

Dispersion and Beating of Bacterial Cellulose and their Influence on Paper Properties

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Three dispersion instruments (a standard laboratory disintegrator, PFI beater, and ultrasonic cell disrupter) were tested to determine their effects on the dispersion of bacterial cellulose (BC) wet films. After treatment with a standard 10000 r disintegrator treatment, there was still a large number of undispersed fiber bundles in the BC suspension. The BC films were dispersed well after PFI beating revolution at 30000 r, and the cationic charge demand of the BC suspension reached 2.4×10^{-4} eq·g⁻¹. The ultrasonic cell crusher was altogether unsuitable for BC dispersion. The ultrasonic cell crusher only separated the BC from the bundles. The properties of the resulting paper indicated that the physical strength of paper containing BC dispersed by the PFI beater was higher than that of the sample produced *via* standard laboratory disintegrator. Well-dispersed BC was distributed evenly among the plant fibers, which benefitted the combination of BC and plant fiber to improve the physical properties of the paper sheets.

Keywords: Bacterial cellulose; Dispersion methods; Cationic charge demand; Paper properties

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INTRODUCTION

Rapid advancements in the paper industry and the increasingly innovative uses for paper have amplified the requirements for the performance of paper products. Plant fiber has an irreplaceable role as the main raw material for papermaking, but it is challenging to meet the performance requirements for certain types of specialty paper. Various fillers are usually added to the pulp to mitigate the drawbacks (Othman *et al.* 2015; Rantanen *et al.* 2015; Ji. *et al.* 2016; Li *et al.* 2016).

Bacterial cellulose (BC) is a general designation of the cellulose synthesized under different conditions (static or dynamic) by various microorganisms (Iguchi *et al.* 2000; Cacicedo *et al.* 2016). It is formed as a white, wet film. BC and natural plant cellulose are very similar in chemical composition and fiber structure. Both are characterized by the similarities between their glucose group and the main chain with the β -1,4-glycosides, which polymerize into a high polymer (Gallegos *et al.* 2016). The differences between the two are mainly reflected by the following aspects. BC does not contain other polysaccharides, and its purity is high. Plant fiber contains hemicellulose, lignin, and other potentially undesirable materials. BC has higher crystallinity and degree of polymerization than plant fibers, with finer fiber width (at the nano scale, 10 to 100 nm). The strength and tear resistance of BC fibers are extremely strong, and the modulus of elasticity can be dozens of times greater than that of ordinary plant fibers. These qualities altogether indicate

that BC is very well suited for papermaking, as its unique structure can enhance the performance of paper (Matsuoka *et al.* 1996; Wada *et al.* 2003; Cacicedo *et al.* 2016; Jozala *et al.* 2016). Further, and perhaps most importantly, BC is an attractive and eco-friendly potential alternative to plant fiber in the pulp and paper industry (Gallegos *et al.* 2016).

Fibers must be dispersed into slurry during papermaking. Most BC applications require fully dispersed BC films (Gao *et al.* 2011). Shinya *et al.* (1997) used self-excited ultrasonic pulverizer to dispersed BC fiber, in addition, Tguchi Masatushi and Morinaga Yasushi used mechanical method (agitation and beating) to treat the BC films (Yasushi *et al.* 1995; Masatushi *et al.* 1996). The focus of their research was the preparation of functional paper from BC and plant fibers. So, the degree of BC dispersion and the measurement of dispersion degree were not discussed. Acid and alkali treatment are another dispersion approach; however, such treatments are not suitable for BC dispersion. After acid and alkali treatment, BC macromolecules would be degraded, and the fiber bundles would not be dispersed (Xiu *et al.* 2005). If the BC wet film is not sufficiently dispersed, the hydroxyl groups on the cellulose molecular chain become encapsulated inside the fiber bundle instead of being exposed. This affects hydrogen bonding between the plant fiber and bacterial fiber. Poor BC dispersion can lead to disadvantageous particle phenomena, which hinder the combination of plant fiber and decrease the physical properties of the resulting paper. BC film dispersion was the primary research object of this study, as discussed below.

According to the mechanism of medium consistency refining, the effect of friction deformation will make the bacterial cellulose disperse during the refining process in a PFI mill (Qian and Tessier 1995). When BC fiber are present between the gears of the PFI mill in the state of fiber bundles, they rub against each other and form a network flocculate with a certain strength. So there are many bundles together under mechanical shear stress rather than a single fiber or fiber bundle. In this way, the stress from the refining teeth is uniformly distributed in each fiber bundle so as to reduce the cutting and crushing to each fiber bundle. Therefore, the integrity of fiber morphology can be maintained. During the refining process, the relative friction between the part of fiber connection makes it difficult to cut the fiber, so the length of the fiber is better retained (Di *et al.* 2004; Ban *et al.* 2011). In summary, PFI beater can preserve the morphology and length of the fibers in the dispersing process.

EXPERIMENTAL

Materials

Bacterial cellulose wet films generated by *Acetobacter xylinum* with solid content of 1% came from Yide Food Company in Hainan, China. Softwood sulfate pulp came from a paper mill in Guangxi, China, and reactive dye came from a textile mill in Guangxi, China.

Methods

BC dispersion - Standard laboratory disintegrator

The BC films were treated with 0.3 M NaOH at 121 °C for 15 min to disrupt and dissolve the embedded microbial cells, which were then washed thoroughly with distilled water until the pH of the water became neutral (Ul-Islam *et al.* 2012). The films were cut into 10 mm × 10 mm pieces. Water was added to adjust the concentration to 0.1% (absolute dry BC to water mass), and then the films were dispersed with a standard laboratory disintegrator.

BC dispersion - PFI beater

BC slurry reached about 10% concentration after standard laboratory disintegrator treatment. A PFI beater (KRK, Tokyo, Japan) was used for the refining process with use of 5000 r, 10000 r, 15000 r, 20000 r, 25000 r, 30000 r, 35000 r, or 40000 r treatment (where “r” refers to the number of revolutions). The BC was placed in the sealing bags to balance the water after the end of the refining.

BC dispersion - Ultrasonic cell crusher

Alternatively, after standard laboratory disintegration, the BC with the consistency of 0.1% was dispersed in an 80 mL beaker using an ultrasonic cell crusher (JY92-IIN, Ningbo, China) for 5, 15, 25, or 35 min.

Degree of dispersion - Apparent morphology observation

Two grams of reactive dye (Reactive Blue 194) and 10 g of NaCl were dissolved in 500 mL of water under vigorous stirring. The mixture was placed in a thermostat-monitored water bath to heat it to 80 °C while the dispersed BC was added to the mixture. The material was washed out of the float after being held at the target temperature for 30 min. The BC samples were then dried, placed in slides, and observed under an optical microscope (Olympus BX51, Tokyo, Japan to determine their respective morphologies.

Degree of dispersion - Cationic charge demand

One g (equivalent to the absolute dry BC) was added into a beaker with 0.01 M HCl to adjust the pH to 2. The mixture was magnetically stirred for 30 min. The HCl-treated BC was washed with deionized water until the conductivity of the filtrate was lower than 5 $\mu\text{s}/\text{cm}$, then 0.01 M NaHCO_3 and NaOH was added to adjust the pH to 9. After magnetically stirring for 30 min, the NaHCO_3 -treated BC was washed with deionized water until the conductivity of the filtrate was lower than 5 $\mu\text{s}/\text{cm}$. A pre-determined volume of cationic standard solution (BTG, Sweden, Germany) marked as V_{cation} plus a certain dosage of BC marked as M_{BC} were then added to deionized water to a total volume of 100 mL. The mixture was magnetically stirred for another 30 min and filtered with a 0.1 μm filter. Ten mL of filtrate was added into a Müttek PCD-5 instrument (BTG) and an anionic standard solution (BTG) was used to titrate the solution until it was electrically neutral. The volume of the anionic standard solution was recorded as V_{anionic} ,

$$\text{Cationic charge demand} = \frac{V_{\text{cation}} \cdot C_{\text{cation}} - 10V_{\text{anion}} \cdot C_{\text{anion}}}{M_{\text{BC}}} \quad (1)$$

where V_{cation} (mL) is the total volume of standard cationic solution, C_{cation} (eq.mL⁻¹) is the standard cationic titrant’s concentration, V_{anion} (mL) is the consumed volume of standard anionic titrant, C_{anion} (eq.mL⁻¹) is the standard anion concentration, and M_{BC} (g) is the dry mass of BC.

Degree of dispersion - Fiber morphology analysis

The bacterial fiber morphology including length, width, kink index, and curl index was analyzed with a Kajaani FS 300 fiber analyzer (Metso, USA).

Making paper

Various dosages of dispersed BC were added to softwood pulp to prepare

handsheets at basis weight of 60 g/m². The BC dosage (relative to absolute dry plant fibers) was 1%, 2%, 3%, 4%, or 5%. The sheets were equilibrated at constant temperature and humidity (25 °C, 50% relative humidity) for 24 h prior to analysis. The physical properties of the paper including tear index, burst index, tensile index, and air permeability were tested according to TAPPI T220 sp-01(2011).

SEM analysis

Paper surface morphology was observed by scanning electron microscope (SEM) (S-3400N, Hitachi, Tokyo, Japan). Gold was sputtered on samples (one cycle of 120 seconds) in advance, and the samples were examined in secondary electron mode at a beam current of 100 mA and an accelerating voltage of 10 kV.

RESULTS AND DISCUSSION

Apparent Morphology of BC under Different Dispersion Methods

BC is a pure cellulose, so the He-type dye typically used for plant fiber dyeing is not suitable. Fibers such as BC can use reactive dye, which is commonly used to color cotton, wool, and other textiles (Cai *et al.* 1999; Montazer *et al.* 2007). Figure 1a shows that BC was suspended in the water after using the standard laboratory disintegrator at 10000 r, but it existed mainly in the form of fiber bundles encapsulating the hydroxyl groups, not as dispersed filamentation. However, in Fig. 1c, most BC fiber bundles were divided into small filaments after treatment with the PFI beater. Compared with Fig. 1a and 1b, there was not much difference between the untreated sample and the sample treated with the ultrasonic cell crusher for about 30 min. There was still a large amount of BC fiber bundles in the suspension.



Fig. 1. Microscopic images of fibers 10×10 times (a) dispersed with a disintegrator, (b) dispersed with an ultrasonic cell crusher, (c) dispersed with a PFI beater

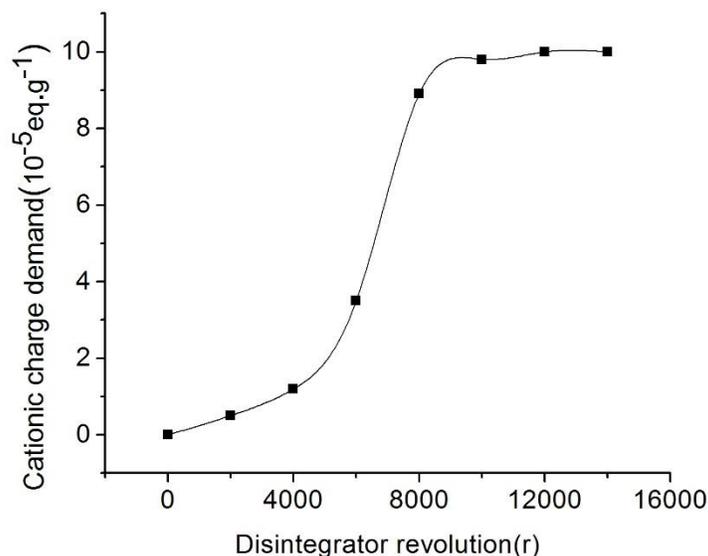


Fig. 2. Cationic charge demand under different disintegrator revolution cycles

Cationic Charge Demand of BC under Different Dispersion Methods

The cationic charge demand reflects the specific surface area through the surface charge of the fibers as well as the dispersion degree of the BC suspension (Bhardwaj *et al.* 2007; Kanjanamosit *et al.* 2010). As shown in Fig. 2, the charge demand of BC dispersion increased rapidly and tended toward stability as the number of disintegrator revolutions increased. The specific surface area was small of BC, so there were few exposed hydroxyl groups and a low demand for cationic charge. As the number of revolutions increased, the specific surface area, as indicated by the relative cationic charge demand of the BC fibers, increased. The cationic demand sharply increased from $3.5 \times 10^{-5} \text{ eq} \cdot \text{g}^{-1}$ to $8.9 \times 10^{-5} \text{ eq} \cdot \text{g}^{-1}$ when the number of revolutions increased from 6000 r to 8000 r, which indicated that the fragmented bacterial fibers had at that point been dispersed into several fiber bundles, and the specific surface area increased dramatically. Therefore, the number of accessible carboxyl groups per unit mass of fiber increased, and so did the cationic charge demand. As the revolutions further increased, the cationic charge demand of the BC dispersion gradually grew stable. At a certain point, most or all of the BC films were dissociated into the fiber bundles, the cationic charge demand was stable, and rotation was no longer necessary to disperse the BC bundles (Spaic *et al.* 2014). At 10000 r, continuing to increase the revolutions no longer increased the effects of dispersion, but instead prolonged the duration of the experiment and consumed more energy.

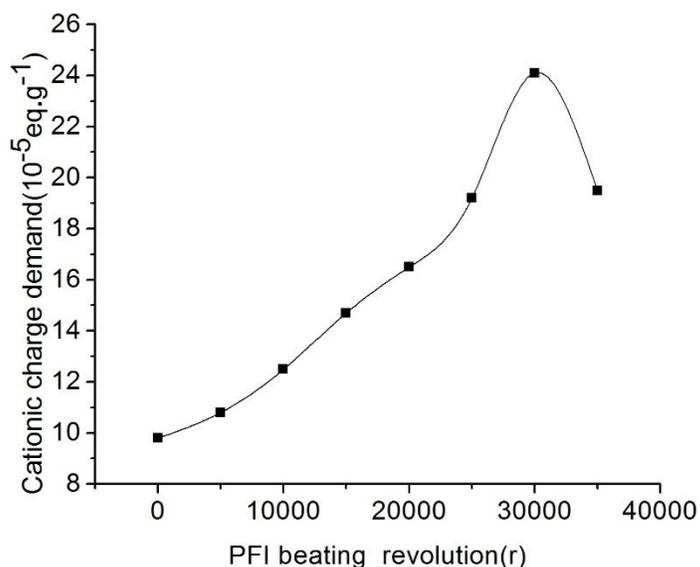


Fig. 3. Cationic charge demand under different PFI revolutions

Figure 3 shows that the cationic charge demand of BC fibers gradually increased as the number of revolutions increased. At the inflection point at 30000 r, the cationic charge demand began to decrease. The PFI beating process indeed dissociated the BC from fiber bundles into small filaments, exposing more hydroxyl groups on the surface of the fibers to increase cationic charge demand. However, excessive revolutions caused unnecessary mechanical refining, which severely fibrillated the BC, caused granulation and flocculation, so the fiber specific surface area was reduced. Therefore, there were lower carboxyl groups to dissociate hydroxyl groups and then the positive charge demand decreased (Iguchi *et al.* 2000; Bhardwaj *et al.* 2007). The optimal PFI beating revolution was judged to be 30000 r.

As shown in Fig. 4, the changes in cationic charge demand were not particularly obvious as the ultrasonic cell crusher treatment progressed. Ultrasound is typically applied to material to create cavitation, *i.e.*, an abundance of small bubbles *via* liquid vibration that ultimately produces a high-energy shock wave (Miller *et al.* 1996; Adam *et al.* 2012). Ultrasound treatment is equivalent to an extremely high temperature and high pressure treatment (Xia and Wang 2002; Bougrier *et al.* 2006). However, due to the solid state, high flexibility, low hardness, and full swelling of the BC fibers in the sample, the ultrasound did not effectively facilitate dispersion. Instead, it only separated the flocculates not closely combined with the fiber bundles. Furthermore, the cavitation effect created by the ultrasound did not readily open the hydrogen bonds between the fibers. The treatment also consumed a great deal of energy and time with very poor results, rendering it altogether unsuitable for BC dispersion.

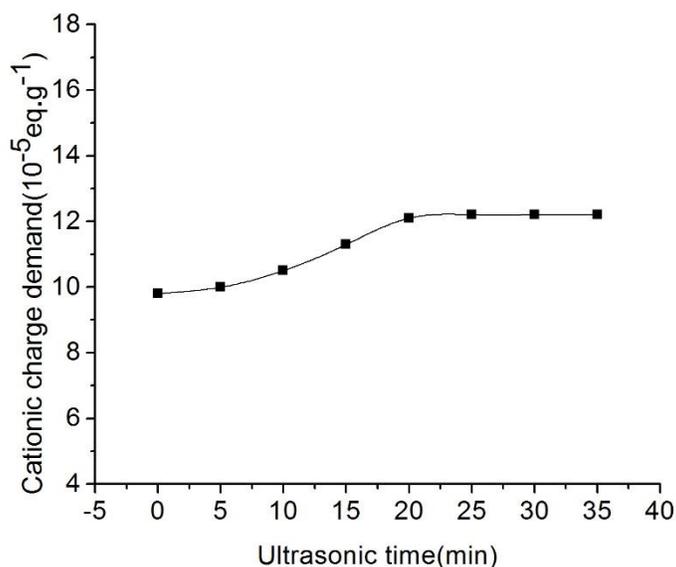


Fig. 4. Cationic charge demand under different ultrasonic time

Table 1. Bacterial Fiber Morphology Analysis of BC under Different Dispersion Methods

	Fiber Length (mm)		Fiber Width (μm)	Fiber Curl Index (%)	Fiber Kink Index (%)
	<i>L</i> (n)	<i>L</i> (w)			
D-Fibers	0.79	0.92	38.90	26.32	20.35
P-Fibers	0.25	0.29	19.51	18.15	11.23

D-Fibers: fibers dispersed with disintegrator; P-Fibers: fibers dispersed with PFI beater

As shown in Table 1, the width of BC fibers was $38.90 \mu\text{m}$, which was similar to that of plant fibers after dispersion with the standard laboratory disintegrator (Yokoyama *et al.* 2002; Tomczak and Satyanarayana 2007; Gao *et al.* 2011). This result suggests that the disintegrator had limited dispersion capability; however, it was helpful as a pretreatment for the wet films. After treatment with PFI, the BC fiber width, curl index, and kink index decreased corresponding to the reduction in fiber length. The physical strength of the paper can be improved by decreased curl index and kink index (Law *et al.* 2001).

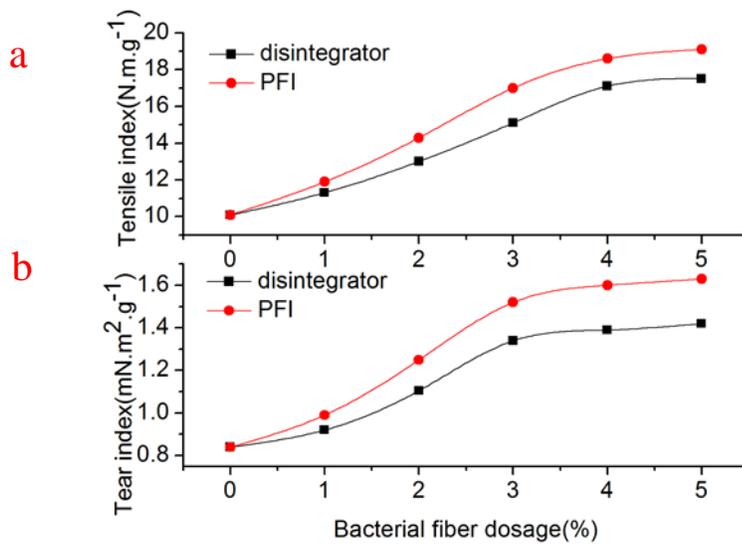


Fig. 5. Effect of BC fiber on the tensile index and tear index of paper

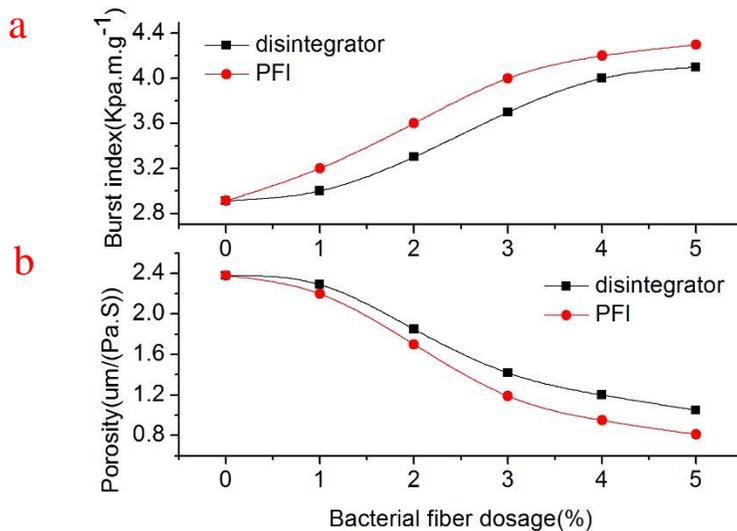


Fig. 6. Effect of BC fiber on the burst index and porosity of paper

Effect of BC on the Properties of Paper

As shown in Figs. 5a, 5b, and Fig. 6a, the paper's tensile index, tear index, and burst index increased as BC increased. The strength of the paper dramatically changed at 3% BC dosage compared with the blank sample (0% BC). The tensile index increased by 52.30%, tear index increased by 49.5%, and burst index increased by 37.45%. Because the elastic modulus of BC is several times (up to 10 times) greater than that of ordinary plant fiber, the tensile strength is high, and Young's modulus is as high as 138 GPa. When BC fibers were dispersed in the pulp, they intertwined with the plant fibers and formed bridging and/or filling effects due to their small size, large specific surface area, and strong adsorption capacity, which filled the holes between the plant fibers, as shown in Fig. 7b. During the sheeting process, the BC attached to the plant fibers to form a compact network. At a 10% BC dosage (Fig. 7c), there was a layer of BC mulch on the surface of the plant

fibers, which may be the main reason for the enhanced physical strength of the paper. The physical strength of paper sheets are influenced by many factors, in addition to the length and strength of the fiber, bonding force between the layers is another factor that affects the strength of the paper. Therefore, when the dosage of BC fiber was more than 3%, the paper physical strength increased slowly and reached a plateau at 4%.

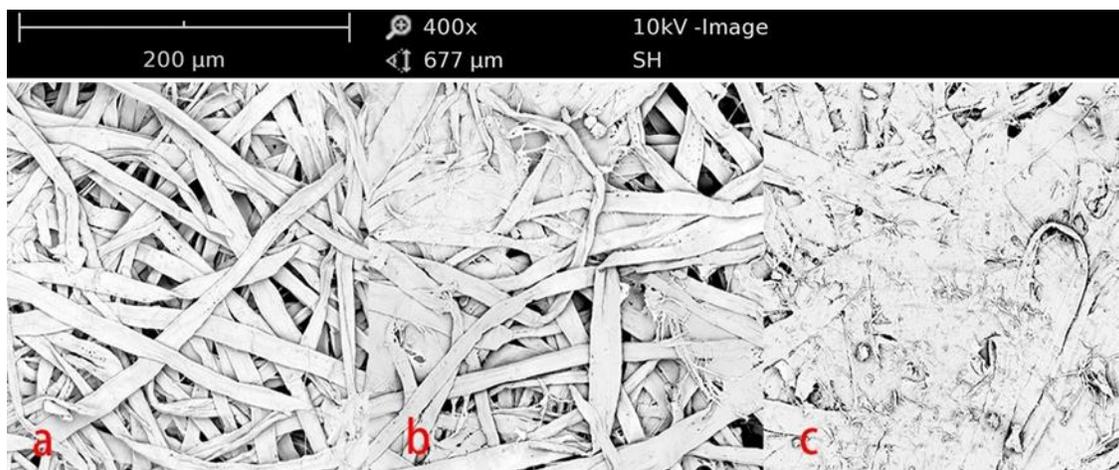


Fig. 7. SEM images of paper sheets (a) without BC, (b) with 3% BC, (c) or with 5% BC

As shown in Fig. 6b, the air permeability decreased as BC dosage increased. The addition of BC improved the bonding force between the fibers, tightening the organization of the paper structure and closing gaps; the thin superficial membrane of BC formed at a certain dosage also gave the paper low air permeability.

Figures 5 and 6 together demonstrate that the physical strength of paper containing BC dispersed by the PFI beater was higher than that of the sample produced *via* standard laboratory disintegrator. Well-dispersed BC was distributed evenly among the plant fibers by the PFI beater. This benefitted the combination of BC and plant fiber to improve the physical properties of the resulting paper sheets.

CONCLUSIONS

1. A standard disintegrator was shown unable to completely disperse bacterial cellulose (BC) wet films to BC fibers but was shown to be an effective pretreatment for the films. The BC was effectively dispersed after treatment with a PFI beater, however. Ultrasonic treatment was not suitable for BC dispersion.

2. Bacterial fibers were shown in this study to effectively improve the physical properties of paper sheets, especially when the BC was dispersed by a 30000 r treatment using a PFI mill.

3. Well-dispersed BC can bridge, fill, cover, and/or combine with plant fibers to improve the properties of the resulting paper.

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