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A method for direct harvest of bacterial cellulose filaments during continuous cultivation of *Acetobacter xylinum*

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Continuous filamentation of bacterial cellulose (BC) was successfully achieved by using shallow pan for the incubation to regulate thickness of the BC gel produced by *Acetobacter xylinum*. The BC filament was harvested and prepared directly by picking up BC pellicles, the thin BC gel, and winding slowly from the surface of the culture medium passed through a preliminary bactericidal washing bath. The X-ray diffraction analysis and scanning electron microscopic observation of the BC filament thus obtained showed that the filament was smooth and the fairly good orientation of BC molecules.

The average tensile strength was 4.4 g denier^{-1} for the filament prepared by hot alkaline treatment and subsequent washing with distilled water and dried under tension (Filament W): 3.4 g denier^{-1} for washing with 10% aqueous ethylene glycol after alkaline treatment followed by drying under tension (Filament E) and 2.4 g denier^{-1} for the treatment with 10% ethylene glycol after normal water-washing followed by drying under tension. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

It has been established that *Acetobacter xylinum* produces bacterial cellulose (BC) under static incubation (Brown, 1986) in Schramm–Hestrin (SH) medium containing glucose as a carbon source (Hestrin and Schramm, 1954). Because BC is a pure cellulose, it has attracted much attention in various manufacturing fields. In our previous studies on the preparation of BC, we developed novel procedures to produce molecular variants of BC. Incorporation of aminosugar residues has been successfully attained by incubation of the bacteria that had been subcultured repeatedly in the medium containing *N*-acetylglucosamine (GlcNAc) and glucose (Glc) or only GlcNAc as carbon source (Ogawa and Tokura, 1992). Since a similar degree of GlcNAc incorporation was found by employing media containing Glc and either galactosamine (GalN) or GlcN but not by mannosamine (ManN), we could assumed that transaminase was activated in the metabolic cycle of the bacteria (Shirai et al., 1994). Under rotatory but not static conditions, a similar degree of GlcNAc incorporation was also observed when cultivation was carried out with air bubbling in the medium containing Glc and ammonium chloride (Shirai et al., 1994).

Although much effort has been devoted to the preparation and utilization of BC and its analogs, these methods and agents have not yet been incorporated as industrial resources, largely because of the high production costs involved. Given that complicated procedures are required for the preparation of fibers and films from these biosynthesized polysaccharides (Hibbert, 1930), a simple method, one that can serve to reduce these high production costs, has been needed. In this paper, we describe the results of our investigations for such a simplified process, which was prompted by recent success with regard to surface polymerization of nylon capable of giving superpolymer films or filaments directly from the interface of organic and aqueous phases (Magat and Strachan, 1995; Wittbecker and Morgan, 1959). We have designed a shallow stainless steel pans to

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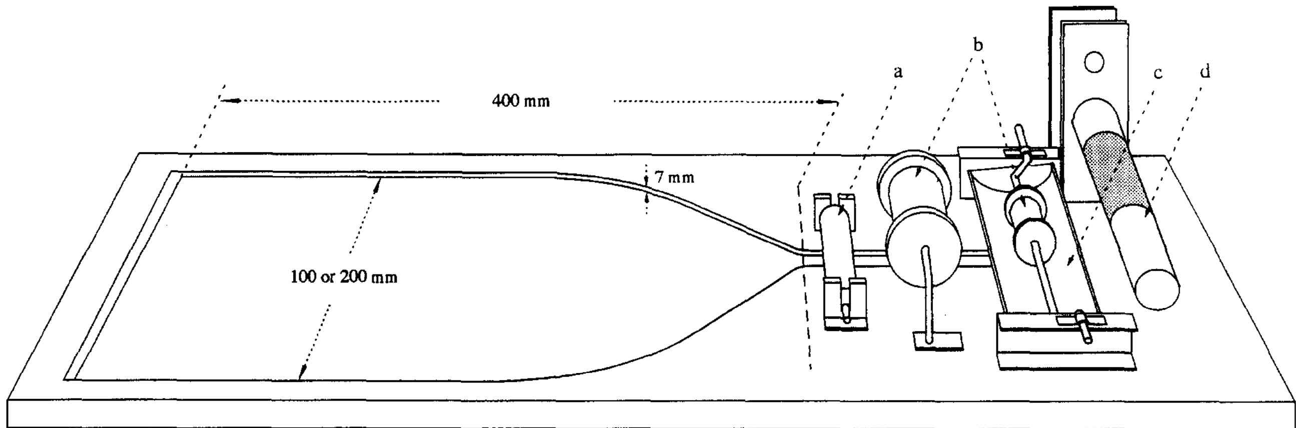

Fig. 1. Outline of the culture pan: (a) sinker; (b) roller; (c) washing pan; (d) wind-up roller.

regulate the gel thickness better during the incubation and to conserve the total amount of the medium used. The tensile strength of the resultant filament was significantly greater than that of the cellulose fibers, and good orientation of molecules was revealed in the X-ray diffraction pattern.

EXPERIMENTAL

Materials

All chemicals were purchased from Wako Pure Chemicals Co. Ltd. (Osaka, Japan) and used further purification. A wild type of *Acetobacter xylinum*, ATCC 10245 strain, was subcultured at 28°C in Schramm–Hestrin (SH) medium containing Glc as a carbon source (Hestrin and Schramm, 1954), and repeatedly transferred to the new culture medium every 3 days.

Incubator

Two culture pans (100 mm wide × 400 mm long × 7 mm deep and 200 mm wide × 400 mm long × 7 mm deep) were worked out by cutting stainless steel board. The inside shape of the pan was specially designed to facilitate harvesting the thin gel smoothly. The pan was equipped with a winding roller and a bath of 2% aqueous sodium dodecyl sulfate (SDS), as shown in Fig. 1. The whole apparatus was set in an incubator in which temperature was maintained at 28°C and filtered air was passed through the incubator (Fig. 2).

Culture medium and cultivation

SH medium (150 ml or 300 ml) was added to the smaller or larger culture pans, respectively. The depth of the culture media in each pan was 3–4 mm. The media were inoculated with the subcultured *Acetobacter xylinum*. The incubation was carried out at 28°C under static conditions. After 2 days of incubation, the edge of the pellicle produced on the surface was picked up, passed through the SDS bath to denature the bacterial cell wall, and set on the winding roller. The winding process was continued for a couple of weeks at the rate of 35 mm h^{-1} at the same temperature. During the incubation, the depth of the culture medium was maintained by the addition of SH medium every 8–12 h.

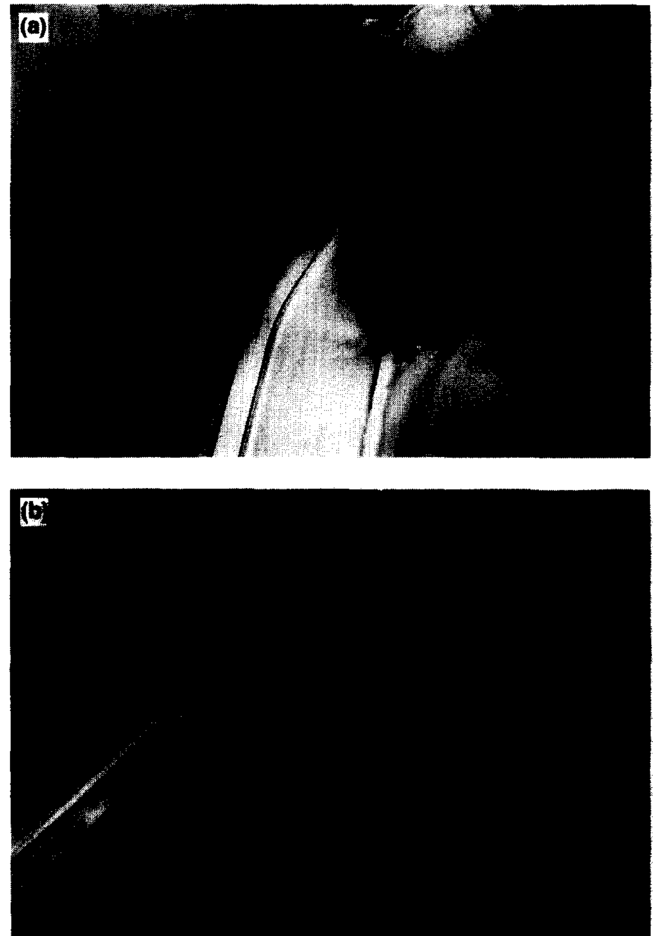

Fig. 2. Winding-up process of BC during incubation of *A. xylinum* in a shallow pan (a) and roller (b).

Purification of filament

The wound filament was boiled for 3 h in 2% SDS solution, washed with distilled water, and boiled again in the 4% aqueous sodium hydroxide solution for 1.5 h. The wet filament was extensively rinsed with distilled water and then air-dried at less than 60°C under tension, giving Filament W. Filament E was prepared by washing of the wet filament three times with 10% aqueous ethylene glycol followed by air-drying at less than 60°C under tension. Filament WE was prepared by rinsing Filament E with distilled water and dried under tension.

Tensile strength

The stress–strain diagrams of the filaments were obtained using a Shimadzu Auto Graph AGS-500D apparatus at a guide distance of 25 mm, a chart speed of 100 mm min^{-1}, and a load cell speed of 2 mm min^{-1}. The force at the breaking point was taken as the tensile stress, which was transferred to tensile strength and Young's modulus.

Wide-angle X-ray diffraction analysis

Wide-angle X-ray diffraction (WAXD) patterns were recorded by using a MAC M18XHF X-ray diffractometer. The X-rays were generated at 40 kV and 100 mA using nickel-filtered Cu$K\alpha$ radiation. A vacuum camera equipped with a 0.5 mm pin-hole collimator was used.

Scanning electron microscopy

Scanning electron microscopy (SEM) was accomplished using an Akashi S-DS 130 microscope with gold-coated sample. Microspheres were sprinkled on to double-sided tape, sputter-coated with gold, and examined in the microscope at 10 kV.

RESULTS AND DISCUSSION

Design of the cultivation system

To make thinner BC gel suitable for direct and continuous filamentation, we devised a shallow pan for the incubation of *A. xylinum*. In a preliminary incubation under static conditions using a pan 10 mm deep, we found that thin BC gel was obtained on the surface of the culture medium, and that the gels were strong and elastic enough to pick up and manipulate. On this basis, we designed a direct filamention system shown in Fig. 1, which consisted of a shallow pan 400 mm long and 100 or 200 mm wide and a winding-up roller. One side of the pan was curved gently to permit harvest of the pellicle through a narrow mouth. The thin BC gel was to be directly passed through a bath containing aqueous SDS solution to reduce the bacterial activity and then the filament would be wound slowly on an attached roller to shallow pan cultivator. The shallow pan would be also effective in conserving the total amount of culture medium used for the incubation.

Direct filamentation

Using the system described above, *A. xylinum* was incubated under static conditions at 28°C in the shallow pan containing SH medium. After 2 or 3 days of static cultivation, thin BC gel was formed and harvest was started on the roller system. Taking the growth of the BC gel into account, we observed the continuous filamentation at the rate of wind-up of less than 40 mm h^{-1}. To maintain the depth of the culture medium, SH medium was supplied in increments during the incubation, without addition of bacteria. From the 100 mm pan, we obtained a filament of more than 5 m by winding-up at a rate of 16 mm h^{-1} for 14 days. Fig. 2 shows the process of the direct filamentation. For purification, the BC filament thus obtained was successively treated with boiling 2% SDS solution and boiling 4% aqueous sodium hydroxide solution. Three samples of the filaments were prepared by washing of the resulting filament with distilled water (W), with 10% aqueous ethylene glycol (E), or with both 10% aqueous ethylene glycol and distilled water (WE). All filaments were subjected to air-drying under tension at less than 60°C.

Physical property of the filament

The BC filament obtained by the direct filamentation was first examined by SEM. A SEM image (Fig. 3) showed that the BC filament with normal water-washing (Filament W) has good alignment cellulose moieties and slightly twisted fiber mode. The cut surface of filament is also shown in Fig. 3(c) which shows the melting of the inner part of filament to form a fiber bundle. The X-ray diffraction pattern of the filaments was also examined. These results suggested that Filament W (Fig. 4) has a higher orientation of molecules than either Filament E or WE. This finding is consistent with those from the SEM observation.

The tensile properties and the stress–strain curves for the filaments obtained by different washing procedures (Filaments W, E, WE) are summarized in Table 1 and Fig. 5, respectively. The stress–strain diagram shows that Filament WE has poor tensile strength. Deniers of ethylene glycol-treated filaments (Filament E) tended to double compared with the filament prepared by normal water-washing (Filament W), a finding that might reflect the slightly disturbed orientation of molecules in the filaments by the trapping of ethylene glycol in the network. Among these three kinds of filaments, normal water-washing filament (Filament W) had good mechanical strength; the best sample revealed 6.1 g $denier^{-1}$ for the tensile strength and 106 g $denier^{-1}$ for Young's module and their average values were 4.4 g $denier^{-1}$ and 90.4 g $denier^{-1}$, respectively. They were significantly stronger than the original BC filament and were comparable to cotton (3.0–4.9 g $denier^{-1}$ for the tensile strength and 68–93 g $denier^{-1}$ for Young's module (Japan

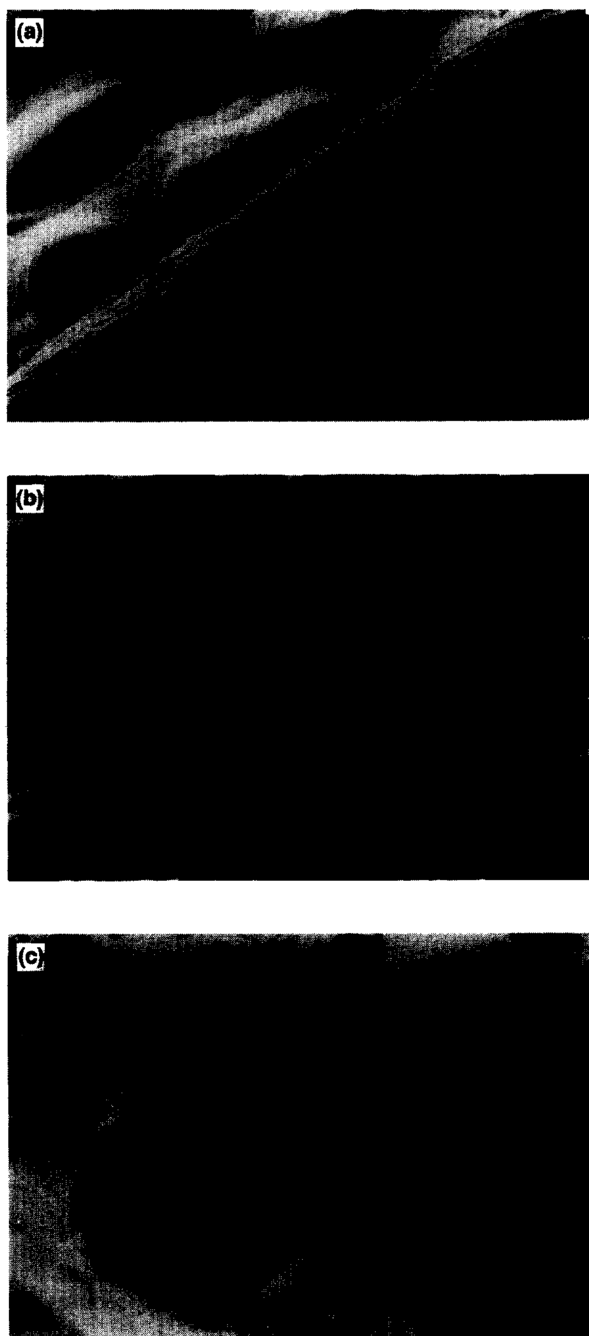

Fig. 3. Scanning electron micrographs of the BC filament obtained by normal water-washing (Filament W) (a: × 109; b: × 319) and (c) its cut surface (× 319).

Chemical Fibres Association (1990)) and other fibers (Meredith, 1956; Rebenfeld and Virgin, 1957). The filaments prepared by ethylene glycol treatment (Filaments E and WE) seem less suitable for practical use; the Young's moduli of these filaments were 81.6 and 51.5 g denier^{-1}, respectively. The low tensile strength and Young's modulus observed for filaments obtained by aqueous ethylene glycol treatment after cleaning of the filaments may be due to obstruction of molecular orientation followed by incomplete crystallization of cellulose molecules.

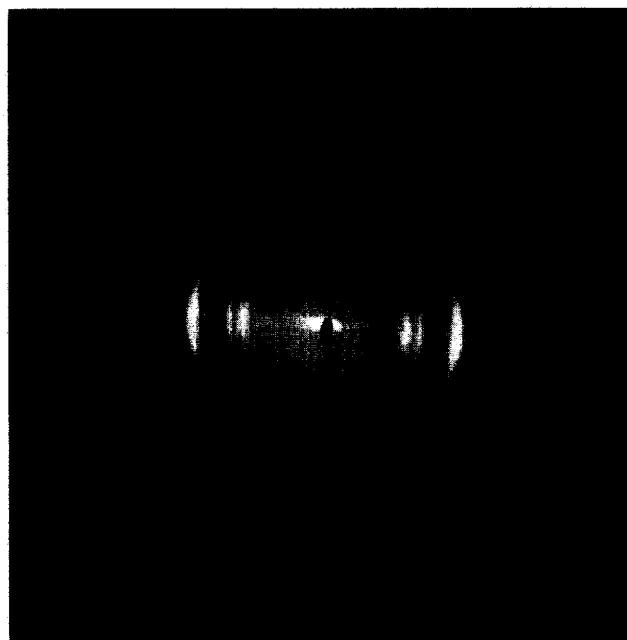

Fig. 4. X-ray diffraction pattern of the BC filament (W).

CONCLUSION

Our design of a simple and direct filamentation system for BC is, we believe, the first such procedure to be reported in the literature. The tensile strength of the filament was found to be significantly stronger than the ordinary cellulose fibers and a good orientation of molecules was shown by both the X-ray diffraction pattern and SEM observation. This simplified methods for producing and harvesting BC, by promising to lower production costs, show the way for its industrial use.

Table 1. Tensile properties of bacterial cellulose filament. The BC filament was treated with boiling 4% aqueous sodium dodecylsulfate solution for 2 h and then 2% aqueous sodium hydroxide for 1.5 h. The resulting filament was rinsed extensively with water (Filament W), with 10% aqueous ethylene glycol (Filament E), or successively with 10% ethylene glycol and water (Filament WE). All filaments were air-dried under tension

Sample	Elongation (%)	Size (denier)	Tensile stress (MPa)	Tensile strength (g denier^{-1})	Young's modulus (g denier^{-1})
W1	4.2	108.0	267.4	3.9	93.8
W2	6.0	169.2	223.3	5.6	93.6
W3	4.5	108.0	431.5	3.6	80.5
W4	3.8	108.0	242.4	3.0	78.9
W5	5.8	140.4	386.4	6.1	105.4
E1	5.2	216.0	310.2	3.6	67.9
E2	3.7	216.0	386.4	3.5	93.7
E3	3.8	216.0	284.7	3.1	83.1
WE1	7.7	180.0	293.6	2.1	27.3
WE2	3.7	180.0	249.3	1.9	52.5
WE3	4.2	180.0	341.3	3.1	74.8

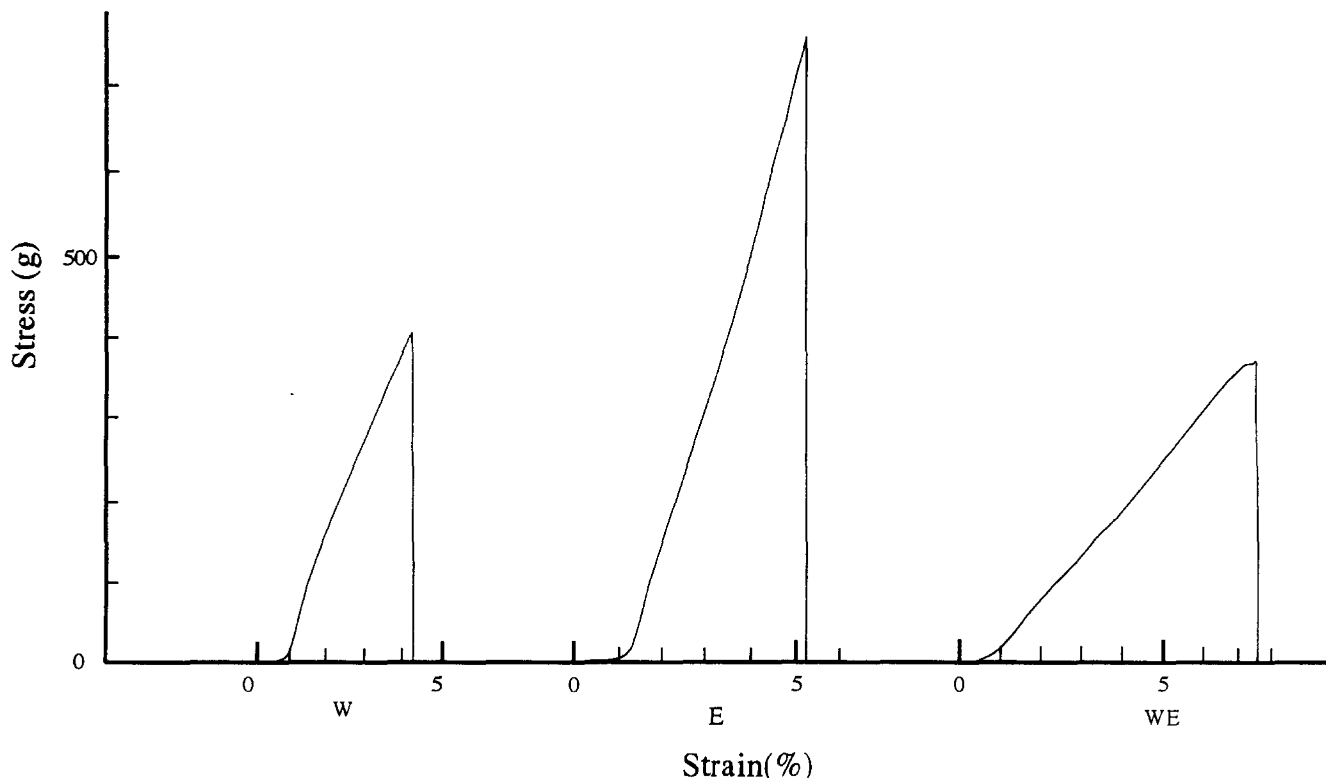

Fig. 5. Stress–strain diagram properties of BC filaments.

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