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PRODUCTION OF CELLULOSE NANOFIBER AND NANOCRYSTALS BY WATER JET SYSTEM

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ABSTRACT

Cellulosic biomass is the most abundant organic compound on earth. The technology for producing cellulose nanofiber and nanocrystal from cellulosic biomass is one of the key to biomass applications. Some technologies for atomizing biomass without strong acid catalysis remain to be developed. Star burst system is one of the atomizing machine using the water jet device that use high-pressure water. On the other hand, the hyperthermophilic endo-type cellulase obtained from archaea can be used for the saccharification of amorphous cellulose in biomass at high temperature. Using star burst system and the hyperthermophilic endo-type cellulase, the production of cellulose nanofiber and nanocrystals can be carried out.

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INTRODUCTION

Cellulose is the most abundant organic compound on earth, and the main building block of trees and plants. Cellulosic biomass has the potential to be a long-term sustainable resource for energy use and chemicals (1). The challenge for scientists is to access this biopolymer and convert it into useful materials or fermentable sugars. An environmentally low-impact pretreatment procedure for producing cellulosic useful materials represents a key technology for the application of cellulosic biomass. Cellulose nanofiber (CNF) and cellulose nanocrystals (CNC) produced from cellulosic biomass exhibit high potential for new materials with notable chemical, optical and electrical properties (2, 3).

Technologies that can economically convert biomass resources into commercially viable materials will be important for the application of biomass. The water jet is a well-known device that is used in machines (e.g., washing machines, cutters, and mills) that use high-pressure water. Recently, Star Bust System was developed for the atomization of materials using a high-pressure water jet system by Sugino Machine Limited (Toyama, Japan) (4). This system places low stress on the environment and provides a new technology for wet atomizing ceramics, stones, crystal, organisms, etc. On the other hand, the hyperthermophilic endo-type cellulase from archaea has been found and studied (5,6) in AIST (Osaka, Japan). In this study, we examined the application of the system to produce CNF and CNC from cellulosic biomass with the aid of the hyperthermophilic endo-type cellulose.

METHODS

Materials

Micronized crystalline cellulose powder Ceolus PH-102 (average particle size 90μm) was supplied by Asahi Kasei Chemicals Corporation (Tokyo, Japan). For the other chemicals, reagent grade were used.

Cellulose atomized by Star Burst System (SBS)

Approximately 200 g of cellulose micronized powder was suspended in 49 times weight of distilled water assisted by a powerful stirring device and placed in the feed tank of the Star Burst System HJP-25080, which was used for suspension jet collision (4). The aqueous cellulose suspension from the feed tank was injected from small nozzles at high speed. The machine automatically permits repeated super high-pressure collision treatments and our samples received 20 collisions (cycles).

Preparation hyperthermophilic cellulases

The hyperthermophilic endo-type cellulase (EGPh, Gene ID: PH1171) from hyperthermophilic archaeon *Pyrococcus horikoshii* was used in this study. The recombinant EGPh was prepared and purified by the methods as follows (5, 6). The recombinant enzyme was expressed in *E. coli* BL21(DE3) cells (Novagen, Madison, Wisconsin) from the T7 promoter of pET11a (Novagen). Cell cultures were grown at 37°C in Luria Broth with 100 mg/mL ampicillin until OD_{600} reached 0.8, and IPTG was added to a final concentration of 0.1 mM for the protein induction. The both enzymes were purified by ammonium sulfate fractionation after heat treatment (30 min at 80°C) and eluted through a HiTrapQ anion exchange column. After confirming the purity of the proteins using SDS-PAGE, the protein concentration of EGPh was determined from UV absorbance at 280 nm, using 136270 as the molar extinction coefficient calculated from their protein sequences, respectively.

Enzyme saccharification

Sacchrification of the cellulose (hyperthermo philic endo-type cellulase) by the cellulase enzyme was examined in 20 mM sodium acetate buffer (pH 5.5) at 85°C. The enzyme reaction was carried out by adding the enzyme solution to the 1% Ceolus suspension (20 mM sodium acetate buffer, pH 5.5).

Electron microscopy

The quick-freeze and deep-etch replicas were prepared as described (7). The samples of cellulose in water with the SBS treatment were quickly frozen by the metal-contact method using liquid helium. The frozen materials were freeze-fractured and deep-etched, and then the exposed surfaces were rotary-shadowed with platinum/carbon at an angle of 25° by using a freeze-replica apparatus (BAF400D, Balzers). The procedure did not include any sample drying processes that might cause re-aggregation of dispersed fine fibers, accurately replicating the structure of the samples dispersed in water. The replicas were observed with HAADF-STEM using Tecnai G2 F20 (FEI Co.) operated at 200 kV. The observation by STEM has an advantage in obtaining images with higher contrast than that by the conventional transmission electron microscopy.

X-ray analysis of the cellulose

The effect of the SBS and enzyme treatments on the crystallinity of the cellulose was examined with X-ray diffractometry using a Rigaku RINT-TTR III diffractometer. The crystallinity was determined and analyzed from the charts by using the method described previously (8).

RESULTS AND DISCUSSION

Application of Star Burst System to crystalline cellulose

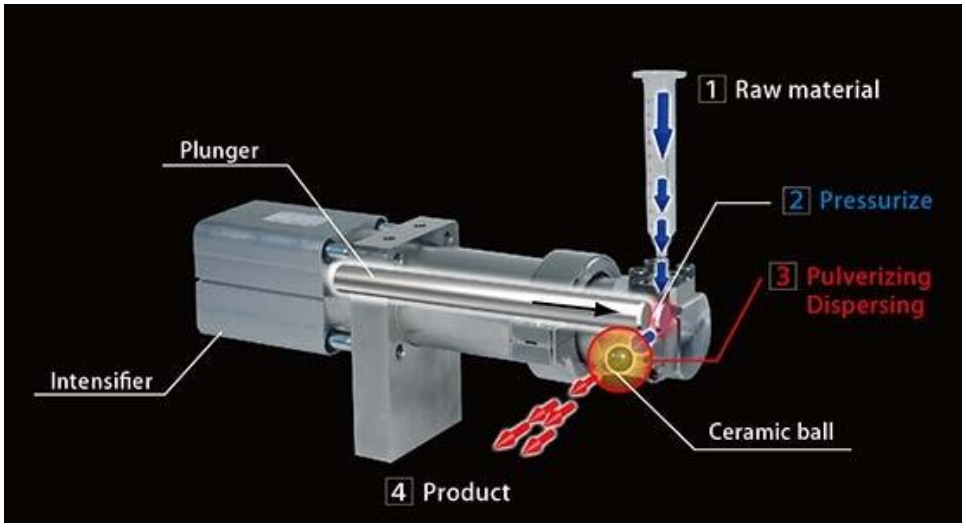

Figure 1. Illustration of SBS.

Sugino Machine has constructed a unique wet atomizing system (Star Burst System; SBS (Figure 1)) using super high pressure water jet system (4). To examine the potential effect of the SBS on biomass materials, we applied the SBS to crystalline cellulose powder (Ceolus PH102) suspended in water (1% w/v). The crystalline cellulose suspension was then fed to SBS. Under the super high pressure (245 MPa), the cellulose suspension was atomized by the SBS. After the treatment, the suspension containing the crystalline cellulose powder was converted into a gel-like form that exhibited remarkably high viscosity (Figure 2).

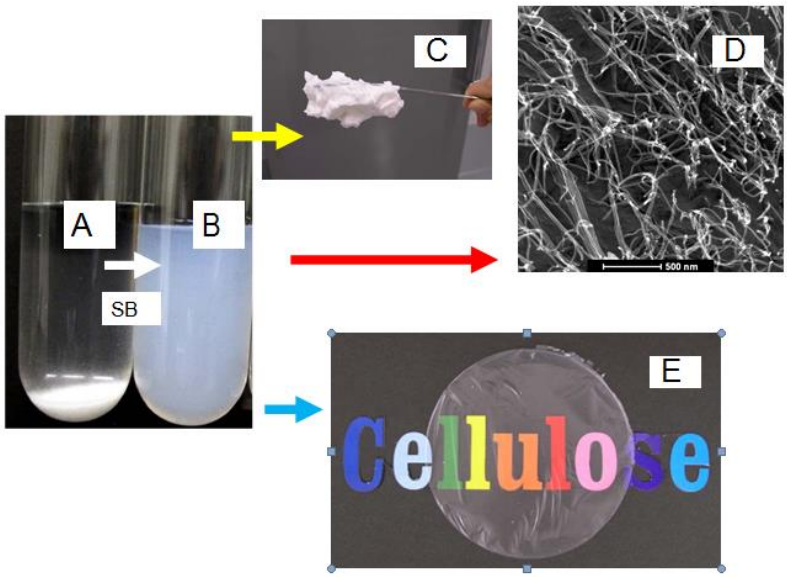

Figure 2. Crystalline cellulose in water and cellulose film. 1% of crystalline cellulose (A) in water and 1% of gel-like cellulose (cellulose nanofiber) (B) prepared by the treatment of SBS. 15% of gel-like cellulose prepared by the treatment of SBS (C). The image of cellulose nanofiber in water reconstructed by HAADF-STEM (D). Cellulose film prepared from 1% of gel-like cellulose (E).

Figure 2 shows images of the cellulose powder suspended in water before (Figure 2A) and after (Figure 2B) the SBS treatment. Using the SBS, we were able to atomize the crystallized cellulose solution at concentrations of up to 15% (w/v) (Figure 2C). This gel-like cellulose is stable and shows no degradation for several months at room temperature. The image of gel-like cellulose in water reconstructed by HAADF-STEM (Figure 2D). This image indicates that cellulose nanofiber can be prepared from cellulose powder by the SBS. Furthermore, we were able to prepare a transparent cellulose film (Figure 2E) from the gel-like cellulose by

drying it at room temperature. The effect of the SBS treatment on the crystallinity of the cellulose was examined with X-ray diffractometry using a Rigaku RINT-TTR III diffractometer. The main crystalline structure was not influenced significantly by the SBS (data not shown).

Saccharification by cellulase to produce cellulose nanocrystals

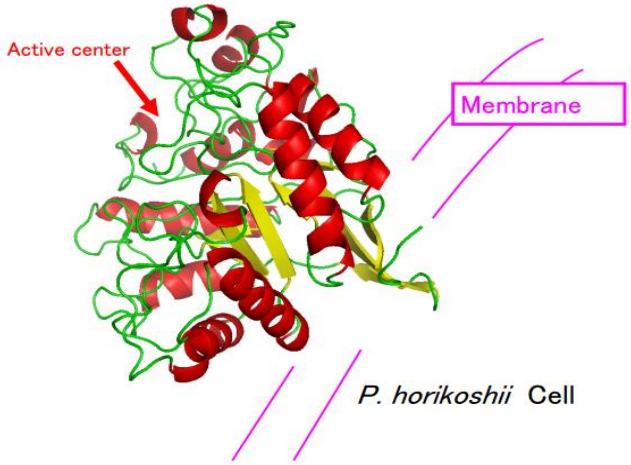

Figure 3. Structural model of hyperthermophilic endo-type cellulase from *P. horikoshii*.

We examined the saccharification of the cellulose nanofiber prepared by the SBS. The amorphous part of cellulose can be saccharified by the endo-type cellulase. *Pyrococcus horikoshii* is one of the hyperthermophilic archaea collected from hydrothermal vent and its membrane was displayed by the hyperthermophilic endo-type cellulase (Figure 3) (6). The enzyme can saccharify the amorphous part of cellulose. The endo-type cellulase (EGPh) from *P. horikoshii* was prepared and used for the sacchrification of the cellulose nanofiber. Figure 4 shows the picture of the cellulose by the treatment of the SBS and EGPh in water. The powder of crystalline cellulose can be converted to cellulose nanofiber and the cellulose film can be prepared from cellulose naofiber (Figure 4). By the saccharification of cellulose nanofiber by EGPh, the gel-like cellulose was converted to clear solution. And the cellulose film can not be prepared from the solution (Figure 4). The crystallinity of the cellulose was not influenced significantly by the saccharification of EGPh (data not shown). Generally crystal part of the cellulose nanofiber was not dedradeted by EGPh. Therefore it indicated that the clear solution contains the water soluble crystal part of the cellulose. The solution must contain the cellulose nanocrystal.

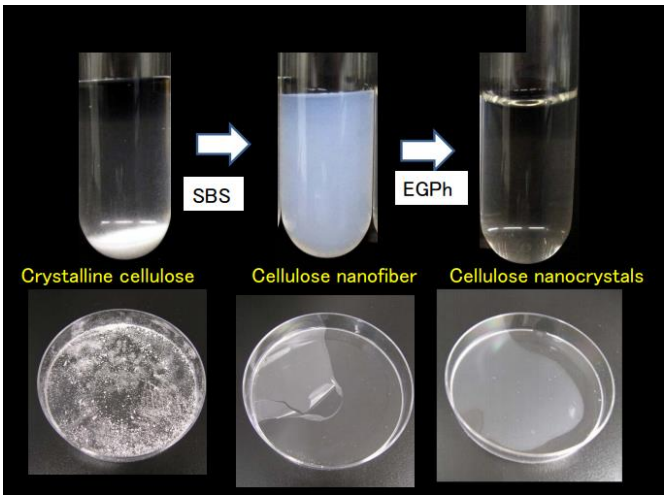

Figure 4. Pictures of the cellulose in water. The powder of crystalline cellulose can be converted to cellulose nanofiber (CNF) and the cellulose film by SBS. The cellulose nanofiber was saccharified by EGPh and converted to the cellulose nanocrystals (CNC).

CONCLUSION

The SBS made by Sugino Machine is a unique system for atomizing materials. With the SBS, we were able to atomize crystalline cellulose at concentrations of up to 15% (w/v). The crystalline cellulose can be converted into a gel-like form (cellulose nanofiber (CNF)) by the system. In addition, cellulose nanocrystals (CNC) can be prepared by using endo-type cellulase

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