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Study on morphology and orientation of cellulose in the vascular bundle of wheat straw

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Abstract

Morphology and orientation of cellulose in the vessels of vascular bundles of wheat straw were studied. The results indicated that cellulose acts as the framework in the vascular bundles and cellulose chains are high in orientation. In the thickening part of the vessels, cellulose exists in the form of cellulose crystalline lamellae but not cellulose microfibrils. The crystalline lamellae are perpendicular to the tangential direction of annular rings and incline clockwise with an angle of about 30–40° to the tangential direction of the spiral line in the spiral vessels. A model of the arrangement of cellulose chains in the vascular bundles was proposed.

Keywords: Cellulose; Orientation; Morphology

1. Introduction

It is known that in the monocotyledonous stem, such as in wheat straw, the conduction takes place inside the vascular bundle of which cellulose acts as the framework. Therefore, the vascular bundle plays a key role in the growth of wheat straw. In the vascular bundle, one of the most important tissues is the vessels. These protoxylem vessels have dense lignified thickenings in the secondary wall, arranged as annular rings or in a spiral, a scalariform, a recticulated or a pitted form. During the early stage of the plant growth, the annular ring and spiral vessels arise in sequence. The non-thickening part of the two types of vessels is primary wall with large areas, which provides the vessels with a strong stretch ability to suit the growth of the plant.

Cellulose is one of the main structural polymers of plant cell walls. It has been suggested that in plant cell walls many parallel cellulose chains are held together by hydrogen bonds between hydroxyl groups of the glucose monomers to form a cellulose fibril with indefinite length and varying degrees of crystallinity, which embedded in a gel matrix composed of hemicelluloses, lignin and other carbohydrate polymers [1–3]. Cellulose microfibrils are of great important in plant tissues because they make the major contribution to the mechanical strength of the cell walls and act as the framework thereof [4].

As the framework in the vascular bundle, few works had been carried out in the morphology, fine structure and orientation of cellulose chains till to now. In this paper, cellulose isolated from wheat straw was presented in its native state. The morphology of cellulose and the orientation of cellulose chains in the annular ring vessel and spiral vessel in the vascular bundles, as well as optical properties and the alignment of cellulose crystalline lamellae were revealed and a model of cellulose in the vessels was proposed.

2. Experimental

Mature wheat straw was kindly provided by China Agriculture University. After being cleaned and dried, the wheat straw was cut into small pieces about 2 mm or less in length. The small pieces of wheat straw were then disencrusted thoroughly with a combination of two

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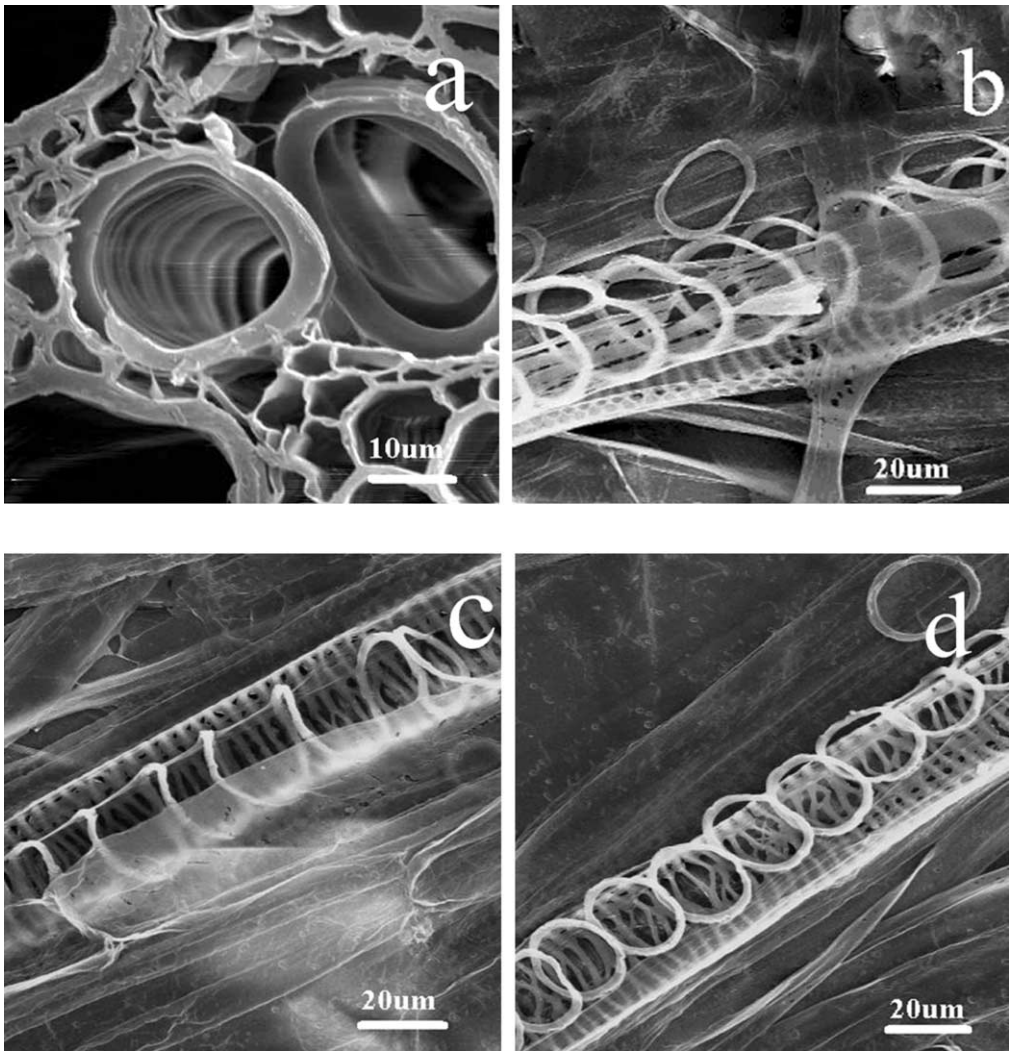

Fig. 1. SEM micrographs of the vascular bundles: (a) cross section of the vascular bundles; (b) and (d) annular rings in the vascular bundles; (c) spiral form in the vascular bundles.

purification methods for the primary wall and the secondary wall to remove pectin, polysaccharides, lignin, and other non-cellulosic substances completely. The details of the purification method were present previously [5,6].

The isolated cellulose from wheat straw was dispersed in distilled water by ultrasonic. The dispersion of cellulose was then transferred to a fresh cleaved mica surface and allowed air-dried. The morphology of the cellulose of the vessels in the vascular bundles was observed using an Olympus BH-2 polarized optical microscope (POM). The optical property was observed under crossing polarized light with a first-order retardation plate.

Scanning electric microscopy (SEM) analysis was performed using a Hitachi S-4300 SEM, operated at 15 kV. The samples were coated with gold using an Eiko IB-3 Incoater.

Tapping-mode atomic force microscopy (AFM) images were obtained by using a NanoScope III MultiMode AFM (Digital Instruments). Both topographic and phase images were recorded simultaneously. Silicon cantilever tips with a resonance frequency of approximately 300 kHz and a spring constant of about 32 N m^{-1} were used. The scan rate varied from 0.5 to 1.5 Hz. The typical value for the amplitude was 2.0 V, and the set point amplitude ratio ($r_{sp}=A_{sp}/A_0$, where A_{sp} is the set-point amplitude and A_0 is the amplitude of the free oscillation) was adjusted to 0.7–0.9. The amplitude and set point ratio were chosen such that the surface was tracked while maintaining the necessary contrast in the phase images. All images were measured in air at 512×512 pixels at ambient condition.

3. Results and discussion

Fig. 1(a) shows the cross section of the vascular bundle in wheat straw. In vascular bundles, some parts of dense lignified thickenings in the secondary wall arrange as the annular rings or the spiral structure to form the vessels. A

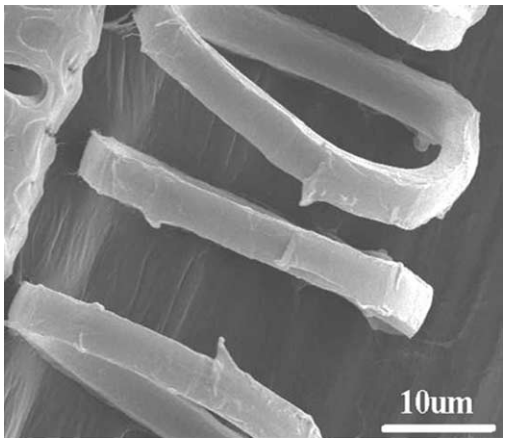

Fig. 2. SEM micrograph of the annular structure in the untreated sample.

strengthening tissue of sclerenchyma fibers surrounds the vascular cells. In wheat straw, these fibers are coarse bast fibers just inside the epidermal layer. These fibers represent the valuable raw material for the paper industry and have potential utilities in composites and building board [7–17].

According to the reference, Lignin, hemicelluloses and other extractive substances were removed from wheat straw by the extraction and purification process, and mainly cellulose was left [5,6]. It can be seen from Fig. 1 that the annular rings (Fig. 1(b) and (d)) and spiral structure (Fig. 1(c)) in the vessels are reserved, and there is a thin film surrounding them. It is indicated that cellulose is the framework of the vascular bundles. It is noted that the rings are almost the same in morphology before (Figs. 1(a) and 2) and after extraction of hemicelluloses and lignin in the wheat straw (Fig. 1(b) and (d)). The surface of the ring in the untreated samples (Figs. 1(a) and 2), however, looks smoother than that in the treated ones (Fig. 1(b) and (d)), which is attributed to the existence of amorphous lignin and hemicelluloses therein. According to above-mentioned, a sketch of spiral and annular vessels in the vascular bundles was shown in Fig. 3. As the arrows directed in Fig. 3, the out layer is the primary wall and the annular and spiral structure are the lignified thickening secondary wall, which mainly consists of cellulose and surrounded by the primary wall.

Under crossing polarized light, the annular rings (Fig. 4(a)) and the spiral structure (Fig. 4(c)) show strong

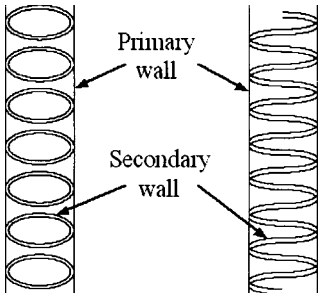

Fig. 3. A schematic model of spiral and annular vessels in the vascular bundles.

birefringence, whereas the birefringence of the surrounding cellulose film is quite weak. It is known that the lignin and hemicelluloses are amorphous carbohydrates in wheat straw, which shows no birefringence under crossing polarized light. Therefore, the birefringence in the vessel is attributed to the orientation and/or crystallization of cellulose [18]. The strong birefringence of the annular rings and the spirals means that cellulose chains has a higher orientation order than those in the surrounding cellulose film. The annular and the spiral vessels arise at the earlier growth stage during which the plant growth is very fast, and a higher stress exists during the formation process of the two types of structure, which leads to a higher orientation order of cellulose chains in the biosynthesis process of cellulose. It had been reported that the metastable triclinic I_α phase is formed due to some types of stress during crystallization, whereas the stable monoclinic I_β phase is formed in a relatively relaxed state [19–22]. There are probably also some types of stress existing during the formation of cellulose I_β crystalline phase, at least during the biosynthesis and subsequent crystallization process of cellulose in the annular rings and the spiral structure in the vascular bundles in wheat straw.

Under crossing polarized light with a first-order retardation plate (λ-plate), a sensitive tint plate, the annular or spiral vessel has a uniform bright yellow color on a red background when the vessel lies in the direction of the first and third quadrants in the cross, while had a uniform blue color when the vessel is oriented in the direction of the second and fourth quadrants (Fig. 4(c) and (d)). For native cellulose from higher plant, such as that from wheat straw, the crystal structure is mainly in its I_β phase [5], the monoclinic two-chain unit cell [23], in which *c*-axis of the unit cell is parallel to the fiber axis and the space group is approximated to $P2_1$ [24]. Therefore, it is suggested, from the optical properties of the sample mentioned above, that the orientation of cellulose chains in the vascular bundle is along the tangential direction of annular or the spiral vessels.

It is very interesting to note that for a single annular ring in the tracheary cells, Maltase cross pattern is shown under crossing polarized light (Fig. 4(a) and (b)), which is similar to the spherulite of synthetic polymers. It is indicated that the crystalline lamellae of cellulose are arranged symmetrically in the annular rings, as those crystalline lamellae of synthetic polymers in spherulites [25]. Insertion of a sensitive tint plate between the crossed polarizers, the annular ring shows yellow color in its first and third quadrants and blue color in its second and fourth quadrants. When the sample is rotated with an angle of 90°, the blue color is changed to yellow and the yellow is changed to blue. It is indicated that the annular rings are negative in optics and the cellulose crystalline lamellae are arranged symmetrically around the ring. Therefore, cellulose chains are oriented along the tangential direction of the annular rings.

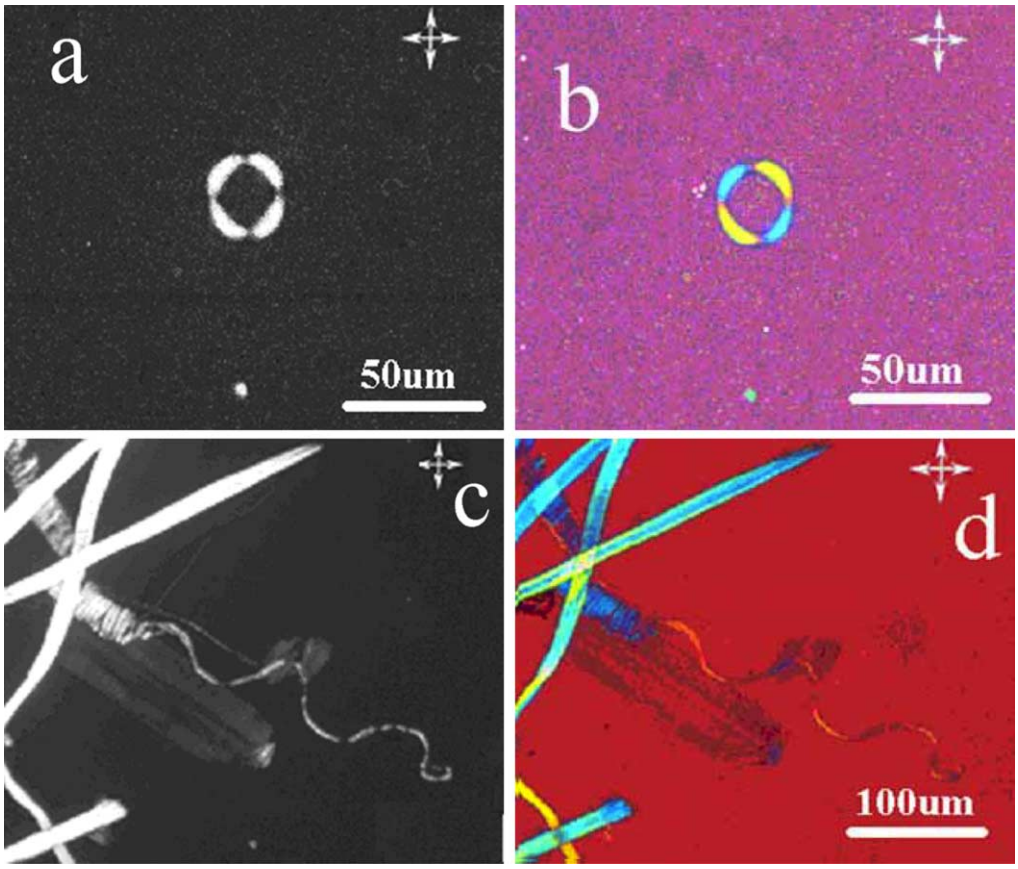

Fig. 4. POM micrographs of annular and spiral cellulose framework in the vascular bundle of wheat straw. Annular vessel (a) and the corresponding photograph with a first-order red plate (b); spiral vessel (c) and the corresponding photograph with a first-order red plate (d). The arrows indicate the direction of polarizer and the analyzer (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

It can be seen from Fig. 4(c) and (d) that, the spiral vessel is partially stretched during the preparation of samples. As result, the properties of the spiral vessel in four quadrants under the crossing polarized light can be easily observed. The optical properties of the stretched spiral vessel indicate that the spiral vessel is negative in optics and therefore it can be deduced that cellulose chains are oriented along the direction of the spiral line, which confirms again that cellulose chains are oriented along the tangential direction in the spiral vessel.

Typical AFM images of cellulose in the annular rings are shown in Fig. 5. Fig. 5(a) is a height image and Fig. 5(b) is a

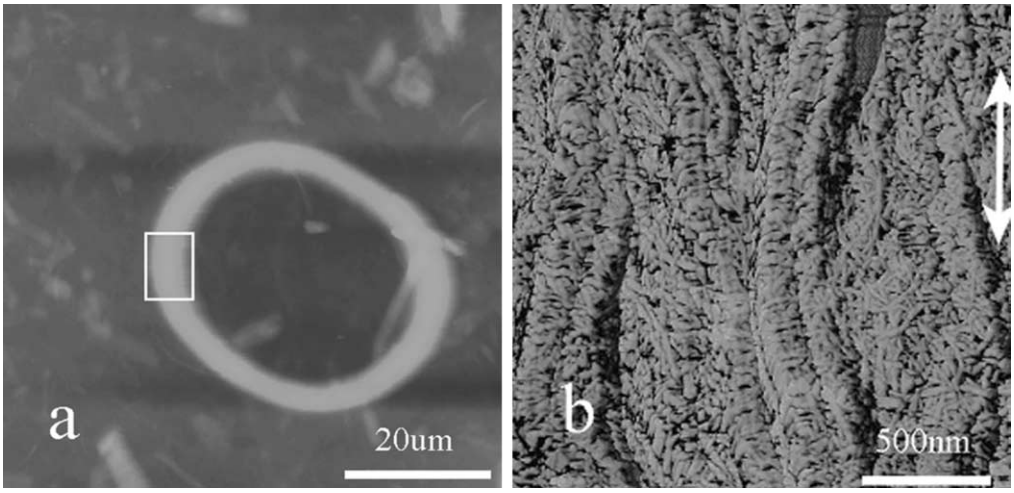

Fig. 5. AFM image of the annular ring: (a) height image, (b) phase image as marked in (a) (the arrow indicates the tangential direction).

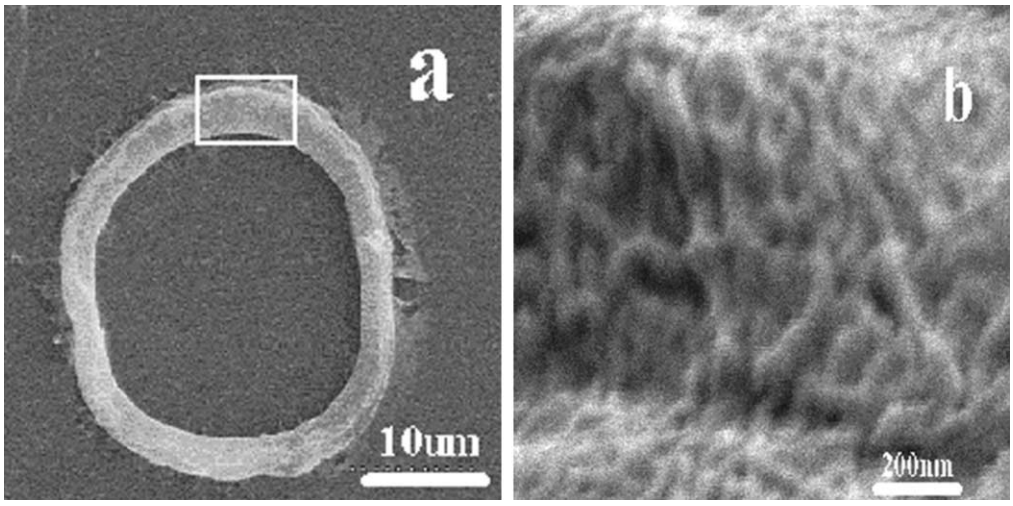

Fig. 6. SEM micrograph of the annular ring in the vascular bundle of wheat straw.

local magnified phase image of the ring. The crystalline lamellar structure can be observed on the surface of the annular ring by AFM. From Fig. 5(b), it can be found that the crystalline lamellae assemble essentially regularly and they are about 20 nm in thickness and most of them are about 100 nm in length [26]. This structure is similar to that in treated spruce kraft pulps [27,28]. Dimensions of this fibrillar structure are consistent with that of a single crystalline lamella [29]. These crystalline lamellae are arranged in sequence to form crystalline lamella bundles that are arranged around the annular rings. The structure observed by AFM should be the edges of the cellulose crystalline lamellae, which are perpendicular to the tangential direction of the annular rings. In the right half of Fig. 5(b), however, some lamellae are irregular arrangement, which may result from the damage of the crystalline lamellae bundle during the preparation of samples. In such area it can be seen that some crystalline lamellae are more than 200 nm in length, even up to 300 nm. It is suggested that some lamellae with 200–300 nm in length exist in the system and they are crossed through more than two lamella bundles. The results of the SEM observation are agreed with that of the AFM image. Fig. 6 is a magnified micrograph of the annular ring captured with a field emission SEM, from which crystalline lamellar structure of cellulose has been observed and the dimension is consistent with that obtained with the AFM image.

Fig. 7 is the typical AFM image of spiral vessel. The crystalline lamellae are about 20–40 nm in thickness and about 100–150 nm in length and assemble regularly. The crystalline lamellae are not perpendicular but incline clockwise with an angle of about 30–40° to the tangential direction of the spiral line. Such assembly of the crystalline lamellae in spirals provided the vessels with higher resistance to the tension and compression stress in the case of the deformation.

Combined with the results above mentioned, a schematic model of cellulose chains in the annular and spiral vessels

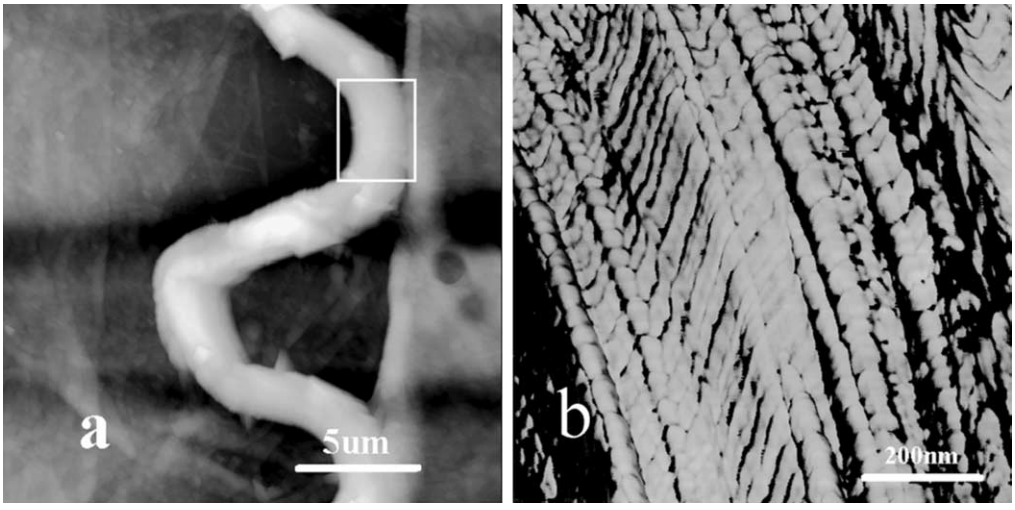

Fig. 7. AFM image of the cellulose in the spiral vessel: (a) height image; (b) phase image as marked in (a).

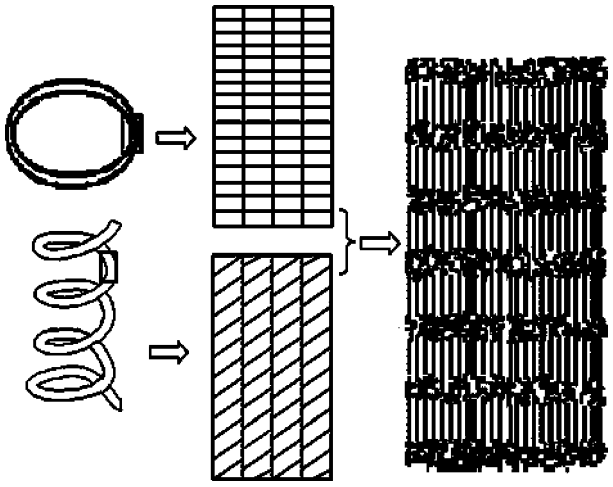

Fig. 8. A schematic model of cellulose molecules in the annular and spiral vessels.

can be proposed, as shown in Fig. 8. The annular and the spiral vessels are formed by the assembled cellulose crystalline lamellae that assembly into crystalline lamellae bundles, in which cellulose molecule chains are parallel to each other. The cellulose crystalline lamellae are perpendicular to the tangential direction in the annular ring and incline clockwise with an angle of about 30–40° to the tangential direction in the spiral line. It can be presumed that in the vascular bundles, cellulose chains arrange parallel to each other and travel through many crystalline lamellae in various sizes and different crystalline regions.

4. Conclusion

POM observations indicate that cellulose chains in the annular and spiral vessels oriented in the tangential direction of the annular or the spiral vessel in the vascular bundle and have a high orientation degree. The AFM observation reveals the crystalline lamellar structure of cellulose chains in the annular and the spiral vessels. In the vessels, cellulose chains exist in the form of cellulose crystalline lamellae but not cellulose microfibrils. The SEM observation confirms the results of the AFM observation. The lamellae of cellulose chains are perpendicular to the tangential direction in the annular vessel and incline clockwise with an angle of about 30–40° to the tangential direction in the spiral vessel.

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References

[1] Hanley SJ, Revol JF, Godbout L, Gray DG. Cellulose 1997;4:209–20.
[2] Campbell NA. Biology. 3rd ed. Redwood City: Benjamin/Cummings; 1993. pp. 68–70.
[3] Carpita NC, Gibeaut DM. Plant J 1993;3:1–30.
[4] Thimm JC, Burritt DJ, Ducker WA, Melton LD. Planta 2000;212: 25–32.
[5] Liu RG, Yu H, Huang Y. Cellulose 2004;12(1):25–34.
[6] Kataoka Y, Kondo T. Macromolecules 1998;31:760–1.
[7] Dufresne A, Dupeyre D, Vignon MR. J Appl Polym Sci 2000;76: 2080–92.
[8] Avella M, Rota GL, Martuscelli E, Raimo M, Sadocco P, Elegir G, et al. J Mater Sci 2000;35:829–36.
[9] Dufresne A, Kellerhals MB, Witholt B. Macromolecules 1999;32: 7396–401.
[10] Dufresne A, Vignon MR. Macromolecules 1998;31:2693–6.
[11] Hornsby PR, Hinrichsen E, Tarverdi K. J Mater Sci 1997;32:1009–15.
[12] Hornsby PR, Hinrichsen E, Tarverdi K. J Mater Sci 1997;32:443–9.
[13] Avella M, Martuscelli E, Pascucci B, Raimo M, Focher B, Marzetti A. J Appl Polym Sci 1993;49:2091–103.
[14] Dufresne A, Cavaille JY, Helbert W. Polym Compos 1997;18: 198–210.
[15] Helbert W, Cavaille JY, Dufresne A. Polym Compos 1996;17:604–11.
[16] Gousse C, Chanzy H, Cerrada ML, Fleury E. Polymer 2004;45: 1569–75.
[17] Liu WJ, Mohanty AK, Askeland P, Drzal LT, Misra M. Polymer 2004;45:7589–96.
[18] Arcidiacono S, Mello CM, Butler M, Welsh E, Soares JW, Allen A, et al. Macromolecules 2002;35:1262–6.
[19] Horii F, Yamamoto H, Kitamaru R, Tanahashi M, Higuchi T. Macromolecules 1987;20:2946–9.
[20] Sugiyama J, Okano T, Yamamoto H, Horii F. Macromolecules 1990; 23:3196–8.
[21] Sugiyama J, Persson J, Chanzy H. Macromolecules 1991;24:2461–6.
[22] Dinand E, Vignon M, Chanzy H, Heux L. Cellulose 2002;9:7–18.
[23] Gardner KH, Blackwell J. Biopolymers 1974;13:1975–2001.
[24] Woodcock C, Sarko A. Macromolecules 1980;13:1183–7.
[25] Bassett DC. Principles of polymer morphology. New York: Cambridge University Press; 1981. pp. 16–36.
[26] Kontturi E, Thune PC, Alexeev A, Niemantsverdriet JW. Polymer 2005;46:3307–17.
[27] Gustafsson J, Ciovica L, Peltonen J. Polymer 2003;44:661–70.
[28] Simola J, Malkavaara P, Alen R, Peltonen J. Polymer 2000;41: 2121–6.
[29] Wunderlich B. Macromolecular physics. vol. 1. New York: Academic Press; 1973.