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Producing Cellulose Microfibrils at a High Solid Content with and without Mechanical or Enzymatic Pretreatment

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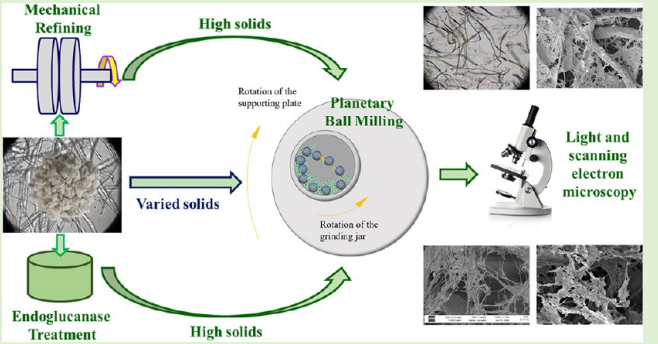
ABSTRACT: This study conducted a detailed evaluation of the feasibility of producing cellulose microfibrils (CMF) from a kraft-bleached hardwood pulp at high solid contents with and without pretreatments. CMFs produced by planetary ball milling at solid contents 17 and 28% were compared with those from 1 to 5% under the same milling conditions. Fiber pretreatments using a commercial endoglucanase and mechanical refining using a laboratory PFI mill were also applied before ball milling at a solid content of 28%. Two mechanisms of fiber fibrillation were identified from the results obtained: (i) ball and fiber/fibril interactions—the primary mechanism and (ii) interfiber/fibril frictional and tensional interactions—the secondary mechanism. The secondary mechanism plays an important role only in early-stage fibrillation and became less important as fibrillation proceeded in the later stage toward nanofibrillation. Improving fiber dispersion at lower solid content facilitated fibrillation. Endoglucanase pretreatment substantially shortened fibers to result in a "pulverized-like" CMF with short fibrils at an extended milling time. Mechanical refining of fibers facilitated fibrillation to result in CMFs with a morphology similar to that from runs without any fiber pretreatment but for a much shorter milling time. Both CMF water retention value (WRV) measurements and CMF suspension sedimentation experiments showed results consistent with imaging observations. The insights gained through this study provide relevant information with commercial significance regarding CMF production at high solids, which is not currently available in the literature.



INTRODUCTION

Microfibrillated cellulose or cellulose microfibrils (CMF) characterized by their entangled and branched fibril network structure with fibril diameters ranging from nanometers to micrometers[1,2] have emerged as a novel and promising cellulosic material for a variety of applications such as barrier coating[3] and polymer reinforcement in paper-based packaging.[4] Based on a recent report,[5] at least five companies have CMF production capacity over 100 tons per year with five additional companies having annual capacities over 10 tons per year globally. While CMF can be produced using a diverse plant biomass such as bamboo, wood, cotton, and agriculture biomass,[6] currently, it is mostly produced at very low solids of approximately 3−5% through mechanical fibrillation with or without prior chemical or enzymatic or mechanical pretreatments.[7−9] Due to the strong hydrophilic nature of cellulose and great CMF surface area, dewatering of CMF is difficult because of the gel-like behavior at concentration as low as 2−3%.[10] High water content not only significantly increases energy cost for shipping wet CMF and dewatering, but the water from dewatering also contains a significant amount of polysaccharides that can induce microbial growth without treatment. All of these issues are environmental concerns and affect sustainability.

Limited studies were conducted on fibrillation of cellulosic fibers at high solid contents, illustrating the difficulties of the subject partly due to limited facilities capable of efficiently fibrillating cellulosic fibers at high solid contents. Milling in a PFI mill was applied to fibrillate bleached birchwood fibers up to solid content of 15% after oxidative pretreatment using sodium periodate and chlorite.[11] Twin-screw extrusion was evaluated for microfibrillating an extensively refined bleached softwood kraft pulp with an ultralow freeness of 10 mL CSF (Canadian standard freeness) at a solid content of 28%.[12] The solid content was increased to 33, 36, 39, and 45% after extrusion for 1, 5, 10, and 14 passes due to water evaporation, and the great numbers of passes applied indicated the inefficiency in fibrillation. Twin screw extrusion was also applied to fibrillate chemically (TEMPO-mediated oxidation) pretreated bleached eucalyptus wood fibers.[13] High shear

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mixing with simultaneous active enzymatic actions was also evaluated for fibrillating bleached and unbleached kraft softwood fibers at a solid content of 25% using a commercial two-shaft sigma mixer[14] and achieved micro- to nanofibrillation with a low energy consumption of 0.6 MWh/ton. While these studies demonstrated the feasibilities of fibrillating cellulose fibers at high solid contents, using chemical treatments or extensive mechanical refining (high energy cost) can result in environmental concerns. Furthermore, these limited studies did not study the effects of solid content on cellulose fibrillation at high solid contents.

Ball milling, though lacking scalability for commercial CMF production, has the advantage of using a small amount of fibers and experimental simplicity in operation for laboratory studies to obtain basic understanding of cellulose fibrillation processes. Ball milling of dry wood or cellulosic materials, especially under cryogenic conditions, are frequently employed for pulverizing wood and cellulose to study basic wood or cellulose properties.[15] However, fibrillation rather than pulverization is essential for CMF production. Earlier studies using ball milling for cellulose fibrillation were conducted at low solid contents less than 5%. For example, a systematic study of various parameters in ball milling on the resultant fibrillated cellulose morphology was carried out at a solid content of 1%.[16] The study found that the ball size is important to produce fibrils rather than powders. Other parameters such as the ball-to-fiber mass ratio and milling time can affect fibrillation productivity. Alkaline pretreatment using carbonate improved productivity.[16] It was found that chemical pretreatment with TEMPO-mediated oxidation greatly facilitated fibrillation at extremely high solid contents of 50−93% (near the dry condition) using a vibrational ball milling.[17] However, earlier studies indicated that fibrillation at high solid contents without chemical and enzymatic treatment of fibers was extremely difficult by ball milling or twin-screw extrusion.[17,18] For fibers without TEMPO oxidation, a significant amount of minimally fibrillated fibers remained after ball milling. The effects of solid content on fibrillation were not elucidated or inconclusive.[17]

The objectives of the present study are (1) to examine the potential of producing CMF at a high solid content without any fiber pretreatment using planetary ball milling with specific focus on understanding the effect of fiber solid content on fibrillation and the morphology of the resultant fibrils and (2) to evaluate the effect of pulp fiber pretreatments, specifically nonchemical pretreatments, i.e., endoglucanase and mild mechanical refining, on cellulose fiber fibrillation at a high solid content. We hypothesize that (1) the interfiber/fibril interaction at very high solid contents can be an important secondary mechanism to facilitate CMF production and (2) fiber pretreatment using mechanical refining can facilitate production of longer fibrils than enzymatic treatment that cuts fibers. The novelty of the present work lies in the elucidation of fibrillation mechanisms in CMF production at high solid contents and the differences between enzymatic treatment—a cellulose chain cutting treatment and mechanical refining—a compressive and shearing treatment on the resultant CMF morphology from high solid ball milling in terms of fibrillation and pulverization.

Recognizing the extreme difficulties in quantitative characterization of CMFs due to their highly branched network structure as discussed in literature[1,2] and avoiding drawing misleading conclusions from the quantitative fibril diameter and/or length distributions obtained using conventional techniques such as fiber imaging analysis and dynamic light scattering, the morphology of the fibrillated fibers was visualized using light microscopy (LM) and scanning electron microscopy (SEM) imaging in a wide range of magnifications. Unlike cellulose nanofibrils (CNF), especially those from fibers with enzymatic pretreatment, that are free of a delaminated fibril sheet structure,[19] CMF contains a significant amount of delaminated thin fibril sheet structures as well as highly branched and entangled fibril structures, which makes quantitative characterization of fibril diameter and length extremely difficult. The images, however, revealed the insights to the difficulties in CMF production at high solid contents and the effectiveness of sustainable fiber pretreatment methods.

MATERIALS AND METHODS

Materials. A never dried commercial bleached kraft hardwood (mixture of birch and maple) pulp (BKHP) at a solid content of 17% was obtained from a U.S. pulp mill. The pulp fibers have a cellulose, xylan, and lignin content of 77.7, 23.1, and 2.7% (Table S1), respectively, determined by the Analytical Chemistry and Microscopy Lab at our Laboratory.[20] Commercial endoglucanase FiberCare (lot CGP20051) was complementarily provided by Novozymes North America (Franklinton, NC). The endoglucanase protein content of 7.53 mg/mL was determined using the Bio-Rad protein assay with bovine serum albumin (BSA) as standard.[21]

Ball Milling. A planetary ball mill (PM100, Retsch Lab Equipment, Germany) was used to fibrillate the BKHP fibers. Planetary ball mills use a rotational ball mill jar to drive the grinding balls to impact the sample through extrusion and frictional interactions. Here, the rotational direction of ball mill main wheel and the rotation direction of the ball mill jar were set in opposite directions (Figure S1), so that the two rotational speeds can be superimposed on the ball mill jar to result in great energy for the grinding balls inside the jar. Unless indicated, the BKHP without any chemical, enzymatic, and mechanical pretreatments was directly used for ball milling. Manual dewatering was applied to result in a pulp fiber solid content of 28 wt % for high solid ball milling. Fiber suspensions with a solid content of 1, 5, 17, and 28 wt % were each separately fed into the ball mill. Milling was conducted at an overall constant rotational speed of 600 rpm for various durations of 0.5, 1, 2, 3, and 6 h. The ball-to-dry fiber mass ratio (w/w), B/F, was maintained at 15:1 utilizing a total of 10 balls with a diameter of 10 mm except for the run at a solid content of 1% with only 3 balls. At the lowest solid content of 1%, a run with B/F = 75:1 was also conducted using 10 balls of 10 mm in diameter. A run with B/F = 10:1 was also conducted at a solid content of 28%.

Enzymatic Pretreatment and Mechanical Refining. To understand fiber pretreatment on high solid ball milling, the as-received BKHP was either enzymatically pretreated or mechanically refined using a PFI mill (No. 160, Testing Machines, Inc., Amityville, Long Island, NY). The enzymatic pretreatment was conducted using a commercial endoglucanase FiberCare (FR) at a protein dosage of 1 mg protein/g fiber (abbreviated as mg/g) in a flask shaker (Eppendorf NB Excella E25) at 200 rpm, 50 °C, and a fiber solid content of 5% in a 50 mM acetate buffer with a pH of 5.0.[22] The pretreatment lasted for 24 h, and endoglucanase was then deactivated by heating the treated fiber suspension at 90 °C for 15 min at the end of the process.[19] FR-pretreated fiber solids were then washed with the same acetate buffer, followed by water wash 3−4 times and vacuum-filtered to remove soluble sugars. The washed fibers were used for ball milling for fibrillation. Cellulose loss was minimum when the fibers underwent endoglucanase treatment with a cellulosic solid yield of 99.7%. The BKHP was also PFI-milled for 3000 revolutions at 1725 rpm to result in a pulp fiber sample of freeness approximately 200 mL of CSF.

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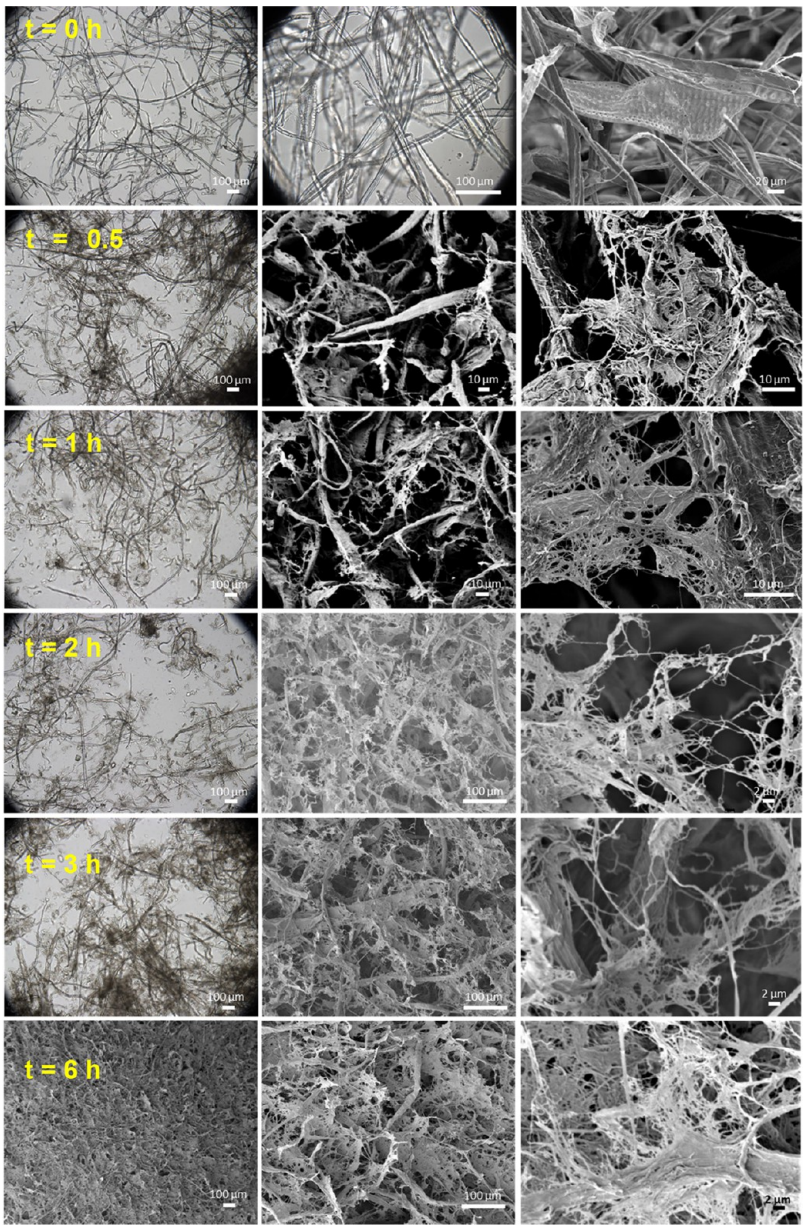

Figure 1. Time-dependent morphology development of fibrils from ball milling at a solid content of 28% with a ball-to-fiber mass ratio of 10:1. Scale bars for all LM images = 100 μm. Middle and right columns (except the top row) are higher-magnification SEM images of the same sample in the same row.

Both the FR-pretreated and PFI mill-refined fibers were dewatered manually and used for ball milling at a solid content of 28% compared with the control run using BKHP without any pretreatments.

Light Microscopic and Scanning Electron Microscopic Imaging. The morphologies of CMFs from ball milling were observed first using light microscopy (LM) which provides an overall morphological characteristic of a fibril sample.[1,23] An aliquot of the aqueous CMF suspension was directly dropped onto a microscope slide. Bright-field images were captured using a Nikon Eclipse Ci microscope (Model 707484, Nikon Corp., Tokyo, Japan) equipped with an FLIR Grasshopper 3 camera. LM images were also obtained by using bright-field optics and a 10x objective lens of a Zeiss Axiophot microscope (D-7082 Oberkohen, Germany). A green filter was placed over the field diaphragm on the illuminating beam path to increase the contrast of the imaging.

To view the fine details of the CMFs, especially those from extended ball-milling time, scanning electron microscopic (SEM) imaging was performed.[1] A drop of an aqueous CMF suspension was placed onto carbon tape, which was mounted on an aluminum stub. The sample was then frozen at −20 °C. It was further frozen in liquid nitrogen (N_2) after 1 h and transferred to a precooled freeze-drier at −60 °C and 0.133 mbar (100 μm Hg). The frozen sample was lyophilized and then sputter-coated with a layer of gold to enhance the conductivity. SEM images were captured by using a Zeiss Leo Evo 40 SEM system operating at a 10 kV accelerating voltage. To ensure that the obtained images were statistically meaningful, many images were taken from the same sample.

Water Retention Value (WRV) Determination. Previous studies indicated that WRV can be an indirect measure for comparing the extents of microfibrillation of CMFs from the same fiber source.[1,24] The WRVs of the ball-milled CMFs were determined according to the Scandinavian standard test method SCAN-C 62:00 as described previously.[25] Approximately 14 g of aqueous suspension of cellulosic materials at a solid consistency of 2% was wrapped in a filtration membrane of 0.1 μm pore size (JVWP14225, Millipore Corporation, USA). The membrane-wrapped samples were centrifuged in a centrifuge tube with the support of a stainless steel screen so that water can be accumulated in the space below the screen at the

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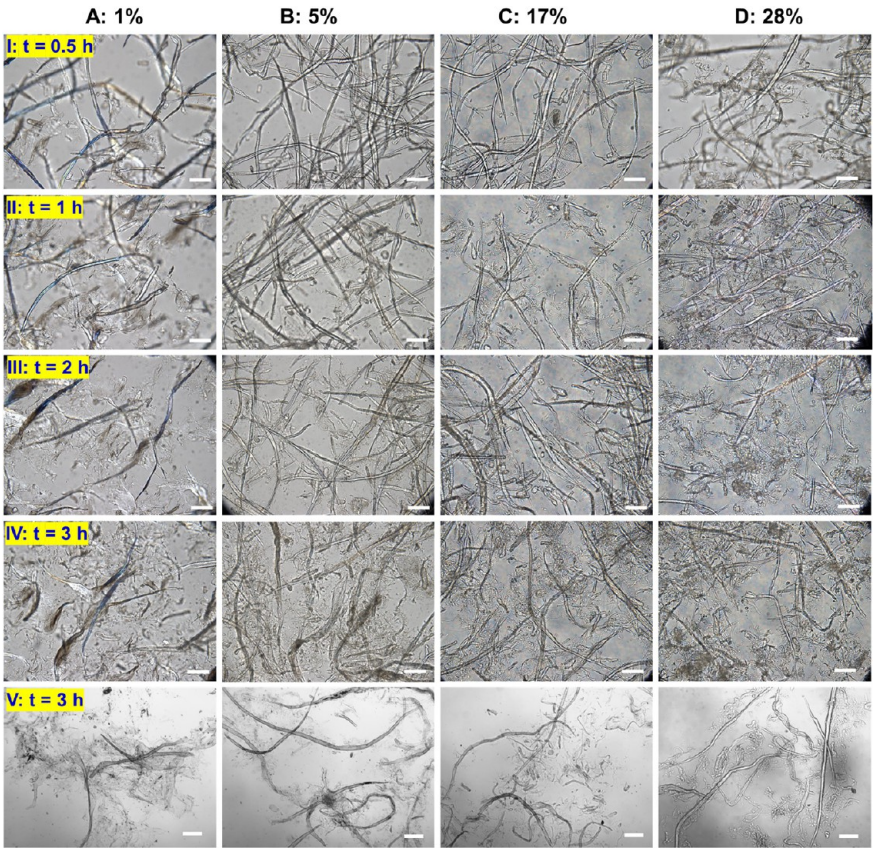

Figure 2. LM images showing the effect of the fiber solid content on time-dependent morphologies of fibrillated cellulosic fibers from planetary ball milling. The mill spinning speed was 600 rpm, and the ball-to-fiber mass ratio (B/F) was 15:1. Scale bars = 100 μm.

bottom of the tube during centrifugation.[1] The centrifugation was carried out at 3000 *g* for 15 min (EXD 4871, International Equipment Co., Chattanooga, TN, USA).

WRV of a cellulosic sample was then calculated according to eq 1:

$$\mathrm{WRV}(\%) = \frac{m_{\mathrm{wet}} - m_{\mathrm{od}}}{m_{\mathrm{od}}} \times 100 \quad (1)$$

where m_{wet} is the weight of the wet cellulosic sample after centrifugation and m_{od} is the oven dry weight of the same wet cellulosic sample.

Suspension Stability Evaluation. The stability of a CMF suspension can be another indirect measure of the extent of fibrillation of CMFs from the same fiber source.[1] It is determined by diluting a CMF suspension to 0.25 wt %[26] and then sonicated to achieve a uniform dispersion. The sedimentation behavior of the dispersion was visually observed over a designated period of time. Images of the dispersion were captured at 0, 1, 2, 18, and 24 h. The stability of the suspensions was determined using the following equation:[27]

$$\text{stability } (\%) = \left(\text{Dispersion height}/\text{Initial height}\right) \times 100 \quad (2)$$

where "Dispersion height" is the height of the dispersion at a given sedimentation time and "Initial height" is the dispersion height before sedimentation.

RESULTS AND DISCUSSION

Time-Dependent Fibril Development by High Solid Ball Milling. The as-received pulp fibers have a length weight-average length of approximately 1.2 mm measured by a fiber analyzer (FQA, LDA02, OpTest Equipment Inc., Canada) as described earlier[1] and a width of 12 μm as shown by the LM images in the top row (t = 0 h) in Figure 1 (also Figure S2). The pulp also contains a lot of fines (particles with lengths less than 100 μm). Vessel elements can be seen. The main feature by ball milling was cell wall delamination to result in significantly flattened cell walls, as observed from both the LM (left column except for t = 6 h) and SEM images (middle and right columns except for t = 0). This suggests that the ball size of 10 mm used was appropriate and did not cause fiber to pulverize.[16] Furthermore, the extent of delamination was increased with an increasing milling time. This is similar to disk milling observed previously,[1,9] indicating that compression force on fibers was created when balls hit fibers. Large fibers remained even after 2 h of ball milling. However, submicrometer fibrils can be clearly seen even after 0.5 h milling. It appears that a fairly uniformly delaminated and highly branched CMF with a substantial amount of submicrometer scale fibrils was obtained after 2 h ball milling based on the SEM images shown, indicating that cellulose fibers can be effectively fibrillated into CMF at a high solid content of 28% without chemical, enzymatic, or mechanical pretreatment. Extending ball milling to over 3 h did not substantially improve fibrillation, indicating that ball milling is not effective for nanoscale fibrillation, similar to disk milling.[1]

Solid Content on Fiber Fibrillation by Ball Milling. While ball milling of dry wood or fibers is effective for producing pulverized cellulosic particles,[15] fibrillation rather than pulverization is required for CMF production. As shown by LM images in Figure 2, the initial fibrillation of fibers can be seen after milling for 0.5 h (row I). This initial fibrillation was more apparent at the lowest (1%) and highest (28%) solid contents with fiber shortening and delamination. Initial

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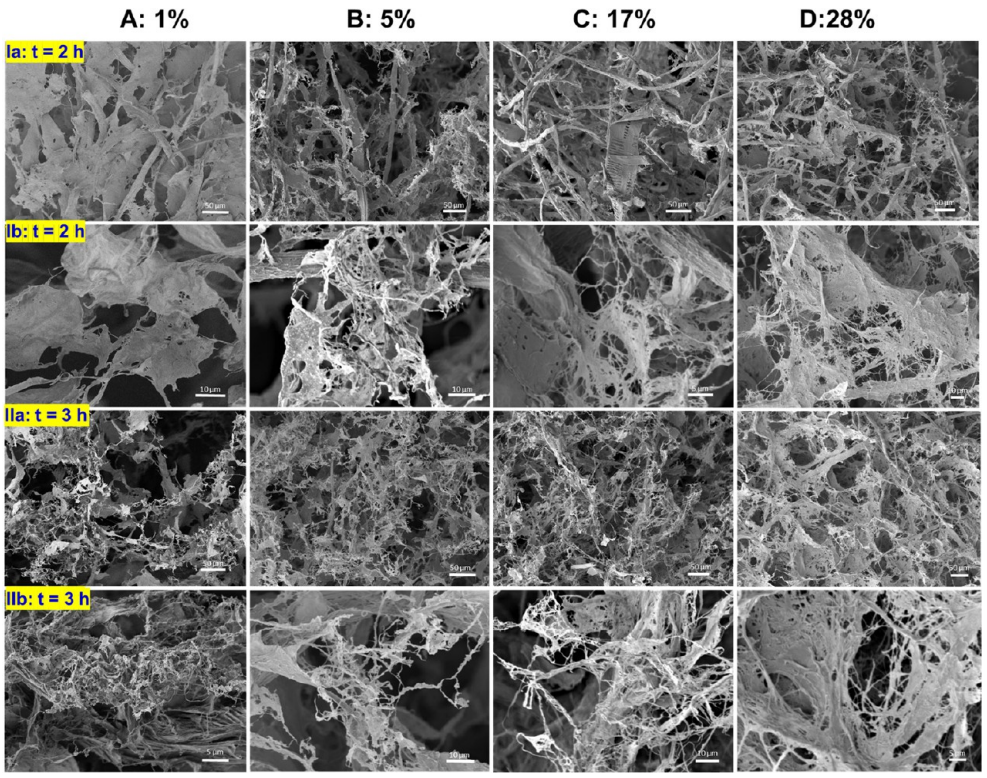

Figure 3. SEM images showing the effect of the fiber solid content on the microstructures of CMFs from planetary ball milling. The mill rotation speed was 600 rpm, and the ball-to-fiber mass ratio (B/F) was 15:1.

fibrillation was less clearly observed at a solid content of 5% compared with that at a solid content of 1 and 28%. Increasing solid content to 17%, the extent of initial fibrillation was slightly increased. This observation of the solid content effect on fiber fibrillation was further observed at 1 h (Figure 2). To ensure that this observation is representative, several rounds LM (Figure S3) as well as green light filter LM (Figure S4) were taken, and all confirmed this observation.

These observed effects of solid content on the morphology of the resulting CMFs through an earlier stage of fibrillation can be explained by the two mechanisms governing high solid fiber/fibril fibrillation: (i) the interactions between the balls and the fibers/fibrils as the primary mechanism and (ii) the interactions among fibers/fibrils themselves as the secondary mechanism. Increasing fiber solid content significantly increased the interactions (frictional and tensional due to entanglement) among fibers and fibrils. Using the crowding factor,[28,29] we can estimate the minimal fiber solid content necessary to form fiber flocks, a necessary condition to create interfiber/fibril interactions—mechanism (ii),

$$N = \frac{2}{3} C_v \left(\frac{L}{d}\right)^2 \tag{3}$$

where N is the crowding factor, C_v is the fiber volumetric concentration, L is the average fiber length, and d is the average fiber diameter. The average fiber length was measured at 1.2 mm, and the average fiber diameter was estimated at 12 μm based on LM imaging (Figure S2) with the assumption of a fiber density of 0.67 g/cm^3 (mixture of birch and maple),

$$C_m = \frac{9}{4} N \left(\frac{d}{L}\right)^2 \tag{4}$$

where C_m is the fiber mass concentration or solid content. It was suggested based on analytical analysis that 3 fiber contacts is the minimal condition to form continuous fiber networks—fiber flocculation, which gives $N = 60$.[29] Often times, the number of contacts up to 5 with $N = 130$ is necessary. Therefore, the minimal initial solid content of 1.35−2.93% is required to form coherent fiber flocks. It should be pointed out that forming fiber flocks is only a necessary condition to produce fibers/fibril interactions. At this critical solid content, the interactive tensional and frictional forces among fibers/fibrils are weak and may not improve fibrillation. A much higher concentration is needed to result in interfiber/fibril interactions with sufficient energy to fibrillate through the secondary mechanism (ii). The greater the solid content, the stronger the interfiber/fibril interactions.

It is worth pointing out that eq 3 has been applied to determining the aspect ratio (L/d) of CMF[30] and CNF (cellulose nanofibrils)[31] samples by assuming CMF and CNF density ~1.5 and C_m being the CMF or CNF gelation concentration obtained from rheology or sedimentation measurements. However, this aspect ratio may not provide additional information for the present CMF samples, especially those from the early stage of fibrillation, due to the significant network and sheet-like structures shown. The CMF samples used in these studies are actually CNF with a distinctive nanofibril morphology based on ISO specification (ISO/TS 20477:2017).

This above analysis indicates that there were no interfiber/fibril interactions at the very low solid content of 1%. The only mechanism of fibrillation was through the interactions between the balls and fibers/fibrils—the primary mechanism (i). A solid content of 1%, however, facilitated the interaction between the balls and the fiber/fibrils due to good fiber/fibril dispersion,

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which resulted in a greater extent of fibrillation than those for the runs at solid contents of 5 and 17% in the earlier stage of milling (row I, Figure 2). The interfiber/fibril interaction—the secondary mechanism (ii)—was probably negligible at a solid content of 5% due to weak fiber flocks formed as discussed earlier and was only increased slightly at 17%. As shown in Figure 2 (rows I to III), the extent of fibrillation at 17% was greater than at 5% but remained lower than at 1%. The worsening fiber dispersion at these elevated solid contents hindered fibrillation. At a high solid content of 28%, the interfiber/fibril frictional and tensional interactions—the secondary mechanism (ii)—became important to result in a greater extent of fibrillation than the runs at 5 and 17% solid contents (comparing Figure 2D with Figure 2B,C in rows I–III) in the earlier stage of fibrillation (up to 2 h). This validated our first hypothesis—interfiber/fibril interaction is important to CMF production at high solid contents.

Extending milling beyond 2 h, the interactions between the balls and fibers/fibrils—mechanism (i)—dominated, and the interaction among fibrils—the secondary mechanism (ii)—became less effective. As a result, lower solid contents produced a greater extent of fibrillations. Comparing Figure 2A (1% solid content) with Figure 2D (28% solid content), the better dispersion at 1% solid content produced more finer fibrils than the run at 28% solid contents with poor dispersion as clearly shown (rows IV and V of Figure 2A,D). Furthermore, the fineness of fibrils follows the order of solid content, i.e., the CMF from the run at 1% being the finest, followed by the CMF from 5, 17, and 28%, as can be clearly seen from the green light microscopy images (row V in Figure 3). This suggests that the interaction between the ball of fiber/fibrils—the primary mechanism (i)—has stronger energy than the interaction among fiber/fibrils—the secondary mechanism (ii). Because nanofibrillation requires higher energy input than microfibrillation, the interactions between fiber/fibrils—secondary mechanism (ii)—or high solid content benefits more for CMF production (earlier stage of fibrillation) than for producing CNFs, in other words, low solid content is better suited for late-stage nanoscale fibrillation for producing CNFs. These results filled the literature gap on understanding the effect of solid content on fiber fibrillation and has practical significance because high solid content can reduce water usage and ease transporting CMFs.

SEM analysis was applied to observe microstructures of the CMF samples from fibrillation at different solid contents for 2 and 3h. Significant fiber delamination was observed for the ball milling run at a very low solid content of 1%, as shown in Figure 3AIa. However, fibers are mostly intact with a low degree of delamination when fibers were ball-milled at solid contents of 5% (Figure 3BIa), 17% (Figure 3CIa), and even at 28% (Figure 3DIa). Fibrillation at 28% (Figure 3DIa) did result in a greater extent of delamination than those at 5% (Figure 3BIa) and 17% (Figure 3CIa). Observations under a greater magnification suggest that the main feature of the CMF from the run at a solid content of 1% (Figure 3AIb) was delaminated fibril sheets with fewer large microfibers (Figure 3AIb) due to better fiber dispersion than at 5% (Figure 3BIb), 17% (Figure 3CIb), and 28% (Figure 3DIb) solid contents. This difference in the microstructure between the CMFs from the very low solid content 1% and higher solid contents resulted from the difference in the milling mechanisms, as discussed in the previous paragraph. Good dispersion at a solid content of 1% significantly promoted the interactions between balls and fibers/fibrils—the primary mechanism (i)—to result in the greatest extent of delamination among all the runs at different solid contents due to compression forces from the ball impacting the fibers. However, the interactions among fibers/fibrils—the secondary mechanism (ii)—promoted interfibril interactions to result in improved fibrillation at the very high solid contents (28%, Figure 3D) compared with the runs at medium solid contents of 5% (Figure 3B) and 17% (Figure 3C).

Increasing fibrillation time to 3h increased the extent of delamination for all samples (rows IIa and IIb in Figure 3). The interaction among fibers/fibrils—the secondary mechanism (ii)—became less important as discussed earlier. The interactions between balls and fibers/fibrils—the primary mechanism (i)—dominated. The better dispersion at lower solid contents facilitated the primary mechanism (i) and produced a greater extent of fibrillation, i.e., the CMF from the run at a solid content of 1% (Figure 3AII) appeared as finer or thinner delaminated fibril sheet fragments than the runs at 5% (Figure 3BII), 17% (Figure 3CII), and 28% (Figure 3DII). More microfibers were observed in CMF samples from higher solid contents, especially at 28%, in agreement with green light microscopy imaging (Figure 2V).

Pulverization versus Fibrillation in Ball Milling. As stated earlier, fibrillation and avoiding pulverization are critical for producing CMFs. The balls and fibers/fibril interactions—mechanism (i)—dominated at a very low solid content of 1%, which can result in pulverization actions during ball milling using large ball sizes, as reported in literature.[16] The effect of the ball-to-fiber mass ratio B/F on CMF production was evaluated at a solid content of 1% with a ball size of 10 mm. The run with a greater B/F of 75:1 resulted in a greater extent of fibril cutting or "pulverization-like" action than the run with B/F of 15:1 at the same solid content (Figure S5). Less long-fibrils were observed, especially at a long milling time of 3 h with B/F = 75:1 (comparing right column Figure S6IIb with Figure 3IIb).

The effect of the ball-to-fiber mass ratio B/F on fiber fibrillation at a high solid content of 28% was also observed by comparing the CMFs obtained from two sets of runs with B/F = 15 and 10 with all other milling conditions being the same. The SEM images did show that the higher B/F run resulted in slightly improved fibrillation, especially in the early stage of fibrillation (Figure S7).

Fibrillation Evaluated by the Water Retention Value. The water retention value (WRV) measures the water accessible volume of a cellulosic material to water molecule,[25] which is often increased with increasing extent of fibrillation and can be used to qualitatively characterize the fibrillation extent of CMFs from the same fiber source.[1,24] The WRV of the as-received BKHP fibers was approximately 108% and increased with increasing milling time, as shown in Figure 4. Ball milling at the lowest solid content of 1% resulted in the greatest WRVs among all CMF samples from different solid contents under the same B/F ratio for the same period of milling time (Figure 4A). This is consistent with the observation from LM and SEM imaging, as discussed previously. Milling at the lowest solid content of 1% also resulted in the greatest increase in WRV with increasing time and achieved the greatest WRV of approximately 450% in 3 h.

Increasing milling solids from 1 to 5% decreased the WRV of the resultant CMFs; however, further increasing solid content above 5% increased WRV in the early stage of fibrillation up to

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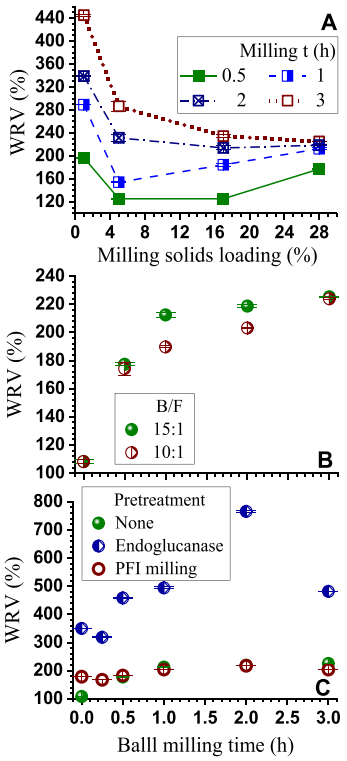

Figure 4. WRV of CMFs from different ball milling and pretreatment conditions. (A) Effect of solid content in milling; (B) effect of the ball-to-fiber mass ratio (B/F) in milling; and (C) effect of fiber pretreatment. The solid content was 28% for both (B,C).

milling time 1 h, i.e., WRVs of the CMF from the solid content of 28% were greater than the WRVs of the corresponding CMFs from 5 to 17% solid contents (Figure 4A). Therefore, high solid content with interfiber/fibril interactions—the secondary mechanism (ii)—facilitated fibrillation in the early stage of milling. This secondary mechanism (ii) became less effective as fibrillation continued toward nanofibrillation, and the primary mechanism (i) dominated. As a result, the rate of increase in WRV with milling time slowed quickly at solid content 28% compared with the runs at 5 and 17% solids. As a result, the WRV of the CMFs from 5 to 17% solid content surpassed the corresponding WRV of the CMFs from the solid content of 28% beyond the milling time of 2 h (Figure 4A). This is again consistent with the imaging results shown in Figures 2 and 3, as discussed previously.

Increasing the ball-to-fiber mass ratio B/F increased energy input in ball milling, which increased WRV, as shown in Figure 4B.

Fibrillation Evaluated by CMF Suspension Stability. The stability of CMF suspensions can also serve as an indicator of the degree of fibrillation.[1] Earlier studies demonstrated a good correlation between the suspension stability and the extent of fibrillation for CMFs derived from the same bleached fibers.[26,27] Figure 5 displays sedimentation images of CMF suspensions standing for varied intervals of up to 24 h. These images provide visual information about the changes in suspension sedimentation over time, allowing for assessing CMF suspension stability. The CMF suspensions shown were produced using a ball-to-dry fiber mass ratio of B/F = 15:1 for milling 0.5 h (bottles 2−5) and 3 h (bottles 6−9). The images show that most CMF suspensions settled very quickly (Figure 5). The CMF produced at a solid content of 28% for milling

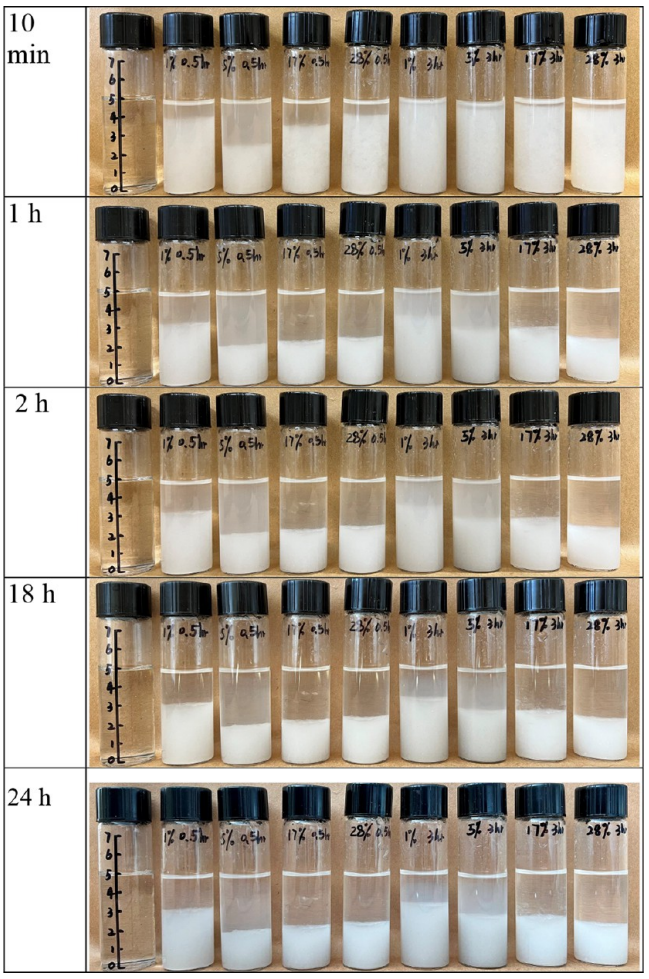

Figure 5. Time-dependent CMF suspension sedimentation images. From left to right: bottle 1 is a dummy bottle to show scale; bottles 2−5 and 6−9 were CMFs from ball milling for 0.5 and 3 h, respectively, and for each set of bottles from left to right, milling solid contents were 1, 5, 17, and 28%, respectively.

time 0.5 h (bottles 2−5, early stage) exhibited better stability or had greater extent of fibrillation than the corresponding CMFs from 5 to 17% solids (Figures 5 and S8). As well explained from the WRV data, the interfiber/fibril interaction—the secondary mechanism (ii)—played an important role in the early stage of fibrillation. This role diminished as fibrillation proceeded, as can be seen from the decreasing CMF suspension stabilities with increasing solid content for CMFs from milling for 3 h (bottles 6−9 in Figures 5 and S8). This is again consistent with WRV measurements (Figure 4A) and LM imaging (Figure 2I,II).

It is interesting to notice that more haze was observed above the settling boundary from the bottles of the CMFs from 3 h milling at 1% (bottle 6) and 5% (bottle 7) solid contents than the corresponding bottles (bottles 8 and 9) with CMFs from high solid contents of 17 and 28% (Figure 5). This suggests that the CMFs from milling at low solid contents 1 and 5% contained more finer and less-aggregated fibrils as observed from imaging (Figures 2 and 3) discussed earlier. These finer fibrils have a lower tendency to settle than the bulk branched fibril network suspension and some of them stuck onto the bottle wall, which created the greater haze. Again, this observation indicates milling at a low solid content for an

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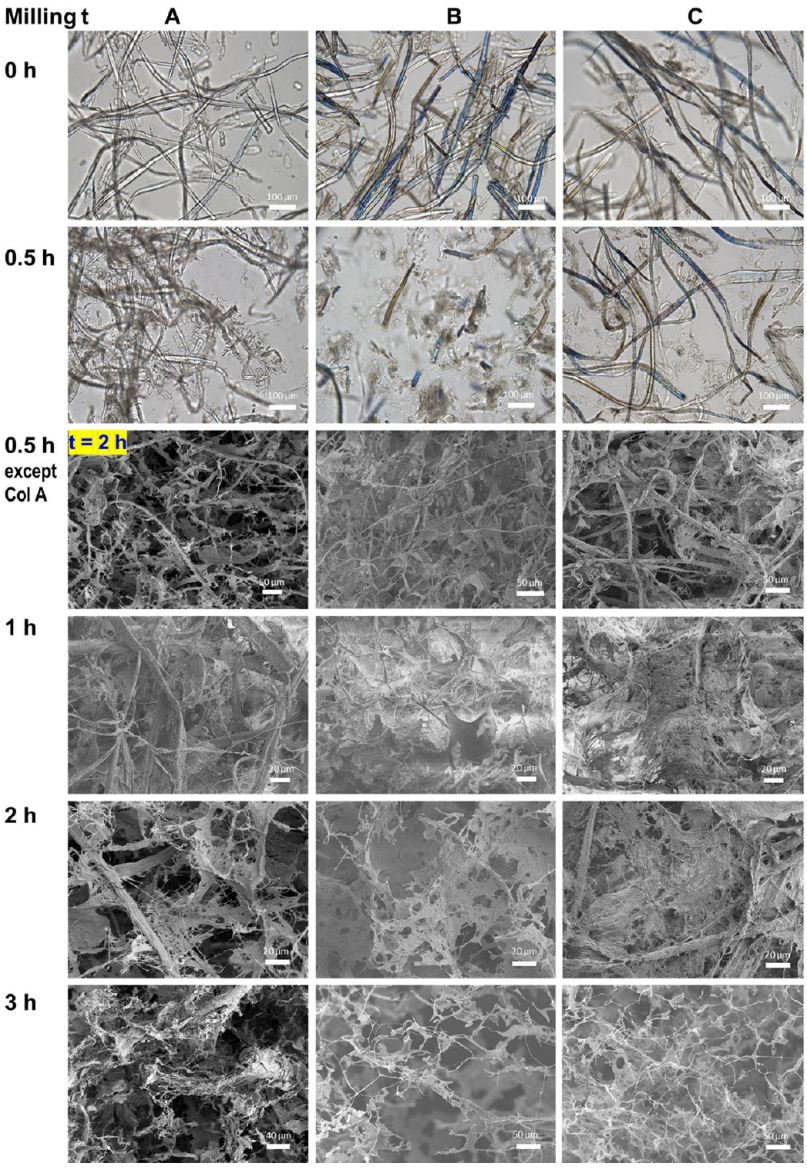

Figure 6. Effects of pulp fiber pretreatment on ball milling BKHP at a solid content of 28% observed from time-dependent imaging analyses. Column A: untreated BKHP; column B: enzymatic pretreated BKHP using FR; column C: mechanically refined BKHP to 200 mL of CSF using a PFI mill. Scale for LM = 100 μm

extended fibrillation time resulted in finer and less-aggregated fibrils than CMFs from milling at higher solid contents due to better dispersion, in agreement with observation from imaging (Figures 2 and 3) and WRV measurements (Figure 4A). These structural differences among CMFs from different solid contents affected their WRV and their suspension stabilities.

Effect of Endoglucanase Pretreatment and Mechanical Refining on High Solid Fibrillation. It is well-known that pretreatment of cellulosic fibers can facilitate fibrillation.[32] Prior studies suggested that fiber length reduction is beneficial to cellulose fibrillation.[7] Here, we evaluated pretreatment using a commercial endoglucanase FiberCare (FR) to enzymatically cut fibers in length and mechanical refining to prefibrillate fibers through compressive and shearing actions on CMF production at a high solid content. As shown in Figure 6 (0 h, top row), FR pretreatment substantially shortened fibers (Column B) to 1 mm or shorter in comparison with the original BKHP fibers (Column A) and PFI mill-refined BKHP fibers (Column C). This fiber length difference is further observed after 30 min of ball milling (0.5 h, row 2). SEM images at a greater magnification show that the CMF from FR pretreatment contains some fairly long fibrils with a diameter of approximately 1 μm together with delaminated fragments (Column B, row 3), while the CMF from PFI mill-refined fibers remained fiber-like with some degree of delamination (Column C, row 3). This validated the effectiveness of FR pretreatment—part of our second hypothesis. Continued ball milling further increased the extent of fibrillation for all three samples (1 h, row 4). The fragments observed in the CMF with FR pretreatment (Column B, row 3) became fibrillated, and micrometer-sized long fibrils remained (Column B, row 4). The CMFs from both the PFI mill-refined and unpretreated BKHP fibers were also significantly delaminated and fibrillated. However, PFI mill refining substantially improved fibrillation and resulted in more fine fibrils of hundred nanometers than the run without pretreatment (comparing Column C with A in row 4). It appears that the morphology or the extent of fibrillation of the CMF from untreated BKHP

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after 2 h of ball milling is similar to that of the CMF from PFI mill-refined BKHP after only 0.5 h of ball milling (comparing Column A with Column C in row 3), indicating that the effectiveness of mechanical refining on microfibrillation—once again validated our second hypothesis. The significance of this finding is that the application of mechanical refining of pulp fibers—a low energy cost step—can be easily implemented and should be implemented to substantially facilitate micro- or nanofibrillation in producing CMFs or cellulose nanomaterials.

At an extended ball milling time of 2 h and beyond, fibrillation continued for all three samples. Furthermore, it appears however that the CMF from FR pretreatment is further shortened and the numbers of micrometer-sized long fibrils decreased (Figures 6 and 2h, Column B row 5). The morphology is "pulverized-like" with increasing nanoscale fibrils and small fibril fragments (Figures 6 and S9, Column B 3h), similar to that observed from ball milling at 1 wt % solid content with a high ball-to-fiber mass ratio of 75:1 (comparing Column B row 5 (2 h) in Figure 6 with Figure S6IIb: t = 3 h). This indicates the effectiveness of FR treatment for nanofibrillation. The efficacy of PFI mill refining on microfibrillation is also clearly shown (comparing Column A with C for 2 and 3 h in Figure 6, also Figure S9). Many long fibrils with diameters on the order of hundreds of nanometers can be seen from the CMF with PFI refining. This illustrates the difference between the effects of FR pretreatment and mechanical refining on the resultant CMF morphology through high solid ball milling—not revealed in the literature.

Endoglucanase pretreatment that enhanced nanofibrillation significantly improved the WRV of the subsequently fibrillated CMFs, as shown in Figure 4C, while mechanical refining did not affect the WRV of subsequently fibrillated CMFs compared with the CMFs from fibers without any pretreatment under the same ball milling conditions. The WRV of the CMF from FR-treated fibers after 2 h ball milling reached over 750% or more than 3 times greater than the corresponding CMFs from the untreated or mechanically pretreated fiber. This is because fiber and fibril shortening and the increasing extent of nanofibrillation substantially increased the cellulose WRV. Further ball milling beyond 3 h of the FR-treated CMF decreased its WRV from 750% to approximately 500% due to the extensive deconstruction of fibrils, which exposed (destroyed) pores in fibrils and decreased its water holding capacity.

CONCLUSIONS

This study provided a detailed evaluation of the fibrillation process of a bleached hardwood kraft pulp by laboratory planetary ball milling at high solid contents up to 28%. It also studied the effects of two frequently applied sustainable fiber pretreatments: endoglucanase and mechanical refining on fiber fibrillation at high solid contents. Light and scanning electron microscopy imaging indicated the greatest extent of fibrillation at the lowest solid content of 1% due to good fiber/fibril dispersion, which facilitated the interactions between the balls and fibers/fibrils—the primary mechanism (i). A high solid content improved fibrillation compared with medium solid contents in the early stage of fibrillation due to the enhanced frictional and tensional interactions among fibers/fibrils—the secondary mechanism (ii). However, the effectiveness of this secondary mechanism (ii) diminished toward nanofibrillation in the late stages to result in a decreasing extent of fibrillation with an increasing solid content. Results from CMF water retention value measurements and suspension stability experiments were consistent with the imaging observations. At a high solid content of 28%, endoglucanase pretreatment of feed pulp fibers shortened fibers/fibrils and resulted in a "pulverization-like" CMF at extended fibrillation times compared with CMFs from feed pulp without pretreatment or with mechanical refining. Longer and finer fibrils were observed from CMFs from both the untreated and the mechanically refined pulp fibers. Mechanical refining substantially improved fibrillation compared with the run without fiber refining pretreatment. Since mild mechanical refining is much more energy efficient than microfibrillation, it is more suitable than endoglucanase pretreatment for producing CMFs for polymer reinforcement applications where long fibrils are desired. The significance of this study is that these findings can be easily implemented in practice for sustainable CMF production.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.biomac.3c01457.

Ball mill operation diagram; LM images of original BKHP fibers and CMFs obtained under different solid contents, ball-to-fiber mass ratio, and milling times; SEM images of CMFs from different ball-to-fiber mass ratios milled at 1% solid content; stabilities of CMF suspension at different sedimentation times; SEM images of CMFs obtained with enzymatic pretreatment or mechanical refining; BKHP chemical composition; ball-milling conditions; and CMF sample labeling (PDF)

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Notes

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