J Wood Sci (2014) 60:99–104
DOI 10.1007/s10086-013-1382-y

ORIGINAL ARTICLE

Change in circularity index of cell lumen in a cross-section of wood induced by aqueous NaOH

Shuhei Nakano · Takato Nakano

Received: 17 September 2013 / Accepted: 18 November 2013 / Published online: 14 December 2013

Abstract We quantitatively examined changes in the morphological features of wood cells treated with aqueous NaOH and their relationship to changes in the micro-structure of wood components. Wood samples were treated with aqueous 0.00–0.20 (w/w) NaOH ([NaOH] = 0.00–0.20). Cross-sectional planes of samples were observed using confocal laser-scanning microscopy and the circularity index of cell lumens in cross-section was evaluated using digital image analysis. At [NaOH] < 0.11, the circularity index was unrelated to crystallinity, while at [NaOH] > 0.12 it was closely related to a decrease in crystallinity, as indicated by X-ray diffraction. Force balance analysis based on a cell model supported experimental results at higher concentrations. We concluded that cells appear circular in cross-section after treatment with NaOH because a decrease in crystallinity results in contraction along microfibrils, resulting in distribution of the central force of the three layers, S1, S2, and S3, in the secondary wall at the corners of cells in cross-section.

Keywords Circularity index · Wood cell wall · Cross-section · Cellulose microfibril · Contraction

Introduction

The aim of this study was to confirm at the mechanism of morphological changes that occur at the cellular level in wood samples treated with aqueous NaOH and to examine the anisotropic changes. Changes in the morphology of wood cells should reflect their high-order micro-structure. In the present study, we observed the features of cell walls in cross-sections of treated wood samples and analyzed the dependence of changes on NaOH concentration using an image analysis method. Both the validity of the mechanism and the anisotropic changes are discussed in this paper.

We have already reported interesting changes in the morphology and physical properties of wood samples treated with aqueous NaOH. Wood samples under wet conditions treated with aqueous NaOH are subjected to longitudinal contraction forces and this mechanical behavior depends on concentration [1, 2]. The morphological change in longitudinal direction reaches 0.07 point in fraction for wood, while nearly 0.30 point for ramie fibers [3, 4]. Figure 1 demonstrates the dimensional change for wood samples and ramie fibers [5, 6]. The mechanism was proposed that the contraction is due to that of microfibrils in tracheids [7, 8]. There is an approximately linear relationship between the degree of shrinkage and the crystallinity of cellulose in wood [7, 8]. Nakano et al. [7] proposed that this shrinkage could be attributed to entropy elastic forces caused by amorphous cellulose chains in microfibrils that were de-crystallized with NaOH treatment, and that these changes are dependent on temperature. A model was later used to explain the anisotropic dimensional changes of treated wood [8]. These changes affect the mechanical properties of wood, especially relaxation behaviors [3, 9].

Stöckmann [10, 11] first investigated the morphological changes of wood samples during pulping, and proposed a model that illustrated changes in fibril position in wood. However, this research was conducted using only high concentrations of NaOH ([NaOH] ≫ 0.20), and cannot directly be compared to our earlier studies. In addition,

S. Nakano · T. Nakano (✉)
Laboratory of Biomaterials Design, Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
e-mail: tnakano@kais.kyoto-u.ac.jp

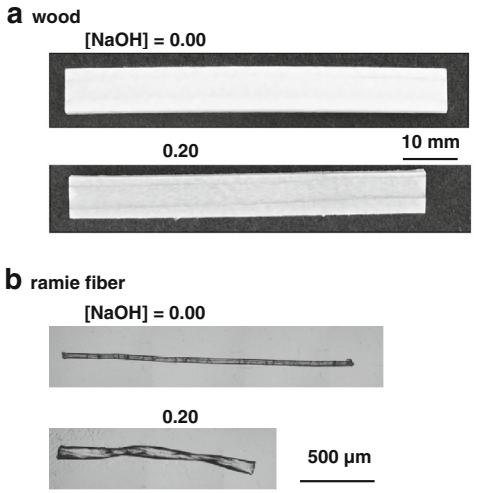

Fig. 1 The morphological change of wood samples treated with aqueous NaOH [5, 6]

Stöckmann did not measure any of the mechanical or thermodynamic properties involved in the contraction forces, and the actual mechanism of microfibril contraction was not addressed. To understand the mechanisms of morphological changes in detail, especially those of anisotropic changes, it is necessary to investigate specific cross-sectional forces at the cell wall level. The circularity index of the lumen in a rectangular cross-section is a useful parameter for investigating the cross-sectional dimensions of wood cells.

In the present study, we quantitatively evaluated the change in the circularity index of the cell lumen in cross-sections of wood that had been treated with aqueous NaOH. In the present paper, we also discuss the relationship between changes in morphology of the cell lumen and crystallinity in microfibrils and the mechanisms that cause changes in the circularity index under NaOH treatment.

Materials and methods

Sample preparation

We prepared 5 (L) × 20 (R) × 20 (T) mm samples of Saghalin fir (*Abies sachalinensis*) and Yezo spruce (*Picea jezoensis*), where L, R, and T correspond to the longitudinal, radial, and tangential axes, respectively. The samples were air-dried, soaked in 1 of 12 concentrations [NaOH] = 0.00–0.20 (fraction concentrations) for 1 h in a vacuum, and stored in the solution at room temperature for 3 days. After NaOH treatment, samples were soaked in distilled water for 1 week. Distilled water was replaced three times a day for the first day and once per day thereafter.

Circularity index

After treatment, the wood samples were cut into blocks measuring 5 (L) × 10 (R) × 10 (T) mm. The RT plane of the samples was smoothed for visualization using a sliding microtome. After removing excess water from the surface, samples were placed on a glass slide and examined using confocal laser-scanning microscopy (CLSM: 1LM15; Lasertech, Yokohama, Japan). Images of the RT plane were obtained at ×20 magnification under wet conditions using the CLSM. The observation area was earlywood, approximately four or five cells from the annual ring. Ten images were obtained for each NaOH concentration. Each image included 15–20 cells, resulting in a total of 150–200 cells per NaOH concentration.

Images from CLSM were analyzed using 2D image analysis software (WinROOF; Mitani Corporation). Images of the wood cells were converted into black and white using software functions and cell lumens were manually delineated. Details of the image processing are shown in Fig. 2.

On the basis of the black and white images, the circularity index of cross-sectional cell lumens was calculated using the following formula:

$$\mathrm{CI} = 4\pi \frac{A}{P^2} \quad (1)$$

where CI, P, and A represent the circularity index, perimeter, and area, respectively. This results in a range of CI values between 0 and 1, with a CI of 1 indicating a perfect circle. This index has been previously used to compare the circularity of earlywood and latewood through cross-sections of trunks from pith to bark [12–14].

Crystallinity

To analyze crystallinity, treated samples were cut into blocks measuring 5 (L) × 10 (R) × 2 (T) mm. These samples were freeze-dried for 1 day (FDU-12AS freeze-dryer; EYELA, Tokyo, Japan), and then vacuum-dried for 1 day (ADP300; Yamato Scientific, Tokyo, Japan). Diffraction patterns of samples were obtained on the LR plane parallel to longitudinal axes at room temperature using an X-ray diffractometer (Ultima IV; Rigaku, Tokyo, Japan) in reflection technique operating at 40 kV and 40 mA over a scanning range of 5°–35° and at a scanning speed of 2°/min. Crystallinity was calculated using the area method from measured diffraction profiles.

Results and discussion

Character of circularity distribution of lumen

Saghalin fir samples cut along the RT plane revealed that cell lumens treated at [NaOH] = 0.20 were rounder than

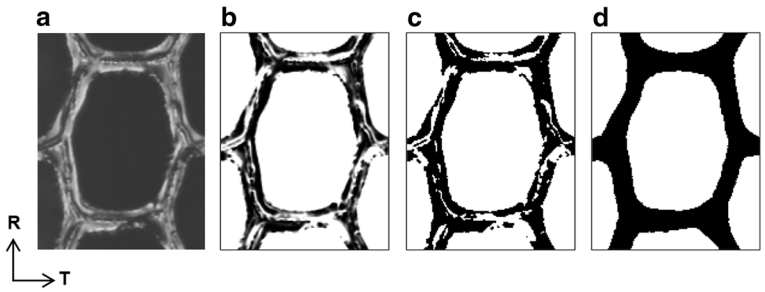

Fig. 2 The procedure for image processing and analysis. **a** Original recorded image; **b** image converted into pure black and white; **c** image after binary processing; **d** completed image. The outlines of lumens were manually drawn by visual assessment

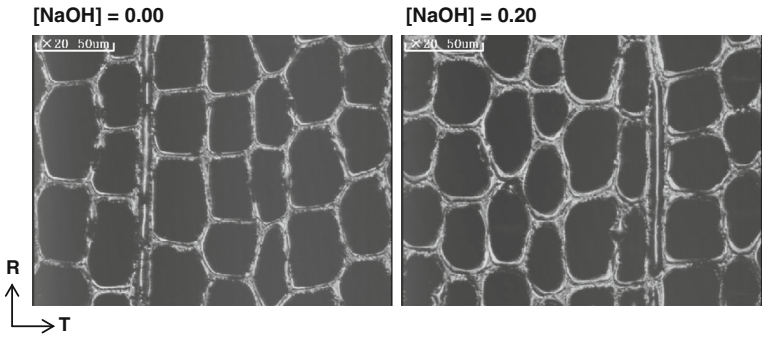

Fig. 3 Transverse image of Saghalin fir after treatment with aqueous NaOH of various concentrations from 0.00 (*left*) to 0.20 (*right*)

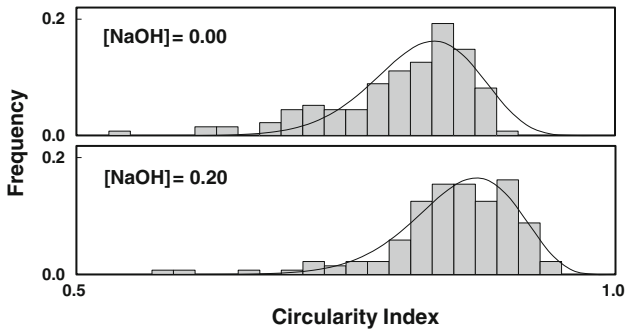

Fig. 4 Frequency distribution of circularity indices of cells in Saghalin fir at NaOH concentrations of 0.00 and 0.20. The *solid line* is the best fit distribution from a beta distribution

those treated with water only (Fig. 3). This morphological change occurred in NaOH solutions [7]. We calculated the circularity index for the 150–200 cells per NaOH concentration. The data were fitted as a beta function, which is shown as a solid line in Fig. 4. The circularity indices for these cells were asymmetrically distributed: there was a long tail of smaller circularity indices. To analyze the concentration dependence of circularity in more detail, new indices which can characterize the asymmetrical circularity distribution are necessary.

Figure 5 shows the change of a series of circularity distributions with increasing NaOH concentrations. The distribution of circularity indices shifted towards higher values as the NaOH concentrations increased, but did not remarkably change the shape of the distribution. This is because the ratio of the standard deviation of the beta distribution (D_{sd}) to the width of the circularity index (D_c, range from minimum to maximum value), D_{sd}/D_c, was relatively stable around 0.1 and did not depend on NaOH concentration. Additionally, the circularity index at the peak of the beta distribution was highly correlated to the mean value of the circularity index, as shown in Fig. 6. Therefore, we decided to use the peak value of the distribution as the new indices characterizing the circularity distribution.

Change in crystallinity

X-ray diffraction profiles of wood samples treated by various concentration of NaOH were obtained. At [NaOH] > 0.12 the intensity of X-ray diffraction decreased with increasing NaOH concentrations. Structural change of cellulose I to II is not observed. The relative crystallinity of cellulose in NaOH-treated wood samples to that in untreated wood samples decreased with NaOH concentration similarly in both species (Fig. 7). Crystallinity changed very little at [NaOH] < 0.11, but decreased markedly at [NaOH] > 0.12. Considering the results shown in Fig. 5, where the circularity index depends upon NaOH concentration in all regions, these results suggest that the circularity index relates to both crystallinity and some other factor. As shown in Fig. 8, the relative circularity index of NaOH-treated wood samples to that of untreated wood samples increased in a concentration-dependent manner that was not dependent on crystallinity at [NaOH] < 0.11, but linearly increased at [NaOH] > 0.12 with decreasing crystallinity.

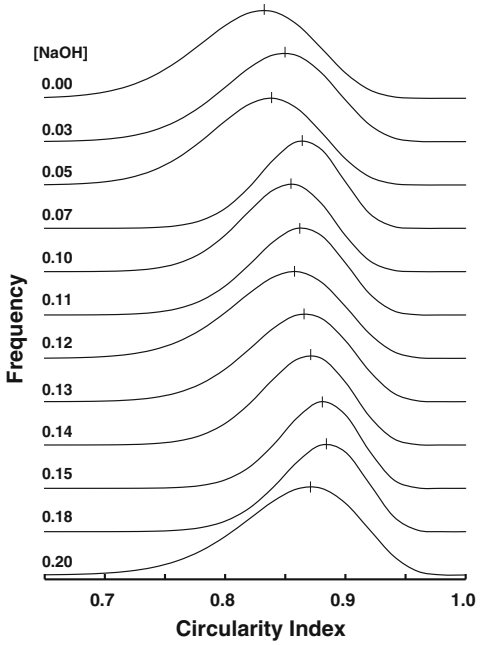

Fig. 5 Change in the distribution of circularity indices for Saghalin fir with increasing NaOH concentration

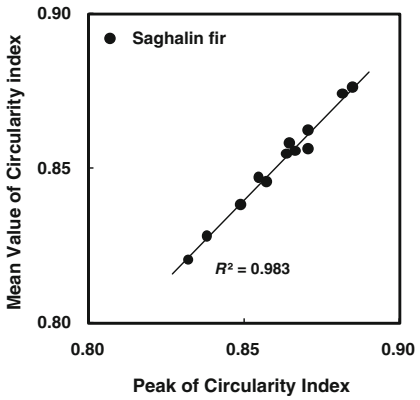

Fig. 6 Relationship between peak and mean values of circularity indices for Saghalin fir. The *solid line* indicates a linear approximation

These two stages of the relationship between the circularity index and crystallinity appear to be the result of two different mechanisms. At low NaOH concentrations, the matrix material of lignin and hemicellulose partially leaches away. Harris [15] revealed leaching of lignin in wood by treatment with dilute alkaline solutions. Ishikura and Nakano [16] measured the weight loss of wood samples treated with [NaOH] = 0.00–0.20, and clarified that weight decreased by 5 % up to NaOH concentrations of 0.05, and was stable at higher concentrations, suggesting that leaching was virtually complete at [NaOH] = 0.05. Rong et al. [17] found that the weight of sisal fiber decreased 13.5 % when treated at [NaOH] = 0.02 for 4 h at 60 °C, and suggested that this decrease was due to the leaching of lignin and hemicellulose. These reports indicate that low concentrations of NaOH are sufficient to induce leaching of the matrix components of wood cell walls. Considering these previous studies, the increase in the circularity index at low NaOH concentrations shown in Fig. 8 is probably due to the leaching of matrix materials, but the details remain unclear.

Formulation of the force acting on cell wall corners

Nakano et al. [7] and Nakano [8] showed experimentally and analytically that microfibril contraction is induced along the longitudinal axis by decreasing crystallinity in the microfibrils due to an entropy elastic force. The observed increase in circularity indices at [NaOH] > 0.12 is probably due to the same process of microfibril contraction along the circumference in cell wall cross-sections.

We used a model to further analyze these results. Stöckmann [10] analyzed the dimensional change in wood cells using a model based on microfibril contraction. Cellulose microfibrils in wood cells are analogous to rubber wrapped around a square pillar. Microfibrils wind around wood cells in a Z-helix. However, Stöckmann's model cannot explain the change in circularity index for cell walls with low microfibril angles (MFA). Our model considers the difference in MFA between radial (R) and tangential (T) walls and includes three distinct layers of secondary walls that may vary in their morphological properties (Fig. 9a).

Our model is described in Fig. 9b, c, which shows the secondary cell walls. The MFA in the LR and LT planes are represented by α_R and α_T, and the contraction force of microfibrils on the LR and LT planes are f_a and f_b, respectively. We assumed $|f_a| = |f_b| = F$ because the contraction force of microfibrils in the LR plane should be equal to that on the LT plane.

We resolved the contraction force f_a into two components along the L and R axes, defined as f_{aL} and f_{aR}, respectively. Thus, $f_{aR} = f_a \sin \alpha_R$. Similarly, f_b is resolved into f_{bL} and f_{bT}, and $f_{bT} = f_b \sin \alpha_T$. A force acting on the corner of the RT plane is defined as f_D, and is described as the resultant force of f_{aR} and f_{bT}. This force faces the center of the cross-section of the cell, and is calculated as follows:

$$f_D = \sqrt{(f_{aR})^2 + (f_{bT})^2} = \sqrt{(f_a \sin \alpha_R)^2 + (f_b \sin \alpha_T)^2} = F\sqrt{\sin^2 \alpha_R + \sin^2 \alpha_T}. \quad (1)$$

Equation (1) describes the contribution of one of the three layers, S1, S2, or S3, in the secondary wall. The overall force toward the center of the lumen in the entire secondary wall can be described as the summation of the contribution of each layer and is calculated as follows:

$$D = \theta_1 f_{D1} + \theta_2 f_{D2} + \theta_3 f_{D3}, \quad (2)$$

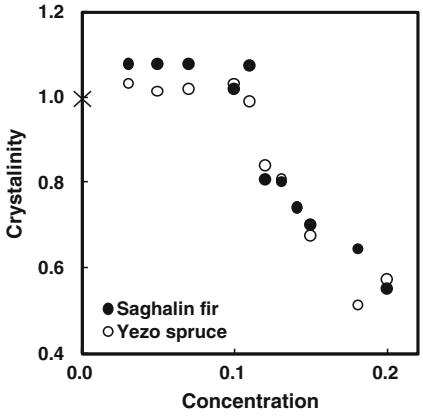

Fig. 7 Crystallinity of NaOH-treated samples relative to the crystallinity of samples treated with water only as a function of NaOH concentration. *Closed circles* represent Saghalin fir and *open circles* represent Yezo spruce

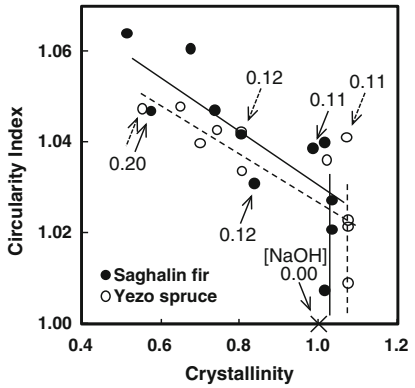

Fig. 8 Relationship between the circularity index relative to the circularity index of samples treated with water only and crystallinity

where θ_i represents the volume fraction of each layer in the secondary wall, f_{Di} is the force toward the center of the cell, and α_{Ri} and α_{Ti} are the MFAs in the LR and LT planes, respectively ($i = 1, 2, 3$ for each layer of the secondary wall). When typical values for the volume fraction of conifers ($\theta_1 = 0.15$, $\theta_2 = 0.80$, $\theta_3 = 0.05$) are used, the resultant equation is as follows:

$$D = \frac{F}{20}\left(3\sqrt{\sin^2\alpha_{R1} + \sin^2\alpha_{T1}} + 16\sqrt{\sin^2\alpha_{R2} + \sin^2\alpha_{T2}} + \sqrt{\sin^2\alpha_{R3} + \sin^2\alpha_{T3}}\right). \quad (3)$$

Equation (3) indicates the dependence of the cross-sectional forces toward the center of the cell on both the contractive force of the microfibrils and the MFA of each layer of the secondary wall. According to the mechanism proposed by Nakano et al. [7] and Nakano [8], the central force described by Eq. (3) occurs at the corners of cells in cross-section when the entropy elastic force of cell wall microfibrils arises due to a decrease in crystallinity. This force should cause a rectangular cross-section of a cell wall to become circular. The change in the circularity index dependent on the crystallinity of cell wall microfibrils is shown in Fig. 8 and can be explained by Eq. (3).

From Eq. (3), the ratio of D to F is given as follows:

$$\frac{D}{F} = \frac{1}{20}\left(3\sqrt{\sin^2\alpha_{R1} + \sin^2\alpha_{T1}} + 16\sqrt{\sin^2\alpha_{R2} + \sin^2\alpha_{T2}} + \sqrt{\sin^2\alpha_{R3} + \sin^2\alpha_{T3}}\right). \quad (4)$$

The magnitude of force toward the center of cell wall cross-sections can be estimated from Eq. (4). Thus, we compared the ratio of D/F among four conifer species (Table 1). Using MFA values determined by Saiki [18], the D/F values for Japanese red pine (*Pinus densiflora*), Japanese cedar (*Cryptomeria japonica*), and Japanese cypress (*Chamaecyparis obtusa*) are 0.72, 0.46, and 0.49, respectively. Moreover, if the average MFA of Yezo spruce, which Ohara [19] reported to be between 29° and 36°, are used and the differences between radial and tangential walls and between the layers in the secondary wall are ignored, Eq. (4) gives D/F ratios of 0.69 and 0.83 for the low and high end of the MFA range for Yezo spruce, respectively. We did not calculate a D/F ratio for Saghalin fir because detailed MFA data were not available.

The contractive forces of microfibrils along their longitudinal axes acted as a force toward the center of cell walls in cross-sections and affected the D/F ratio for species with different MFA (Table 1). The magnitude of this force was more than half of the overall contractive force of the microfibrils. Considering Eqs. (3) and (4), the contractive force of microfibrils along their longitudinal axes that is associated with alkaline treatment is extremely large. This strongly supports the results of Nakano et al. [7].

As discussed above, changes in the circularity index of the lumen of cell walls in cross-sections of wood treated with aqueous [NaOH] < 0.11 were caused by leaching of matrix materials in the cell wall. The appearance of contractive forces of microfibrils in the cross-sectional direction was related to decreasing crystallinity at [NaOH] > 0.12, and this change depended on MFA.

Conclusion

We analyzed photographs of wood cell lumens in cross-sections of wood samples after NaOH treatment and washing. We found that the circularity index of the lumen increased with NaOH concentration. The circularity index was independent of microfibril crystallinity at [NaOH] < 0.11, but linearly increased with decreasing

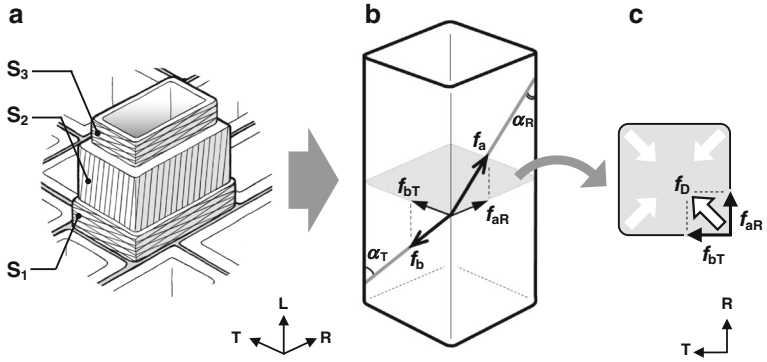

Fig. 9 Cell model of morphological change induced by alkaline treatment. **a** Image of secondary wall structure; **b** model of single layer (square pillar); **c** resultant force f_D acting at the corner of the RT plane in cross-section

Table 1 Microfibril angles of softwood secondary walls and *D*/*F* values

Species	Microfibril angle (°)			References	*D*/*F*
	S1	S2	S3		
Japanese red pine				[18]	0.72
Radial wall	71	26.5	83		
Tangential wall	68	20	83		
Japanese cedar					0.46
Radial wall	68	14	81		
Tangential wall	64	3.5	85		
Japanese cypress					0.49
Radial wall	81	14	82		
Tangential wall	68	6.5	79		
Yezo spruce				[19]	0.69–0.83
Average	29–36				

crystallinity at [NaOH] > 0.12. The results at low NaOH concentrations were due to leaching of matrix materials in wood cells, and the effects at higher NaOH concentrations were due to the contractive force of microfibrils along the longitudinal axis in the cross-section of the cell. Force balance analysis based on a cell model supported experimental results for [NaOH] > 0.12. We conclude that circulation is primarily due to the central force of the three layers, S1, S2, and S3, in the secondary cell wall that occurs at the corner of cells in cross-section.

Acknowledgments The authors thank Prof. Keiji Takabe, Associate Prof. Arata Yoshinaga, Division of Forest and Biomaterials Science, Kyoto University for useful advice and technical help.

References

1. Nakano T (1988) Plasticization of wood by alkali treatment effects of kind of alkali and concentration of alkaline aqueous solution on stress relaxation (in Japanese). J Soc Rheol 16:104–110
2. Nakano T (1989) Plasticization of wood by alkali treatment. Relationship between plasticization and the ultra-structure (in Japanese). Mokuzai Gakkaishi 35:431–437
3. Tanimoto T, Nakano T (2013) Side-chain motion of components in wood samples partially non-crystallized using NaOH–water solution. Mater Sci Eng C 33:1236–1241
4. Nakano T, Tanimoto T, Hashimoto T (2013) Morphological change induced with NaOH–water solution for ramie fiber: change mechanism and effects of concentration and temperature. J Mater Sci 48:7510–7517
5. Tanimoto T, Nakano T (2012) Effects of alkali treatment on ramie cellulose fibers. In: Abstracts of the 62nd annual meeting of the Japan wood research society. Sapporo Hokkaido K16-06-1030
6. Hashimoto T, Nakano T (2013) Effects of alkali treatment and cooling on ramie fibers. In: Abstracts of the 63rd annual meeting of the Japan wood research society. Morioka Iwate K27-09-1700
7. Nakano T, Sugiyama J, Norimoto M (2000) Contractive force and transformation of microfibril with aqueous sodium hydroxide solution for wood. Holzforschung 54:315–320
8. Nakano T (2010) Mechanism of microfibril contraction and anisotropic dimensional changes for cells in wood treated with aqueous NaOH solution. Cellulose 17:711–719
9. Tanimoto T, Nakano T (2012) Stress relaxation of wood partially non-crystallized using aqueous NaOH solution. Carbohydr Polym 87:2145–2148
10. Stöckmann VE (1971) Effect of pulping on cellulose structure Part I. Tappi 54:2033–2037
11. Stöckmann VE (1971) Effect of pulping on cellulose structure Part II. Tappi 54:2038–2045
12. Jagels R, Dyer MV (1983) Morphometric analysis applied to wood structure. I. Cross-sectional cell shape and area change in red spruce. Wood Fiber Sci 15:376–386
13. Diao X-M, Furuno T, Fujita M (1999) Digital image analysis of cross-sectional tracheid shapes in Japanese softwoods using the circularity index and aspect ratio. J Wood Sci 45:98–105
14. Sarén M-P, Serimaa R, Andersson S, Saranpää P, Keckes J, Fratzl P (2004) Effect of growth rate on mean microfibril angle and cross-sectional shape of tracheids of Norway spruce. Trees 18:354–362
15. Harris EE (1933) Effect of alkali treatment on the yield of lignin. Ind Eng Chem 5:105–106
16. Ishikura Y, Nakano T (2005) Adsorption properties and structural features of alkali treated wood (in Japanese). Mokuzai Gakkaishi 51:364–371
17. Rong MZ, Zhang MQ, Liu Y, Yang GC, Zeng HM (2001) The effect of fiber treatment on the mechanical properties of unidirectional sisal-reinforced epoxy composites. Comp Sci Technol 61:1437–1447
18. Saiki H (1970) Influences of wood structure on radial variations in some physical properties within an annual ring of conifers. Memoir Coll Agric Kyoto Univ 96:47–73
19. Ohara K (1947) Microstructure of plant cell walls (in Japanese). Seibutsugaku Souhou 1:1–51