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Optimal reaction conditions for modifying dialdehyde bacterial cellulose

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Abstract

Bacterial cellulose (BC) has been extensively studied and applied as scaffold in tissue engineering due to its unique properties such as high mechanical strength, high water adsorption capacity, non-toxicity and biocompatibility. However, non-biodegradability *in vivo* of BC is a main drawback which limits its application. Strong oxidizing agent such periodate is used to oxidize the secondary hydroxyl groups of BC (locating at C2 and C3) to form aldehyde groups for reducing its strongly crystalline structure and improving its biodegradability. Unfortunately, oxidizing agents are costly and many parameters affect the periodate oxidation reaction. The aim of this research is to optimize the reaction conditions such as mole ratio of BC and periodate, temperature and reaction time for obtaining the highest aldehyde content in dialdehyde bacterial cellulose (DBC). Chemical and physical properties of DBC were characterized by Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscope (SEM). After periodate oxidation, DBC with 89% of aldehyde content was obtained using 1:2 of mole ratio between BC and periodate at 60°C for 6 h. The FTIR bands at 1729 cm^{-1} of aldehyde functional group and at 886 cm^{-1} of hemiacetal bonds between aldehyde groups and neighboring hydroxyl groups were noticed in DBC spectrum. Moreover, fibrils of DBC were much smaller and shorter than those of BC.

Keywords: Bacterial cellulose, Dialdehyde bacterial cellulose, Periodate oxidation

Introduction

BC, a natural polymer, is composed of D-glucose by β-1,4 linkages. BC can be produced by gram-negative bacteria such as *Gluconacetobacter*, *Acetobacter*, *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Sarcina*, *Azobacter*, *Rhizobium*, *Pseudomonas*, *Salmonella* and *Alcaligenes* [1]. During bacteria metabolism, sub-elementary fibrils are extruded through cell membrane and then aggregate to form cellulose ribbon in a web-like network structure. Consequently, BC is chemically pure (without hemicelluloses and lignin), high crystallinity index (above 60%), high tensile strength in both dry and wet states and high water holding capacity [2].

BC has been successfully applied in various fields such as food industries, separation process and paper products. Moreover, BC has been used in medical applications as an artificial skin for temporary covering of wounds and artificial blood vessels [3]. Currently, BC has been interested to use as scaffold in tissue engineering. BC scaffold is claimed that various human cells such as embryonic kidney cells, osteoblasts cells, fibroblast cells and smooth muscle cells can grow and proliferate on the matrix of BC scaffold [4]. However, BC is not biodegradable in the human body which limits its applications in tissue engineering.

In order to improve biodegradability of BC, DBC is synthesized by oxidation reaction using periodate as an oxidizing agent. Periodate oxidation is performed by cleavage of the C2-C3 bond to form the two aldehyde groups per one glucose unit [5]. The aldehyde functional groups locating at C2 and C3 of DBC reduce its crystallinity and improve its biodegradability [6]. To obtain DBC with high aldehyde content, many parameters involving in the oxidation must be studied. The aim of this research is to optimize the reaction conditions such as mole ratio of BC and periodate, temperature and reaction time for obtaining the highest aldehyde content of DBC. Chemical and physical characteristics of DBC were examined.

Materials and methods

Materials

BC pellicles synthesized by *Acetobacter xylinum* AGR 60 were kindly provided by Pramote Thammarad from the Institute of Research and Development of Food Product, Kasetsart University, Bangkok, Thailand. Sodium metaperiodate (98% purity) was purchased from Fisher Scientific.

Preparation of BC

BC pellicles were treated with 1 wt.% NaOH and washed with DI water until they were neutral. Then, BC pellicles were disintegrated by a blender to obtain BC slurry.

Preparation of DBC

150 g of BC slurry was mixed with sodium metaperiodate in different mole ratios (1:1 and 1:2). The mixture was stirred by an overhead stirrer and controlled at 40-60°C for 2-6 h. The reaction was conducted in dark. After that, oxidized cellulose was rinsed with DI water to remove excess of periodate and then was homogenized by ultrasonic homogenizers (SONOPULS HD 2200 homogenizer, BANDELIN, Germany) to obtain DBC.

Determination of aldehyde content

The aldehyde content of DBC was evaluated by converting the aldehydes to oximes by a Schiff base reaction with hydroxylamine hydrochloride. 0.1 g dry weight of DBC slurry was mixed with 30 ml of DI water and was adjusted pH to 4. Then, 25 ml of 0.25 M hydroxylamine hydrochloride was added into the mixture. The mixture was stirred at room temperature. After 24 h, the mixture was titrated with 0.1 M of sodium hydroxide solution using methyl orange as an indicator. A blank was also performed using native BC. The aldehyde content (AC%) was calculated by the following equation.

$$AC\% = [M_{NaOH} (V_s - V_b) 160 \times 0.1]/ m \quad (1)$$

M_{NaOH} is concentration of sodium hydroxide solution (Molarity), m is dry weight of DBC (g), V_s and V_b are sodium hydroxide consumptions (ml) of the sample and the blank, respectively, and molecular weight of DBC is 160 g/mole [7].

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of DBC and BC were performed by Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy (Spectrum One, Perkin Elmer, USA). in the wavelength range of 4000-515 cm^{-1} at a resolution of 2 cm^{-1}.

Morphology

Morphological structure of DBC was investigated by Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectrometer (JSM-IT-500HR, JEOL and JED-2300, JEOL, Tokyo, Japan). DBC was sputtered with gold and then was photographed.

Results and discussion

Aldehyde content of DBC

The conditions of periodate oxidation were optimized in this study to obtain DBC with the highest aldehyde content. The oxidation reaction of BC using periodate as an oxidizing agent were illustrated in figure 1. Effects of mole ratio of BC and periodate, reaction temperature and time were examined. The aldehyde content of all conditions are summarized in figure 2. For BC and periodate mole ratio of 1:1, the aldehyde content of DBC were only in the

range of 10-37% even the temperature and time increased. It was obvious that the aldehyde content of DBC significantly increased when periodate mole was twice of BC mole.

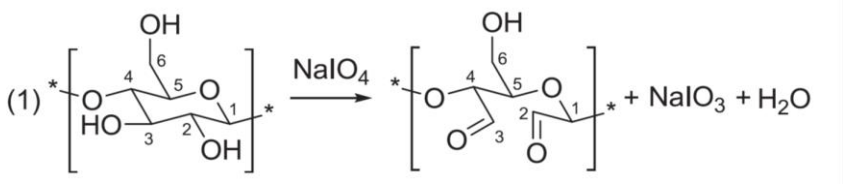

Figure 1 Schematic diagram of periodate oxidation of BC [10]

High periodate concentration in the reaction could produce numerous anions (IO_4^-) which rapidly oxidized the secondary hydroxyl groups of BC resulting in the aldehyde content up to 89% of DBC synthesized by mole ratio of 1:2 at 60°C for 6 h. Keshka et al. (2015) [8] was also obtained DBC with 82.9% aldehyde content by using 1:2 of mole ratio at 55°C for 2 h.

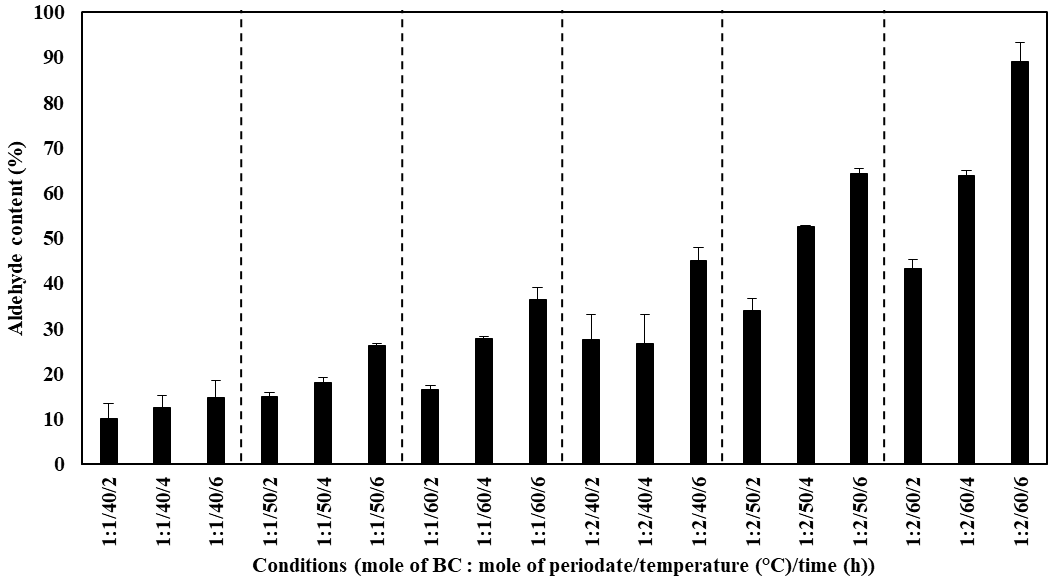

Figure 2 Aldehyde contents of DBC oxidized under different conditions

Effect of reaction temperature was investigated. Results demonstrated that the aldehyde content increased when temperature increased. Liua, X., et al. [9] reported that a higher temperature could accelerate the rate of periodate oxidation. However, there was not significant difference of the aldehyde contents of DBC oxidized under mild conditions (mole ratio of 1:1 and duration of 2 and 4 h). Low yield of fiber and decomposition of periodate were observed at reaction temperature over 85°C [10]. For reaction time, no effect of longer duration for mild conditions (mole ratio of 1:1 and reaction temperature of 40 °C). Longer duration could increase the aldehyde content of DBC, especially under strong conditions such as 1:1/60°C, 1:2/50°C and 1:2/60°C. It could be concluded that the condition of mole ratio between BC and periodate of 1:2, reaction temperature of 60 °C and reaction time of 6 h produced DBC with the highest aldehyde content of 89%.

FTIR analysis

FTIR spectra of BC and DBC are shown in figure 3. The characteristic bands of BC were observed at 3341 cm^{-1} of OH stretching, 2896 cm^{-1} range of C-H and 1107 cm^{-1} of C-O stretching [8]. New peaks at 1729 cm^{-1} and 886 cm^{-1} of DBC were noticed which represented aldehyde group (C=O), and formation of hemiacetal bonds between aldehyde groups and neighboring hydroxyl groups, respectively. Jian, et al. (2009) [5] also observed aldehyde peak and hemiacetal bonds peak of dialdehyde cellulose at 1740 cm^{-1} and 880 cm^{-1}, respectively. Moreover, the intensity

of hydroxyl group at 3342 cm^{-1} of DBC was lower compared with that of BC. It confirmed that hydroxyl group locating at C2 and C3 of D-glucose structure were oxidized by IO_4^- to form aldehyde groups.

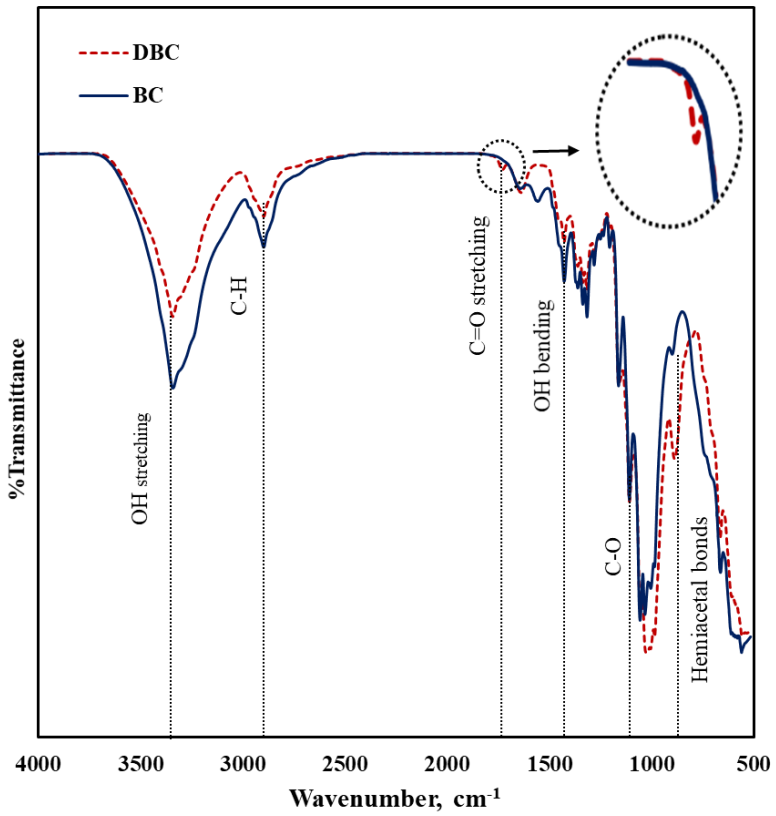

Figure 3 FT-IR spectra of BC and DBC

Morphology of DBC

Structure of BC and DBC are compared in figure 4. Fibrous web-like network structure was observed in BC (figure 4A). BC fibers were approximately 36.5±7.3 nm diameter. After BC was oxidized by periodate, ultrafine fibers, which was much shorter than theirs original length, were noticed in figure 4B.

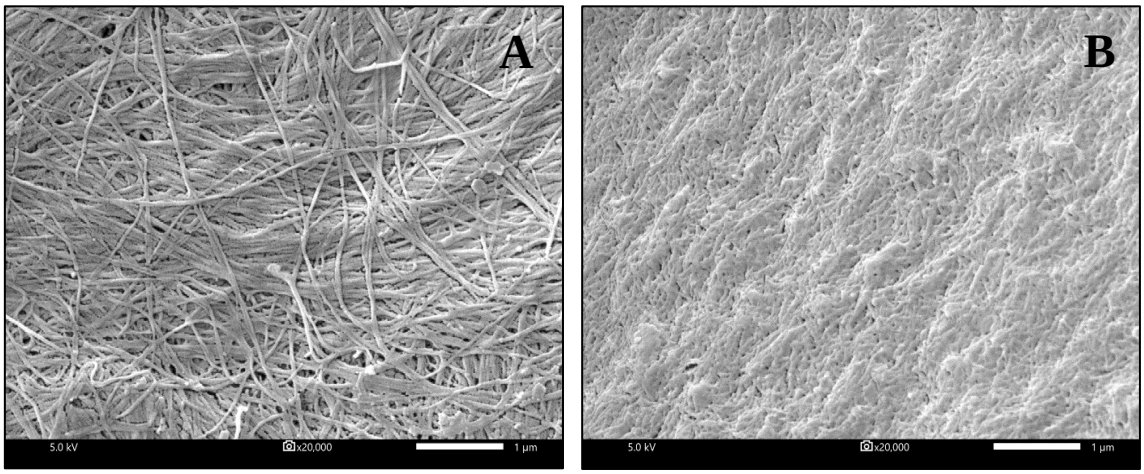

Figure 4 SEM images of BC (A) and DBC (B)

The specific cleavage of C2 and C3 in glucose monomers to form aldehyde groups led to the weakness of the BC polymeric backbone. Moreover, high periodate concentration could strongly oxidize and also degrade some amount of fibers.

Conclusion

The reaction conditions of periodate oxidation were optimized to obtain DBC with the highest aldehyde content. The mole ratio of BC and periodate and reaction temperature mainly affected the aldehyde content of DBC. The optimal reaction conditions to obtain DBC with 89% aldehyde content were mole ratio of 1:2 (BC:periodate), 60°C and 6 h. FTIR spectrum of DBC confrimed the formation of aldehyde functional groups. The morphology of DBC was ultrafine fibers.

Acknowledgements

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