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Review
Bacterial cellulose—a masterpiece of nature's arts

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Ever since its remarkable mechanical properties were found fifteen years ago, interest has grown in bacterial cellulose for which the use had been more or less limited to the manufacture of *nata-de-coco*, an indigenous food of South-East Asia. This paper reviews the progress of relevant studies including the production of cellulose by bacteria, the formation of microfibrils and gel layer, the properties of gel and processed sheets, and some aspects of applications.

1. Introduction

Whilst "cellulose" is a word originally given, in early last century by Anselme Payen, to the substance which constitutes the cell wall of higher plants [1], bacterial cellulose is an ex-cellar product of vinegar bacteria which was described by Louis Pasteur as "*a sort of moist skin, swollen, gelatinous and slippery*. . ." [2]. Although the solid portion in the gel-like stuff is less than one percent, it is almost pure cellulose containing no lignin and other foreign substances.

Most familiarly, bacterial cellulose has long been useful as the raw material of *nata-de-coco*, an indigenous dessert food of Philippines, for which one-centimetre thick gel sheets fermented with coconut-water are cut into cubes and immersed in sugar sirup. Similar food can be prepared from other saps or fruit juices, e.g., *Nata-de-pina* from pineapple. That the major component of *nata-de-coco* gel was cellulose, not dextran as assumed in the past, was proved in 1960s [3]. *Nata-de-coco* is now manufactured in a large quantity at the level of home industry also in Indonesia and exported as a healthy diet. *Teekvass*, or tea-fungus, grown in tea-cups and served in some parts of Russia and Middle-Asia is said to be a similar ferment [4].

Scientifically, a substance known as "vinegar plant" or "mother" and of use for vinegar brewery in old days in Europe was cultured in pure condition and identified by Brown [5, 6] to be the same as cell-wall cellulose from its chemical composition and reactivity, although contemporary means of microscopy only gave a picture of "*bacteria lying embedded in a transparent structureless film*".

With the emergence of X-ray diffraction early this century, it was observed that bacterial cellulose belonged crystallographically to Cellulose I, common with natural cellulose of vegetable origin, in which two cellobiose units were arranged parallel in a unit cell, and that cellulose molecules tended to have a specific planer orientation in dried film [7]. The change of orientation in drying process was also studied in early days [8]. After the advent of electron microscope, the water-swollen cellulosic gel was revealed to comprise random assembly of microfibrils of less than 100 Å diameter [9] such as seen in a scanning electron micrograph of freeze-dried gel surface in Fig. 1, whereas cell-wall

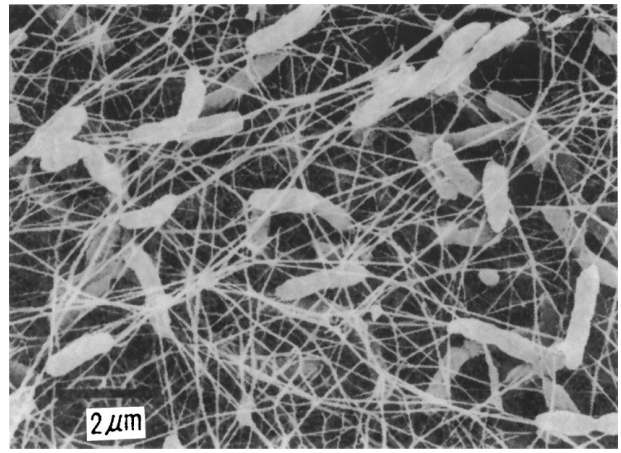

Figure 1 A scanning electron micrograph of freeze-dried surface of bacterial cellulose gel.

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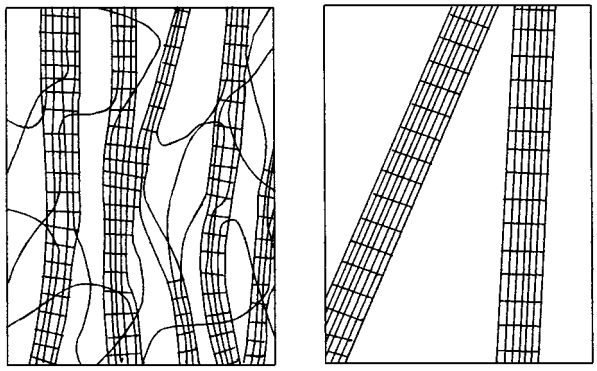


Figure 2 Schematic model of bacterial cellulose microfibrils (right) drawn in comparison with "fringed micelle" (left).

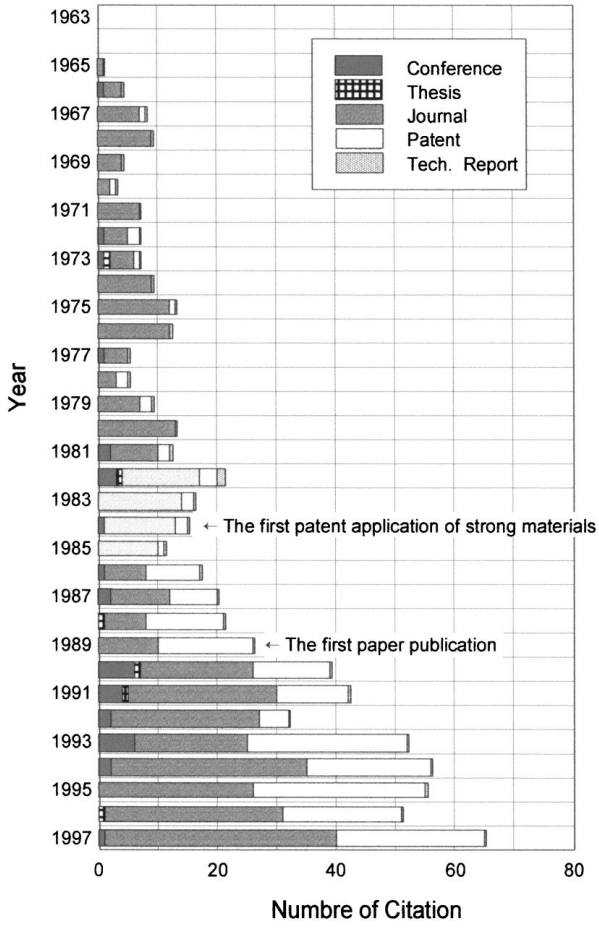


Figure 3 Chemical Abstract citation of bacterial-cellulose related articles.

cellulose had much complicated structure. The structure of microfibril should be as simple as drawn in Fig. 2 compared to the "fringed micelle" envisaged in old days for the texture of vegetable fibres. Indeed, it is one of the finest examples of Nature's arts in which long chain molecules are aligned parallel in the extended form. One may recall that such an oriented structure had been idealized in fibre spinning ever since the early days of artificial silk manufacture, or at least after the recognition of macromolecular hypothesis, and only simulated in the last few decades in the efforts of developing super-strong fibres and growing extended-chain single-crystals.

According to Brown [5, 6], the pellicle of bacterial cellulose was "*very tough, especially if an attempt was made to tear it across its plane of growth*". There were a number of people who obtained films from the pellicle and studied the structure, but somehow no attention had been paid to the physical properties of films until mid-1980s when stress-strain measurement was first conducted by the present authors [10–13]. The Young's modulus recorded, 16–18 GPa isotropically across the surface of plane, was extraordinarily large for two-dimensional materials of organic substances, and further improved up to 30 GPa. The fragments of bacterial cellulose were also found effective for reinforcing pulp papers and useful for other purposes. Among various possible applications, these materials have become of use for acoustic diaphragms of high-fidelity loudspeakers and headphones.

Interest in bacterial cellulose has grown rapidly in the past decade as seen in the statistics of publications shown in Fig. 3. This paper is aimed at reviewing the development of bacterial cellulose study with special reference to its use as materials.

2. Production of cellulose by bacteria

2.1. Bacterial species

The species of bacteria which produces cellulose is generally called *Acetobacter xylinum*, although bacteria of different names are often of use in literatures. The vinegar-plant bacterium originally used by Brown was obtained from a pellicle appeared on the surface of beer. In nature, the kind of bacteria are found, for instance, in rotten fruits and vegetables as more than thirty cases have been reported [14]. The reason why the microorganisms generate cellulose has been a quest of biologists. One considers that the aerobic bacteria produce pellicle to maintain their position close to the surface of culture solution [15, 16]. Another assumes that the bacteria generate cellulose to guard themselves from ultraviolet [17]. The authors prefer to imagine that they construct such a 'cage' and confine themselves in it to protect themselves from enemies and heavy-metal ions, whereas nutrients can be supplied easily by diffusion.

2.2. Culture methods

The source substance of bacterial cellulose is saccharides. A typical culture medium widely of use in laboratories is prepared by dissolving, 50 g sucrose, 5 g yeast-extract, 5 g $(NH_4)_2SO_4$, 3 g KH_2PO_4, and 0.05 g $MgSO_4 \cdot 7H_2O$ in a litre of water [15]. According to the experience of the authors, the recipe can be more complete if a small amount of vitamins is added. Although the addition of inorganic nutrients are not necessarily required when natural saps and juices are used, it is a common practice in *nata-de-coco* industry to add a small amount of nitrogen-containing compounds, such as ammonium sulphate and di-ammonium hydrogen phosphate.

Culture is carried out normally in static condition at around 28–30 °C by adding an aliquot of activated seed broth to the culture medium. The system becomes turbid and, after a while, a white pellicle appears on the surface and its thickness increases steadily with time, reaching over 25 mm in four weeks, as demonstrated in Fig. 4. It is important to note that in the process of gel

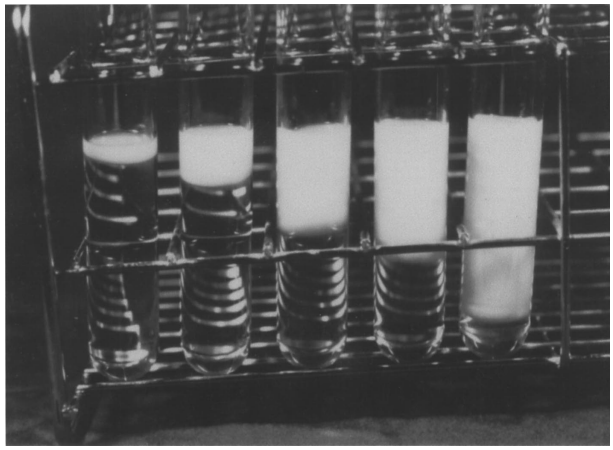


Figure 4 Bacterial cellulose layers grown with different culture time (maximum 4 weeks).

growth the aerobic bacteria generate cellulose only in the vicinity of surface, so that the productivity depends primarily on the surface area, not on the volume of vessel [18]. As long as the system is kept unshaken, the disc-shaped gel is suspended by the cohesion to the interior wall of vessel and slides steadily downwards as it thickens. It was experienced by the authors that the growth of continuous gel layer tended to fail if a vessel with tapered wall like a conical flask was used. In *nata-de-coco* home industries, plastic vessels of ca. $50^{w} \times 35^{d} \times 10^{h}$ cm^3 are employed. After the inoculation, the vessels are covered with an old newspaper and kept in a storehouse for 8–10 days. If purification is necessary, *viz.* for scientific purpose, bacteria contained in the nascent gel can be conveniently removed by immersing it in dilute alkaline solution and washing with water as originally conducted by Brown [5, 6]. For further purification, treatment by oxidant was found effective as described below [12].

With the aim of enhancing the productivity, culture in agitated conditions has been studied recently in [19], although a flat gel is no longer obtained and the use has to be limited to such applications as papermaking.

2.3. Formation of microfibrils

The mechanism of formation as well as the structure of microfibril has been studied extensively in recent decades combining the knowledge of biogenesis [20, 21]. Today, it is believed as illustrated in Fig. 5 that cellulose molecules synthesized in the interior of bacterial cell are spun out of 'cellulose export components' or nozzles to form a protofibril of ca. 2–4 nm diameter, and the protofibrils are bundled in the form of a ribbon-shaped microfibril of ca. 80×4 nm [22].

The kinetics of cellulose production by bacterium has been studied since 1950s and it has been established that the yield of cellulose increases almost exponentially with time, at least in low conversion ranges, when culture is carried out in agitated condition and sufficient oxygen is supplied from air. It is commonly assumed that a bacterial produces a certain number of chain initiators during its generation time to which monomer units are added to form cellulose and that the population of bacteria obeys the law of bacterial growth. Thus,

$$N_t = N_0 e^{\alpha t} \tag{1}$$

where N_t and N_0 are the number of bacteria at time t and 0, respectively, and a constant, α is related to the mean generation time of bacteria, τ by;

$$\alpha = \left(\frac{1}{\tau}\right) \ln 2 \tag{2}$$

A theory to express the yield and the degree of polymerization on account of the average lifetimes of bacteria and chain-growth was derived [23] and $\tau = 220$–330 min was estimated from the data of yield and average molecular-weight measurements. Similar

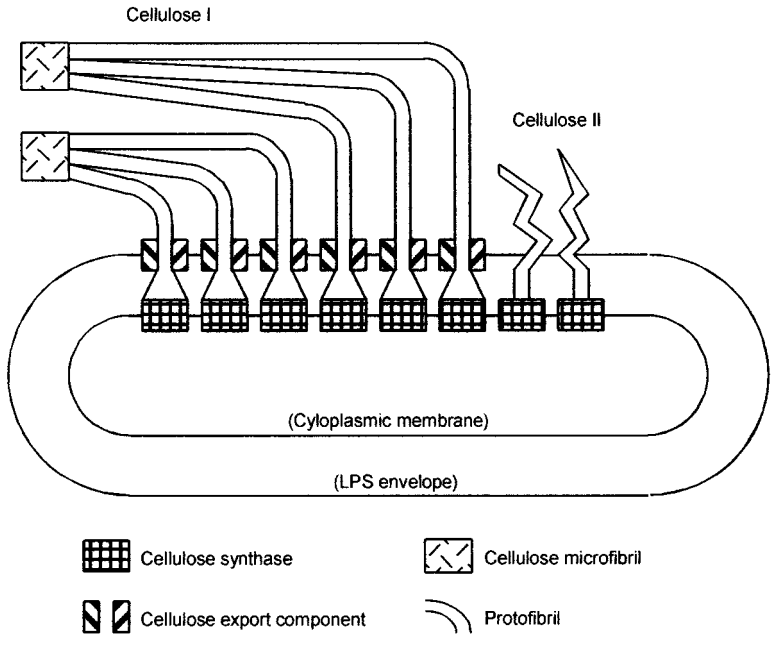


Figure 5 Schematic illustration of bacterial cellulose biogenesis and fibril formation [22].

experiments were carried out and $\tau = 290$–480 min and 380–900 min were reported by other authors [2, 24]. Regarding the lifetime of chain growth itself, evidences have been given by the same authors in that the average molecular weight of cellulose continues to increase over generation change, possibly up to several generations or more, although contradictory results were raised rather recently [25].

What happens to the fibril structure during the cell division is a question. It was considered that the formation of a three-way branching was inevitable, if the extrusion of fibril continued beyond generation from mother to two daughter cells [11]. The fibrils may be narrower at the branching point, if not normal number of nozzles are provided at the stage of cell division, but the recovery of normal diameter has to be a matter of time. In fact, fibrils on magnified photographs appear not necessarily and not always linear. The segmental length between branching points was estimated as 580–960 μm [26] from the lifetime of bacterium [24, 27] and the growth rate of fibril [20, 28], and 200–700 μm from the counting of bacteria in the product. The existence of such branches, if true, may relate to the toughness or the resistance against stretching of gel sheets.

2.4. Formation of gel layer

The mechanism of gel formation was considered as follows [11, 29]. In the initial stage, the bacteria increase their population by taking dissolved oxygen and produce a certain amount of cellulose in the entire liquid phase as observed by the appearance of turbidity. When the dissolved oxygen is used up, bacteria existing only in the vicinity of surface can maintain their activity to produce cellulose. Although they may undergo cell division, the population in the surface region does not increase exponentially but should reach a certain equilibrium number, as excess others are occluded in the gel and brought into the depth. Those bacteria below the surface are not 'dead' but 'asleep', so that they can be reactivated and used as the seed for new culture operation. Whether oxygen pressure higher than in air accelerates the cellulose production is different matter and rather complicated [30].

Regarding the growth of gel layer in static condition, it is a general trend observed [11, 18, 31] in that the thickness as well as the yield of cellulose increases sharply, after a few days of induction period, until the rate tended to slow down after a week or ten days. Fig. 6 reproduces the results of recent experiment in which the base medium was coconut-water [32]. The thickness, wet weight and dry weight followed similar trend and the addition of sugar did not give much difference at least when it was above 1%. As saccharides, fructose which should have been generated by the hydrolysis of sucrose was not detected due possibly to the conversion to some other substances. As shown in Fig. 7, the concentration of glucose did not necessarily decrease monotonically, particularly when the concentration of added sugar was high, whereas the concentration of sucrose decreased monotonically towards zero. It was considered that glucose was the

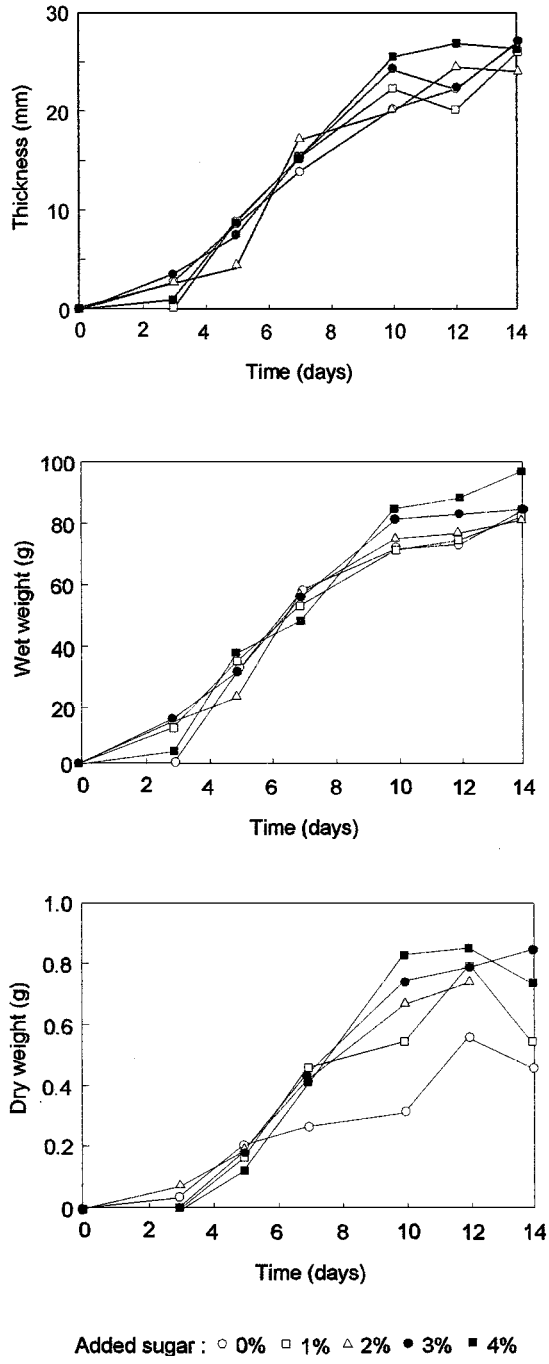


Figure 6 Changes of the thickness, wet weight and dry weight of gel with culture time. The base medium was coconut-water and 1% $(NH_4)_2HPO_4$ and 1–4% sugar was added [32].

kind of saccharide which was digested by bacteria and converted to cellulose.

Fig. 8 shows computer-simulated curves of glucose consumption, or cellulose production corresponding to Fig. 7 (bottom), in the second stage of reaction obtained on the following equations [32].

$$-\frac{\partial C_o}{\partial t} = -\frac{D_o \partial^2 C_o}{\partial x^2} + K C_o C_g \qquad (3)$$

$$-\frac{\partial C_g}{\partial t} = \frac{D_g \partial^2 C_g}{\partial x^2} + K C_o C_g \qquad (4)$$

where C_o and C_g are the concentrations of oxygen and glucose, D_o and D_g are the diffusion coefficients of

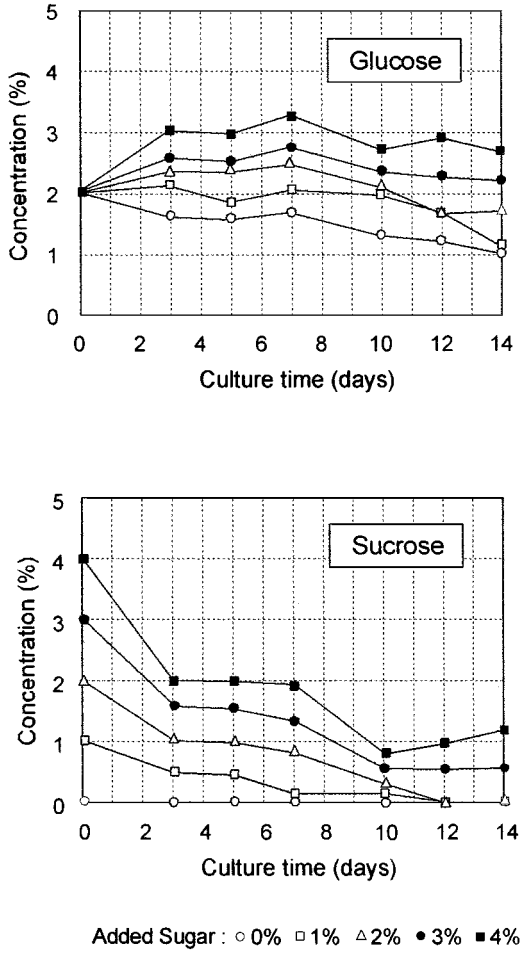


Figure 7 Changes of glucose and sucrose concentrations with culture time. The conditions are the same as in Fig. 6 [32].

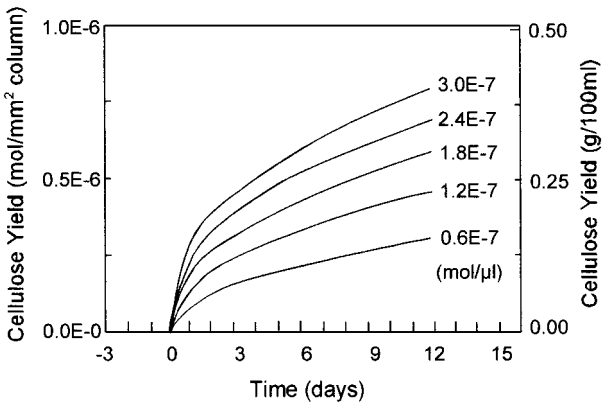


Figure 8 A set of cellulose yield vs. time traces calculated on Equations 1 and 2. ($C_{g,0} =$ 0.6–3.0 × 10–7 mol/μl, $D_o = 9.0 \times 10$–12 mm^2/h, $D_g = 4.0 \times 10$–12 mm^2/h, $C_{o,0} = 1.6 \times 10$–8 mol/μl and $K = 1.5 \times 10$–8 μl/mol/h, $\Delta t = 1$ h) [32].

oxygen and glucose, respectively, x is the depth from the surface, and K is an integrated rate constant of glucose consumption. There, mass-transfer by convection was neglected, and the effect of gel layer on diffusion was not taken into account as the solid fraction was less than one percent in volume. In a plot of glucose and oxygen concentration against the depth from the surface, it was recognized that glucose diffuses gradually from the interior, whereas oxygen diffusing from air did not penetrate deep consumed by the reaction.

3. Properties of bacterial cellulose

3.1. Elastic properties of gel

Although bacterial cellulose is obtained in the form of a highly swollen gel, the texture is quite unique and different from typical hydro-gels. Those readers who have tasted *nata-de-coco* should know that the original elasticity would never recover once the gel is crashed. One may remember the flesh of squid, a typical oriental seafood, which hardly swell again after dried. These are ascribed to the fact that the elements which constitute the gel are microfibrils, not the segments of chain molecules, such as in agar or gelatin gels, which can take thermodynamically stable form. Fig. 9 shows an example of compression stress relaxation curve in which the stress continued to fall beyond the period of measurement [33]. With three-element Maxwell model, it was fit by:

$$f = 8.5\exp(-3.64 \times 10^{-3}) + 14.9 \times \exp(-7.00 \times 10^{-2}) + 45.2\exp(-5.40 \times 10^{-1}) \quad (5)$$

Fig. 10 (top) and (bottom) show the changes of complex viscosity, η^*, storage modulus, G' and loss modulus G'' measured by a parallel plate rheometer as a function of strain and frequency, respectively [34]. The response is linear up to strains of 5% and the fact that G' is significantly higher than G'' implies that the material has characteristics of rubber in the deformation range. The complex viscosity decreased monotonically but the storage and loss moduli maintained a certain level against the increase of oscillation frequency.

Since the gel is hard to be stretched beyond several percent, efforts of orienting fibrils such as made in the past [8] have been virtually in vain. Attempts of cold extrusion was not successful either [34]. A roll device to wind up thin gel, in the form of a continuous ribbon, from the surface of culture medium was invented and applied, but the orientation observed by X-ray diffraction was not necessarily high [35].

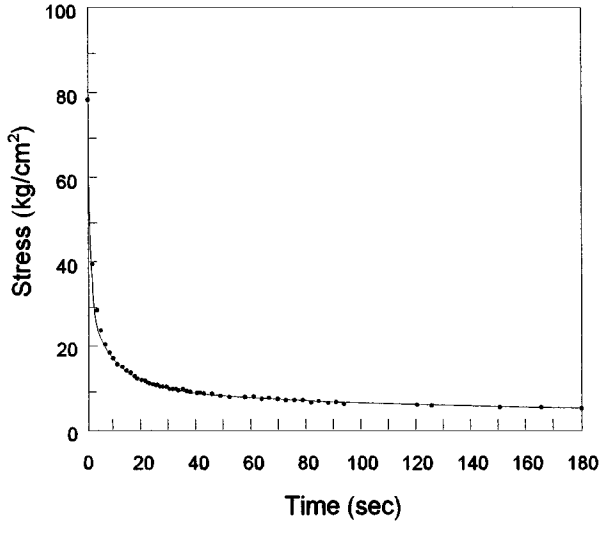
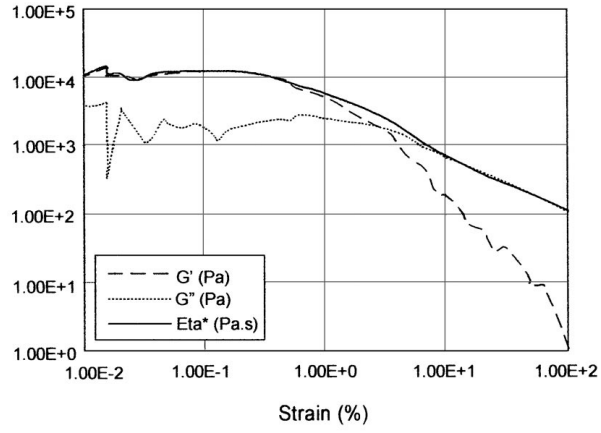


Figure 9 Compression stress relaxation of bacterial cellulose gel [33].



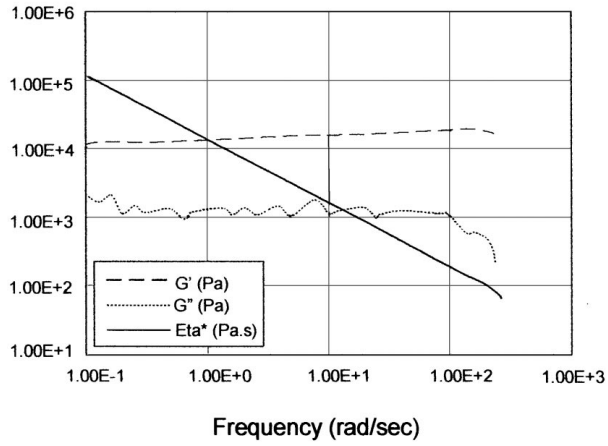
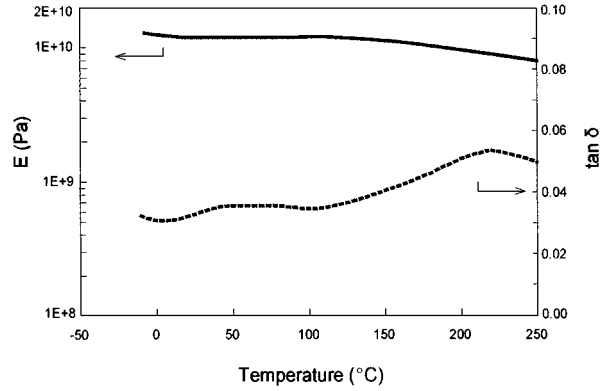


Figure 10 Complex viscosity, η^*, storage modulus, G' and loss modulus G'' measured by a parallel plate rheometer as a function of strain (top) and frequency (bottom) [34].



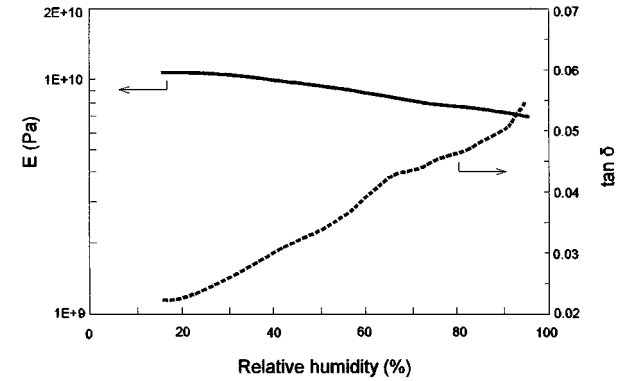


Figure 11 Dynamic viscoelastic properties (E' and tan δ) of typical film measured as a function of temperature (top) and relative humidity (bottom) [33].

3.2. Mechanical properties of films

Traditionally, films were prepared by drying a gel sheet in air on a flat surface, e.g., glass plate, by fixing the area. In the newly developed heat-press method [10], it is important to place a gel sheet sandwiched between stainless-steel meshes and/or non-woven fabrics to facilitate the escape of water. The results of tensile measurements of films prepared in various conditions are summarized in Table I. As far as the Young's modulus is concerned, the values obtained were much the same without regard to the preparative condition, and the tensile strength as well as elongation tended to decrease when excess pressure was applied, due presumably to the introduction of defects.

Fig. 11 (top) shows dynamic viscoelastic properties of typical film measured as a function of temperature [33]. Whilst the dynamic modulus, E' decreased slowly

TABLE I Mechanical properties of bacterial cellulose films prepared in various conditions

Culture time (days)	Preparation method[a]	Temperature (°C)	Pressure (kPa)	Film thickness (μm)	Young's modulus (GPa)	Tensile strength (MPa)	Elongation (%)
7	Air-dry	20	0	—	16.9	256	1.7
7	Heat-press (⊥)	150	49	—	17.4	224	1.8
7	Heat-press (⊥)	150	49	—	18	231	1.8
7	Heat-press (⊥)	200	49	—	16.4	243	1.9
7	Heat-press (⊥)	150	49	—	16.9	260	2.1
7	Heat-press (⊥)	150	196	—	16.7	216	1.7
7	Heat-press (⊥)	150	490	—	17.5	155	1.4
7	Heat-press (⊥)	150	980	—	17	129	0.9
7	Heat-press (⊥)	150	1470	—	16.6	102	0.8
7	Heat-press (⊥)	150	1960	—	18.1	91	0.8
7	Heat-press (⊥)	150	49	—	16.1	221	1.9
7	Heat-press (‖)	150	49	—	15.9	205	1.8
5	Heat-press (⊥)	150	49	14	16.5	246	1.9
7	Heat-press (⊥)	150	49	37	16.1	217	1.7
14	Heat-press (⊥)	150	49	63	16.2	255	2
28	Heat-press (⊥)	150	49	159	15.1	199	1.7

[a] Press direction: (⊥); normal to the plane of growth, (‖); parallel for a cut-out strip.

from 15 to 9 GPa with the increase of temperature, tan δ showed two maxima at around 50 and 230 °C, corresponding to the desorption of water and the degradation of cellulose, respectively. The specimen showed a typical water-sorption isotherm in which the water regain at 100%RH was 9.3%. As a function of relative humidity, E' decreased and tan δ increased gradually as seen in Fig. 11 (bottom).

Morphologically, fibrils in the sheets appear to constitute a pile of thin layers, as seen in Fig. 12, regardless to the press direction. This magnified picture reminds one of the structure of pulp papers in which hydrogen-bond between fibrils is believed to be the source of strength [36]. In the case of bacterial cellulose, the density of inter-fibrillar hydrogen-bonds must be much higher, as the diameter of fibrils is much smaller, and

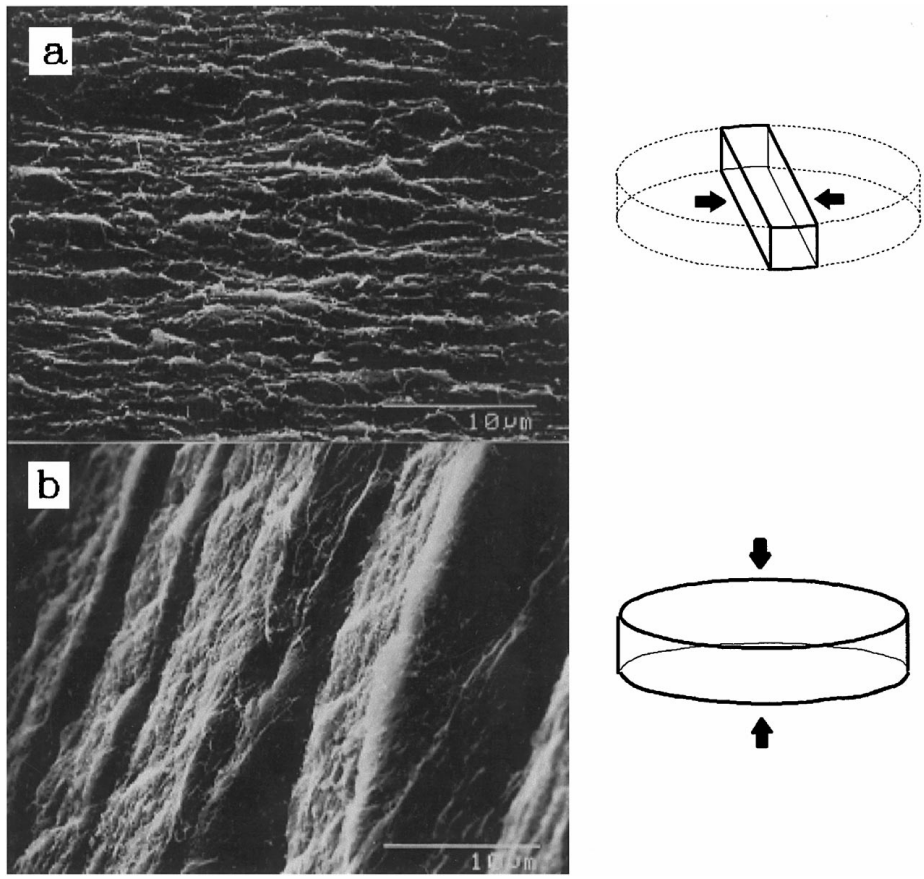


Figure 12 Scanning electron-micrographs of fracture edge of bacterial cellulose film [11].

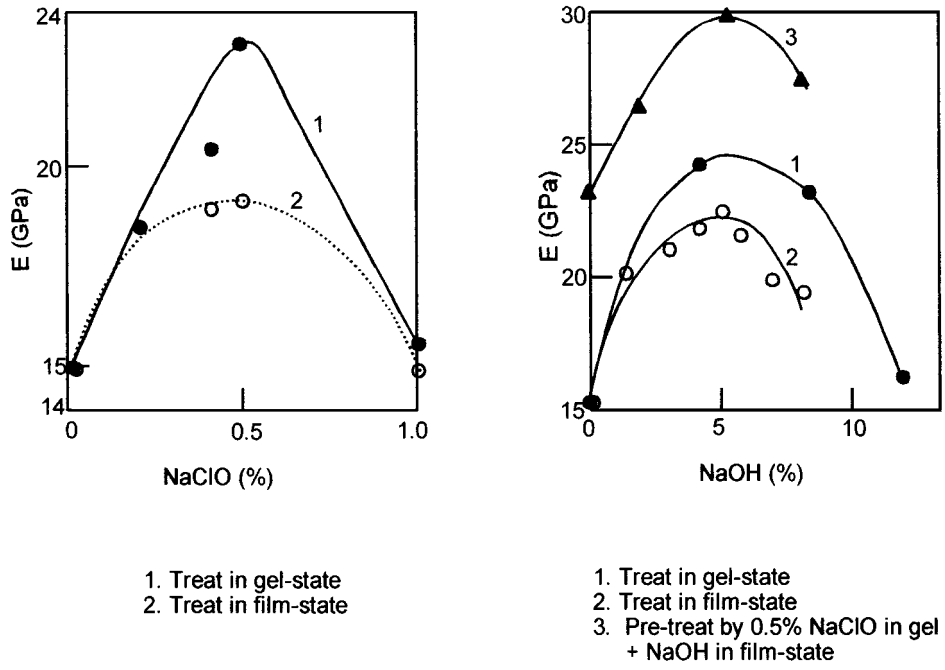


Figure 13 Change of Young's modulus by chemical treatment [12].

this can be the reason why such a high Young's modulus develops with this material.

Since traces of contaminants were suspected to affect the formation of hydrogen-bond, treatment with oxidant and alkaline solutions have been attempted parallel with careful chemical analysis [12]. Fig. 13 (left) and (right) show the change of the Young's modulus with the concentration of NaClO and NaOH, in which maxima are found at around 0.5%, and 5%, respectively, before cellulose is damaged at higher concentrations. In terms of the Young's modulus, the best result was obtained when the material was soaked in 0.5% NaClO solution in the stage of gel and treated with 5% NaOH solution after processing into film. The Young's modulus attained, 30 GPa isotropically across the film plane, is quite large compared to the theoretical value of cellulose along the chain direction, 173 GPa [37]. It is remarked that the value is several times higher than those attained by synthetic polymers, e.g., two-dimensionally stretched polyester film.

3.3. Properties of sheets prepared with fragmented bacterial cellulose

Suspension of fragmented bacterial cellulose gel was obtained by means of a bladed-blender. The Young's modulus and tensile strength of composite sheets prepared by filtering the mixture of cotton lint pulp and fragmented bacterial cellulose is plotted against the fraction of the latter in Fig. 14, in which the reinforcing effect is clear. The pure suspension gave a sheet like parchment paper which measured a Young's modulus, 4.9 GPa.

More practical data [38] for applying bacterial cellulose to papermaking is shown in Fig. 15. While the increase in Young's modulus and tensile index is reproduced in (top) and (middle), respectively, it is another interesting effect that the folding endurance of pulp papers can be significantly improved (bottom). The is due presumably to the peculiar property of fragmented bacterial cellulose that it tends to stick on other substance [10, 11]. Fig. 16 shows a scanning-electronmicrograph of glass-fibre on which bacterial cellulose fragments are entangled on the surface and binding the fibres. Thus, one can prepare self-supporting sheets from non-cellulosic fibres without adhesive by adding small amount of disintegrated bacterial cellulose as shown in Table II.

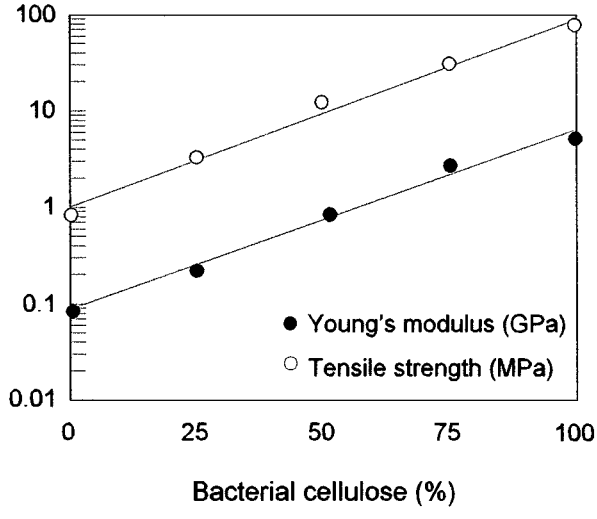


Figure 14 Young's modulus and tensile strength of sheets prepared from the mixture of cotton lint and fragmented bacterial cellulose (data from [11]).

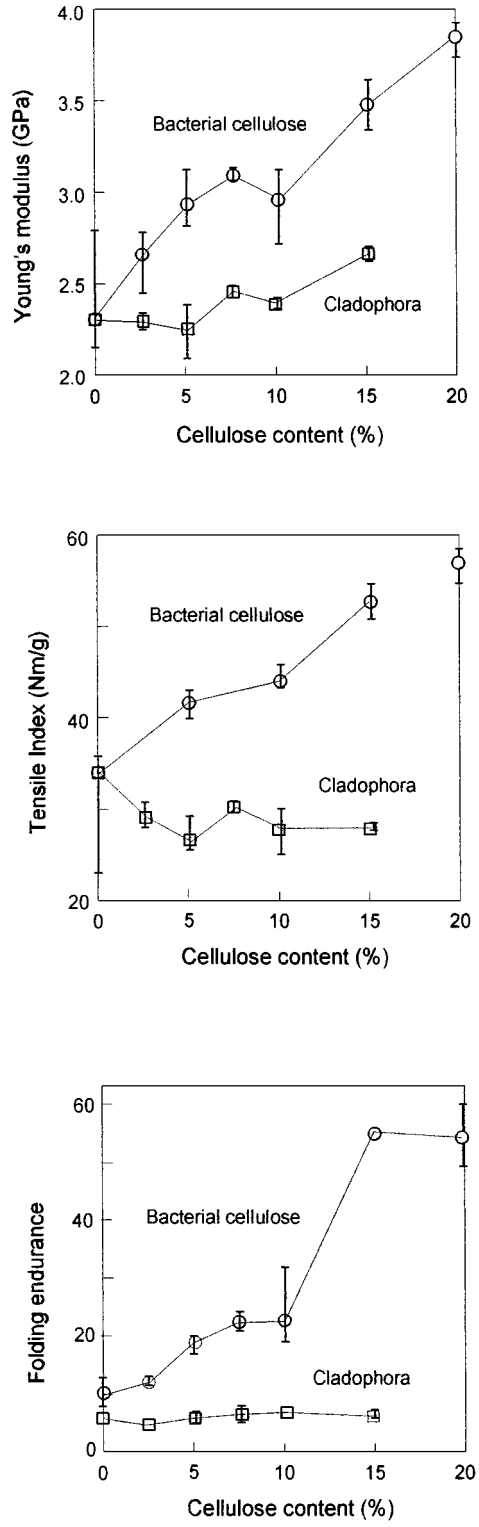


Figure 15 Properties of papers prepared by mixing bacterial cellulose [38].

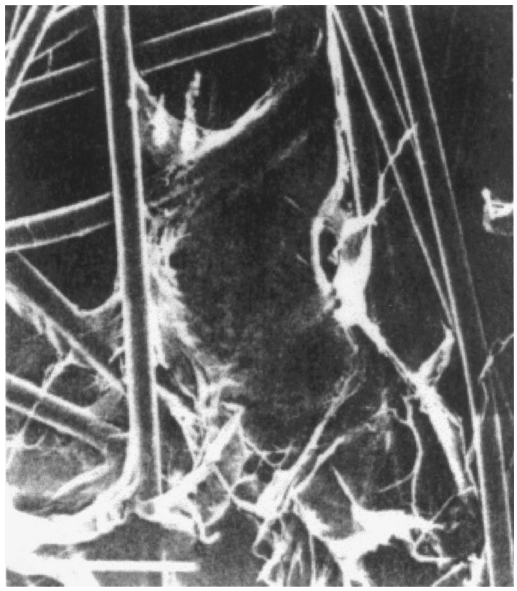


Figure 16 Scanning electron-micrograph of glass-fibre on which bacterial cellulose fragments are entangled.

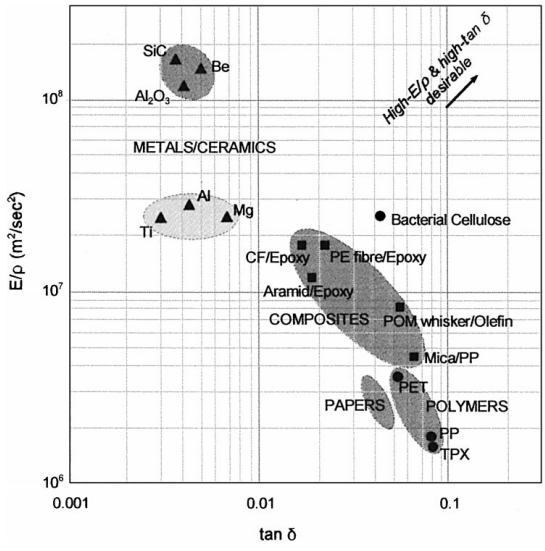


Figure 17 Specific Young's modulus vs. internal-loss of various acoustic materials.

TABLE II Breaking length of sheets from non-cellulosic fibres prepared by mixing disintegrated bacterial cellulose

Material	Fibre (parts)	BC (parts)	Bk length (km)
Novoloid fibre (Kainol® KP0203)	95	5	0.33
Novoloid fibre (Kainol® KP0203)	90	10	0.79
Novoloid fibre (Kainol® KP0203)	80	20	1.67
Carbon fibre (Toreca T008® 6mmL)	90	5	0.64
Alumina fibre (Denka Arecen Bulk®)	90	5	0.24

4. Applications

Among various applications studied so far, that which has reached the level of practical use is for acoustic diaphragms as bacterial cellulose has been found to bear the two essential properties, i.e., high sonic velocity and low dynamic loss (see, Fig. 17). In fact, the sonic velocity of pure film was almost equivalent to those of aluminium and titanium, while the tangent-delta was in a low range, 0.4–0.5. In the sound-pressure-level curves of a composite-paper cone diagram, shown in Fig. 18, it is seen that both frequency response and second harmonic distortion are smoothed and extended to higher frequency regions. Thus, hi-fidelity loudspeakers and headphones have been marketed by Sony Corp.

The use of films as the raw material of conductive carbon film was investigated and found excellent, although it still stays in the cradle of laboratory [39].

The use of fragmented bacterial cellulose for papermaking is promising and test pieces of flexure-durable papers and high filler-content papers, ideal for banknote papers and bible papers, have been prepared by Mitsubishi Paper Mills Co. Fancy-papers with low-portion bacterial cellulose has been also prepared but it is not an application aimed at improving the physical properties.

Other ideas raised include the use of sheet or film as a temporary skin for medical care [40] and separation membrane [41], the use of fragmented suspension as an viscosity enhancing agent for various purposes, etc.

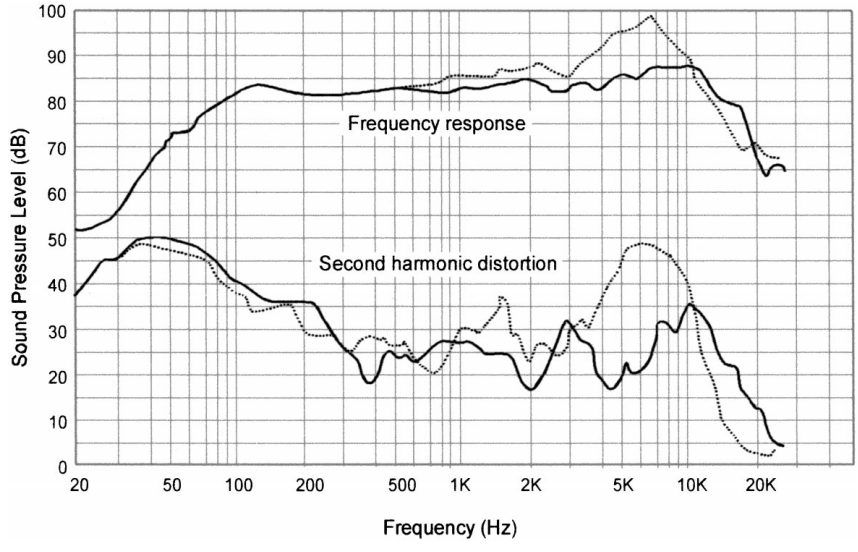


Figure 18 Frequency characteristics of test speakers (16 cmϕ cone-type full-range). Solid-line: bacterial cellulose composite, Dotted-line: conventional paper.

5. Conclusion

The finding that the unique structure of bacterial cellulose offers interesting properties has taught us that more useful substances are left in Nature unknown to mankind. It is an expectation of authors that bacterial cellulose can contribute Indonesia and other low-latitude countries to promote high-tech industries based on their indigenous materials. The cost of the material estimated as about US$30/kg at dry-base is expected to be lowered by the use of agricultural wastes as carbohydrate resources and the rationalization of production process.

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References

1. T. P. NEVELL and S. H. ZERONIAN, in "Cellulose Chemistry and Applications," edited by T. P. Nevel and S. H. Zeronian (Howrwood Ltd., 1995) p. 15.
2. G. J. F. RING, in "Cellulose and Other Natural Polym. System," edited by R. M. Brown (Plenum, New York, 1982) p. 299.
3. M. M. LAPAS, E. G. GALLARDO and M. A. PALO, *Philipp. J. Sci.* **96** (1967) 91.
4. G. CAMPBELL-PLATT, in "Fermented Foods of the World—A Dictionary and Guide" (Butterworth, 1987).
5. A. J. BROWN, *J. Chem. Soc.* **49** (1986) 172.
6. *Idem., ibid.* **49** (1986) 432.
7. H. MARK and G. V. SUSICH, *Z. Physikal. Chem. (B)* **4** (1929) 431.
8. A. W. SISSON, *J. Phys. Chem.* **40** (1936) 343.
9. E. FRANTS and E. SCHIEBOLD, *J. Makromol. Chem.* **1** (1943) 4.
10. M. IGUCHI, S. MITSUHASHI, K. ICHIMURA, Y. NISHI, M. URYU, S. YAMANAKA and K. WATANABE, US Patent 4,742,164 (1988).
11. S. YAMANAKA, K. WATANABE, N. KITAMURA, M. IGUCHI, S. MITSUHASHI, Y. NISHI and M. URYU, *J. Mater. Sci.* **24** (1989) 3141.
12. Y. NISHI, M. URYU, S. YAMANAKA, K. WATANABE, K. KITAMURA, M. IGUCHI and S. MITSUHASHI, *ibid.* **25** (1990) 2997.
13. M. IGUCHI, S. YAMANAKA, K. WATANABE, Y. NISHI and M. URYU, in Proc. Int. Meet. Polym. Sci. Technol, Rolduc Polym. Meet. 5, May 1990, edited by P. J. Lemstra and L. A. Kleintjens 44-50, London, 1991) p. 371.
14. E. G. JESUS, R. M. ANDRES and E. T. MAGNO, *Philipp. J. Sci.* **100** (1971) 41.
15. M. SCHRAMM and S. HESTRIN, *J. Gen. Microbiol.* **11** (1954) 123.
16. S. VALLA and J. KJOSBAKKEN, *ibid.* **128** (1982) 1401–1408.
17. W. S. WILLIAM and R. E. CANNON, *Appl. Env. Microbiol.* **55** (1989) 2448.
18. S. MASAOKA, T. OHE and K. SAKOTA, *Ferment. Bioeng.* **75** (1993) 18.
19. F. YOSHINAGA, K. TONOUCHI and K. WATANABE, *Biosci. Biotechnol. Biochem.* **61** (1997) 219.
20. R. M. BROWN JR., J. H. M. WILLISON and C. L. RICHARDSON, *Proc. Natl. Acad. Sci. USA* **73** (1976) 4565.
21. K. ZAAR, *Cytobiologie* **16** (1977) 1.
22. R. M. BROWN JR., in "Cellulose: Structural and Functional Aspects" (Ellis Howoodide, 1989) p. 145.
23. A. M. BROWN, *J. Polymer Sci.* **59** (1962) 155.
24. M. MARX-FIGINI and B. G. PION, *Biochimica Biophysica Acta* **338** (1974) 382.
25. K. OKAJIMA, Y. MATSUDA and K. KAMIDE, *Polymer International* **25** (1991) 145.
26. K. WATANABE, PhD desertation, Tokyo University, 1995.
27. M. MARX-FIGINI and B. G. PION, *Makromol. Chem.* **177** (1976) 1013.
28. A. KAI, J. KOGUSURI and Y. KOBAYASHI, *Bull. Chem. Soc. Jpn.* (1982) 1394.
29. M. IGUCHI and S. YAMANAKA, (1997) in Proc. Int. Workshop Green Polym., Bandung-Bogor 1996, edited by H. Adisessa (Indonesian Polymer Association, 1997) p. 47.
30. K. WATANABE and S. YAMANAKA, *Biosci. Biochem.* **59** (1995) 65.
31. W. BORZANI and S. J. DE SOUZA, *Biotechnol. Lett.* **17** (1995) 1271.
32. A. BUDHIONO, B. ROSIDI, H. TAHER and M. IGUCHI, *Carbohydrate Polymers*, in press.
33. S. YANO, A. BUDHIONO and A. SUGIHARTO, Report on the International Research Cooperation Project: The industrial utilization of bacterial cellulose, AIST, Tokyo, 1998.
34. T. E. BUSTNES, R. J. MURRAY and M. MACKLEY, Project Report, Department of Chemical Engineering, University of Cambridge, 1998.
35. N. SAKAIRI, H. ASANO, M. OGAWA, N. NISHI and S. TOKURA, *Carbohydr. Polym.* **35** (1998) 233.
36. D. H. PAGE and R. S. SETH, *Tappi* **63** (1980) 113–116.
37. K. TASHIRO and M. KOBAYASHI, *Polymer Bull. (Berlin)* **14** (1985) 213.
38. H. SHIBAZAKI, S. KUGA and F. ONABE, *Tappi, Jpn.* **48** (1994) 93.
39. K. YOSHINO, R. MATSUOKA, K. NOGAMI, H. ARAI, S. YAMANAKA, K. WATANABE, M. TAKAHASHI and M. HONDA, *Synthetic Metals* **41–43** (1991) 1593.
40. J. D. FONTANA, A. M. DE SOUZA, C. K. FONTANA, I. L. TORRIANI, J. C. MORESCHI, B. J. GALLOTTI, S. J. DE SOUZA, G. P. NARCISCO, J. A. BICHARA and L. F. X. FARAH, *Appl. Biochem. Biotechnol.* **24–25** (1990) 253.
41. H. SHIBAZAKI, S. KUGA, F. ONABE and M. USUDA, *J. Appl. Polym. Sci.* **50** (1993) 965.

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