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The structure and mechanical properties of sheets prepared from bacterial cellulose

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A preliminary experiment has shown that a sheet-shaped material prepared from bacterial cellulose has remarkable mechanical properties, the Young's modulus being as high as >15 GPa across the plane of the sheet. The mechanical properties were little affected by the fermentation conditions of pellicles and the preparation conditions of the sheets, i.e. the pressing and drying of pellicles. From structural investigations, the high Young's modulus has been ascribed to the unique super-molecular structure in which fibrils of biological origin are preserved and bound tightly by hydrogen bonds. It has also been found that a "pulp" obtained from bacterial cellulose gives a strong paper and is useful for reinforcing conventional pulp papers and enabling paper-making from some fibrous materials.

1. Introduction

It was one century ago that the first scientific paper was written in 1886 by Brown [1] on a peculiar fermentative substance which would have been known in many places on earth and was particularly popular in his country under the name of "vinegar plant". Under pure cultivation in carbohydrate media, it was observed that "the whole surface of the liquid is covered with a gelatinous membrane, which, under very favourable circumstances, may attain a thickness of 25 mm. . . . On removing the membrane from the liquid, it is found to be very tough, especially if an attempt is made to tear it across its plane of growth". From chemical analysis and various reactions, the substance was concluded without doubt to be cellulose, although microscopy at that time only gave a picture of bacteria lying embedded in a transparent structureless film.

During the following one hundred years, a number of studies have been made on the structure of the pellicle* as well as its production, the biogenesis of cellulose, etc. Today, it is known that the pellicle comprises a random assembly of fibrils, < 130 nm wide, which are composed of a bundle of much finer microfibrils, 2 to 4 nm diameter [2, 3]. It is also known that the pellicle gives a film or sheet when dried if shrinkage across the plane is restricted. The crystallographic form of this cellulose has been revealed to be almost the same as that of "cellulose I", commonly found in vegetable cellulose [4], and the molecular orientation is believed to be parallel to the direction of the length of the fibrils.

Such a peculiar super-molecular structure engineered by nature has come to be of great interest to the present authors when compared with that of synthetic polymers which have been created during the last two decades in the development of super-strong fibres. It is rather surprising that nothing has been reported on the mechanical properties of dried pellicles or the processing of this substance into structural materials. To the authors' knowledge, the bacterial cellulose is only commonly used for indigenous desert food in the Philippines. A patent covers its use for production of medical pads but the aim is quite different [5].

In this paper, it has been found that the sheets prepared from bacterial cellulose have excellent mechanical properties, the Young's modulus being as high as > 15 GPa across the plane of the sheet, whereas the highest values attained in the past by polymeric films or sheets (having no preferential molecular orientation in one direction and/or another) is < 10 GPa at most. Thus, the structure of sheets obtained from the pellicles as well as the process of fermentative generation of the pellicles have been investigated to ascertain

*The word "pellicle" is preferred to membrane in this paper for describing the fermentation product.

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their mechanical properties. It has also been found that a "pulp" obtained from bacterial cellulose (BC) gives a strong paper and is useful for making paper from other fibrous materials.

2. Experimental procedure

2.1 Fermentative production of bacterial cellulose

The strain of *Acetobacter aceti* mainly used for the present study was AJ 12368 (a stock from Central Research Laboratories, Ajinomoto Co. Inc., Tokyo, Japan) which has been proved to produce cellulose in a preliminary study. The culture medium was 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 $MgSO_47H_2O$ in 1 litre of water and the pH was adjusted to 5.0. The seed broth was prepared by adding a loopful of stock culture to 10 ml culture medium and incubating, without stirring, at 30° C for 7 d. Culture for producing the pellicle was carried out in various types of vessel by inoculating 1%(vol/vol) of the seed broth into the culture medium and incubating under the same conditions for various numbers of days. The pellicles thus obtained were washed thoroughly in running water, boiled in 10 times their volume of 2%(wt/vol) NaOH solution for 1 h, to eliminate bacterial cells and other ingredients, and then washed again in running water until the pH of water became neutral.

For analytical purposes, pellicles were dried in an oven at 105° C to constant weight and the cellulose content was calculated from the ratio of dried and wet weight. The alpha cellulose content measured by the conventional method for several samples was 97%(wt/wt) on average [6].

The X-ray crystallinity of the dried sample, ~63%, was several per cent higher than those of cotton lint or wood pulp, measured under the same conditions.

2.2. Processing of pellicles into sheets and papers

The details will be described below. The hydraulic press machine used for making sheets from pellicles was Model T-1, from Toho Machinery Co. Ltd., Tokyo, Japan and the pulp-disintegrator used for making pulp from the same was a 2 litre model, from Kumagai Riki Kogyo Co. Ltd., Tokyo, Japan.

2.3. Structural and physical analysis

The scanning electron microscope used for morphological observation was S-530, from Hitachi Ltd, Tokyo, Japan. X-ray diffraction was carried out by conventional methods using an apparatus, DF-3, from Rigaku Co. Ltd, Tokyo, Japan, equipped with a conventional goniometer, a Weissenberg Camera, etc.

Tensile and dynamic mechanical measurements were carried out under the standard conditions (20° C, 65% r.h.) with, respectively, an Instron-type mechanical tester (Tensilon UTM-II-100), and a longitudinal oscillation tester (Rheovibron DDV-II-EA), both manufactured by Orientec Co., Tokyo, Japan. For these measurements, rectangular specimens were cut from sheets or papers.

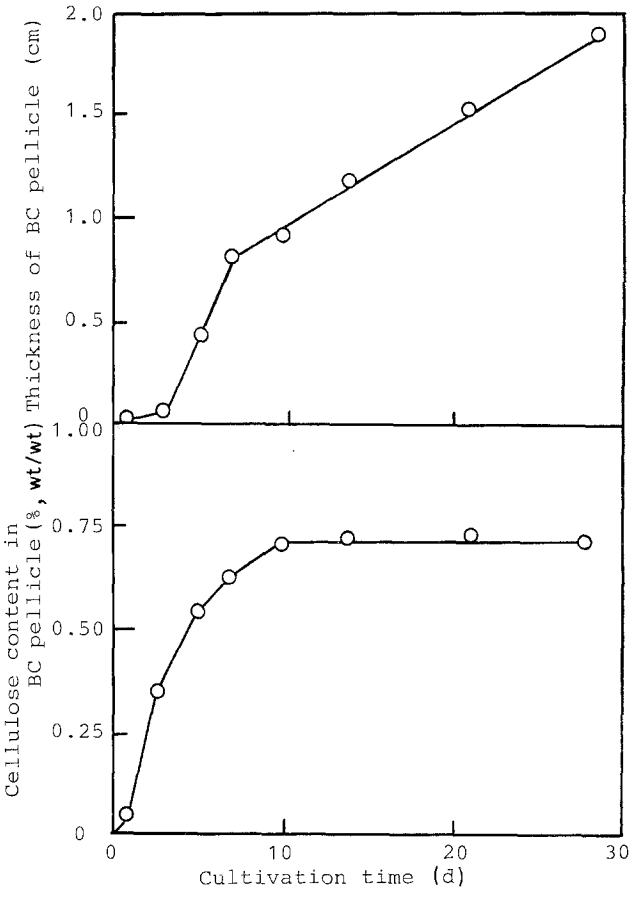

Figure 1 Change of thickness and cellulose content of BC pellicle with cultivation time.

3. Results and discussion

3.1. Generation and morphology of pellicles

Fig. 1 shows a typical example of the changes over time in thickness and cellulose content of BC pellicle cultivated with a strain of AJ 12368. After an inducing and a rapid growth period, the thickness increases steadily at a constant rate of ~0.5 mm d^{-1}, reaching ~2 cm in 1 mo. The cellulose content of the pellicle also increases with cultivation time but became saturated at the level of 0.7% (wt/wt) at around 10 d, when the thickness was ~ 10 mm. Pellicles with such a saturated cellulose content were used in the following experiments unless otherwise specified.

A scanning electron micrograph of unwashed BC is shown in Fig. 2. The morphology is essentially the same as observed before and comprises slender fibrils, 20 to 50 nm wide, lying in various directions and giving an image of a network structure [7]. The length of the fibrils is at least 10 μm, exceeding the visual area of the photograph [2, 3]. When the picture is observed carefully, the fibrils appear to be not necessarily linear but contain some "three-way branching points" along their length. This type of branching is considered to be related to the unique characteristics of this material, as will be discussed in the next section.

Although more evidence is needed, the mechanism of branch formation can be tentatively described in terms of the incidence of cell division, as drawn schematically in Fig. 3. As observed by Brown *et al.* [2] and Zaar [3], the fibrils of BC are considered to be formed by the bunching of microfibrils which are excreted from pores aligned on the surface of cells in a row

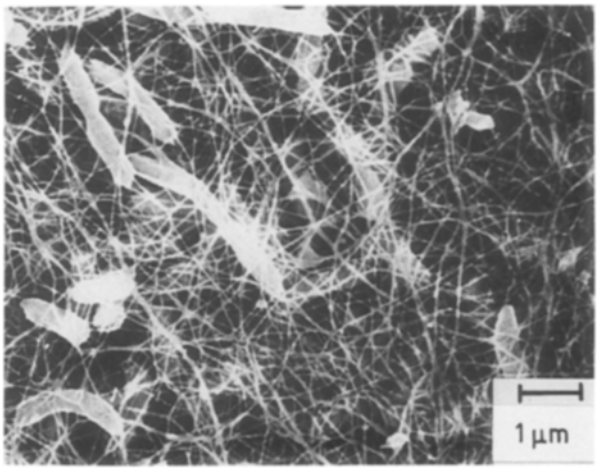

Figure 2 Scanning electron micrograph of the surface of a freeze-dried BC pellicle.

along their longitudinal axis. If the excretion continues beyond cell generation, the microfibrils of parent cells will be inherited one-half by each of the daughter cells, forming a branching point on the fibril. In this case, the diameter of fibrils may be narrower after cell division, although it must recover as the new cell matures. Alternatively, the normal number of pores for excreting microfibrils will be provided in the new cells at the stage of division as suggested by Brown *et al.* [2]. In any case, the repetition of this process will generate a cascade of branchings.

The doubling time of this type of bacteria is known to be 1.5 to 8 h [8]. The growth rate of fibrils is not well known but $\sim 2\,\mu m\,min^{-1}$ has been reported as the rate observed for isolated cells during the initial stage of cultivation [2]. From these data, the length of fibrils between branching points is calculated to be 180 to 960 μm. This value may be too large, however, because the growth rate of fibrils is thought to be retarded by many factors, e.g. the delaying of nutrient supply with the development of networks, a decrease in the activity of enzyme with the ageing of the cell, etc.

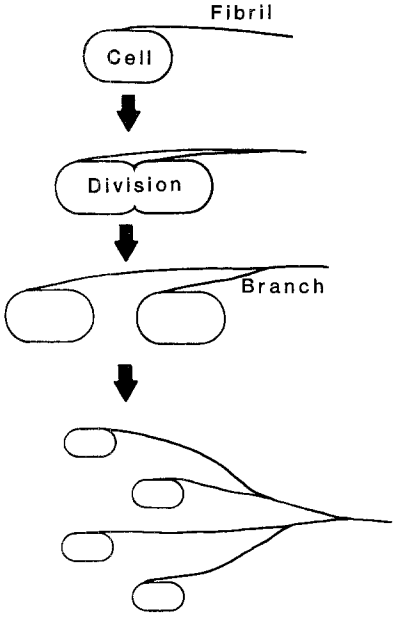

Figure 3 Schematic drawing to explain the formation mechanism of branches in fibrils.

3.2. Structure and mechanical properties of the sheet

As described above, pellicles of bacterial cellulose as produced, form gel-like material, more or less similar to the flesh of squid: when air-dried on a glass plate by fixing the area, it shrinks in the thickness direction and forms a solid sheet. Preliminary measurement has shown the Young's modulus to be as high as > 15 GPa, in any direction across the plane of the sheet, which is almost one-tenth of the theoretical value of cellulose, 173 GPa, calculated by Tashiro and Kobayashi [9].

Optimum conditions for preparing sheets were investigated, with respect to the mechanical properties, using a hydraulic press machine equipped with a heating device. Throughout the experiments, the native pellicle was sandwiched between a pair of stainless-steel meshes (200 meshes/inch) to squeeze out water, and pressed for 5 min under various pressures (49 to 1960 MPa) at elevated temperature (120 to 200° C).

Table I summarizes the results of the mechanical measurements. As far as the Young's modulus is concerned, neither pressure nor temperature affected it within the range of the experiments, and the values were similar to that measured for the air-dried sheet. The tensile strength and the elongation at break decreased, however, when the pressure was as high as ~ 500 MPa or more, presumably due to some mechanical damage. The dynamic Young's modulus measured for some specimens by the longitudinal oscillation method was 18.8 GPa (110 Hz), agreeing in magnitude with the static value. The effect of varying cultivation time, and hence of varying cellulose content, on the mechanical properties was examined, but no difference was observed. A sheet made from pellicles obtained using a different bacterial strain (ATCC 10821, from the American Type Culture Collection, Maryland, USA) did not show much difference either.

The very high Young's modulus of this material must be ascribed to its super-molecular structure. The molecular arrangement in the sheet, confirmed by X-ray diffraction, was such that the crystallographic *c*-axis or the molecular chain axis lay randomly perpendicular to the thickness and the (1 1 0) plane was oriented parallel to the surface [10]. Scanning electron microscopy of a fractured edge has revealed a pile of

TABLE I Effect of preparation conditions on the mechanical properties of BC sheet

Preparation method	Temperature (°C)	Pressure (MPa)	Young's modulus (GPa)	Tensile strength (MPa)	Elongation (%)
Air-dried	20	0	16.9	256	1.7
	120	49	17.4	224	1.8
	150	49	18.0	231	1.8
	200	49	16.4	243	1.9
	150	49	16.9	260	2.1
Hot-pressed	150	196	16.7	216	1.7
	150	490	17.5	155	1.4
	150	980	17.0	129	0.9
	150	1470	16.6	102	0.8
	150	1960	18.1	91	0.8

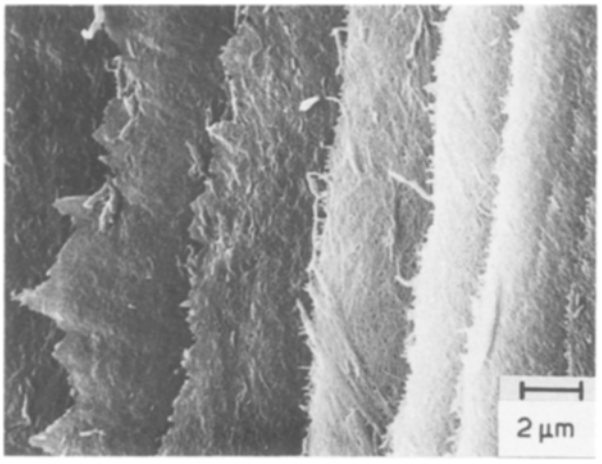

Figure 4 Scanning electron micrograph of a fractured edge of BC sheet.

very thin layers, as shown in Fig. 4, which consist of the fibrils observed in the native pellicle. It is considered that these fibrils in layers are bound through interfibrillar hydrogen bonds, just as in pulp-papers, but the density of the interfibrillar hydrogen bonds must be much higher as the fibrils are finer, hence the contact area is larger. As will be reported in a subsequent paper, the Young's modulus becomes even higher when further purification of the material is carried out [11]. Whether the thin layer found in the sheets was related to the layer structure often observed in the native pellicle [1] is not certian. However, an experiment has shown that a sheet obtained by pressing a parallel slice of thickly grown pellicle perpendicular to the direction of the original thickness comprised the same type of thin layers and the mechanical properties were not very different.

Various attempts to align fibrils in a direction during or after the sheet preparation were not very successful, only giving a partial orientation such as attained by Takai *et al.* [10], because the gel-like material was so tough that it resisted large-scale deformation. Isolation of fibrils, e.g. by fragmentation in a kitchen mixer, was never easy either. Such difficulty is considered to be related not only to the entanglement of fibrils but also to the existence of branchings, as pointed out above.

3.3. Properties and use of disintegrated BC

The fragments of BC obtained by mechanical disintegration in water retained the original nature of the pellicle, consisting of entangled fibrils, but fragmentation itself was considered to be useful as another means of processing the material. Thus, one part of wet pellicle was added to 10 parts of water and disintegrated in a laboratory-size pulp-disintegrator. The suspension of "pulp" obtained was filtered through a stainless-steel mesh (100 meshes/inch) and then processed into "paper" by means of a heat-press machine as used above. Experiments to prepare composite paper with other fibrous substances were also conducted.

Table II shows the results of mechanical measurements for a paper of pure BC pulp, as well as papers containing various proportions of cotton lint pulp. The Young's modulus and the tensile strength of BC paper decreased to the level of one-third of those attained by the sheets from un-disintegrated pellicles, due presumably to the loss of the continuity of the original network structure. It is seen, however, that paper made from BC is much stronger than papers made from ordinary pulps and the mixing of BC has a remarkable reinforcing effect on the latter.

TABLE II Reinforcement effect of BC for composite sheets

Composition (BC : cotton lint pulp)	Young's modulus (GPa)	Tensile strength (MPa)	Density ($g\,cm^{-3}$)
4 : 0	4.9	85	0.99
3 : 1	2.7	30	0.48
1 : 1	0.61	12	0.43
1 : 3	0.21	3.3	0.20
0 : 4	0.085	0.83	0.19

It has also been found that such fibrous substances as carbon fibre, phenol-resin fibre, SiC whiskers, etc., can be successfully processed into the form of a paper by the addition of a small amount of bacterial cellulose pulp, e.g. 5%(wt/wt) or less by weight.

4. Conclusion

While vegetable cellulose has been one of the most important materials for mankind ever since the dawn of its history, little attention has been paid to a bacterial product which has also long been in existence and was identified one hundred years ago to be of the same chemical composition. It has been found for the first time that the white gel-like substance comprising fine cellulosic fibrils can be easily processed, retaining its unique super-molecular structure, into a very strong sheet-form material whose mechanical properties are scarcely rivalled by synthetic polymers. By virtue of its high modulus and low density, the sheet is expected to be useful for industrial purposes, e.g. in acoustic diaphragms, as will be described in a subsequent paper. It has also been found that a "pulp" obtained from bacterial cellulose gives a strong paper and is useful for paper making with other fibrous materials.

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