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Influence of cellulose nanocrystal addition on the production and characterization of bacterial nanocellulose

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ABSTRACT

Bacterial nanocellulose (BNC) is characterized by high purity and excellent mechanical properties; however, its production is constrained by low yield. Therefore, efforts aimed at improving its yield and material properties are imperative. This study investigated the effect of adding different concentrations (0%, 0.5%, and 1.0%) of cellulose nanocrystal (CNC) in Hestrin–Schramm modified medium on the yield and properties of BNC produced by *Komagataeibacter* sp. SFCB22-18. The BNC yield increased as following an increase in added CNC concentration. Also, the morphology, structure, crystallinity, thermal stability, and mechanical properties of BNC improved after CNC incorporation. A low CNC concentration (0.1%) favored mechanical strength, whereas 0.5% gave the optimum morphology, structural, and thermal stability. These results showed that modifying BNC with CNC could help increase yield and improve its properties, and thus; the potentiality of BNC in various applications would be much enhanced.

1. Introduction

Cellulose is an abundant resource, and it is attracting research and industrial attention as a replacement for petroleum products [1]. The application of cellulose cuts across all human activities, and this is attributable to its ability to produce elongated rod-shaped crystalline fibrils when subjected to mechanical shear [2]. The refined forms of cellulose, categorized as cellulose nanocrystals (CNC), nanofibrillated cellulose (NFC), and bacterial nanocellulose (BNC), are characterized by this basic property, and high degradability, low density, and nontoxicity [3]. The interest in BNC production is increasing because of its high purity and high specific surface area [4,5]. Besides, the properties of BNC can be easily manipulated during production to meet a specific purpose [6].

Bacterial nanocellulose is produced by certain acetic-acid bacteria, especially those belonging to the genera *Komagataeibacter*, *Acetobacter*, and *Achromobacter* [7,8]. Cellulose is extruded from the cell membrane of the organisms following sequential elaboration of enzymes, including phosphoglucomutase, uridine diphosphate-glucose pyrophosphorylase, and cellulose synthase, through the hexose phosphate pathway or indirectly through the pentose and gluconeogenic pathways [9]. The produced cellulose during fermentation consists of nanofibers with an average diameter of 20–100 nm and is characterized by high crystallinity, tensile strength, Young's modulus, water absorption capacity, and porosity [10].

However, the production of BNC is constrained by low yield, poor antimicrobial, and electrical conductivity; therefore, limiting its application [11]. Several options have been considered in the past few decades to ameliorate these challenges. Such options include identifying new microbial strains with high BNC-producing capacity, screening new substrates, optimizing microbial growth conditions, and reinforcing with other materials to form a composite with improved properties [7,12–16]. A cost-effective approach involves the *in situ* incorporation of microbial growth inducers for improved BNC yield and properties [9]. Accordingly, previous studies have demonstrated an improvement in the yield and properties of BNC by the incorporation of different

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biomaterials *in situ* [17–22]. However, BNC properties were considerably different depending on the inducing substance. Therefore, there is a need to search for more biomolecules that could enhance BNC production and its properties, proving CNC to be effective in this regard.

Cellulose nanocrystals are long crystalline rod-like nanocellulose with little or no amorphous region, and this is responsible for its high rigidity and mechanical strength [1]. Use of CNC to reinforce bio-based polymers to give stable and strong composites is well documented [23–25]. The material properties of the polymers following their blend with CNC were improved. Furthermore, Niamsap et al. [18] reported the suitability of CNC in improving the stability and dispersion of a microbial growth inducer, hydroxyapatite, in the culture medium during BNC production. However, there are only a few studies investigating the inducing influence of CNC on acetic acid-producing organisms during BNC production. Therefore, this current study investigated the effect of CNC incorporation during fermentation on the yield, morphology, crystallinity, thermostability, and structural and mechanical properties of BNC. The microbial growth and concentration changes in glucose, acetic acid, and ethanol were monitored, using high-performance liquid chromatography (HPLC) during fermentation. Besides, the BNC samples were characterized using scanning electron microscopy (SEM), Fourier-transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), thermogravimetry, and deformation testing.

2. Materials and methods

2.1. Microorganism and materials

Komagataeibacter sp. SFCB22–18 used in this study was isolated from ripe persimmon (*Diospyros kaki*). The characteristic features of the organism have been reported previously [26]. The organism was kept in 50% glycerol solution (*v*/v) and stored (−80 °C) until further use. The red alga (*Gelidium amansii*) used for CNC production was supplied by Milyang Agar Co. Ltd., Gyeongsangnam-do, Korea. All reagents used in this study were of analytical grade.

2.2. Production of cellulose nanocrystals

Cellulose nanocrystals were synthesized from the red alga based on the procedure outlined by So et al. [27]. First, the alga was pretreated by boiling with distilled water for 2 h, and then dried at room temperature (20 ± 2 °C), milled, and sieved using a 300 μm sieve. Subsequently, the pretreated dried alga (20 g) was treated with 350 mL of 60% sulfuric acid at 45 °C for 45 min. After dilution with distilled water, neutralization with 10 M NaOH, and centrifugation at 7000 ×*g* for 15 min was done; then, the precipitate was washed with distilled water several times until a turbid supernatant was obtained; this was considered CNC.

2.3. Production of bacterial nanocellulose

BNC was produced based on the fermentation procedure described by Park et al. [26]. In brief, 1 mL aliquot of the organism from a 3-d old previously prepared culture was inoculated on 100 mL Hestrin–Schramm modified (HSM) medium in different 250 mL flasks with varying CNC concentrations (0%, 0.1%, 0.5%, and 1.0%) at 30 °C for 7 d. After fermentation, the BNC pellicles formed were carefully removed from the medium and purified with 0.1 N NaOH. Next, the purified BNC was washed severally with distilled water till the pH became neutral. Finally, the BNC was freeze-dried, and its yield was calculated and expressed in g/L.

2.4. Determination of bacterial growth

Optical density was determined by measuring the absorbance at 600 nm using a UV/Vis spectrophotometer (1709111A, Biotek Instruments Inc., Winooski, VT, USA). The pH of the medium was measured using a pH meter (Orion Star A215, Thermo Fisher Scientific, Waltham, MA, USA). Glucose, acetic acid, and ethanol concentrations were determined using a HPLC (L6000, Futecs Co. Ltd., Daejeon, Korea) equipped with an HPX-87H column at a 0.5 mL/min flow rate. Sulfuric acid (0.005 M) was used as a mobile phase.

2.5. Determination of morphology and structural properties of bacterial nanocellulose

The surface morphology of BNC was determined using a field-emission scanning electron microscope (SU8220; Hitachi, Japan) at an acceleration voltage of 16 kV [27]. The structural property was determined using an FT-IR spectrometer (Nicolet iS5, Thermo Fisher Scientific, Waltham, MA, USA) [24]. The FT-IR spectrum range obtained was between 500 and 4000 cm^{-1} at 4 cm^{-1} and 16 scan resolution. OMNIC v.9.7.46 and firmware v.2.03 (Thermo Fisher Scientific, Waltham, MA, USA) were used to analyze the data obtained.

2.6. Determination of crystallinity of bacterial nanocellulose

The freeze-dried BNC was analyzed on an X-ray diffractometer (D/Max-2500, Rigaku, Japan) using a copper X-ray source to determine the crystallinity. Scans were collected at 2θ = 5°–50°. The crystallinity index of cellulose (CrI) was obtained using Eq. (1) [28].

$$\mathrm{CrI}\ (\%) = [(I_{200} - I_{am})/I_{200}] \times 100 \quad (1)$$

where, I_{200} and I_{am} represent the total intensity at 2θ = 22.7° and the baseline intensity at 2θ = 18°, respectively.

2.7. Thermogravimetric analysis

BNC's thermal properties were evaluated using an automatic thermal analyzer (Q500, TA Instruments, New Castle, DE 19720, USA). Samples were heated from 50 °C to 600 °C at a rate of 10 °C/min under a nitrogen atmosphere of 40 mL/min.

2.8. Mechanical properties of bacterial nanocellulose

BNC's mechanical properties were analyzed using a universal testing machine (Z010, Zwick-Roell, 89,079 Ulm, Germany). The freeze-dried BNC sample was cut into 60 × 10 mm portions and mounted between the upper and the lower clamps of the equipment. The experiment was performed at 1 mm/min.

3. Results and discussion

3.1. Growth of Komagataeibacter sp. SFCB22-18 in CNC-modified culture medium

The growth rate of *Komagataeibacter* sp. SFCB22–18, as influenced by the addition of CNC during BNC production, is presented in Fig. 1 (a–g). In the pure HSM medium, an increase in OD was observed on the third day of fermentation and continued until the seventh day. Accordingly, glucose was continuously consumed during fermentation (Fig. 1b), implying that glucose is an energy source for acetic acid bacteria and a precursor for cellulose production [10]. With CNC incorporation in the growth medium, a reduction in lag phase, as indicated by a visible increase in OD after the first day of fermentation (Fig. 1c), was observed. Since BNC production became faster after CNC addition, the CNC might play a role as a nucleating filler. Also, this could be due to improved water binding by the CNC added, enhancing microorganism's adaptability in the microenvironment [17]. As the CNC concentration increased to 0.5%, the OD considerably increased, whereas a further increase in CNC concentration to 1% did not show much improvement in OD. This reduction in OD might be because of the increased viscosity,

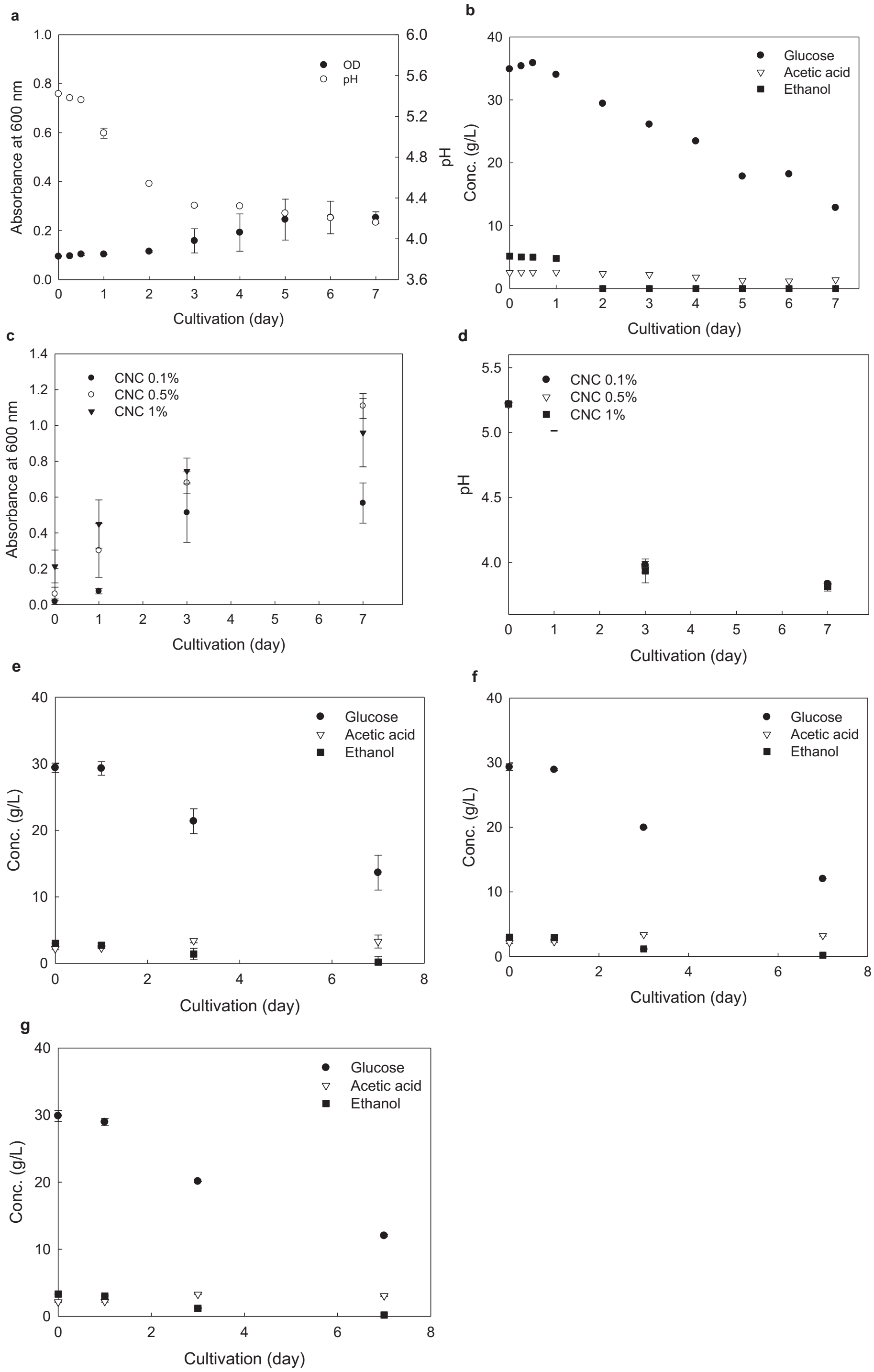

Fig. 1. Growth indicators of *Komagataeibacter* sp. SFCB22–18 in HSM medium (a) optical density and pH of pure HSM medium (b) glucose, acetic acid, and ethanol concentrations in pure HSM medium, (c) optical density of HSM medium with different CNC concentrations, (d) pH of HSM medium with different CNC concentrations, (e) glucose, acetic acid, and ethanol concentration in HSM medium with 0.1% CNC, (f) glucose, acetic acid, and ethanol concentration in HSM medium with 0.5% CNC, (g) glucose, acetic acid, and ethanol concentration in HSM medium with 1.0% CNC.

which probably limited the organism's proliferation [29].

There was a consistent reduction in pH from the first day of fermentation (>5.2) to the last day (<4.5). The reduction in pH could be due to the production of organic acids, including D-gluconic and 2-keto-gluconic acids, during BNC production [30,31]. There was a further reduction in pH (Fig. 1d) of the growth medium following the incorporation of CNC, and this could be due to the increased rate of bacterial metabolism. This synergistic effect of the substrates (HSM medium and CNC) probably resulted in increased organism growth, implying an increase in BNC yield.

3.2. *Yield of bacterial nanocellulose*

The yield of BNC as influenced by the addition of CNC in the growth medium is presented in Fig. 2. *Komagataeibacter* sp. SFCB22–18 in the pure HSM medium produced 0.58 g/L of cellulose. With 0.1% CNC addition into the medium, 0.64 g/L of cellulose was produced. As CNC concentration increased to 1%, BNC yield increased to 1.4 g/L, which is 2.55-fold higher than that obtained in the pure HSM medium. This increase in BNC yield following CNC addition could be attributed to the enhancement of substrate's crystallinity, resulting in an increased affinity of the organism on the substrate [32] and the increased rate of β-1, 4 glucan formation [4]. Machado et al. [14] also reported an increase in

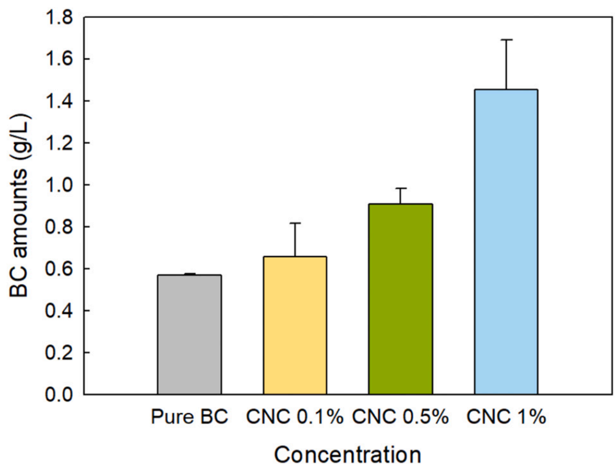

Fig. 2. Yield of BNC produced by *Komagataeibacter* sp. SFCB22-18 in HSM medium containing different CNC concentrations.

BNC yield (18%) by *Komagataeibacter rhaeticus* after adding sugarcane molasses. Also, carboxyl methylcellulose (CMC) addition gave a 213.43% increase [21]. The concentration of CNC influenced the yield of BNC probably because of variation in substrate's composition and surface properties, such as crystallinity and dispersibility, which influenced rate of organism proliferation [6].

3.3. Morphology of bacterial nanocellulose

The morphology of BNC samples as depicted by the SEM images is presented in Fig. 3. Pure BNC (Fig. 3a) showed thread-like dense fibrils with clumps. These clumps were generated probably due to high microfibril aggregation [32] during the parallel stacking of several cellulose chains by van der Waals forces among adjacent micromolecules [33]. After incorporating CNC during BNC production, an alteration in the morphology of BNC was observed. This might be due to the reduction in the density of fibers, probably caused by the dispersion of CNC into BNC, thus enhancing intermolecular interactions of the carbonyl and hydroxyl groups of the polymers [34]. This indicated an increase in surface area and porosity, which implied an improvement in the adhesion potential of BNC [11]. Accordingly, 0.5% and 1.0% CNC addition showed a finer network of BNC without clumps relative to 0.1% CNC. This indicated that 0.5–1.0% CNC addition resulted in its optimum dispersion on the surface of BNC during synthesis.

3.4. Structural properties and crystallinity of bacterial nanocellulose

The FT-IR spectra of BNC and CNC-modified BNC are presented in Fig. 4. All samples showed common typical bands, such as 1429 cm^{-1} (CH_2 stretching), 1035–1060 cm^{-1} (C—O stretching), and 893 cm^{-1} (β-1, 4 glycosidic linkage), indicating cellulose I [35,36]. The addition of 0.5–1.0% CNC caused a noticeable increase in absorption intensity at the 1572 cm^{-1} (carboxylic group) and 2700–2886 cm^{-1} (C—H stretching) bands, which is related to an increase in intramolecular bonding [11]. This could be due to the reduction of the amorphous region of BNC by CNC's presence, which probably led to the generation of a hydroxyl group and its consequential cleavage to BNC microfibrils. These findings suggested an infiltration of CNC into the BNC microfibril; thus, implying an enhancement in mechanical properties [37].

The crystallinity index of BNC as influenced by CNC addition is

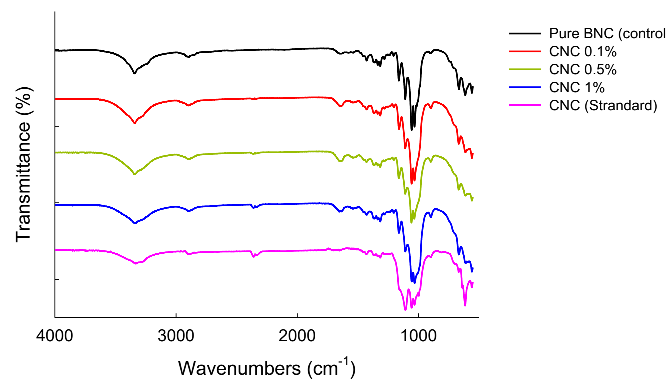

Fig. 4. FT-IR spectra of BNC produced by *Komagataeibacter* sp. SFCB22-18 in HSM medium containing different CNC concentrations

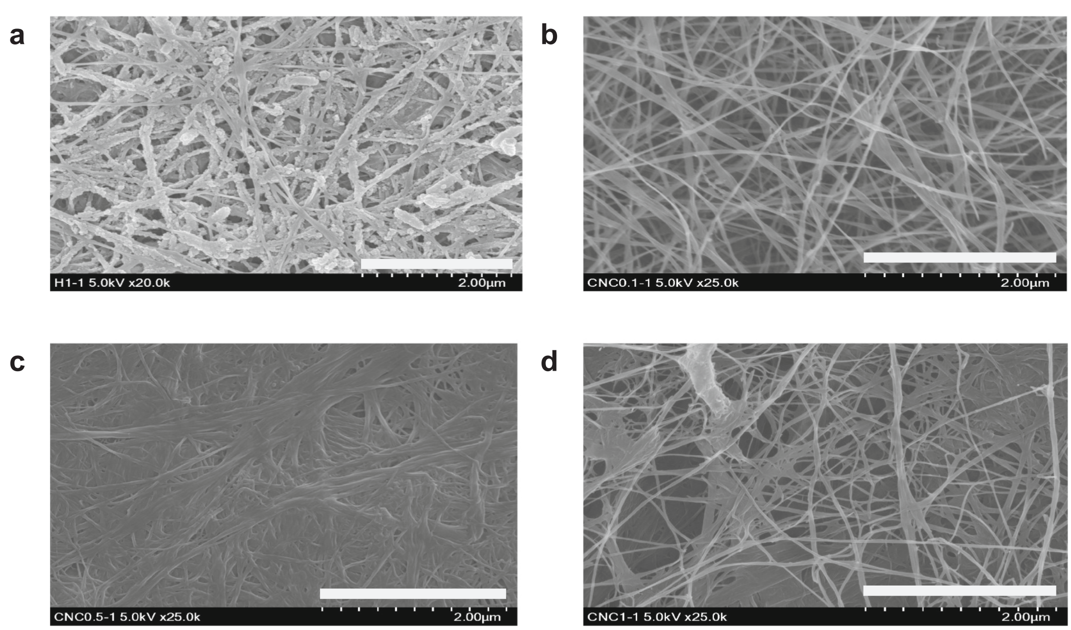

Fig. 3. SEM images of BNC produced by *Komagataeibacter* sp. SFCB22-18 in HSM medium containing different CNC concentrations: (a) pure BNC, (b) 0.1% CNC, (c) 0.5% CNC, (d) 1.0% CNC.

presented in Table 1. As CNC concentration increased from 0.1% to 1.0%, CrI and TCI (ratio of FT-IR absorbance at 1375 and 2900 cm^{-1}) increased from 51.2% and 0.929% to 60.2% and 0.961%, respectively. CNC has high crystallinity [1] and this probably influenced BNC's crystallinity. The increase in crystallinity of BNC reflects an improvement in its mechanical properties [18]. The result aligned with the findings of Yu and Yao [38], who reported an increased crystallinity of bacterial polyester following its reinforcement with cellulose nanocrystals. Dayal and Catchmark [17] had reported that the crystallinity of BNC is influenced by the type and concentration of polysaccharides added to the culture medium during fermentation. Meanwhile, XRD patterns of all BNC samples showed similar diffraction peaks at 22.7°, 14.5°, and 16.8° of 2θ (Fig. 5), which were related to the type Iα cellulose structure that characterizes BNC [39].

3.5. Thermal stability of bacterial nanocellulose

The thermal stability of BNC as influenced by CNC addition during fermentation is presented in Fig. 6. The thermogravimetric patterns (Fig. 6a) show a two-step degradation, at 100 °C due to moisture evaporation and < 400 °C due to cellulose decomposition [40]. The initial weight loss, which occurred around 100 °C, might be related to the volatilization of water or residual bacterial cells. BNC and CNC-modified BNC were stable at 200 °C. Beyond this temperature, rapid BNC deterioration, as indicated by a reduced percentage weight, was observed. This might be related to cellulose degradation. However, the stability of CNC-modified BNC was extended up to 300 °C. The improvement in thermal stability of BNC, following the addition of CNC during fermentation, could be related to the dispersion of CNC into the BNC microfibrils, thereby reducing the amorphous region [33]. This was similar to a previous study, [41], which showed an improvement in thermal properties of polyvinyl alcohol-containing cellulose nanomaterials. Above 400 °C, there was a significant weight loss of the BNC with 0.1% CNC; however, the BNC samples containing 0.5–1.0% CNC were still stable. At elevated temperatures (400–650 °C), less than 75% of the BNC containing 0.5–1.0% CNC was degraded. These findings showed that the concentration of CNC remarkably influenced the thermal properties of BNC. Furthermore, the derived thermogravimetric curve (Fig. 6b), indicating the maximum degradation rate temperature (Tmax), showed that the pure BNC had its maximum peak at 280 °C while CNC-modified BNC showed a maximum peak at 320–350 °C. This validated the higher thermal stability of CNC-modified BNC, as previously discussed using the thermogravimetric pattern. This variation could be due to differences in the rate of CNC dispersion on the surface of BNC microfibrils, hence the difference in intermolecular interactions [41].

3.6. Mechanical properties of bacterial nanocellulose

The mechanical properties of BNC regarding the stress-strain curves are presented in Fig. 7. The BNC and CNC-modified BNC exhibited variable mechanical properties. The curve of the CNC-modified BNC showed an initial linear region, which depicted a high Young's modulus. However, this region was absent in pure BNC. This result indicated an improvement in the mechanical strength, rigidity, and compatibility of BNC following its reinforcement with CNC, due to increased water

Table 1
The crystallinity of modified BNC produced in HSM medium with different CNC concentrations.

CNC concentration (%)	CrI (%)	TCI (%)
0.1	51.208	0.9286
0.5	52.693	0.9596
1	60.162	0.9613

CrI (XRD: 22.7–18.0/22.7), TCI (FT-IR: 1375/2900).

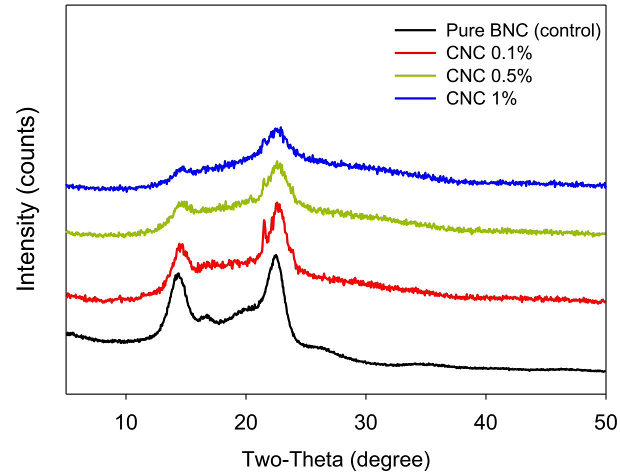

Fig. 5. XRD patterns of BNC produced by *Komagataeibacter* sp. SFCB22-18 in HSM medium containing different CNC concentrations.

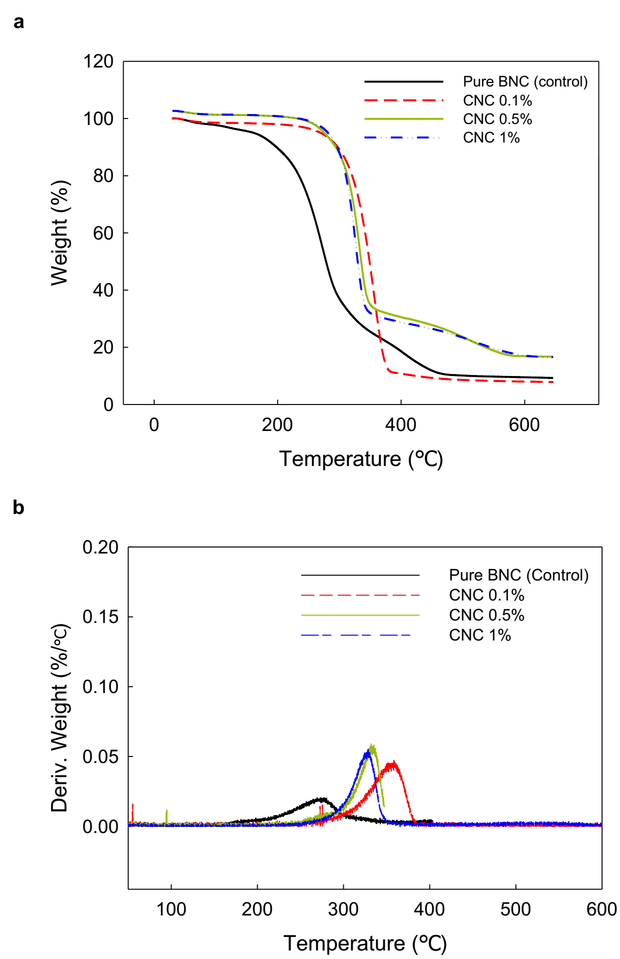

Fig. 6. Thermogravimetric patterns of BNC produced by *Komagataeibacter* sp. SFCB22-18 in HSM medium containing different CNC concentrations (a) thermogravimetric curves (b) derivative thermogravimetric curves.

holding capacity [5]. The high water holding capacity (value not presented in this study) could be linked with intermolecular interactions, such as hydrogen bonding, static electricity, hydrophobicity, and van der Waals, implying higher entanglement of CNC on the BNC fibers. Also, the addition of 0.1% CNC increased the tensile modulus and this

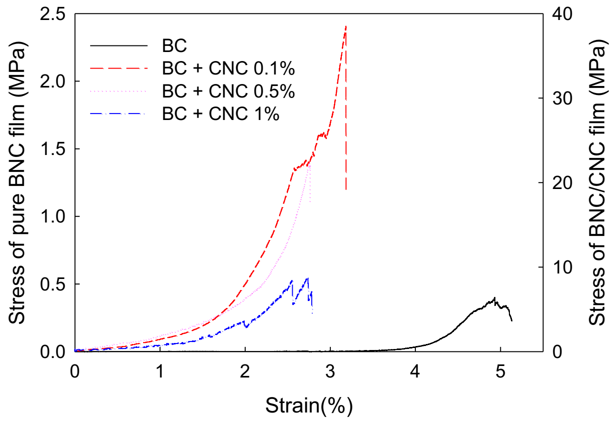

Fig. 7. Stress *vs.* strain patterns of BNC produced by *Komagataeibacter* sp. SFCB22-18 in HSM medium containing different CNC concentrations.

could imply an improvement in BNC's structure due to the alignment of its fibers along the loading direction. However, as CNC concentration increased (0.5%–1.0%), the tensile modulus decreased. It can be thought that higher CNC concentration weakened the BNC network by excluding cellulose–cellulose-binding during synthesis [17]. This result agrees with Cheng et al. [42] who reported that the inclusion of 1% CMC reduced the modulus of BNC.

4. Conclusions

This study showed that the addition CNC in HSM medium enhanced BNC yield. There was an improvement in the morphology, structure, crystallinity, thermal stability, and mechanical properties of BNC following CNC addition during fermentation. The concentration of CNC influenced the yield and properties of BNC. The highest BNC yield was obtained using 1.0% CNC. A low CNC concentration (0.1%) favored mechanical strength, whereas 0.5% gave the best morphology, structural, and thermal stability.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Won Yeong Bang: Methodology, Investigation, Writing – original draft. **Olajide Emmanuel Adedeji:** Methodology, Writing – original draft. **Hye Jee Kang:** Methodology, Investigation. **Mi Dan Kang:** Software, Methodology, Investigation. **Jungwoo Yang:** Writing – review & editing. **Young Woon Lim:** Conceptualization, Writing – review & editing. **Young Hoon Jung:** Conceptualization, Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] B. Thomas, M.C. Raj, K.B. Athira, M.H. Rubiyah, J. Joy, A. Moores, G.L. Drisko, C. Sanchez, Nanocellulose, a versatile green platform: from biosources to materials and their applications, Chem. Rev. 118 (2018) 11575–11625, https://doi.org/10.1021/acs.chemrev.7b00627.
[2] M. Skocaj, Bacterial cellulose in paper making, Cellulose 26 (2019) 6477–6488, https://doi.org/10.1007/s10570-019-02566-y. (0123.
[3] C. Molina-Ramírez, C. Enciso, M. Torres-Taborda, R. Zuluaga, P. Gañán, O.J. Rojas, C. Castro, Effects of alternative energy sources on bacterial cellulose characteristic produced by komagataeibacter medellinensis, Int. J. Biol. Macromol. 117 (2018) 735–741, https://doi.org/10.1016/j.ijbiomac.2018.05.195.
[4] P. Jacek, F. Dourado, M. Gama, S. Bielecki, Molecular aspects of bacterial nanocellulose, Biosynthes. Microb. Biotechnol. 12 (2019) 633–649, https://doi.org/10.1111/1751-7915.13386.
[5] H. Khan, A. Kadam, D. Dutt, Studies on bacterial cellulose produced by a novel strain of lactobacillus genus, Carbohydr. Polym. 229 (2020), 115513, https://doi.org/10.1016/j.carbpol.2019.115513.
[6] I. Reiniati, A.N. Hrymak, A. Margaritis, Kinetics of cell growth and crystalline nanocellulose production by komagataeibacter xylinus, Biochem. Eng. J. 127 (2017) 21–31, https://doi.org/10.1016/j.bej.2017.07.007.
[7] S.S. de Souza, F.V. Berti, K.P.V. de Oliveira, C.Q.P. Pittella, J.V. de Castro, C. Pelissari, C.R. Rambo, L.M. Porto, Nanocellulose biosynthesis by komagataeibacter hansenii in a defined minimal culture medium, Cellulose 26 (2019) 1641–1655, https://doi.org/10.1007/s10570-018-2178-4.
[8] Y. Wan, J. Wang, M. Gama, R. Guo, Q. Zhang, P. Zhang, F. Yao, H. Luo, Biofabrication of a novel bacteria/bacterial cellulose composite for improved adsorption capacity, Compos. Part A Composites Part A 125 (2019), 105560, https://doi.org/10.1016/j.compositesa.2019.105560.
[9] C. Molina-Ramirez, C. Castro, R. Zuluaga, P. Gañán, Physical characterization of bacterial cellulose produced by komagataeibacter medellinensis using food supply chain waste and agricultural by-products as alternative low-cost feedstocks, J. Polym. Environ. 26 (2018) 830–837, https://doi.org/10.1007/s10924-017-0993-6.
[10] C. Campano, A. Balea, A. Blanco, C. Negro, Enhancement of the fermentation process and properties of bacterial cellulose: a review, Cellulose 23 (2016) 57–91, https://doi.org/10.1007/s10570-015-0802-0.
[11] K. Naloka, K. Matsushita, G. Theeragool, Enhanced ultrafine nanofibril biosynthesis of bacterial nanocellulose using a low-cost material by the adapted strain of komagataeibacter xylinus MSKU 12, Int. J. Biol. Macromol. 150 (2020) 1113–1120, https://doi.org/10.1016/j.ijbiomac.2019.10.117.
[12] S. Dubey, J. Singh, R.P. Singh, Biotransformation of sweet lime pulp waste into high-quality nanocellulose with an excellent productivity using komagataeibacter europaeus SGP37 under static intermittent fed-batch cultivation, Bioresour. Technol. 247 (2018) 73–80, https://doi.org/10.1016/j.biortech.2017.09.089.
[13] Y. Gupte, A. Kulkarni, B. Raut, P. Sarkar, R. Choudhury, A. Chawande, G.R. K. Kumar, B. Bhadra, A. Satapathy, G. Das, B. Vishnupriya, S. Dasgupta, Characterization of nanocellulose production by strains of komagataeibacter sp. Isolated from organic waste and kombucha, Carbohydr. Polym. 266 (2021), 118176, https://doi.org/10.1016/j.carbpol.2021.118176.
[14] R.T.A. Machado, A.B. Meneguin, R.M. Sábio, D.F. Franco, S.G. Antonio, J. Gutierrez, A. Tercjak, A.A. Berretta, S.J.L. Ribeiro, S.C. Lazarini, W.R. Lustri, H. S. Barud, Komagataeibacter rhaeticus grown in sugarcane molasses-supplemented culture medium as a strategy for enhancing bacterial cellulose production, Ind. Crop. Prod. 122 (2018) 637–646, https://doi.org/10.1016/j.indcrop.2018.06.048.
[15] R. Mangayil, S. Rajala, A. Pammo, E. Sarlin, J. Luo, V. Santala, M. Karp, S. Tuukkanen, Engineering and characterization of bacterial nanocellulose films as low cost and flexible sensor material, ACS Appl. Mater. Interfaces 9 (2017) 19048–19056, https://doi.org/10.1021/acsami.7b04927.
[16] A.C. Rodriguesa, A.I. Fontão, A. Coelho, M. Leal, F.A.G. Soares da Silva, Y. Wan, F. Dourado, M. Gama, Response surface statistical optimization of bacterial nanocellulose fermentation in static culture using a low-cost medium, New Biotechnol. 49 (2019) 19–27, https://doi.org/10.1016/j.nbt.2018.12.002.
[17] M.S. Dayal, J.M. Catchmark, Mechanical and structural property analysis of bacterial cellulose composites, Carbohydr. Polym. 144 (2016) 447–453, https://doi.org/10.1016/j.carbpol.2016.02.055.
[18] T. Niamsap, N.T. Lam, P. Sukyai, Production of hydroxyapatite-bacterial nanocellulose scaffold with assist of cellulose nanocrystals, Carbohydr. Polym. 205 (2019) 159–166.
[19] Y.Z. Wan, S.R.Jia Long, Y. Huang, Y. Zhu, Y.L. Wang, H.J. Jiang, Synthesis and characterization of hydroxyapatite–bacterial cellulose nanocomposites, Compos. Sci. Technol. 66 (2006) 1825–1832.
[20] S. Gea, E. Bilotti, C.T. Reynolds, N. Soykeabkeaw, T. Peijs, Bacterial cellulose–poly (vinyl alcohol) nanocomposites prepared by an in-situ process, Mater. Lett. 64 (2010) 901–904.
[21] K.C. Cheng, J.M. Catchmark, A. Demirci, Effect of different additives on bacterial cellulose production by acetobacter xylinum and analysis of material property, Cellulose 16 (2009) 1033–1045.
[22] J. Kim, Z. Cai, Y. Chen, Biocompatible bacterial cellulose composites for biomedical application, J. Nanotechnol. Eng. Med. 1 (2010), 011006.
[23] Y. Li, Q. Fu, M. Wang, J. Zeng, Morphology, crystallization and rheological behavior in poly(butylenesuccinate)/cellulose nanocrystal nanocomposites

fabricated bysolution, Carbohydr. Polym. 164 (2017) 75–82, https://doi.org/10.1016/j.carbpol.2017.01.089.
[24] I.T. Seoane, P. Cerrutti, A. Vazquez, L.B. Manfredi, V.P. Cyras, Polyhydroxybutyrate-based nanocomposites with cellulose nanocrystals and bacterial cellulose, J. Polym. Environ. 25 (2017) 586–598, https://doi.org/10.1007/s10924-016-0838-8.
[25] P. Panyasiri, N.T. Lam, P. Sukyai, Efect of hydroxyapatite prepared by in situ synthesis on the properties of poly (vinyl alcohol)/cellulose nanocrystals, Biomater. J. Polym. Environ. 28 (2020) 141–151, https://doi.org/10.1007/s10924-019-01599-5.
[26] M.S. Park, Y.H. Jung, S.Y. Oh, M.J. Kim, W.Y. Bang, Y.W. Lim, Cellulosic nanomaterial production via fermentation by Komagataeibacter sp. SFCB22-18 isolated from ripened persimmons, J. Microbiol. Biotechnol. 29 (2019) 617–624, https://doi.org/10.4014/jmb.1801.01005.
[27] B.R. So, H.J. Yeo, J.J. Lee, Y.H. Jung, S.K. Jung, Cellulose nanocrystal preparation from Gelidium amansii and analysis of its anti-inflammatory effect on the skin in vitro and in vivo, Carbohydr. Polym. 254 (2021), 117315, https://doi.org/10.1016/j.carbpol.2020.117315.
[28] H. Foner, N. Adan, The characterization of papers by X-ray diffraction (XRD): measurement of cellulose crystallinity and determination of mineral composition, J. Forensic Sci. Soc. 23 (1983) 313–321, https://doi.org/10.1016/S0015-7368(83)72269-3.
[29] N. Yin, S. Chena, Y. Caob, H. Wanga, Q. Wub, Improvement in mechanical properties and biocompatibility of biosynthetic bacterial cellulose/lotus root starch composites, Chin. J. Polym. Sci. 35 (2017) 354–364, https://doi.org/10.1007/s10118-017-1903-z.
[30] S.M.A.S. Keshk, Vitamin C enhances bacterial cellulose production in gluconacetobacter xylinus, Carbohydr. Polym. 99 (2014) 98–100, https://doi.org/10.1016/j.carbpol.2013.08.060.
[31] M. Ul-Islam, J.H. Ha, T. Khan, J.K. Park, Effects of glucuronic acid oligomers on the production, structure and properties of bacterial cellulose, Carbohydr. Polym. 92 (2013) 360–366, https://doi.org/10.1016/j.carbpol.2012.09.060.
[32] D. Mikkelsen, B.M. Flanagan, G.A. Dykes, M.J., Gidley1Influence of different carbon sources on bacterial cellulose production by gluconacetobacter xylinus strain ATCC 53524, J. Appl. Microbiol. 107 (2009) 576–583, https://doi.org/10.1111/j.1365-2672.2009.04226.x.
[33] R.J. Moon, A. Martini, J. Nairn, J. Simonsen, J. Youngblood, Cellulose nanomaterials review: structure, properties and nanocomposites, Chem. Soc. Rev. 40 (2011) 3941–3994, https://doi.org/10.1039/C0CS00108B.
[34] H. Yu, Z. Qin, Y. Liu, L. Chen, N. Liu, Z. Zhou, Simultaneous improvement of mechanical properties and thermal stability of bacterial polyester by cellulose nanocrystals, Carbohydr. Polym. 89 (2012) 971–978, https://doi.org/10.1016/j.carbpol.2012.04.053.
[35] C.J. Grande, F.G. Torres, C.M. Gomez, M.C. Bañó, Nanocomposites of bacterial cellulose/hydroxyapatite for biomedical applications, Acta Biomater. 5 (2009) 1605–1615.
[36] R. Auta, G. Adamus, M. Kwiecien, I. Radecka, P. Hooley, Production and characterization of bacterial cellulose before and after enzymatic hydrolysis, Afr. J. Biotechnol. 16 (2017) 470–482.
[37] H. Kurniawan, Y. Yea, W. Kuo, J. Lai, M. Wang, H. Liu, Improvement of biofouling resistance on bacterial cellulose membranes, Biochem. Eng. J. 78 (2013) 138–145, https://doi.org/10.1016/j.bej.2013.03.021.
[38] H. Yu, J. Yao, Reinforcing properties of bacterial polyester with different cellulose nanocrystals via modulating hydrogen bonds, Compos. Sci. Technol. 136 (2016) 53–60, https://doi.org/10.1016/j.compscitech.2016.10.004.
[39] S.P. Lin, C.T. Liu, K.D. Hsu, Y.T. Hung, T.Y. Shih, K.C. Cheng, Production of bacterial cellulose with various additives in a PCS rotating disk bioreactor and its material property analysis, Cellulose 23 (2016) 367–377.
[40] M.L. Cacicedo, G. Pacheco, G.A. Islan, V.A. Alvarez, H.S. Barud, G.R. Castro, Chitosan-bacterial cellulose patch of ciprofloxacin for wound dressing: preparation and characterization studies, Int. J. Biol. Macromol. 147 (2020) 1136–1145, https://doi.org/10.1016/j.ijbiomac.2019.10.082.
[41] A.A. Rowe, M. Tajvidi, D.J. Gardner, Thermal stability of cellulosenanomaterials and their composites with polyvinyl alcohol (PVA), J. Therm. Anal. Calorim. 126 (2016) 1371–1386, https://doi.org/10.1007/s10973-016-5791-1.
[42] K.C. Cheng, J.M. Catchmark, A. Demirci, Effects of CMC addition on bacterial cellulose production in a biofilm reactor and its paper sheets analysis, Biomacromol. 12 (2011) 730–736.