Transaction

Creation of Bacterial Cellulose-Fabric Complexed Material

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Abstract：The acetic acid bacterium *Gluconacetobacter xylinus* synthesizes a unique cellulose molecule called bacterial cellulose (BC) as an extracellular polysaccharide. The cellulose ribbons of BC are very fine and form a three-dimensional knitted structure. To add properties to the surfaces of fabrics, we produced hybrid materials composed of BC and fabric by culturing *G. xylinus* with fabrics as scaffolds. Nine kinds of fabric were soaked in medium, and then *G. xylinus* was statically cultured with the fabric at 25 ℃. After the cultivation, the fabrics were purified by alkaline treatment and press-dried. BC displayed greater affinity for cotton and viscose rayon than for wool, silk, nylon, polyester, and Kevlar®. The surfaces of all fabrics were uniformly coated with a BC sheet and became shiny. However, when viscose rayon was used and the fabric was inverted during the cultivation process, both surfaces were coated with the BC sheet. It is suggested that the *G. xylinus* cells adhered to the surface of the fabric and produced BC in the vicinity of the air-water interface. During the inversion, the cells entered the interior of the fabric, and the resultant BC sheets surrounded each fabric.

1. Introduction

Cellulose is one of the most abundant organic macromolecules in the biosphere and has maintained a dominant position in many material fields including the pulp, paper, and textile industries. It is synthesized by a great diversity of living organisms [1]. While most cellulose is produced as a plant cell wall component, some other organisms such as bacteria, insects, and animals can also produce cellulose. The cellulose produced by bacteria is called bacterial cellulose (BC) and has a different structure and mechanical properties from plant cellulose. For instance, it possesses an extremely fine and pure fiber network structure. The microfibrils extruding from bacterial cells, whose diameter ranges from 2 to 4 nm, are bundled together, forming cellulose ribbons. Furthermore, these cellulose ribbons are randomly assembled depending on the movement of each bacterium, resulting in a complex network structure. These networks generate high hydrophilicity as well as high tensile strength. The water retention of a never dried cellulose pellicle leads to values in the range of 1000%, while the value of cotton linter, a plant cellulose, is about 60% [2]. On the other hand, when the cellulose pellicle is press-dried, the cellulose ribbons interact strongly through hydrogen bonds, and the pellicle

is converted into a sheet with high tensile strength.

Recently, various attempts have been made to create natural and artificial polymer complexes such as bio-composites and modified polymers. Due to its high biocompatibility, BC has been used to produce composites with collagen type I [3] or hydroxyapatite [4]. These compounds are expected to be useful for medical applications such as the treatment of chronic wounds [3] and as a scaffold for the tissue engineering of cartilage [5]. Moreover, a composite of BC and multiwalled carbon nanotubes has been reported to enhance electrical conductivity [6], and the applications of BC are expected to expand in the near future.

Gluconacetobacter xylinus is the most common cellulose producing bacterial species and is used as a model bacterium of cellulose biosynthesis. Juntaro et al. reported the nanocomposite composed of bacterial cellulose and sisal faibers by the co-cultivation with *G. xylinus* [7, 8]. In this composite, BC was introduced as a nanoscale reinforcement by attaching it to the surface of sisal fibers. In this study, to create a new bio-composite composed of BC and various fabrics, *G. xylinus* was cultured on fabric soaked in culture medium.

2. General instructions

2.1 Bacterial strain

G. xylinus ATCC53582 was used in this study and was grown in Schramm-Hestrin (SH) medium [9], which contained 20 g/L glucose, 5.0 g/L yeast extract, 5.0 g/L polypeptone, 0.27 g/L $Na_2HPO_4 \cdot 12H_2O$, and 0.115 g/L citric acid, at 25 ºC.

2.2 Fabrics

As model fabrics, pieces of JIS Test Fabric-Multifiber (Standard adjacent fabrics for staining of colour fastness test, JIS L 0803) composed of wool, silk, cotton, acetate, nylon, or polyester were used together with two non-woven fabrics, viscose rayon (8890CR, 93 g/m^2, Japan Vilene) and Bemlise® (110 g/m^2, Asahi Chemical Co. Ltd.). The size of each fabric was 8 cm × 8 cm. To increase the thickness of the bio-composite, four sheets of viscose rayon were layered and used as scaffolds.

2.3 Modification of fabrics

The cellulose pellicles obtained from the subcultures were added to 40 mL of SH medium. Then, 1 mL of Celluclast® (Novozymes), which was diluted 2-fold with 10 mM sodium acetate buffer (pH5.0) and then filtrated using an syringe filter (0.45 μm, ADVANTEC), was added to the culture medium and rotary cultured at 115 rpm and 25 ºC for 3 days. After centrifugation, the collected cells were suspended in 200 mL of SH medium, and the turbidity of the suspension (OD660) was adjusted to 0.13 with sterilized SH medium. Forty ml of this suspension were dispensed into a Petri dish (140 × 100 × 15.5 mm). Then, fabrics that had been preliminarily sterilized by autoclaving at 121 ºC for 20 min were soaked in the suspension, and static cultivation was carried out at 25 ºC.

2.4 Purification of the modified fabrics

After the cultivation, the modified fabrics were separated and washed using deionized water, before being soaked in 2wt% NaOH solution for 1 day to remove the bacterial cells and the other components of the culture medium. After the modified fabrics had been washed with deionized water to neutralize them, they were dried at 55 ºC.

2.5 Field emission scanning electron microscopy (FE-SEM)

The modified fabrics were observed using an S-4100 field emission scanning electron microscope (Hitachi Ltd.) at high magnification. After being purified with NaOH, the dried samples were sputter-coated with gold using a twin coater JEC-550 (JEOL). The spattering of gold particles was carried out at 1.5 kVA for 1.5 min.

Table 1 Affinity of BC for various fabrics

Cloth type	Affinity
Fabric	
Wool	-
Silk	-
Cotton	+
Cellulose acetate	-
Nylon	-
Polyester	-
Kevlar®	-
Nonwoven fabric	
Viscose rayon	++
Bemlise®	++

+: weak interaction, ++: strong interaction

2.6 Measurements of the dry weight of BC and the glucose concentration

The dry weights of the BC and fabric composites were measured after they had been purified with 2wt% NaOH and dried at 55 ºC. The net weight of dried cellulose was calculated by subtracting the dry weight of the fabric before its cultivation from the total dry weight of the cellulose/fabric complex. The glucose concentration of the culture medium was assayed according to the mutarotase-glucose oxidase/peroxidase method using a Glucose CII-test WAKO (Wako).

3. Results and discussion

3.1 Affinity of BC for the fabrics

To examine the affinity between BC and the various fabrics, six kinds of fabric, wool, silk, cotton, cellulose acetate, nylon, and polyester, and two types of non-woven fabric, viscose rayon and Bemlise®, were simultaneously cultured with *G. xylinus* in the culture medium. A cellulose pellicle is usually produced at the air-liquid interface of the culture medium by static cultivation under normal conditions without fabrics. When the fabric was completely immersed in the culture medium, a cellulose pellicle was only produced at the surface of the culture medium, and no cellulose fibers were observed in the fabric. Thus, it was necessary to adjust the amount of culture medium so that there was just enough to cover the fabric.

The affinity of BC for the fabrics is described in Table 1. Although cellulose pellicles were produced on the surfaces of all fabrics, they were easily peeled away from the non-cellulose fabrics ; i.e., wool, silk, nylon, polyester, and Kevlar® during the NaOH washing step. Since wool and silk are protein fibers, they might have been denatured by the alkali treatment with 2% NaOH.

For polyester, the BC pellicle covered the surface of the fabric but did not display any interaction with fabric due to the low water adsorption ability of polyester. On the other hand, the fabrics derived from cellulose such as cotton, viscose rayon, and Bemlise® were coated with cellulose fibers and could not be peeled away after the washing step. In particular, the non-woven fabrics ; i.e., viscose rayon and Bemlise®, showed high affinity for the BC.

3.2 Modification of fabric surface

Cotton was cultivated in SH medium for 3 days and observed by FE-SEM (Fig. 1). Fig. 1a shows the surface of the cotton before the extraction of cell bodies with a 2% NaOH solution. Bacterial cells were attached to the surface of the cotton fabric and had produced cellulose fibers. After the purification and press-drying steps, a cellulose pellicle was produced on the surface of the cotton fabric. After the press-drying, the cellulose-coated surface was very shiny and smooth. Fig. 1b shows a tear in the BC sheet coating the fabric surface. Since the *G. xylinus* cells mainly moved across the surface of the cotton fabric as a cellulose producing scaffolding, it was assumed that the BC pellicle produced at the surface was converted to a comparatively solid and thick BC sheet by the press-drying.

On the other hand, BC fibers were also formed inside the fabric close to the surface (Fig. 1b, 1c). The width of the BC fibers ranged from 40-100 nm, whereas that of the cotton fibers was about 10 μm. Although the density of BC fibers was lower than those produced at the surface, a three-dimensional knitted structure was formed that connected each cotton fiber. It is suggested that this knitted structure contributes to the adhesion of the BC sheet to the fabric surface.

3.3 Effect of fabric inversion during cultivation

In the experiment using cotton fabric described above, BC modification was only observed on the surface facing the air-liquid interface. To modify both surfaces of the fabric, it was inversed during the cultivation. Since the spaces between the fibers of the non-woven fabrics were not tight compared with those of the woven fabrics, *G. xylinus* cells were able to enter the interior of the fabric. Thus, a stack of non-woven fabrics ; i.e., four layered pieces of non-woven viscose rayon fabric, was cultivated with *G. xylinus* for 3 days. After only 1 day, the surface of the fabric stack had already been coated with a BC sheet (Fig. 2a). The fabric stack was then inverted and cultivated for an additional 4 days. When a stack of viscose rayon fabrics was cultured for 7 days without inversion, only the surface that was initially
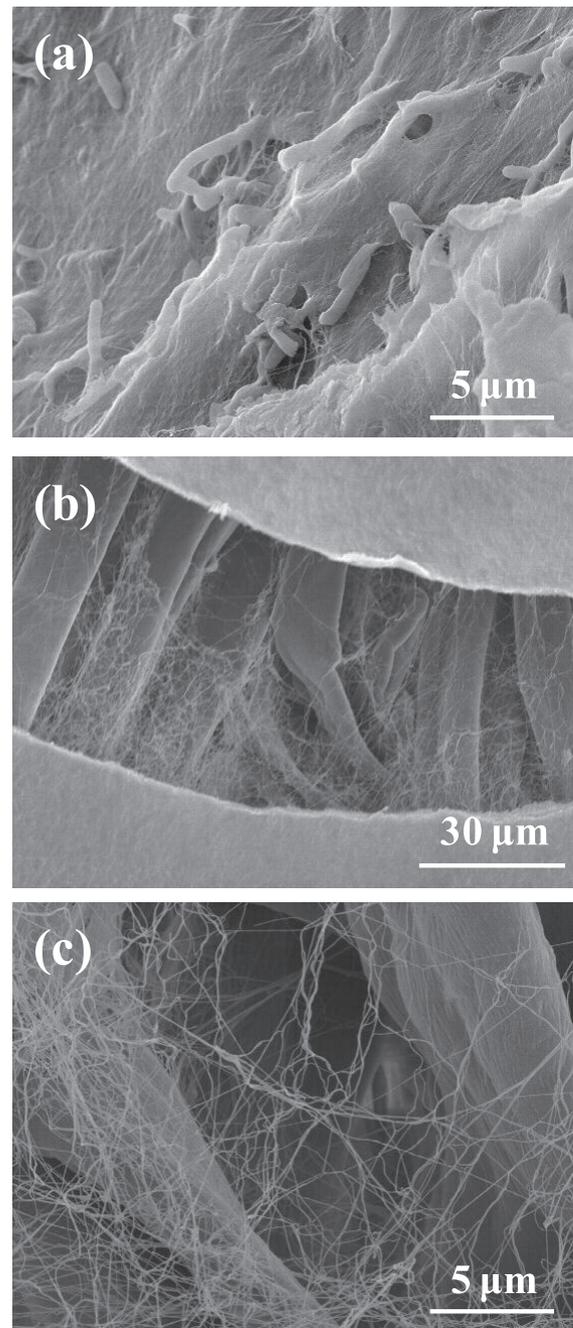

Fig. 1 SEM images of the surface of the cotton fabric modified with BC. (a) After 3 days cultivation, bacterial cells had become attached to the fabric surface. (b) A nick observed in the fabric surface, which has been covered with a BC sheet, after purification with NaOH. (c) Three-dimensional knitted structure of the BC (thin) and cotton fibers (thick).

facing the liquid was fully coated with a BC sheet (Fig. 2b). Because the fabric stack was not separated into each viscose rayon fabric after cultivation, it is thought that bacterial cells moved through the interior of the fabric stack with producing cellulose. Although BC was observed on the bottom surface, the amount was significantly lower than that on the top surface (Fig. 2e).
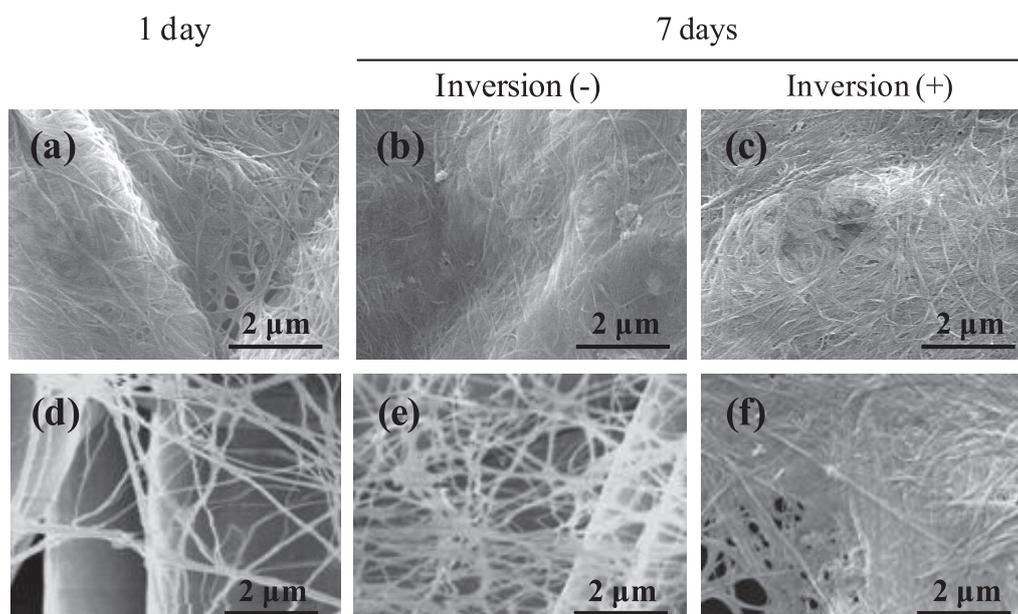
1 day · 7 days

Inversion (-) · Inversion (+)

(a) · (b) · (c)

2 µm · 2 µm · 2 µm

(d) · (e) · (f)

2 µm · 2 µm · 2 µm

Fig. 2　SEM images of the surface of the non-woven fabric viscose rayon after its modification with BC. The rayon fabrics were soaked in the culture medium and cultured for 1 day and 7 days. In 7 days cultivation, the fabrics were cultured without inversion and with inversion after the first 3 days. Images of the initial upper surface of the fabric are shown in (a), (b), and (c), and those of the initial bottom surface are shown in (d), (e), and (f).

On the other hand, when the stack of viscose rayon fabrics was inverted at 3 days, both surfaces were uniformly coated with a BC sheet (Fig. 2c, 2f).

In the first stage, *G. xylinus* cells stayed on the surface near to the air-liquid interface ; i.e., the initial upper surface, and produced a cellulose pellicle. After the inversion of the stack, it was assumed that the cells on the bottom surface moved through the interior of the fabric to the new upper surface ; i.e., the initial bottom surface, in order to remain in the vicinity of the air-liquid interface. As *G. xylinus* produced BC in the interior of the viscose fabric during the inversion treatment, each of the four pieces of viscose rayon fabric was tightly connected with BC fibers after cultivation for 7 days. The resultant bio-composite was compressed to a thickness of 2 mm by press-drying and had a moderate hardness that the unmodified viscose rayon fabric did not possess (Fig. 3). Interestingly, the dried bio-composite composed of BC and non-woven viscose rayon showed water absorbability and recovered its initial thickness (about 7 mm) after being soaked in solution. It is suggested that the BC fibers produced in the interior of the viscose rayon fabric acted as adhesive fibers connecting each viscose rayon fiber and producing a spring like structure.

To observe the interactions between BC and viscose rayon fibers, SEM analysis of the bio-composite produced by the cultivation with inversion treatment was carried out on different locations from the surface to the inside area. The BC ribbons directly interacted with the viscose rayon fibers and held them together (Fig. 4a). This morphology was frequently observed on the surface of the fabric and in the vicinity of the surface. A cross-section of a stack of viscose fabric is shown in Fig. 4b and 4c. Meanwhile, a distinctive BC sheet morphology was observed in the internal region of the stack. The BC surrounded the viscose rayon fibers and its cross-section view looks like irregular honeycomb pattern. It is considered that this irregular structure was formed during the translocation of the *G. xylinus* along the viscose rayon fibers after the inversion of the fabric.
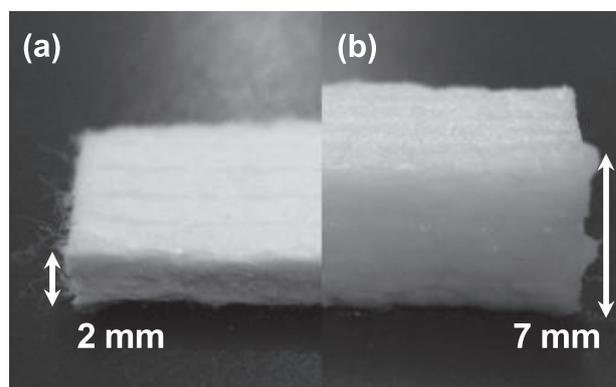

(a) · (b)

2 mm · 7 mm

Fig. 3　Water absorbability of the dried bio-composite composed of BC and non-woven viscose rayon fabric. (a) The dried bio-composite composed of BC and non-woven viscose rayon fabric. The bio-composite was about 2 mm thick. (b) After water absorption, its thickness recovered to 7 mm.
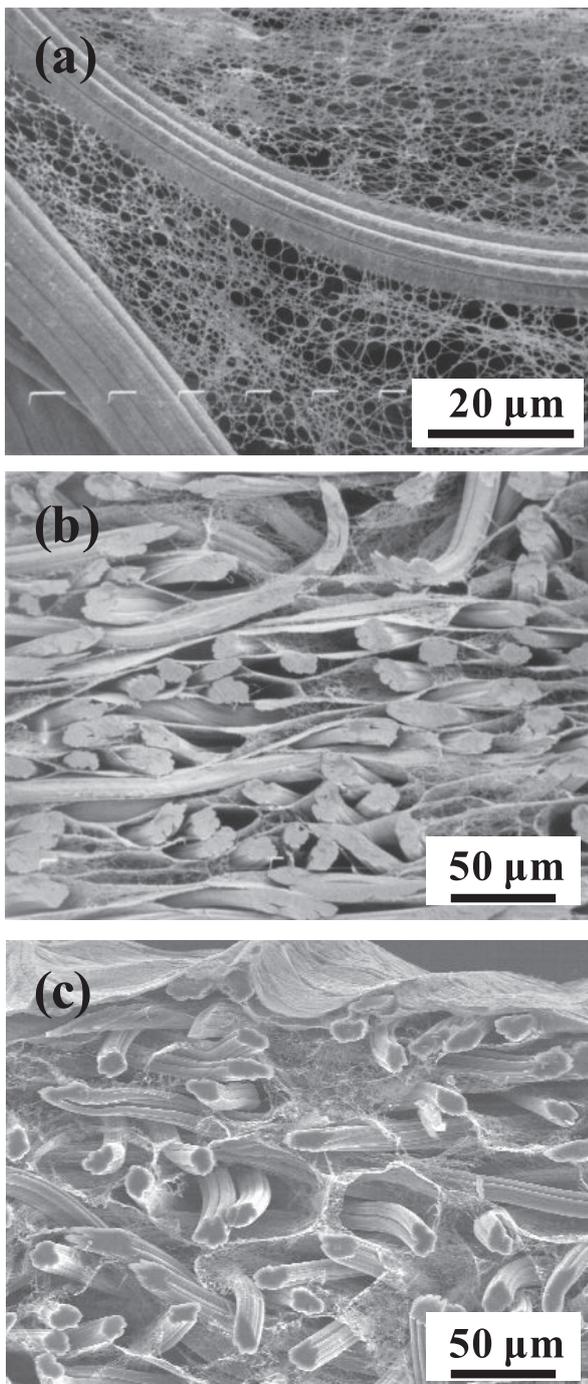
Fig. 4 SEM images of the bio-composite of BC and non-woven viscose rayon fabric. (a) The morphology of the BC in the vicinity of the bio-composite surface. (b) and (c) The BC produced in the interior of the rayon fabric. The BC sheet surrounded the rayon fibers.

3.4 Changes in the pH and glucose concentration of the medium and the production of BC

As mentioned above, we found that it was possible to modify both surfaces of the fabric by inverting it during the cultivation. The time courses of the glucose concentration and pH of the culture medium, in addition to that of BC production are shown in Fig. 5. While nearly 75 mg of cellulose were produced by *G. xylinus* in the absence of viscose rayon fabric, the amount of cellulose was increased by about 3-fold by the addition of fabric (Fig. 5a). In particular, when the fabric was inverted at 3 days, about 270 mg of cellulose were obtained, and the conversion rate relative to the amount of glucose present reached 37%. In the static cultivation of *G. xylinus* using SH medium, the conversion rate was approximately 10-15%. Thus, the addition of fabric to the culture medium as a scaffold material increased BC production.

The glucose contained in the culture medium was consumed within the first two days (Fig. 5b), and the pH of the culture medium dropped rapidly to about 3.5 as the glucose concentration decreased (Fig. 5c). While the pH of the medium during the cultivation of *G. xylinus* without fabric gradually increased to 4.2 over 4 days, a rise in the pH to 5.2 was observed when the *G. xylinus* cultivation was performed with fabric. It has been reported that glucose is converted to gluconic acid by oxidative enzymes such as glucose oxidase and glucose dehydrogenase bound to the membrane of *G. xylinus* [10, 11], resulting in a decrease in the pH. Subsequently, the pH is increased, since gluconic acid is taken into the cell and metabolized to cellulose via the pentose phosphate cycle and Entner-Doudoroff pathway [12]. It is considered that the consumption of gluconic acid during the cultivation of *G. xylinus* with fabric accelerates cellulose fiber production rather than basal metabolism or energy production. However, the reason for the change in the metabolism of gluconic acid remains unclear.

4. Conclusions

In this study, a non-woven fabric made of viscose rayon was found to be the most suitable scaffold for creating a bio-composite composed of BC and fabric. We were able to coat the surface of the fabric with a BC sheet by adjusting the amount of culture medium, and inverting the fabric during the cultivation procedure allowed us to modify both surfaces of the fabric. Furthermore, BC modifications were also observed inside the fabric. As a result, after being press-dried, the bio-composite composed of BC and layered fabric sheets had a shiny surface, physical intensity, and high water absorption ability. The moderate hardness of the dried bio-composite allows it to be subjected to compression molding, and the original form of products composed of this material could be restored through water absorption. Bio-composites are expected to be employed in various fields, for example, as medical materials.
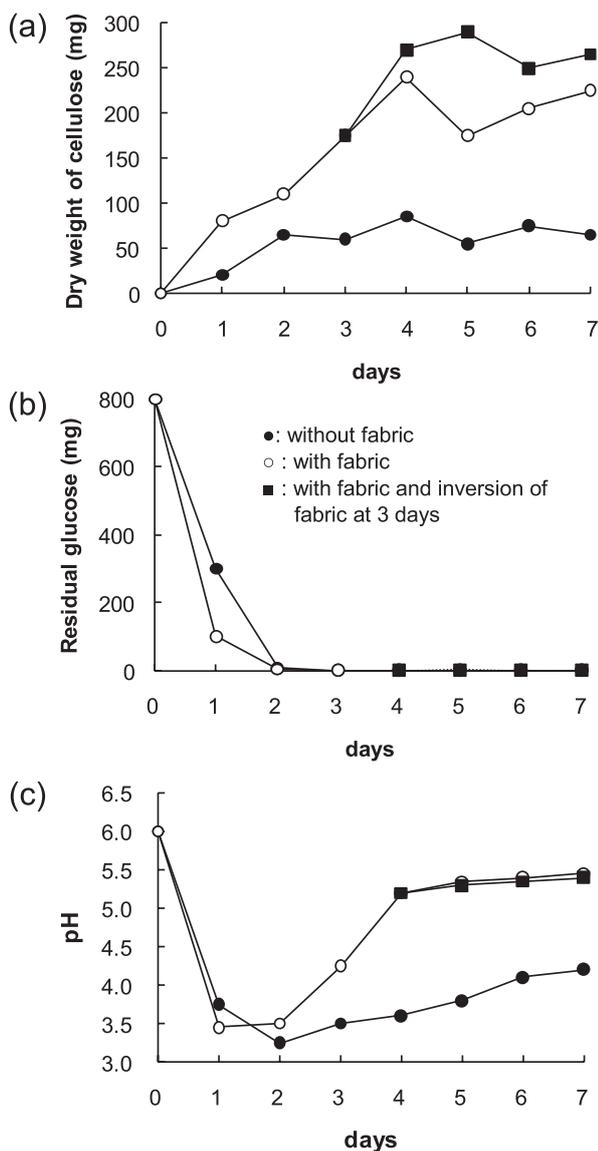
(a)

(b)

● : without fabric
○ : with fabric
■ : with fabric and inversion of fabric at 3 days

(c)

Fig. 5 Time courses of BC content, glucose concentration, and pH. (a) Dry weight of cellulose after purification. (b) Residual amount of glucose in the culture medium. (c) pH of the culture medium.

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