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Crystallinity Improvements in Cellulose II after Multiple Posttreatment Cycles with a Dilute NaOH Solution

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Crystallinity improvement in Cell-II by posttreatment

Keywords

Cellulose II/Crystallinity improvement/Mercerization/Low-concentration alkali treatment

Abstract

The low crystallinity of cellulose II makes industrial use a major challenge. In our previous report, we proposed a mechanism for improvement of the crystallinity of cellulose II during posttreatment with a dilute NaOH solution. Specifically, rearrangements of the cellulose molecules occurred only near inaccessible surfaces through which NaOH penetrated, resulting in conversion into crystalline regions. To verify our proposed mechanism and to improve the crystallinity further, we performed both long-term single-cycle and multicycle posttreatments with dilute NaOH solutions. Multiple posttreatment cycles under optimal conditions increased the crystallinity by up to approximately 20%. In contrast, long-term single-cycle posttreatment led to crystallinity improvements of only up to approximately 10%. Changes in the proportions of the regions during multiple cycles after treatment supported our proposed mechanism. During multiple posttreatment cycles, the products were converted to crystalline regions only on the inaccessible surfaces enlarged by the previous posttreatment cycle, which increased the crystal sizes in each cycle. Optimization of the treatment times used during the multiple posttreatment cycles led to crystallinity improvements with fewer posttreatment cycles. Our investigations of the mechanism for crystallinity improvement will contribute to structural elucidation of cellulose II and the efficient preparation of highly crystalline cellulose II.

Introduction

Cellulose is the most abundant biopolymer in the world [1], and it has received considerable attention due to its renewable, sustainable, and biodegradable utilization in industrial applications. When natural cellulose is treated with a strong alkaline solution, a process called mercerization occurs [2]. In this process, a transition from cellulose I crystals to cellulose II crystals occurs. Cellulose II crystals are also formed by the regeneration of dissolved cellulose. Cellulose II has excellent luster, dyeability, and drape properties, as well as other advantageous properties; thus, it is an indispensable material in various industrial products, such as fibers and films. However, the crystallinity of cellulose II decreases during the crystal transition process. Many strategies, including the preparation of cellulose II via saponification of cellulose acetate and regeneration of more ordered cellulose with highly polar coagulation media [3,4] and ionic liquids [5], have been proposed to address this problem. The preparation of highly crystalline cellulose II via simpler methods could expand the range of cellulose applications.

Posttreatment with dilute sodium hydroxide (NaOH) aqueous solutions [6–11] has long been used to increase the crystallinity of cellulose II. Our group previously proposed a mechanism for improving the crystallinity of cellulose II via NaOH posttreatment [12]. As shown in Fig. 1(a), the surface areas of cellulose II fibrils can be divided into accessible and inaccessible regions,

depending on whether solvent molecules, especially water, can access them [13,14]. These surface regions can be distinguished by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy and confirmed by dynamic nuclear polarization solid-state NMR spectroscopy [15]. Discussions of the posttreatment should address crystallization of the inaccessible surfaces because they are easily crystallized owing to their soft structures and high mobility. In our previous study, NaOH posttreatment was performed with aqueous NaOH solutions of various concentrations. We found that posttreatment with a 10 wt% NaOH solution resulted in the maximum cellulose II crystallinity and that the proportion of inaccessible surfaces/amorphous regions decreased as the cellulose crystallinity was increased during the posttreatment. Based on these results, we proposed a mechanism for crystallinity improvement in which rearrangements of the cellulose molecules on inaccessible surfaces occur via the penetration of NaOH into these surfaces, followed by conversion of these surfaces into crystalline regions. Furthermore, the crystallinity of cellulose II has been reported to be greater after repeated alkali posttreatments than after a single posttreatment [9–11]. Thus, more highly crystalline cellulose II could be obtained by optimizing the posttreatment conditions. However, a mechanism focused on surface regions for improving crystallinity during these repeated posttreatments has not been reported. A detailed analysis of the compositional changes occurring in the cellulose II fibrils during repeated posttreatments would be useful for efficient preparation of highly crystalline cellulose II and high-strength cellulose II

74 materials.

75 The objective of this study was to elucidate the mechanism for improvements in
76 crystallinity during dilute alkali posttreatment. The mechanism for improving the crystallinity
77 was verified with two posttreatment methods: long-term single-cycle posttreatment and
78 multicycle posttreatment with a 10 wt% NaOH solution. Fig. 1(b) shows a schematic diagram of
79 the crystallinity improvements expected for long-term single-cycle posttreatment and multicycle
80 posttreatment. If our proposed mechanism is correct, multicycle posttreatment should lead to
81 greater crystallinity improvements than single-cycle posttreatment for a longer duration. The
82 upper panel in Fig. 1(b) illustrates the expected mechanism for the long-term single-cycle
83 posttreatment. Crystallization occurs from inaccessible surfaces toward amorphous regions
84 during the posttreatment; however, the areas in which crystallization can occur are limited. After
85 a certain degree of crystallization has occurred on all inaccessible surface regions, further
86 prolonged posttreatment is not likely to enhance the crystallinity. On the other hand, during
87 multiple cycles after treatment, as depicted in the lower panel in Fig. 1(b), the crystallinity is
88 expected to increase in stepwise fashion with each posttreatment cycle, as crystallization proceeds
89 from the inaccessible surfaces that were expanded during the previous cycle. Therefore,
90 crystallization of the amorphous regions away from the initial crystals is anticipated to occur,
91 resulting in a more substantial improvement in the crystallinity than that seen with a single

92 treatment over an extended period. These posttreatment methods were employed in this study.
93 Changes in the proportions of crystalline regions, accessible surfaces, and inaccessible
94 surfaces/amorphous regions in the obtained samples were tracked, and their crystallinities were
95 compared. The obtained results were used to assess the mechanism for crystallinity improvement.

96

97 (Figure 1)

98 **Materials and methods**

99 **Materials**

100 Whatman CF11 cellulose powder (Whatman International Ltd., Kent, England) was
101 used to prepare the initial mercerized cellulose II powder. Cellulose II fibers, Fortisan (Celanese
102 Corp., Dallas, USA), Polynosic (Teijin Limited, Tokyo, Japan), and a commercial rayon for
103 handicrafts (Perugia Corp., Osaka, Japan) were used for the multicycle posttreatments. NaOH
104 (>97.0%) and sulfuric acid (H_2SO_4; >95.0%) were purchased from FUJIFILM Wako Pure
105 Chemical Corp. (Osaka, Japan). Deionized water (Elix@Essential3, Merck KGaA, Darmstadt,
106 Germany) was used to prepare the NaOH and H_2SO_4 aqueous solutions and wash the cellulose II
107 samples.

108 **Preparation of the initial cellulose II powder**

The cellulose powder was mercerized with a 25 wt% NaOH solution for 2 h at room temperature (24 °C). The mercerized sample was neutralized with a 20 vol% H_2SO_4 solution at room temperature (24 °C) to bring its pH below 7.0. The sample was washed with deionized water and air-dried at room temperature (24 °C).

Long-term single-cycle posttreatment

The initial cellulose II powder was treated with a 10 wt% NaOH solution for 2–16 h at room temperature (24 °C) to observe the effect of treatment time on the results of the low-concentration alkali posttreatment. A 10 vol% H_2SO_4 solution was used for neutralization. The samples were washed with deionized water, air-dried at room temperature (24 °C), and subsequently analyzed via solid-state ^{13}C cross-polarization magic angle spinning (CP/MAS) NMR and X-ray diffraction (XRD).

Multicycle posttreatment

For each posttreatment cycle, the initial cellulose II powder was treated with a 10 wt% NaOH solution for 2 h at room temperature (24 °C). Neutralization was performed with a 10 vol% H_2SO_4 solution at room temperature (24 °C) to bring the pH of the powder below 7.0. The sample was washed with deionized water and air-dried at room temperature (24 °C). The next posttreatment was performed after the sample was dried. The posttreatment process was repeated

nine times with the same sample. After each cycle, the dried samples were analyzed by solid-state ^{13}C CP/MAS NMR and XRD.

Multicycle posttreatment of cellulose II fibers

Multiple treatment cycles were performed on Fortisan, Rayon, and Polynosic fibers. One side of the fiber was taped to the bottom of a vat to stabilize it. For each posttreatment cycle, the fiber sample was treated with a 10 wt% NaOH solution at room temperature (24 °C) for 2 h. Neutralization was performed with a 10 vol% H_2SO_4 solution at room temperature (24 °C) to bring the pH below 7.0. The sample was washed with deionized water and air-dried at room temperature (24 °C). All posttreatments were performed after the samples were dried. The multicycle treatments were repeated up to three times for Fortisan and Rayon and up to four times for Polynosic. The samples were analyzed by solid-state ^{13}C CP/MAS NMR and XRD.

Multicycle posttreatment, including neutralization with H_2SO_4 solutions of various concentrations

In a series of multicycle posttreatments, the low-concentration NaOH treatment and neutralization treatment with a H_2SO_4 aqueous solution were repeated. Multiple posttreatment cycles were performed with the initial cellulose II powder by changing the concentration of the H_2SO_4 aqueous solution used for neutralization to evaluate the effect of neutralization on the

crystallinities of the samples. Each multicycle posttreatment was performed for 2 h at room temperature (24 °C) with a 10 wt% NaOH solution. The neutralization treatments were performed with 0 (water), 5, and 20 vol% H_2SO_4 solutions at room temperature (24 °C) to bring the pH of the powder below 7.0. The samples were washed with deionized water and air-dried at room temperature (24 °C). After each subsequent posttreatment cycle, the samples were dried, and the process was repeated up to three times for the same sample. The dried samples were analyzed by solid-state ^{13}C CP/MAS NMR after each posttreatment cycle.

Multiple posttreatment cycles for various durations

Multiple posttreatment cycles were performed with the cellulose II powder for various durations ranging from 2 to 12 h during the second to fourth cycles with a 10 wt% NaOH solution at room temperature (24 °C) to evaluate the effect of the posttreatment time on the crystallinities of the samples. The treatment time was set to 2 h in all cycles prior to that in which the treatment time was changed. For example, when the treatment time was changed in the fourth cycle after treatment, the treatment times for cycles 1–3 were 2 h.

Furthermore, the total posttreatment time was divided into several cycles of 6, 8, and 10 h to optimize the treatment time and number of posttreatment cycles. Each posttreatment cycle on the cellulose II powder was performed with a 10 wt% NaOH solution at room temperature

(24 °C). The samples were neutralized with a 10 vol% H_2SO_4 solution at room temperature (24 °C) to bring their pHs below 7.0. The samples were washed with deionized water and air-dried at room temperature (24 °C). Each cycle was performed after the sample was dried. The dried samples were analyzed by solid-state ^{13}C CP/MAS NMR.

Solid-state ^{13}C CP/MAS NMR spectroscopy

Solid-state ^{13}C CP/MAS NMR spectra were measured at the Instrumental Analysis Support Office, Frontier Chemistry Center, Faculty of Engineering, Hokkaido University, Japan, with an Avance 500 spectrometer (Bruker, Germany) operated at 125 MHz with a 2.5 mm rotor as well as a DSX300 spectrometer (Bruker) operated at 75 MHz with a 4 mm rotor. The MAS frequency was 10 kHz. All spectra were obtained with a ^{1}H NMR 90° pulse, which had a length of 4.0 μs, a contact time of 1.5 ms, and a repetition time of 4 s. The spectra were calibrated with the carbonyl-carbon peak of glycine (176.46 ppm). A ramp sequence was employed for the contact pulse, and a tppm-15 sequence was utilized for decoupling. Deconvolution of the C4 resonance lines was performed with the Dmfit2017 line-fitting program [16]. A Lorentzian geometry was utilized for each line, and the C4 resonance lines were divided into crystalline regions, accessible surfaces, and inaccessible surfaces/amorphous regions [14,17], as shown in Fig. 2[12]. The chemical shifts for the crystalline regions, accessible surfaces, and inaccessible

177 surfaces/amorphous regions of cellulose II crystals were 88.9 and 87.8 ppm, 86.9 and 85.9 ppm,
178 and 84.2 ppm, respectively. The proportion of each region was calculated from the integrated area
179 under the resonance line.

180

181 (Fig. 2)

182 **XRD analyses**

183 XRD was performed with a SmartLab (Rigaku Co. Ltd., Tokyo, Japan) X-ray
184 diffractometer equipped with a Cu Kα anode (λ = 1.5418 Å) powered at 45 kV. The scan range
185 was 5°–40°, and the step size was 0.02°. Peak fitting was performed with the fitting software
186 SmartLab Studio II (Rigaku Co. Ltd.) with three pseudo-Voigt functions to obtain the peak
187 positions, peak heights, half-widths, and crystal sizes from the $(1\bar{1}0)$, (110), and (020) peaks.

188 **Results and discussion**

189 **Long-term single-cycle posttreatments**

190 Fig. 3 shows the proportions of the crystalline regions, accessible surfaces, and
191 inaccessible surfaces/amorphous regions of the samples after the long-term single-cycle
192 posttreatments; the data were calculated by deconvoluting the C4 resonance line of each ^{13}C
193 CP/MAS NMR spectrum. The crystallinities of the samples increased during posttreatment for up

194 to 8 h but did not change with further treatment. This suggested that in the case of a long-term
195 single-cycle posttreatment, the improvement in crystallinity was limited even with longer
196 posttreatment times. The maximum crystallinity achieved was 59%, which was approximately
197 10% greater than the crystallinity of the initial cellulose II powder. In terms of the surface area,
198 the proportion of inaccessible surface/amorphous regions decreased with increasing crystallinity.
199 This result confirmed our previously reported mechanism in which inaccessible surface regions
200 were converted into crystalline regions by rearrangement of the cellulose molecules, ultimately
201 increasing their crystallinity. During the posttreatment process, the following changes were
202 observed within the cellulose fibrils. First, rearrangement of the cellulose molecules occurred on
203 the inaccessible surfaces, and as previously noted, crystallization and growth occurred from the
204 boundaries between the crystalline and inaccessible surface regions. When all accessible surfaces
205 were completely rearranged, the crystallinities of the fibrils did not increase further, even after
206 longer posttreatment times. When the maximum crystallinity was achieved, the accessible and
207 inaccessible surfaces expanded alternately. This observation suggested that NaOH did not
208 penetrate the crystalline regions during the posttreatment process; instead, it penetrated only the
209 amorphous regions, thereby affecting the rearrangement of cellulose molecules only in these
210 regions.
211

(Figure 3)

Fig. 4 shows the crystal sizes of the samples obtained after long-term single-cycle posttreatment, as determined by XRD measurements. The crystal sizes of the samples increased by approximately 10%–20% for each plane over the course of 2–8 h but did not increase further during longer posttreatments. The NMR and XRD results were consistent, at least in terms of their trends.

In general, high concentrations (20%–25%) of the NaOH aqueous solution must be used for mercerization at room temperature (24 °C); a 10% NaOH solution cannot be used for this purpose. As shown by this behavior, the NaOH in a 10 wt% NaOH solution did not penetrate the crystalline regions but did penetrate the inaccessible surface regions. However, a certain amount of time was required for NaOH to penetrate all areas of the inaccessible regions. These results also clearly demonstrated that the long-term single-cycle posttreatment increased the maximum crystallinity by no more than approximately 10%.

(Fig. 4)

Multicycle posttreatments

228 Fig. 5 shows the proportions of crystalline regions, accessible surfaces, and inaccessible
229 surfaces/amorphous regions determined by fitting of the C4 resonance lines in the ^{13}C CP/MAS
230 NMR spectra of the samples subjected to multiple posttreatment cycles. The crystallinities of the
231 samples increased over up to three posttreatment cycles, reaching a maximum value of 69%. This
232 value was 20% greater than that of the initial cellulose II powder and 10% greater than that of the
233 sample subjected to one long-term posttreatment cycle. These findings confirmed the
234 effectiveness of multiple posttreatment cycles in improving the crystallinity of cellulose II and
235 verified the accuracy of our proposed mechanism for the improved crystallinity. In terms of
236 surface area, the proportion of inaccessible surface/amorphous regions decreased with increasing
237 crystallinity up to the third posttreatment cycle. This result also verified the accuracy of our
238 proposed mechanism for improving the crystallinity, in which the inaccessible surfaces penetrated
239 by NaOH underwent crystallization. After the maximum crystallinity of cellulose II was achieved
240 in the fourth to ninth posttreatment cycles, no significant change in the crystallinity was observed.
241 This was attributed to the following characteristics. Crystallization on the inaccessible surfaces
242 could only occur in the amorphous regions. As the number of amorphous regions was decreased
243 by multiple treatment cycles, the inaccessible surface regions at which crystallization could
244 proceed gradually diminished, and the improvement in crystallinity was saturated. After reaching

245 saturation, a slight decrease in crystallinity occurred from the seventh cycle onwards, suggesting

246 the possibility of minor crystallinity degradation.

247

248 (Fig. 5)

249 Fig. 6 shows the crystal sizes determined from the XRD profiles of samples obtained
250 with multiple posttreatment cycles, and Fig. S1 shows the XRD profiles of the mercerized initial
251 cellulose II and the samples obtained after one to three posttreatment cycles. When up to three
252 cycles were performed, the crystal sizes increased in all planes as the number of posttreatment
253 cycles increased. This was confirmed by the decreased peak width in the XRD profile shown in
254 Fig. S1, which indicated that the crystal sizes increased with increasing crystallinity, as estimated
255 from the solid-state ^{13}C CP/MAS NMR spectra. The crystal sizes of the samples obtained after
256 multicycle posttreatment were larger than those of the samples obtained after a single long-term
257 posttreatment cycle. This was consistent with the fact that the crystallinities estimated from the
258 solid-state ^{13}C CP/MAS NMR spectra were greater for samples subjected to multiple
259 posttreatment cycles than for those subjected to a single long-term posttreatment cycle. After the
260 seventh cycle, when the crystallinity decreased, as shown in Fig. 5, the intensity of the peak for
261 the hydrophobic (110) plane decreased slightly, while those of the (020) and hydrophilic ($1\bar{1}0$)
262 planes increased. This suggested that the orientations of the molecular chains may have changed
263 slightly.

264

(Fig. 6)

Crystallinity improvement in cellulose II fibers following multicycle posttreatment

Fig. 7 shows the proportions of crystalline regions, accessible surfaces, and inaccessible surfaces/amorphous regions determined from fitting the C4 resonance lines in the ^{13}C CP/MAS NMR spectra of Fortisan, Rayon, and Polynosic after multicycle posttreatment. The crystallinities of the Fortisan samples increased up to the second posttreatment cycle and did not significantly improve with additional posttreatment cycles. The crystallinity improvement in this sample was approximately 15%. The crystallinities of the Rayon samples also increased up to the third posttreatment cycle, an improvement of approximately 25%. The crystallinities of the Polynosic samples improved after up to the fourth posttreatment cycle by approximately 36%. These results showed that multiple posttreatment cycles improved the crystallinities of the cellulose II fibers. In all multicycle posttreatments, the proportion of inaccessible surfaces/amorphous regions decreased as the sample crystallinity improved, similar to the behavior of the mercerized cellulose II powder. In other words, the mechanisms for crystallinity improvement via multicycle posttreatment appeared to be similar, regardless of the shape of the cellulose II sample.

(Fig. 7)

Fig. 8 shows the crystal sizes of Fortisan and Rayon after multiple cycles and posttreatment, as determined via XRD. Polynosic had smaller fiber widths than the other cellulose II fiber samples and experienced greater deformation due to shrinkage after multiple cycles. Consequently, arranging yarns of polynosic in the measurement container used for XRD proved challenging, leading to gaps that hindered the XRD measurements. As a result, only NMR measurements were performed for Polynosic, and the XRD measurements were omitted. The crystal sizes of the hydrophobic (110) plane increased significantly. Hydrophobic interactions have been reported to play important roles in sheet stacking of cellulose II crystals [18]. Although the crystal sizes in all planes of the cellulose II powder increased after multiple posttreatment cycles, they increased in a specific direction, that is, in the hydrophobic plane direction, in the case of cellulose II fibers with high orientation.

(Fig. 8)

Multicycle posttreatment with neutralization using various concentrations of H_2SO_4 solutions

297 In a series of multicycle posttreatments, the low-concentration alkali treatment and
298 neutralization treatment with a H_2SO_4 aqueous solution were repeated. Repeated neutralization
299 may have improved the crystallinity of cellulose II. Thus, to evaluate this effect, multiple
300 posttreatment cycles, including neutralization with 0 (deionized water), 5, 10 (same as in Fig. 5),
301 and 20 vol% H_2SO_4 solutions, were performed. As shown in Fig. 5, the crystallinity of the
302 cellulose II continuously improved up to the third posttreatment cycle when a 10 vol% H_2SO_4
303 solution was used for neutralization. Therefore, multiple posttreatment cycles with H_2SO_4
304 solutions of different concentrations were performed for up to three cycles. Fig. 9 shows the
305 proportions of the crystalline regions, accessible surfaces, and inaccessible surfaces/amorphous
306 regions of the samples obtained after multiple cycles posttreatment. The crystallinity of the
307 sample subjected to a single posttreatment improved the most when a 20 vol% H_2SO_4 solution
308 was used, which was consistent with our previous report [12]. However, the crystallinity of the
309 sample subjected to two posttreatment cycles decreased, and that of the sample subjected to three
310 posttreatment cycles was identical to that of the sample subjected to one posttreatment cycle. The
311 use of a 20 vol% H_2SO_4 solution for neutralization led to hydrolysis of the crystalline regions
312 after two posttreatment cycles, which suggested that some parts of the inaccessible surface were
313 less crystallizable than others. On the other hand, multiple posttreatment cycles including
314 neutralization with 0, 5, or 10 vol% H_2SO_4 solution led to continuous improvements in the

315 crystallinity for up to three posttreatment cycles. Furthermore, higher crystallinities were
316 observed when 5 and 10 vol% H_2SO_4 solutions were used than when a 0% H_2SO_4 solution was
317 employed. This suggested that the use of a low-concentration H_2SO_4 solution for neutralization
318 improved the crystallinity during multiple posttreatment cycles.

319

320 (Fig. 9)

321 **Multicycle posttreatments with varying treatment times in the second to fourth cycles**

322 Multicycle posttreatments of the cellulose II powder were performed with treatment
323 times varying from 2 to 12 h in the second to fourth cycles to evaluate the effect of the
324 posttreatment time in each cycle. Fig. 10(a) shows the proportions of crystalline regions,
325 accessible surfaces, and inaccessible surfaces/amorphous regions for samples that underwent the
326 first posttreatment cycle for 2 h and the second posttreatment cycle for 2–12 h. The crystallinities
327 of the samples increased up to 2 h but did not improve during subsequent posttreatments. The
328 maximum crystallinity achieved was 63%, which was higher than that of the sample obtained
329 after a single posttreatment cycle for 2 h. Figs. 10(b) and 10(c) show the proportions of the
330 crystalline regions, accessible surfaces, and inaccessible surfaces/amorphous regions for samples
331 that underwent multiple posttreatment cycles for 2 h in the first and second cycles and 2–12 h in

the third cycle and samples that underwent multiple posttreatment cycles for 2 h in the first to third cycles and 2–12 h in the fourth cycle, respectively. As demonstrated in Fig. 10(b), during the three posttreatment cycles, the crystallinities of the samples increased up to 2 h, after which they were not affected by further posttreatment. The maximum crystallinity achieved was 69%, which was higher than those of the samples obtained after one and two posttreatment cycles. The crystallinities of the samples subjected to four posttreatment cycles for varying treatment times differed from those of the previous samples. As shown in Fig. 10(c), the crystallinities of the samples did not increase significantly after four posttreatment cycles. The maximum crystallinity achieved was 69%, which was identical to those of the samples subjected to multiple posttreatment cycles, as depicted in Fig. 5, and those of the samples subjected to three posttreatment cycles for varying treatment times, as shown in Fig. 10(b). This indicated that there was an upper limit to crystallinity improvement and that once this limit was reached, further repeated or prolonged posttreatments did not lead to additional improvements. These findings suggested that there were surface regions that were difficult to crystallize or difficult to reach with NaOH. In addition, our previous report [12] showed that crystallinity was greatly enhanced by posttreatments at temperatures higher than room temperature, and the most significant improvement in crystallinity was achieved with posttreatment at 80 °C for 2 h and subsequent neutralization at room temperature, which resulted in a crystallinity of 62.7%. In comparison, in

this study, an upper limit was observed for crystallinity improvement, and the crystallinity at this limit was 69%. A significant improvement in crystallinity was achieved by optimizing the posttreatment time even at room temperature.

(Fig. 10)

Mechanism for crystallinity improvement during multiple posttreatment cycles

The experimental results confirmed that the inaccessible surface regions were converted into crystalline regions after each posttreatment cycle. Fig. 11 provides a schematic diagram showing the mechanism for crystallinity improvements during multiple treatment cycles, and the bottom part of Fig. 11 shows the structural changes occurring in a single-crystal region. In the first posttreatment cycle, NaOH penetrated only the inaccessible surfaces of the initial structure, and crystallization occurred on the inaccessible surfaces that NaOH penetrated. In the second and subsequent posttreatment cycles, NaOH penetrated only the inaccessible surfaces enlarged by the previous posttreatment cycle and converted them into crystalline regions. Consequently, the crystal sizes increased with each posttreatment cycle, and ultimately, crystallization occurred even in distant amorphous regions beyond the limit of the crystal sizes observed in the first posttreatment cycle. This feature explains why the crystallinity was improved more by multiple

posttreatment cycles than by a single long-term posttreatment. However, after two posttreatment cycles with a concentrated H_2SO_4 solution used for neutralization, the crystallinity of the sample decreased. Furthermore, an upper limit was observed for the improvements in crystallinity; once this limit was reached, additional or prolonged posttreatments did not improve the crystallinity of the sample. These results suggested that cellulose II contained inaccessible surface regions that were difficult to crystallize.

(Fig. 11)

Highly efficient posttreatment method for increased crystallinity

With more posttreatment cycles, more NaOH solution was used for posttreatment and more deionized water was utilized for washing. Thus, reaching the upper limit for crystallinity with fewer posttreatment cycles is important in optimizing the production of high-crystallinity cellulose II and mitigating the environmental impact. The experimental results showed that both the three-cycle and the 8 h single-cycle posttreatments led to the greatest improvements in crystallinity. Based on these results, an ideal and efficient process for crystallinity improvement was realized by optimizing the treatment time and number of posttreatment cycles. For this purpose, the total posttreatment time was divided into several cycles of 6, 8, and 10 h. In the following discussion, the samples obtained after multiple posttreatment cycles for various

385 treatment times were labeled according to the treatment time for each cycle. For example, a
386 sample obtained after three posttreatment cycles for a total time of 6 h, with each cycle performed
387 for 2 h, is denoted as 6 h (2-2-2). Fig. 12 shows the proportions of crystalline regions, accessible
388 surfaces, and inaccessible surfaces/amorphous regions for samples prepared with a total
389 posttreatment time of 6 h, and Figs. S2(a) and S2(b) present the proportions of each region for
390 samples prepared with total posttreatment times of 8 and 10 h, respectively. Fig. 12 shows that
391 the 6 h (4-2) sample had the highest crystallinity among the samples prepared with a total
392 posttreatment time of 6 h. Fig. S2(a) shows that the 8 h (4-4) sample had the highest crystallinity
393 among the samples prepared with a total posttreatment time of 8 h. The 8 h (6-2) sample had
394 higher crystallinity than the 8 h (2-6) sample. Fig. S2(b) reveals that the 10 h (6-4) sample had
395 the highest crystallinity among the four samples obtained after two posttreatment cycles with
396 various treatment times. Furthermore, the 10 h (8-2) sample had a higher crystallinity than the 10
397 h (2-8) and 10 h (4-6) samples. These results confirmed that when the posttreatment time for the
398 first cycle was sufficiently long and the posttreatment time for the second cycle was relatively
399 short, the crystallinity of the sample tended to increase with fewer posttreatment cycles. The
400 conversion of inaccessible surface regions during the first posttreatment cycle is believed to play
401 a crucial role in obtaining highly crystalline samples. A possible explanation for this tendency is
402 that a longer first posttreatment cycle converted a wider area of the inaccessible surface into a

403 crystalline region, and the second posttreatment cycle converted this enlarged inaccessible region
404 into a crystalline region. In previous studies [10,11], posttreatment with a 12 wt% NaOH aqueous
405 solution for 2 h at 4 °C was repeated four or five times. On the other hand, in this study, substantial
406 improvement was achieved in the crystallinity of cellulose II with two cycles by adjusting the
407 posttreatment time.
408
409 (Fig. 12)
410 **Conclusions**
411 In this study, the mechanism underlying the improvement in crystallinity after low-
412 concentration alkali treatment was elucidated by tracking the structural changes, primarily in the
413 surface regions. The crystallinity of cellulose II was improved by up to approximately 10% during
414 a single long-term posttreatment and up to approximately 20% during multicycle posttreatment.
415 Thus, multicycle posttreatment was more effective than single-cycle posttreatment for improving
416 the crystallinity of cellulose II. During multicycle posttreatment, the crystallinities and crystal
417 sizes were improved after each cycle, and the proportion of inaccessible surfaces/amorphous
418 regions decreased as the crystallinity increased. This was explained by the following mechanism:
419 in the second and subsequent posttreatment cycles, rearrangement of the cellulose occurred only
420 at the inaccessible surface expanded by the previous posttreatment cycle, and crystallization

proceeded toward amorphous regions far from the initial crystalline regions. In this mechanism, the NaOH in a 10 wt% NaOH solution penetrated only the inaccessible surface regions during the posttreatment, and the rearrangement of cellulose occurred only in these regions. Moreover, we confirmed that when the treatment time for the first posttreatment cycle was relatively long and that for the second posttreatment cycle was somewhat shorter, the crystallinity was increased even with fewer posttreatment cycles. The mechanism elucidated in this study explains this observation as follows: the longer first posttreatment cycle converted a wider area of the inaccessible surface into a crystalline region, and the second cycle converted this enlarged inaccessible surface region into a crystalline region. In addition, we believe that this mechanism is applicable for the fabrication of high-strength cellulose II materials because posttreatment improved the crystallinities of cellulose II fibers.

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Author contributions

Yuki Kugo: Conceptualization, Methodology, Investigation, Writing – Original Draft. Satoshi Nomura: conceptualization and methodology. Takuya Isono: Supervision. Masashi Fujiwara: Supervision. Toshifumi Satoh: Supervision. Hirofumi Tani: Supervision. Tomoki Erata: Supervision, Writing – Review & Editing. Kenji Tajima: Supervision, Writing – Review & Editing

Declaration of competing interest

The authors declare that they have no financial interests or personal relationships that could be considered potential competing interests.

Data availability

The data will be made available upon reasonable request.

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Figure captions

Fig. 1. (a) Suggested model for cellulose II [12]. (b) Schematic images of the anticipated crystallinity improvement mechanisms for a long-term, single-cycle posttreatment and multiple posttreatment cycles. In the long-term, single-cycle posttreatment, the area transformed into crystals (pink line) was limited. After the crystallinity was improved to some extent, further increases in the treatment time was not expected to lead to additional improvements in the crystallinity. During multicycle posttreatment, further crystallization was expected to progress from the expanded inaccessible surface regions in the previous cycle. Crystals were expected to accumulate with each posttreatment cycle, thereby enhancing the crystallinity compared to that achievable with a long-term, single-cycle posttreatment.

Fig. 2. Deconvolution of the solid-state ^{13}C CP/MAS NMR C4 resonance of dry cellulose II.[12]

Fig. 3. Proportions of crystalline regions (blue line), accessible surfaces (orange line), and inaccessible surfaces/amorphous regions (green line) determined from the ^{13}C CP/MAS NMR spectra of cellulose fibrils subjected to a long-term single-cycle posttreatment.

Fig. 4. Crystal sizes in the $(1\bar{1}0)$ (gray line), (110) (purple line), and (020) (pink line) planes estimated from the X-ray diffraction profiles of cellulose II after a long-term single-cycle posttreatment.

Fig. 5. Proportions of crystalline regions (blue line), accessible surfaces (orange line), and inaccessible surfaces/amorphous regions (green line) evaluated from the solid-state ^{13}C CP/MAS NMR spectrum of cellulose II after multiple posttreatment cycles.

Fig. 6. Crystal sizes in the $(1\bar{1}0)$ (gray line), (110) (purple line), and (020) (pink line) planes estimated from the XRD profiles of cellulose II after multicycle posttreatment.

Fig. 7. Proportions of the crystalline regions (blue line), accessible surfaces (orange line) and inaccessible surfaces/amorphous regions (green line) of (a) Fortisan, (b) Rayon, and (c) Polynosic after multicycle posttreatment.

Fig. 8. Crystal sizes in the $(1\bar{1}0)$ (gray line), (110) (purple line), and (020) (pink line) planes estimated from the XRD profiles of (a) Fortisan and (b) Raymond following multicycle posttreatment.

538 Fig. 9. Proportions of crystalline regions (blue line), accessible surfaces (orange line), and
539 inaccessible surfaces/amorphous regions (green line) evaluated from the ^{13}C CP/MAS NMR
540 spectra of samples obtained after multiple posttreatment cycles involving neutralization with (a)
541 0% (water) and (b) 5 vol%, (c) 10 vol%, and (d) 20 vol% H_2SO_4 solutions.

542 Fig. 10. Proportions of the crystalline regions (blue line), accessible surfaces (orange line), and
543 inaccessible surfaces/amorphous regions (green line) evaluated from the ^{13}C CP/MAS NMR
544 spectra of samples that underwent (a) two-, (b) three- and (c) four-cycle posttreatments with
545 treatment times varying from 2 to 12 h.

546 Fig. 11. Schematic diagram showing the mechanism for crystallinity improvement with multiple
547 posttreatment cycles of low-concentration NaOH. The bottom part of Fig. 11 shows the structural
548 changes focused on a single crystal. In subsequent posttreatments after the second posttreatment
549 cycle, NaOH permeated only the inaccessible surface enlarged by the previous posttreatment
550 cycle and converted it into a crystalline region. Consequently, the crystal sizes increased with
551 each posttreatment cycle. The crystallinity continued to improve until reaching the upper limit.

552 Fig. 12. Proportions of crystalline regions (blue bars), accessible surfaces (orange bars), and
553 inaccessible surfaces/amorphous regions (green bars) as evaluated from the ^{13}C CP/MAS NMR
554 spectra of samples obtained via multiple posttreatment cycles with a total posttreatment time of 6
555 h. When the posttreatment time for the first cycle was sufficiently long and the posttreatment time
556 for the second cycle was relatively short, the crystallinity tended to increase with fewer
557 posttreatment cycles.

558

559

Graphical Abstract

The study on the improvement of the crystallinity of cellulose II by post-treatment with dilute NaOH solution showed that the crystallinity was significantly improved by post-treatment with multiple cycles. The NaOH in an aqueous NaOH solution penetrated only inaccessible surface regions, and cellulose rearrangement occurred only in these regions during post-treatment, improving crystal size. In the second and subsequent posttreatment cycles, cellulose rearrangement occurred only at the inaccessible surfaces expanded during the previous post-treatment cycle, crystallization progressed toward amorphous regions away from the initial crystalline regions.