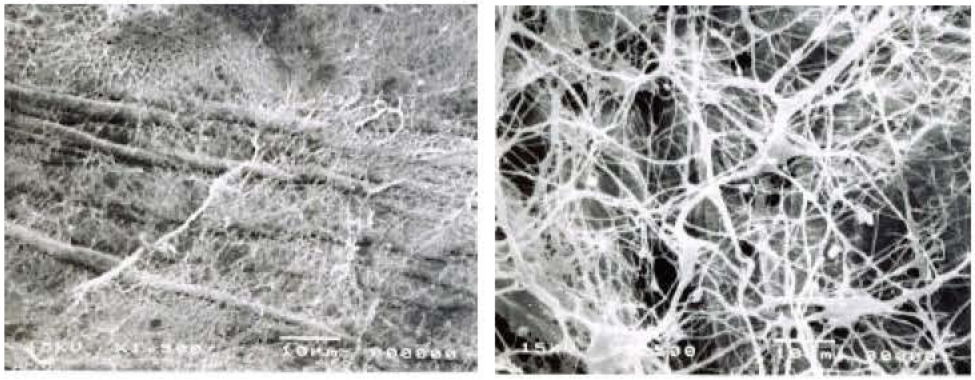
Morphological properties: Dried samples were cutted in size of 0.7×0.7 cm and put on sample holder using carbon double tape. In order to attainment of conductived samples, gold was coated on the sample surfaces by Sputter JFC 1100. Morphological properties of dried samples were observed by Scanning Electron Microscope Jeol T330A. The observation was conducted on voltage of 10 kV, current of 0.5 mA and magnification of 1000 times.

Observation of hydrated samples by Atomic Force Microscope (AFM) was also conducted. High resolution of AFM is utilized to observed diameter fibres of gellous materials which predicted to have diameter in nanoscale.

RESULTS AND DISCUSSION

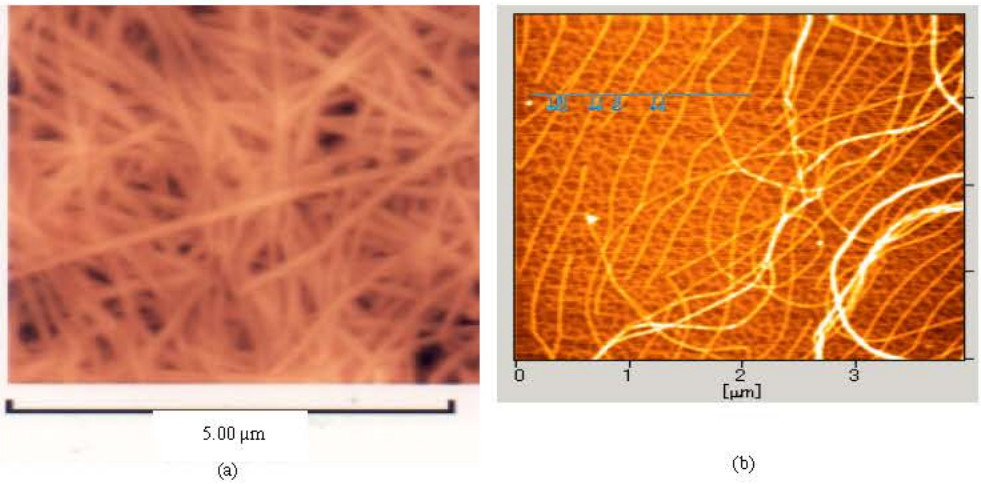
Bacterial Cellulose (BC) and hydrogel constitute inexpensive cellulose resources in nature. They have appearance white gellous-like displaying high water content (98-99%) in wet state. After drying, morphological surface of sheets was observed by Scanning Electron Microscope (SEM), shown in Fig. 1. The arrangement of BC and hydrogel fibres is irregular forming three-dimensional fibre network. Three-dimensional network consists of fine fibres in random orientation. Fibres of BC and hydrogel have length of 1-5 and 3-12 μm, respectively in forming a dense reticulated structure as three-dimensional network.

Actually, arrangement of fibres in forming three-dimensional network consists of ultrafine fibres, evaluated by observation of hydrated fibres by Atomic Force Microscope (AFM) (Fig. 2). These images elucidated that BC (Fig. 2a) and hydrogel (Fig. 2b) have ultrafine fibres having diameter in nanometer scale, 7-10 nm (BC) and 2-3 nm (hydrogel). This is predicted that the presence of nanofibres in BC and hydrogel should be utilized as strategic polymer in the future.



(a) (b)

Fig. 1: Observation of Morphology Surface of BC and Hydrogel Sheets by Scanning Electron Microscope (SEM) (a. BC; b. Hydrogel)



(a) (b)

Fig. 2: Observation of Morphological Surface of Hydrated Fibres of BC and hydrogel by Atomic Force Microscope (AFM) (a. BC; b. Hydrogel)

Difference in water absorption ability between BC and hydrogel is present as well. In wet state, BC forming hard gel and hydrogel forming soft gel contain about ~99% water. After drying, the entrance of water into BC structure have inhibition, shown by unchanged dimension of sheet and low swelling ability about 6.2%. Otherwise, the entrance of water into dried hydrogel structure run easily inducing changed dimension sheet to form a soft gel indicated in high swelling ability about 102.2%. Ability of dried hydrogel in water absorption much higher than that's of dried BC. Ability of structure to absorp water determines amount of water absorbed by structure. Amount of absorbed water stated as Water Holding Capacity (WHC) pointed out 5.5 mL g^{-1} (BC) and 39.2 mL g^{-1} (hydrogel). Swelling ability and WHC presumably correspond to the structure. Structure of BC and hydrogel was evaluated by X-ray diffraction (Fig. 3). Presence of cellulose within structure affects order-disorder structure dued to completely linear structure of cellulose. Author compared d-spacing of these gels referring to Iα, β cellulose calculated by Sugiyama *et al.* (1991) in determination lattice diffraction present in these gels. Based on calculation conducted by Sugiyama *et al.* (1991), Iα cellulose has lattice diffractions (100), (010), $(\bar{1}\bar{1}2)$ and (110) correlated to d-spacing 0.621, 0.528, 0.438 and 0.397 nm, respectively and Iβ-cellulose has lattice diffractions $(1\bar{1}0)$, (110), (102), (002) correlated to d-spacing 0.607, 0.535, 0.435 and 0.397 nm, respectively. The strength of the (100) as Iα lattice diffraction and $(1\bar{1}0)$ as Iβ lattice diffraction is easily influenced by changing of cellulose structure. BC is established as Iα cellulose dued to presence of d-spacing 0.617, 0.526, 0.438, 0.392 nm as

lattice diffractions (100), (010), $(\bar{1}\bar{1}2)$ and (110), respectively. Hydrogel isolated from *Ocimum americanum* containing 27.6% Iα cellulose and 72.4% hemicellulose (Azuma *et al*., 2000) is rich in Iβ cellulose. Consequently, d-spacing present in hydrogel of 0.466 and 0.393 nm correlated to two peaks at 2θ = 17.6° and 2θ = 22.56°, respectively. Dued to broadening peaks of X-ray diffraction profile of hydrogel (Fig. 3b) indicates that peak at 2θ = 22.56° as a cluster of lattice diffractions (200) and (102) while peak at 2θ = 17.6° as a cluster of $(1\bar{1}0)$ and (110). Forming clusters in X-ray diffraction profile of hydrogel dued to broadening peaks indicate that hydrogel is more amorphous than that's of BC.

Index crystallinity of cellulose is affected on diffracted intensity at 2θ = 18° as amorphous region and the highest intensity at 2θ = 22.56° as combination of crystalline and amorphouse region. After calculation, obtained index crystalinity of cellulose in BC and hydragel should be about 75 and 35%, respectively as the reason that BC should be more crystalline that's of hydrogel. Crystalline structure of dried BC induce inhibition in water absorption in which affected by hydrogen bonding of OH-groups of inter and intramolecular cellulose. Hydrogen bonding of OH groups make up primary structure of BC to have high mechanical strength and high crystallinity which dominated by cellulose content. The H-bond are indeed and important factor for arrangement the cellulose chain within the crystall lattice. The low crystallinity of hydrogel is attributed to non-cellulosic compound dominated in the structure inducing linkage of cellulose to non-cellulosic occurred. Presence of non-cellulosic compound is proved by neutral sugar composition analysis.

Neutral sugar composition analysis by HPAEC is performed to know contribution of structure in gel forming. After calculation and compared with standard sugars containing arabinose, rhamnose, galactose, glucose, xylose and mannose, obtained that BC has less 0.1% arabinose and rhamnose, 1.1% galactose, 98.5% glucose, 0.2% xylose and 0.2% mannose (Fig. 4b). The result shows that BC is dominated by glucose as representative of cellulose. Otherwise, hydrogel has sugar compositions such as arabinose (11.9%), rhamnose (4.5%), galactose (18.6%), glucose (50.5%), xylose (13.2%) and mannose (1.3%) (Fig. 4c) elucidating that hydrogel is rich in hemicellulosic compounds. The results proved that BC has higher cellulose content than that's of hydrogel. Forming soft gel in hydrogel may be affected by presence of arabinogalactan as branching structure and xylan as main chain attaching to cellulose linearly.

Ability in water absorption depends on presence of structure. Therefore, the behaviour of water within gel material is important to understand because it dominates

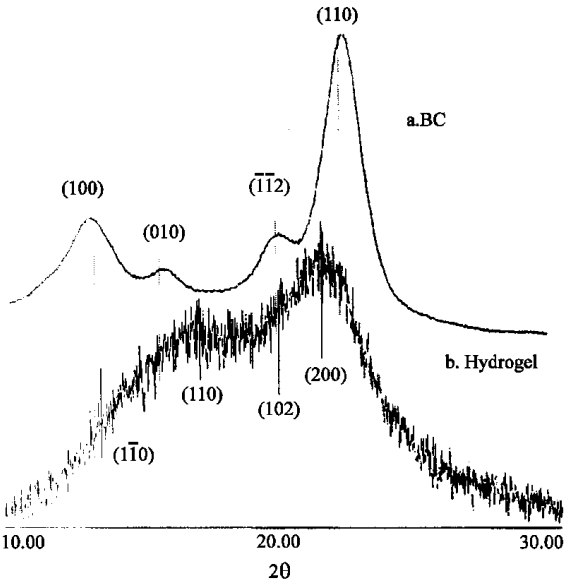


Fig. 3: X-ray diffraction profiles of BC and hydrogel by HPAEC

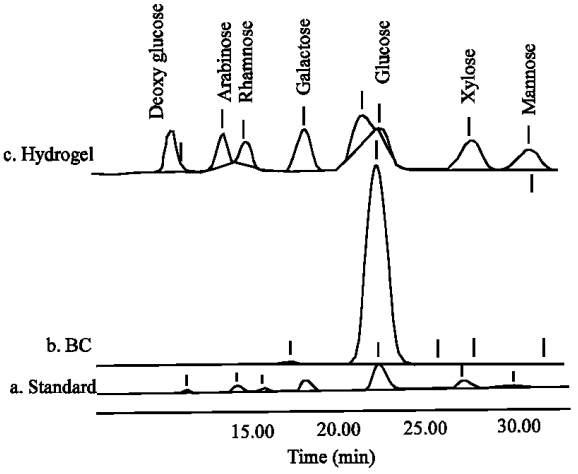


Fig. 4: Neutral sugar composition analysis of BC and hydrogel

the physical properties. As the results above, elucidated that amount of cellulose present in the structure affect crystallinity and ability of structure to absorp water. Understanding of these properties is usefull in controlling hydrophobicity and hydrophilicity by modification of structure. This is one of most important mechanism on enhancing behaviour of gellous material for numerous application.

CONCLUSION

High crystalline structure of BC causes inhibition in water absorbtion ability includes amount of absorbed

water in structure, indicated by WHC and swelling ability of BC much lower than that's of hydrogel.

Branching in hydrogel structure decreases ordered structure and increases water absorbtion ability include amount of absorbed water.

The mechanism elucidates that modification of structure controls hydrophilicity and hydrophobicity of materials.

ACKNOWLEDGMENTS

The authors are thankful to the financial support of the Indonesian Institute of Sciences Research Project and Japan Society for Promotion Sciences (JSPS) on Collaborative Program.

REFERENCES

Azuma, J., M. Sakamoto, H. Takeda, R. Yudianti and L. Indrarti, 2000. Surface Structure and Gellous Polysaccharides of *Ocimum americanum* Seed. In: Proceeding of the 2nd International Workshop on Green Polymer, 15-20 October 2000, Bandung-Bogor, Indonesia.

Azuma, J. and M. Sakamoto, 2003. Cellulosic hydrocolloid system present in seed of plants. Trends in Glycosci. Glycotechnol., 81: 1-14.

Indrarti, L., J. Azuma, R. Yudianti and M. Sakamoto, 2004. Characterization and Properties of Cellulosic Hydrogel from Various Kinds of Basil Plants in Indoneisa. In: Proceedings of the 5th International Wood Sci. Sym. in the Fields of Wood Science, 17-19 September 2004, Kyoto, Japan.

Indrarti, L., J. Azuma, R. Yudianti and M. Sakamoto, 2005. Hemicellulosic Polysaccharide Present in the Cellulosic Hydrogel of Ocimum Seed. In: Proceedings of the 6th Int. Wood Sci. Sym. in The Fields of Wood Science, 29-31 August 2005, Bali, Indonesia.

Melo, J.S. and S.F. D'Souza, 2004. Removal of chromium by mucilaginous seeds of *Ocimum basilicum.* Bioresour. Technol. J., 92: 151-155.

Nishino, T., K. Takano and K. Nakamae, 1995. Elastic modulus of the crystalline region of cellulose polymorph. J. Polymer sci., 33: 1647-1651.

Shah, J. and M.R. Brown, 2005. Towards electronic paper displays made from microbial cellulose. Applied Microbiol. Biotechnol., 66: 352-355.

Segal, L., J. Creely, A. Martin and C. Conrad, 1959. An empirical method for estimating the degree of crystallinity of native cellulose using the x-ray diffractometer. Text. Res. J., 29: 786-794.

Sugiyama, J., R. Vuong and H. Chanzy, 1991. Electron diffraction study on the two crystalline phases occurring in native cellulose from an algal cell wall. Macromolecules, 24: 4168-4175.

Tajima, K., M. Fujiwara, Takai, Mitsuo and J. Hayashi, 1995. Synthesis of bacterial celluloe composite by *Acetobacter xylinum* I. First Annual Meeting of the Cellulose Society, 41: 749-757.

Yudianti, R., L. Indrarti, M. Sakamoto and J. Azuma, 2004. Morphological properties of seed coat of *Salvia* sp., In: Proceedings of the 5th International Wood Sci. Symposium in The Fields of Wood Science, 17-19 September 2004, Kyoto, Japan.

Yudianti, R., L. Indrarti, J. Azuma and M. Sakamoto, 2005. Cellulose-Hemicellulose present in hydrocolloids from *Salvia* sp., Proceedings of the 6th International Wood Sci. Symposium in The Fields of Wood Science, 29-31 August 2005, Bali, Indonesia.