DIRECT FILATURE OF BACTERIAL CELLULOSE FROM CULTURE MEDIUM

Seiichi Tokura*, Hisashi Asano, Nobuo Sakairi and Norio Nishi

Graduate School of Environmental Earth Science,
Hokkaido University, Sapporo 060, Japan

Abstract: The continuous Filature of bacterial cellulose has been achieved directly from culture medium using shallow culture pan newly designed. A remarkable progress of fiber properties was observed comparing with regenerated cellulosic filaments on the filament of bacterial cellulose dependently on the rinsing process with water or ethylene glycol. The progress of fiber properties was also shown on the filament of N-acetylglucosamine(GlcNAc) incorporated bacterial cellulose probably due to enforcement of fiber interactions. A copolymer of carboxymethyl-glucose (CM-Glc) and glucose has been achieved successfully to increase the adsorption capacity for lead ion comparing with original carboxymethyl cellulose (CMC).

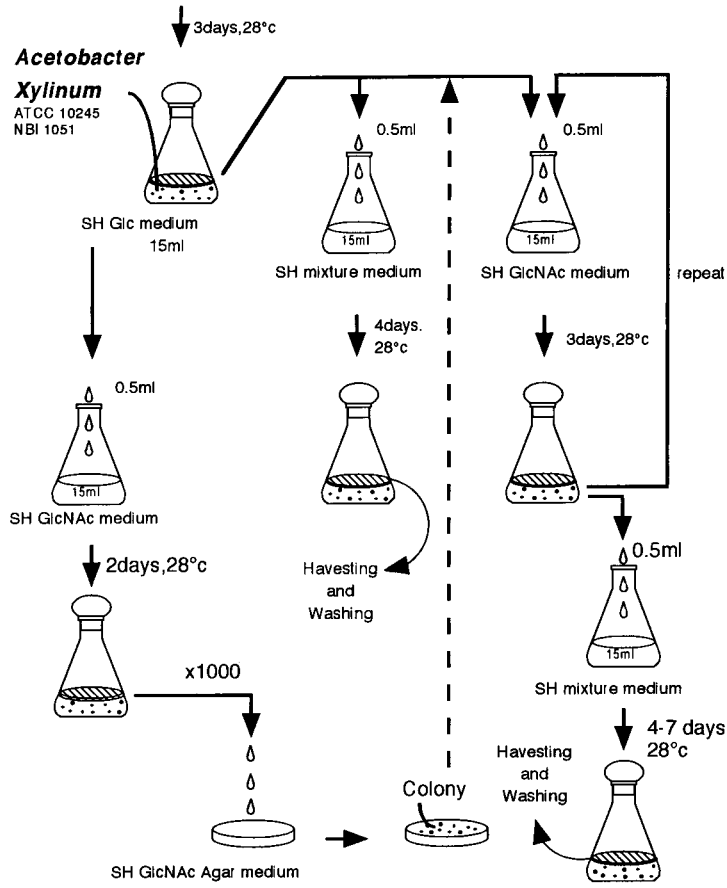
INTRODUCTION

A bacterial cellulose is known to be the cellulose of high purity and high crystallinity which is produced by *Acetobacter xylinum* in Schramm-Hestrin (SH) medium containing glucose as a carbon source(Refs.1-2). The functionalization of bacterial cellulose has been requested to overcome such a high production cost of BC.

A generation of BC filament has been done directly from culture medium using newly designed shallow culture pan (7mm of depth, 400mm of length and 100 or 200mm of width), when the gel is thin and strong enough for wind up to the roller(Ref.3).

We have also investigated on the functionalization of bacterial cellulose only by the change of carbon source for *A. xylinum* in which several glucose analogues were applied as mixed carbon source with glucose to introduce residual glucose analogues into BC(Ref.4). N-Acetylglucosamine (GlcNAc) residue was incorporated into BC, when *A. xylinum* subcultured repeatedly with mixed carbon sources was cultured in the medium containing glucose and GlcNAc, glucosamine (glcN) or galactosamine (GalN). But there was hard to find amino sugar incorporation with use of mannosamine (ManN) as mixed carbon source even after the repeated subculture against ManN. As several enzyme systems were suggested to regulate the GlcNAc incorporation, ammonium salts were mixed with glucose in SH medium instead of aminosugar(Ref.5). A similar level of GlcNAc incorporation was found only when ammonium chloride was applied with glucose under the aerobic rotary cultivation, but not under static condition. As better properties were observed on the BC filament than those of cellulosic filaments, GlcNAc incorporation was expected to enforce the tensile strength of BC filament.

 CCC 1022-1360/98/$ 10.00



Scheme 1. Innovation and subculture of *Acetobacter xylinum.*

A carboxymethyl glucose(CM-Glc) residue was also found in BC whose ion exchange capacity was enhanced remarkably only for lead ion, when *A. xylinum* was cultured in SH medium containing CM-Glc, CM-cellulose oligomer or CMC and glucose(Ref.6).

RESULTS AND DISCUSSION

Cultivation mediums: A Shramm-Hestrin medium (SH medium) was applied to prepare BC fundamentally as shown in Table 1 A and B. Total sugar concentration was kept as 2%. In the case of ammonium salts mixing to original SH medium, salt concentration was shifted from 0 to 2.0% without change of glucose concentration.

Table 1-A. Components of Schramm-Hestrin Medium

Components	Concentration(w/v%)
Glucose	2.0
Bacto peptone	0.5
Yeast extract	0.5
Disodium hydrogenphosphate	0.27
Citric acid	0.115
(Ammonium salts	0--2.0)
(CMC	0--2.0)

Initial pH of the medium : 6.0

Table 1-B. Components of Schramm-Hestrin Medium containing Aminosugar

Components	Concentration(w/v%)
Glucose	2.0--0
Aminosugar	0--2.0
Bacto peptone	0.5
Yeast extract	0.5
Disodium hydrogenphosphate	0.27
Citric acid	0.115

Initial pH of the medium: 6.0

Design of apparatus for rotary cultivation system: Several rotary fermenters were designed including aerobic rotary incubator to increase the surface area for aerobic bacteria as shown Figure 1. The aerobic rotary fermenter was effectively applied on the preparation of GlcNAc incorporated BC in the presence of ammonium salts. Hollow fiber net work was also applied with slow rate of rotation (20-50 rpm) under the similar air pressure as that of static cultivation. However, only a slight increase of the yield was observed except on the case of the mixed SH medium with ammonium chloride.

Design of shallow culture pan: Shallow pans (100mm or 200mm of width, 400mm of length and 7mm of depth) were designed to prepare a thin BC gel first and then wind up to roller at the rate of 35mm/hr directly from cultivation medium as shown in Figure 2. The cultivation of *A. xylinum* was proceeded for a couple of days under static condition and

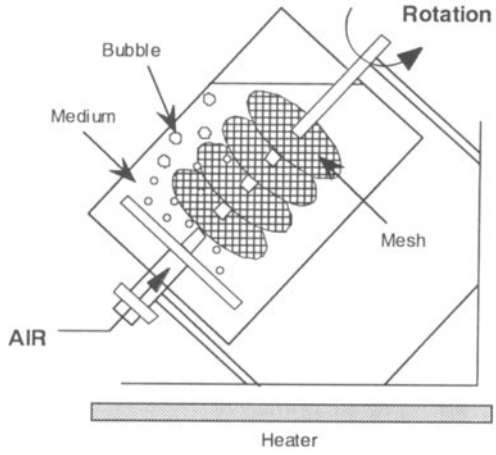


Figure 1. Outline of Aerobic Rotary Fermenter.

then thin gel formed on the surface of medium was picked up to wind up roller continuously for a week. A separator was attached to prepare the strips of 30mm width for the pan of 200mm width.

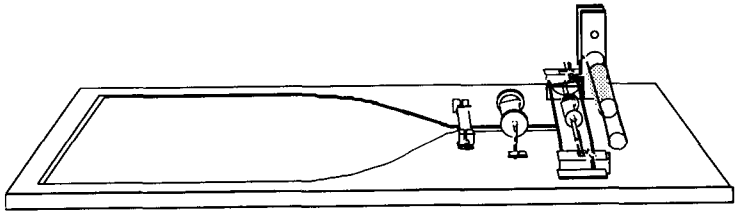


Figure 2. Outline of shallow culture pan. Maximum volume was 350ml for the pan of 200mm width.

Direct Filature of bacterial cellulose from culture medium: Since BC tends to form thick three dimensional gel during fermentation, thinner gel was expected to form on the

surface of culture medium by the regulation of the depth of culture medium. Thus a shallow culture pan was designed as shown in Figure 2. The shallow pan cultivation is a mixed type, 2 days of static and 7 days of stirring fermentation. Produced fibrous gel was stretched under slightly twisted mode to remove water following to boiling treatments in 2% sodium dodecyl sulfate(SDS) aqueous solution for 3 hr and in 4% sodium hydroxide aqueous solution for 1.5hr, respectively. The profiles and tensile strengths are shown in Table 2. The tensile strength of filament is comparatively higher than those of regenerated cellulosic filament such as rayon or acetate, although bigger filament diameter was shown than those of cellulosic filaments. As the filament was too thick to prepare filament in the case of wider pan (200mm width), a separator was attached to prepare the strips of 30mm width. The separator was so effective to prepare thinner filament that the tensile strength was increased remarkably as shown in Figure 3 of practical runs. The molecular weights of these bacterial cellulose were partly confirmed to be more than 50,000 through the viscosity measurement after O-acetylation. The tangling effect was shown to be main factor to give such a high tensile strength, since tensile strength tended to be reduced by the treatment the filament with ethylene glycol as seen in the Table 2. The ethylene glycol treatment seems to disturb the generation of hydrogen bondings which may accelerate the tangling of fibrous bacterial celluloses. The recovery of tensile strength was shown through the removal of ethylene glycol with water rinsing which suggested the participation of interactions between filament surfaces to the tangling of filaments.

Incorporation of N-acetylglucosamine residue into bacterial cellulose: A wild type *A. xylinum* ATCC 10245 has been subcultured to adopt for a new culture medium containing aminosugar as one of carbon sources in Schram-Hestrin medium. The renovation and subculture of bacteria are achieved by the procedures as shown in Scheme 1. *A. xylinum* starts to incorporate N-acetylglucosamine (GlcNAc) residue into BC, when a mixed SH culture medium containing glucose and GlcNAc was applied following to repeated subcultures of bacteria in GlcNAc SH medium. The GlcNAc content in BC depends significantly on the number of subculture. Although the highest content of GlcNAc is 4-5 mole % in bacterial cellulose so far, GlcN and GalN achieved almost similar level of GlcNAc incorporation when they mixed with glucose in SH medium. The existence of epimerization and deamination steps was assumed to serve glucose as a metabolic substrate for the bacteria in the case of GalN and GlcN. Deacetylation step was also assumed for GlcNAc consumption to give GlcN into culture medium. The regeneration of amino group by the use of released ammonium ion was the second hypothesis on the incorporation of GlcNAc residue into bacterial cellulose. The addition of ammonium salts to SH medium was unsuccessful under static cultivation. But ammonium chloride was the most effective salts among several other ammonium salts on the incorporation of GlcNAc residue into BC as shown in Table 3, when aerobic rotary fermenter was applied, though the mechanism of amination step is still unknown. The influence of GlcNAc incorporation was shown on the higher orientation calculated from X-ray diffraction pattern as shown in Table 4.

Table 2. Size and tensile strength of filaments on different rinsing procedures.

Sample	Elongation	Size	Tensile stress	Tensile strength
	Young's modules			
	(%)	(denier)	(Map)	(g/denier)	(g/denier)
W-1	4.2	108.0	267.4	3.9	93.8
W-1	6.0	169.2	223.3	5.6	93.6
W-2	4.5	108.0	431.5	3.6	80.5
W-3	3.8	108.0	242.4	3.0	78.9
W-3	5.8	140.4	386.4	6.1	105.4
E-1	5.2	216.0	303.0	3.6	67.9
E-2	3.7	216.0	386.4	3.5	93.7
E-3	3.8	216.0	284.7	3.1	83.1
WE-1	7.7	180.0	209.8	2.1	27.3
WE-2	3.7	180.0	249.3	1.9	52.5
WE-3	4.2	180.0	341.3	3.1	74.8
Nac-1	1.5	26.3	568.0	7.4	125.4
Nac-2	1.2	26.3	520.2	5.9	119.1
NAc-3	0.9	60.5	373.6	1.3	39.4
NAc-4	2.1	60.5	585.7	4.6	54.8

W: Alkaline treated filament was rinsed directly with distilled water followed by air-drying. E: Alkaline treated filament was rinsed with ethylene glycol followed by air-drying. WE: Filament E was rinsed extensively with distilled water followed by air-drying. NAc: BC filaments incorporated with GlcNAc.

Table 3. Influences of ammonium salts on GlcNAc incorporation into BC

Cultivation	Ammonium Salts	GlcNAc Content (mol%)
Aerobic	Ammonium Sulfate	1.36
Rotary	Ammonium Chloride	4.51
	Ammonium Sulfate	0.13
Static	Ammonium Phosphate Dibasic	0.16
	Ammonium Chloride	0.16
	Ammonium Citrate	0.21

Direct Filature of the bacterial cellulose containing GlcNAc residue: SH medium containing 0.2% of GlcNAc was applied to shallow pan fermentation to prepare a BC filament incorporated with GlcNAc residue. The filament was purified and rinsed by similar procedures those for bacterial cellulose filament. The profile and properties of the filaments are

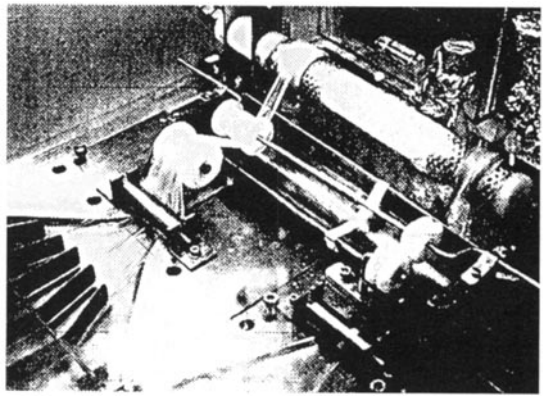


Figure 3. Practical run of BC filamentation.

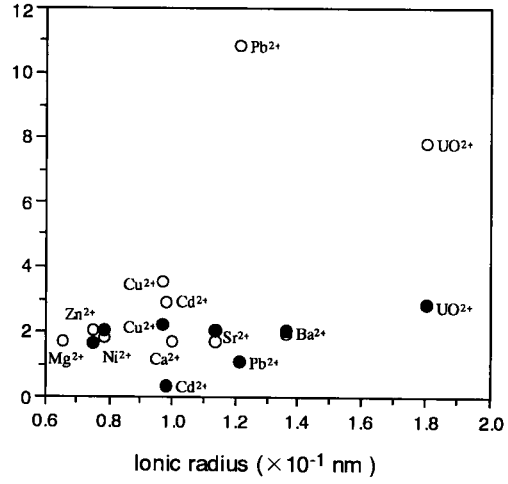


Figure 4. Metal ion adsorption profile of novel CMC(○)
(Degree of carboxymethylation : 11.1%) and that of original CMC(●)

listed in the bottom of Table 2. As seen in the Table, Young's modulus is enhanced by the incorporation of GlcNAc residue probably due to the formation of strong hydrogen bondings between acetamide and hydroxyl groups or acetamide groups, but it seems to be hard to show the influence of GlcNAc incorporation on tensile strength clearly. But the progress of these properties is expecting by the increment of GlcNAc incorporation.

Table 4. Influence of GlcNAc incorporation into BC Pellicle on X-ray diffraction Intensity

Sample Number	GlcNAc content (mol%)	(101/002)
BC-1	0.0	1.07
BC-2	0.0	0.95
BC -3	0.0	1.08
GlcNAc-BC-1	0.2	0.87
GlcNAc-BC-2	0.4	1.30
GlcNAc-BC-3	2.8	1.34
GlcNAc-BC-4	2.8	1.43

Incorporation of carboxymethyl-glucose(CM-G) residue: A water soluble carboxymethyl cellulose(CMC), applying as food expander mainly in the food technology, was mixed into glucose SH medium on the cultivation of BC. Around 10% of residual incorporation of CM-G was the maximum at 0.5%(w/v) of CMC mixing in SH medium, though there are maximum incorporation by the mixing ratio of CMC. Produced CM-bacterial cellulose was applied to investigate the ion exchange properties following to extensive washing with SDS and sodium hydroxide aqueous solutions at boiling temperature, respectively. A significant increase of adsorption for lead ion was observed on the CM-bacterial cellulose as shown in Figure 4 comparing with ion exchange profile of original CMC. The mechanism and molecular conformation of specific adsorption of lead ion is now under investigation.

REFERENCES

(1) A.J.Brown, J. Chem. Soc., **49**, 432(1886)
(2) H. Hibbert, Science, **71**, 419(1930). H. Hibbert, J. Barsha, Can. j. Res., **5**, 580(1931)
(3) N, Sakairi, H. Asano, M. Ogawa, N. Nishi , S. Tokura, Carbohydr. Polym., in submission.
(4) A. Shirai, N. Sakairi, N. Nishi, S. Tokura, Carbohydr. Polym., in press.
(5) A. Shirai, M. Takahashi, H. Kaneko, S-I. Nishimura, M. Ogawa, N. Nishi, S. Tokura Int. J. Biol. Macromol., 16, 297(1994)
(6) N. Sakairi, S. Suzuki, K. Ueno, S-M. Han, N. Nishi, S. Tokura, Carbohydr. Polym., in presss.