LIGNIN DISTRIBUTION IN SODA-OXYGEN AND KRAFT FIBERS AS DETERMINED BY CONVENTIONAL ELECTRON MICROSCOPY[1]

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ABSTRACT

The lignin distribution in loblolly pine (*Pinus taeda* L.) fibers produced by soda-oxygen and kraft pulping was investigated by electron microscopy of $KMnO_4$ stained ultrathin sections and direct carbon replicas. A more extensive delignification of outer layers of fibers was found in soda-oxygen pulps than in kraft pulps. However, kraft pulping is more effective than soda-oxygen pulping in removing lignin from the lumen side of fibers. These observations were in good agreement with an earlier study of the lignin distribution within pulp fibers performed with SEM-EDXA technique.

Keywords: Soda-oxygen pulping, kraft pulping, Kappa number, lignin distribution, fiber, SEM-EDXA, $KMnO_4$ staining, ultrathin section, direct carbon replica.

INTRODUCTION

From a papermaking point of view, the residual lignin distribution during and after pulping is of considerable interest. Therefore, both electron microscopy (Norberg 1969; Parham 1974) and ultraviolet (UV) microscopy (Kerr and Goring 1976; Wood and Goring 1973) have been widely used for studies on lignin distribution.

Conventional electron microscopy techniques for detecting lignin involve preparation of lignin skeletons (Bentum et al. 1969; Parham and Côté 1971; Sachs et al. 1963) or the use of potassium permanganate ($KMnO_4$) stains (Bland et al. 1971; Hepler et al. 1970; Kutscha and Schwarzmann 1975). Both of these techniques provide only qualitative estimates of the lignin distribution. A recently developed technique for lignin detection, however, allows a quantitative assay of lignin in the various layers of the cell wall (Saka et al. 1978). This method involves scanning electron microscopy coupled with energy dispersive X-ray analysis (SEM-EDXA).

UV microscopy has been most frequently used for quantitative studies of lignin distribution in cell walls with satisfactory results (Fergus and Goring 1970; Fergus et al. 1969). It was shown that in kraft pulps the secondary wall lignin is preferentially removed and that significant amounts of lignin remain in the primary wall and cell corner regions (Wood and Goring 1973). Studies employing the SEM-EDXA method confirmed this type of lignin removal in kraft pulping and also revealed that in soda-oxygen (soda-O_2) pulping, lignin is preferentially removed from the outermost portion of the fibers. These observations suggest that lignin

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TABLE 1. *Description of pulps used in this study.*

Pulps	Kappa no. (K#)	Yield (%)	Delignification (%)[5]
Soda-O_2	96	55.3	72
	75	52.8	79
	48	47.1	88
	26	45.9	94
Kraft	100	54.9	71
	74	51.0	80
	51	47.9	87
	27	44.1	94

is more susceptible to oxidative degradation than to hydrolysis as in kraft pulping and that significant differences exist in the topochemistry of lignin removal between these pulping processes (Saka et al. 1978).

In order to obtain additional information, comparative studies on lignin distribution in soda-O_2 and kraft pulp fibers with different lignin contents were performed via conventional electron microscopy. Concomitantly, the results obtained in this study were compared with the previous results with the SEM-EDXA technique.

MATERIALS AND METHODS

Loblolly pine (*Pinus taeda* L.) pulp fibers[3] with comparable lignin contents were prepared by soda-O_2 and kraft pulping (Table 1).

For the $KMnO_4$ staining study, four different pulps, two soda-O_2 pulps with Kappa number (K#)[4] 96 and 26 and two kraft pulps with K# 100 and 27, were chosen. Because the lignin distribution of these four pulps had been previously determined by the SEM-EDXA technique (Saka et al. 1978), it was possible to compare both methods. Following the routine procedures, the samples were stained with 2% aqueous solution of $KMnO_4$ and embedded in epoxy resin (Luft 1961). Ultrathin sections (700Å) were subsequently cut with a diamond knife mounted in a Porter-Blum MT-2 ultramicrotome.

For detailed characterization of the external surface, a direct carbon replica technique was employed. Handsheets from each of the eight pulps were made, air-dried, and replicated (Côté et al. 1964).

Both the ultrathin sections and replicas were studied with a Siemens Elmiskop 1A electron microscope at an accelerating voltage of 80 kV.

RESULTS AND DISCUSSION

The $KMnO_4$ staining has been widely utilized for lignin detection (Bland et al. 1971; Hepler et al. 1970; Kutscha and Schwarzmann 1975). The results obtained by this technique were consistent with the data from UV microscopy or lignin

[3] The term "fiber" refers to the longitudinal tracheid which constitutes about 90% of the cells found in softwoods.

[4] Kappa number (K#) is a measure of the lignin content.

[5] Calculated assuming 0.15 × (K#) × (Yield) = (Residual lignin).

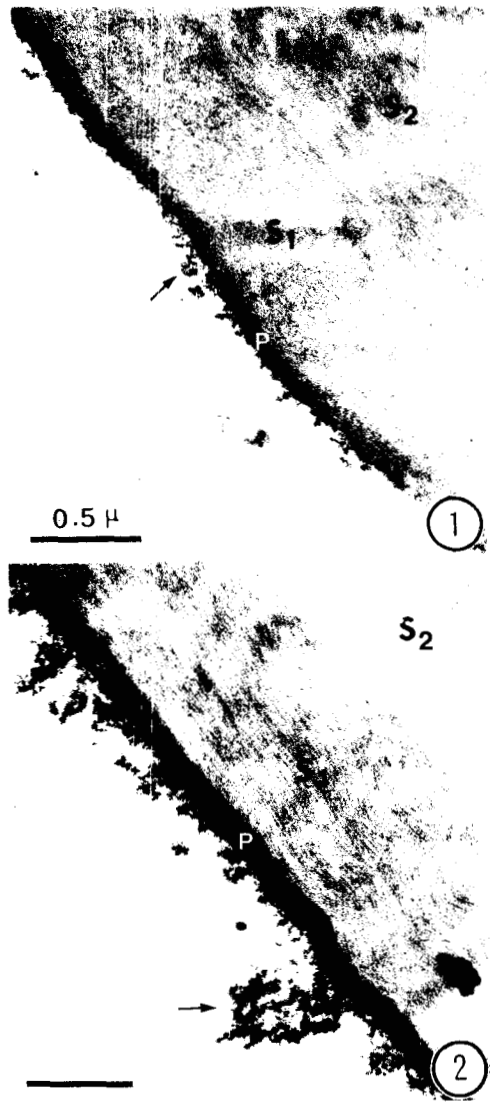

FIG. 1. Ultrathin cross section of the outer cell wall of a soda-O_2 pulp fiber (K# = 96) showing the primary wall (P) and the S_1 and S_2 layers of the secondary wall. Arrow indicates residual middle lamella lignin. 2% $KMnO_4$. Line on all micrographs represents 0.5 μm.

FIG. 2. Ultrathin cross section of the outer cell wall of a kraft pulp fiber (K# = 100). Arrow indicates residual middle lamella lignin. 2% $KMnO_4$.

skeleton technique (Parham 1974). Therefore, in order to perform comparative studies on lignin distribution in soda-O_2 and kraft pulp fibers with different lignin contents, the $KMnO_4$ staining technique was employed.

In the examination of ultrathin sections, lignin-rich areas are revealed as dark zones because $KMnO_4$ is an electron-dense stain specific for lignin. Thus, in Figs. 1 and 2 (soda-O_2, K# = 96; kraft, k# = 100) the various layers of the cell wall can be detected because of their different lignin contents. The more intense stain-

FIG. 3. Ultrathin cross section of a soda-O_2 pulp fiber (K# = 96). Note that the boundaries between the primary wall (P) and contiguous layers are not distinct. 2% $KMnO_4$.

FIG. 4. Ultrathin cross section of a kraft pulp fiber (K# = 100). Note the intense staining of the cell corner (CC). 2% $KMnO_4$.

FIG. 5. Ultrathin cross section of a soda-O_2 pulp fiber (K# = 26). Note the absence of residual lignin in the cell corner. 2% $KMnO_4$.

FIG. 6. Ultrathin cross section of a kraft pulp fiber (K# = 27). Note that residual middle lamella lignin can be detected in the cell corner. Also, the primary wall still contains lignin. 2% $KMnO_4$.

ing of the primary wall, as compared to the secondary wall, is the result of the higher lignin content of the primary wall. However, note that in soda-O_2 fiber (Fig. 1), the contrast between the primary and secondary wall is such that the boundary between them is not distinct, whereas the more intense staining of the kraft fiber primary wall results in a distinct boundary (Fig. 2). In addition, the

figures show much less residual middle lamella lignin in the soda-O_2 fiber than in the kraft fiber (compare Figs. 1 and 2).

Lignin in the cell corner (CC) also indicates a topochemical difference between these two pulps during lignin degradation (Figs. 3 and 4); although both pulp fibers still retain cell corner lignin at this stage of delignification (soda-O_2, K# = 96; kraft, K# = 100), the primary wall is much more delignified in the soda-O_2 fiber (Fig. 3) so that the boundaries between the primary wall and contiguous layers are not obvious. However, the primary wall in the kraft pulp fiber (Fig. 4) is sharply differentiated from both the cell corner and S_1 layer.

At lower lignin contents (soda-O_2, K# = 26; kraft, K# = 27), the outermost part of soda-O_2 pulp fibers presents a rather uniform appearance since most of lignin has been removed. Note in Fig. 5 that the primary wall cannot be distinguished from the S_1 layer. In addition, note the rather complete removal of cell corner and primary wall lignin. On the other hand, the kraft pulp fiber still has more lignin in the outer fiber wall, especially in the cell corner (Fig. 6). The presence of cell corner lignin in kraft pulp fibers even at the lowest lignin content is in agreement with earlier studies (Kerr and Goring 1976; Norbert 1969; Parham 1974; Saka et al. 1978).

Also examined was the amount of residual lignin in the cell-wall areas near the lumen. Figures 7 and 8 depict the S_3 and part of the S_2 layer for fibers from soda-O_2 and kraft pulp (K# = 96 and 100, respectively). At these Kappa numbers, the lamellated structure of the S_2 and S_3 layers can be easily observed for both pulp types because of the residual lignin. Although uniform staining is apparent within the S_2 and S_3 layers, the S_2 layer is stained more intensely than the S_3 layer. SEM-EDXA observations have also shown that the innermost portion of the fiber wall has a lower lignin concentration than the middle of the fiber wall (Saka et al. 1978). However, this observation differs from results obtained with UV microscopy which indicated no concentration gradients in the secondary wall of spruce tracheids during pulping (Procter et al. 1967).

At the highest level of delignification (soda-O_2, K# = 26; kraft, K# = 27), the S_3 layer in both soda-O_2 pulp fiber (Fig. 9) and kraft pulp fiber (Fig. 10) appears to retain slightly more lignin than the S_2 layer; however, in kraft pulp fibers, the more uniform removal of lignin from the S_2 and S_3 layers obscured these morphological regions (Fig. 10). These observations suggest that with kraft pulping, more lignin is removed from the innermost part of the fiber wall than with soda-O_2 pulping.

Further evidence of the more complete removal of lignin from the external fiber surface in soda-O_2 pulping was revealed from replicas of the external fiber wall. As delignification proceeds, microfibrils that were embedded in the matrix of lignin and some hemicelluloses become more visible. Thus, the clarity with which microfibrils can be seen provides a qualitative measure for the removal of matrix substances. Figures 11 through 18 show the external fiber walls at different levels of delignification (Figs. 11, 13, 15, 17 for soda-O_2 pulps and Figs. 12, 14, 16, 18 for kraft pulps). Figures 11 and 12 reveal that matrix substances obscure most of the microfibrils in the primary wall in both soda-O_2 and kraft pulps (soda-O_2, K# = 96; kraft, *K#* = 100). However, the microfibrils in kraft pulp fibers appear more encrusted with matrix materials than those in soda-O_2 fibers. Thus, the microfibrils in soda-O_2 fiber can be more easily detected. As delignification

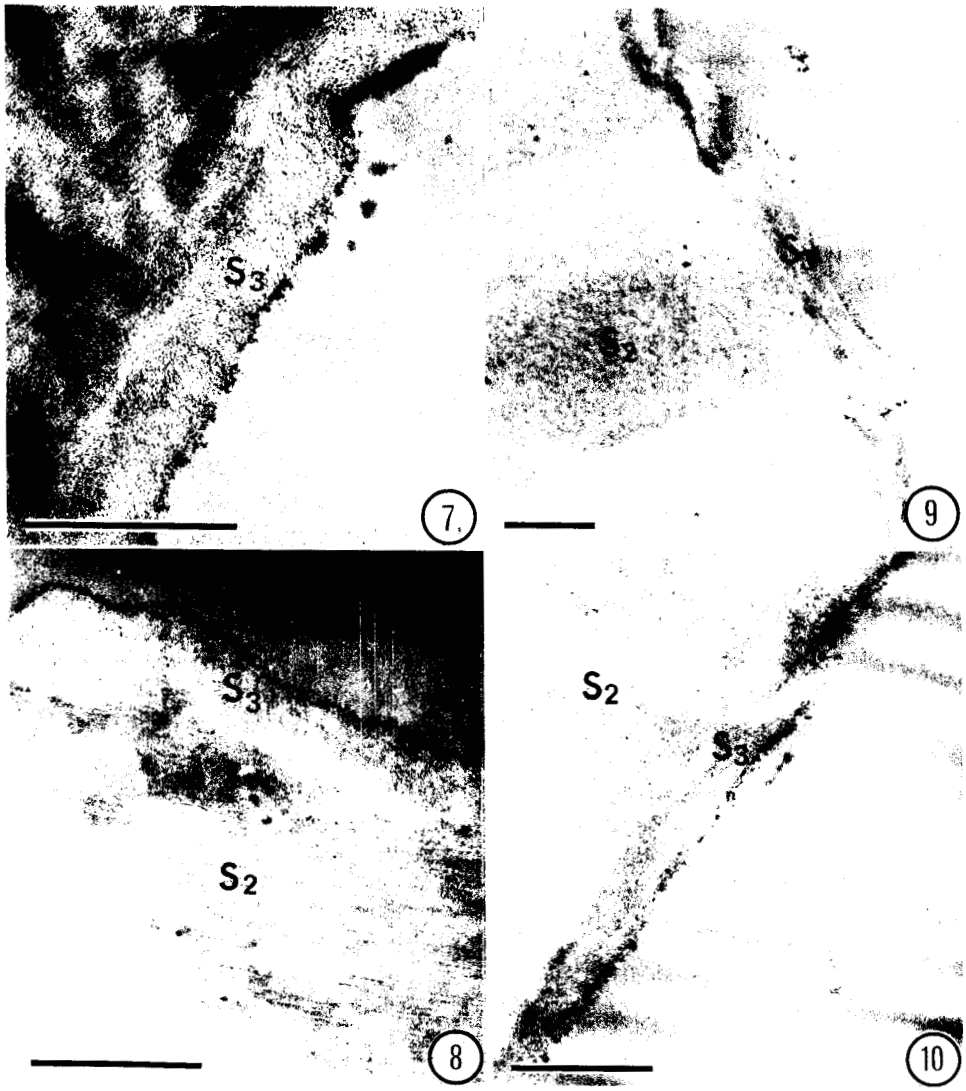

FIG. 7. Ultrathin cross section of the inner cell wall of a soda-O_2 pulp fiber (K# = 96) showing the S_2 and S_3 layers. Note that the S_2 layer is stained more intensely than the S_3 layer. 2% $KMnO_4$.

FIG. 8. Ultrathin cross section of the inner cell wall of a kraft pulp fiber (K# = 100). Note that the S_2 layer is stained more intensely than the S_3 layer. 2% $KMnO_4$.

FIG. 9. Ultrathin cross section of the inner cell wall of a soda-O_2 pulp fiber (K# = 26). 2% $KMnO_4$.

FIG. 10. Ultrathin cross section of the inner cell wall of a kraft pulp fiber (K# = 27). 2% $KMnO_4$.

proceeded to the next stage (soda-O_2, K# = 75; kraft, K# = 74), the microfibrils in soda-O_2 pulp (Fig. 13) are more clearly delineated than in kraft pulp (Fig. 14) because of more extensive removal of matrix substances from the outer surface. Although the primary wall microfibrils in kraft pulps also become more visible as delignification proceeds, the residual substance of matrix can still be detected

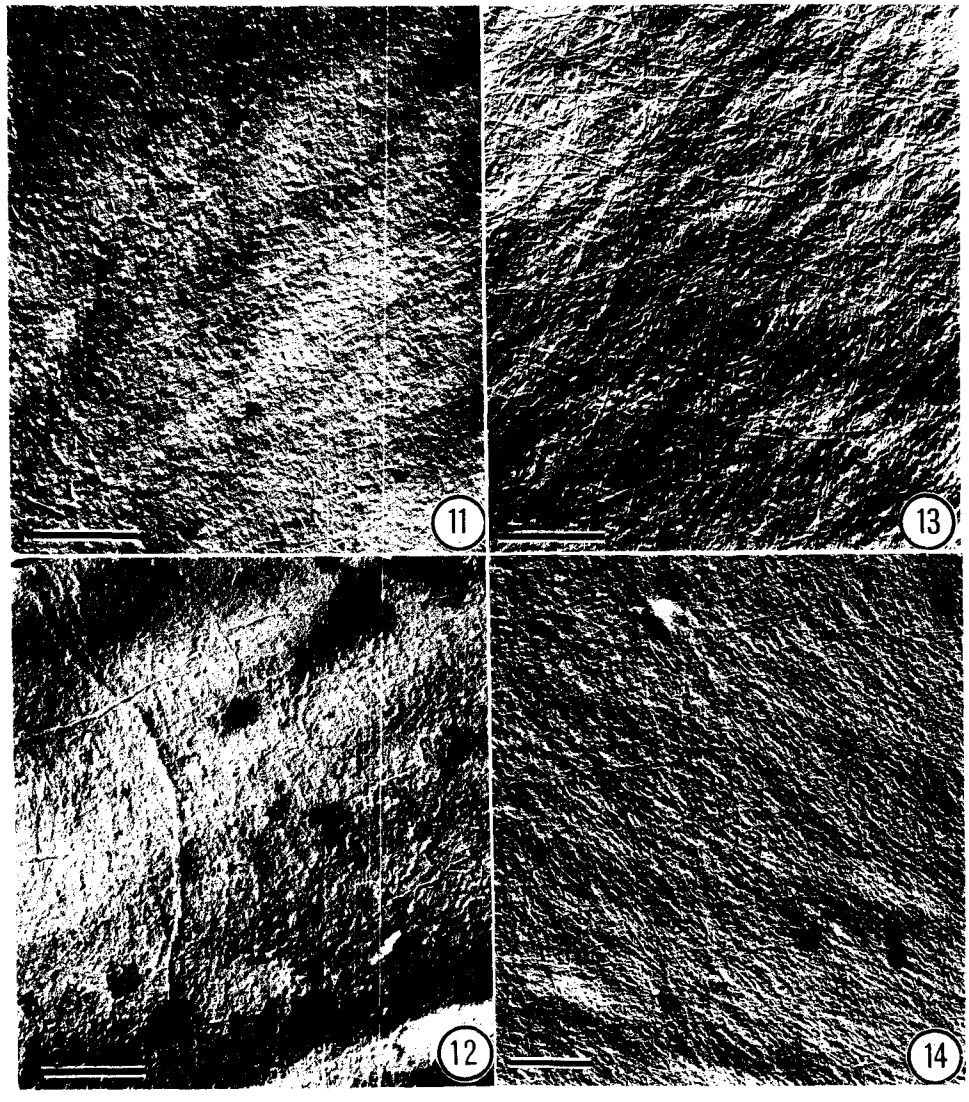

FIG. 11. Replica of a primary wall encrusted with the matrix substances. Soda-O_2 pulp fiber (K# = 96).

FIG. 12. Replica of a primary wall encrusted with the matrix substances. Kraft pulp fiber (K# = 100).

FIG. 13. Replica of the primary wall of a soda-O_2 pulp fiber (K# = 75). The microfibrils are very distinct compared to those in Fig. 14.

FIG. 14. Replica of the primary wall of a kraft pulp fiber (K# = 74). Microfibrils are still partially encrusted with residual matrix substances.

even in the lower Kappa numbers (K# = 51 and 27, Figs. 16 and 18, respectively). However, note that at the equivalent delignification levels (K# = 48 and 26), no residual substances can be detected in soda-O_2 pulps (Figs. 15 and 17).

The matrix substances observed in replicas of the outer surface of fibers are presumably the residual materials detected in the middle lamella region in Figs.

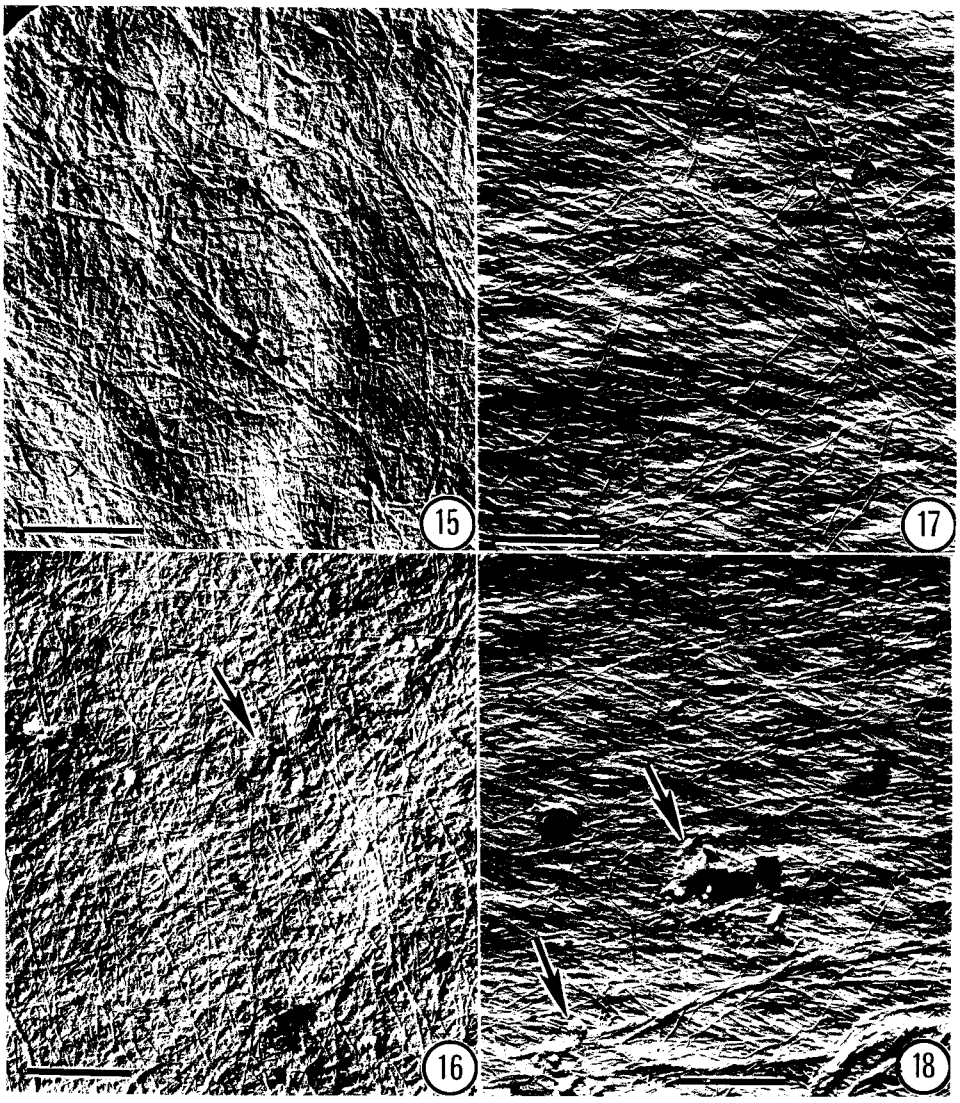

FIG. 15. Replica of the primary wall of a soda-O_2 pulp fiber (K# = 48).

FIG. 16. Replica of the primary wall of a kraft pulp fiber (K# = 51). Microfibrils have been exposed, but residual substance still exists (arrow).

FIG. 17. Replica of the primary wall of a soda-O_2 pulp fiber (K# = 26). Note that the residual matrix substance is completely removed.

FIG. 18. Replica of the primary wall of a kraft pulp fiber (K# = 27). Note that the residual matrix substance still exists (arrows).

1 and 2. In addition, these were extensively stained with $KMnO_4$ so that the matrix substances detected in replicas can be generally regarded as the residual lignin.

Thus, the conventional electron microscopy studies utilized in this work, particularly the ultrathin sections, clearly revealed a more extensive delignification

from the external fiber wall in soda-O_2 pulp fibers than in kraft pulp fibers. These observations are in good agreement with the results obtained from SEM-EDXA studies, indicating the comparability of the two techniques.

The observed differences in lignin distribution pattern seem to be caused by two factors, namely (a) accessibility of reactive sites in lignin in the various layers, and (b) different chemical degradation reactions. In two stage soda-O_2 processes, soda pulp is refined and the separated individual fibers are subsequently exposed to oxygen. Therefore, the lignin in the outer portion of fibers is more accessible to oxygen than the inner portion (Phillips and McIntosh 1975). As a result, lignin from the outer fiber wall is removed first. In kraft pulping, the fibers do not separate until the end of the pulping process; thus, diffusion of cooking liquors is predominantly from the lumen to the middle lamella region. Consequently, the middle lamella and primary wall are not as accessible to the pulping chemicals as the fiber walls adjacent to the lumen. Therefore, the residual lignin distribution appears to be the result of accessibility to the pulping chemicals as well as the type of chemical degradation reactions.

SUMMARY AND CONCLUSIONS

1. Observations of the sequence of the lignin removal from the outer portion of fibers with $KMnO_4$ staining and direct carbon replica techniques revealed the following: (i) a more extensive delignification from the outer layers of the fiber in soda-O_2 pulps than in kraft pulps, (ii) at low lignin contents, cell corner lignin was present in kraft pulps but absent from soda-O_2 pulps, and (iii) kraft pulping was more effective than soda-O_2 pulping in removing lignin from the lumen side of fibers.

2. The lignin distribution found with conventional electron microscopy techniques, particularly the $KMnO_4$ staining technique, is in good agreement with results obtained from SEM-EDXA studies.

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