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Effect of Residual Lignin Type and Amount on Bleaching of Kraft Pulp by *Trametes versicolor*

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The white rot fungus *Trametes* (*Coriolus*) *versicolor* can delignify and brighten unbleached hardwood kraft pulp within a few days, but softwood kraft pulps require longer treatment. To determine the contributions of higher residual lignin contents (kappa numbers) and structural differences in lignins to the recalcitrance of softwood kraft pulps to biobleaching, we tested softwood and hardwood pulps cooked to the same kappa numbers, 26 and 12. A low-lignin-content (overcooked) softwood pulp resisted delignification by *T. versicolor*, but a high-lignin-content (lightly cooked) hardwood pulp was delignified at the same rate as a normal softwood pulp. Thus, the longer time taken by *T. versicolor* to brighten softwood kraft pulp than hardwood pulp results from the higher residual lignin content of the softwood pulp; possible differences in the structures of the residual lignins are important only when the lignin becomes highly condensed. Under the conditions used in this study, when an improved fungal inoculum was used, six different softwood pulps were all substantially brightened by *T. versicolor*. Softwood pulps whose lignin contents were decreased by extended modified continuous cooking or oxygen delignification to kappa numbers as low as 15 were delignified by *T. versicolor* at the same rate as normal softwood pulp. More intensive O_2 delignification, like overcooking, decreased the susceptibility of the residual lignin in the pulps to degradation by *T. versicolor*.

Kraft pulps need to be bleached to achieve the high level of brightness expected of fine papers, but conventional bleaching with chlorine is becoming unpopular because of the chlorinated organic by-products involved. New bleaching processes which do not depend on chlorine compounds are being developed. The brown color of unbleached kraft pulp is mainly due to residual lignin, which must be removed to produce pulp with a high level of brightness. Modifications to the kraft pulping process, such as extended modified continuous cooking (EMCC) (17), can lower the lignin content of pulp entering a bleach plant. Delignification of pulp with pressurized O_2 is widely used as a first step in bleaching, but this process can remove only about one-half of the residual lignin before attack on polysaccharides causes unacceptable losses in the strength of the pulp (14). Ozone is also an effective delignifying chemical, but its reactivity with polysaccharides means that pulp strength can be damaged unless the process is carefully controlled (6).

The need for highly selective removal of lignin without depolymerization of the cellulose in pulp presents an opportunity for biotechnological methods, which typically have high levels of specificity (24). Treatment of unbleached pulps with xylanase increases the effectiveness of subsequently applied bleaching chemicals, including ozone (3), allowing chemical savings of up to 20% (24). Another biological approach, with potentially greater impact, is using white rot fungi or their enzymes to remove the colored residual lignin from kraft pulp brownstock (11, 24). Pulps delignified with appropriate white rot fungi have adequate strength properties (21, 22, 25). The fungus *Trametes* (*Coriolus*) *versicolor* can substantially decrease the residual lignin content and increase the brightness of hardwood kraft pulp (HWKP) within 3 to 5 days (16, 18, 19, 22, 27). This fungus also delignifies softwood kraft pulp (SWKP) but does not significantly increase its brightness within 14 days (25). The difference in the responses of hardwood and softwood pulps to fungal bleaching is of both practical and scientific interest. SWKPs are produced in greater amounts and command higher prices than HWKPs, and chlorine bleaching of SWKP generates more chlorinated organic compounds than bleaching of HWKP does (25). Therefore, it is important for any biological bleaching method to work well with SWKP. Furthermore, the differences between HWKP and SWKP could provide clues to the mechanism by which *T. versicolor* delignifies and brightens pulps.

Softwoods and hardwoods differ in both the amount and the structure of their lignins; softwood lignins contain mostly guaiacyl units, and hardwood lignins contain both guaiacyl and syringyl units. The hardwood lignins are more easily solubilized during kraft pulping, and HWKPs typically contain less than one-half as much residual lignin as SWKPs (9). The higher lignin contents of SWKPs could contribute to their resistance to brightening by *T. versicolor*.

Many white rot fungi, including *T. versicolor*, naturally occur more commonly on hardwoods than on softwoods, and these organisms decay wood with hardwood type lignin better than they decay wood with softwood type lignin (15). Guaiacyl-syringyl lignins are more rapidly depolymerized by these fungi and their enzymes than guaiacyl lignins are (10, 26). The lack of syringyl units in the residual lignin of a SWKP may increase its resistance to delignification by *T. versicolor*.

This study was undertaken to determine the relative importance of the differences in residual lignin structure and amount in the different responses of HWKP and SWKP to biobleaching by *T. versicolor*. We prepared SWKP with a low lignin content by prolonged cooking and HWKP with a high residual lignin content by abbreviated cooking and compared delignification of these preparations by *T. versicolor* with delignification of normal HWKP and normal SWKP, respectively. Because we observed more brightening of SWKP in these experiments than in previous studies (22, 25), we compared the results of bleaching of several batches of SWKP by *T. versi-*

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TABLE 1. Sources and properties of kraft pulps used

Pulp	Kappa no.	Source
A	12	Mixed hardwoods, 10% spruce, eastern mill
B	26	Aspen, Paprican pilot plant
C	12	Black spruce, Paprican pilot plant
D	26	Black spruce, Paprican pilot plant
E	30	Black spruce, Paprican pilot plant
F	30	White spruce and pine, western interior mill
G	27	50% cedar, 50% spruce-pine-fir, west coast mill
H	32	Mixed softwoods, western interior mill
I	24	Mixed softwoods, west coast mill
J	29	Black spruce, Paprican pilot plant
K	17	Pulp J, O_2 delignified
L	21	Softwood, EMCC, eastern mill
N	31	Mixed softwoods, Paprican pilot plant
O	21	Black spruce, O_2 delignified, Paprican pilot plant
Q	16	Black spruce, O_2 delignified, Paprican pilot plant
S	12	Black spruce, O_2 delignified, Paprican pilot plant
T	11	Black spruce, O_2 delignified, Paprican pilot plant

color. The effect of lowering the residual lignin content of SWKP by EMCC or O_2 delignification on subsequent delignification and brightening by *T. versicolor* was also investigated.

MATERIALS AND METHODS

Cultures. The strains of *T. versicolor* (L.:Fr.) Pilát used in this study were strain 52P, a dikaryon used in previous studies (16, 18, 19, 22, 25, 27), and 52J, a monokaryon derived from strain 52P (1). Stock cultures were stored at −80°C (1). Inocula were grown in mycological broth (Difco) or a defined medium containing (per liter of water) 10 g of D-glucose, 0.26 g of L-asparagine, 0.68 g of KH_2PO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 15 mg of $CaCl_2 \cdot 2H_2O$, 100 μg of thiamine, 1 ml of a trace element solution, and 0.5 g of Tween 80; the pH of the defined medium was adjusted to 5.5 with 6 M KOH. The trace element solution contained 2 mM $FeSO_4$, 1 mM $CuSO_4$, 10 mM $MnSO_4$, 5 mM $ZnSO_4$, 5 mM $CoCl_2$, 0.5 mM Mo added as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 95 mM HCl. Inoculum cultures containing 200 ml of medium in 500-ml plastic Erlenmeyer flasks, each with a glass marble to disperse mycelial clumps, were each seeded with three 1.2-cm-diameter plugs cut from the margin of a colony on agar and were incubated at 27°C on a gyratory shaker with a radius of 9 mm at 200 rpm for 5 days.

Pulps. The various pulps used in this study are listed in Table 1. The pulps were either obtained from Canadian mills or prepared in the Paprican pilot plant. Most of the pulps were prepared by conventional kraft cooking, with the cooking time adjusted to obtain the desired kappa number (a measure of residual lignin content, approximately equal to seven times the weight percentage of lignin in the pulp). Some pulps were further delignified by treatment with O_2 at 100 lb/in^2/g at 90°C and pH 11 for 10 to 60 min. Pulp L was prepared by the EMCC process (17) at an eastern Canadian mill. The pulps were thoroughly washed, pressed so that they contained ca. 25% solids, and fluffed by passing them through a 2.5-mm-pore-size screen before use.

Biobleaching conditions. In experiments to compare bleaching of low-lignin-content softwood pulp (pulp C) and hardwood pulp (pulp A), 4-g (dry weight) portions of pulps were placed in 500-ml Erlenmeyer flasks and suspended in 200-ml portions of water. The flasks were stoppered with foam plugs, autoclaved, and inoculated with 5-day-old shake cultures of *T. versicolor* 52P in mycological broth containing 0.1 g (dry weight) of mycelium. In all other experiments the pulp was suspended at a concentration of 2% in the defined medium. After autoclaving, the pulp suspensions were inoculated (15%, vol/vol) with a shake culture of *T. versicolor* 52J grown in the defined medium. The flasks were incubated at 27°C on a gyratory shaker with a radius of 9 mm at 200 rpm.

At intervals, ca. 50-ml samples of the pulp suspensions were aseptically removed from the flasks. The pulp was recovered by filtration through stainless steel mesh with fines recycling and washed with 500 ml of H_2O. The pulp was treated with *T. versicolor*, and then one-half of each pulp sample (concentration, 2%) was extracted with alkali (2% NaOH on pulp, 120°C, 15 min) (25). Brightness and residual lignin (kappa number) values were determined for the pulps before and after the alkali extraction step.

For chemical brightening (ED sequence) after the fungal treatment, unextracted pulps were suspended at a concentration of 10% with 1% NaOH on pulp, heated at 70°C for 1.5 h, filtered, treated at a concentration of 10% with 0.5% ClO_2 at 60°C for 3 h, soured with SO_2, and filtered.

Analytical methods. Miniature handsheets (2 by 4 cm) were formed by filtering 200-ml portions of a pulp suspension (concentration, 0.25%) through a stainless steel mesh. The wet sheets were transferred to a blotter, pressed between two blotters, and air dried. Brightness was measured as the reflectance, compared with a $BaSO_4$ standard, of the dry sheets at 457 nm with a Perkin-Elmer model Lambda 3B spectrophotometer equipped with an integrating sphere. The residual lignin content was determined by the micro kappa number method of Berzins (5). The total and phenolic methoxyl contents of pulp samples were determined by the Zeisel method and by periodate oxidation as described by Paice et al. (23).

RESULTS

Biobleaching of pulps at kappa number 12. A low-lignin-content SWKP (pulp C), prepared by prolonged cooking, was delignified by *T. versicolor* 52P much more slowly than a hardwood pulp (pulp A) with the same initial kappa number (Fig. 1A). It also exhibited a much smaller increase in brightness, both directly and after ED bleaching, than the hardwood pulp (Fig. 1B).

Biobleaching of pulps at kappa number 26. There was a lag of about 3 days in the removal of lignin from the normal SWKP (pulp D), but not in the removal of lignin from the high-lignin-content HWKP (pulp B) (Fig. 2A). Subsequently, the kappa numbers of the two pulps decreased at comparable rates. Before extraction the kappa number of the aspen pulp was lower than the kappa number of the spruce pulp, but after treatment for 6 to 15 days the two pulps had equal kappa numbers after extraction. The kappa number of the aspen pulp reached a minimum of 8 after 15 days of fungal treatment, but the kappa number of the spruce pulp continued to decrease until day 18 and reached a minimum value of 6.5. For the spruce pulp and for the aspen pulp after up to 15 days of fungal treatment, alkaline extraction significantly decreased the kappa number.

The aspen pulp had a considerably higher initial brightness value than the spruce pulp, and it maintained this brightness advantage over the course of the fungal treatment (Fig. 2B). The brightness values of both pulps decreased during the first 3 days of incubation with the fungus and then began to increase. Alkaline extraction slightly increased the brightness of aspen pulp treated for 3 to 9 days with the fungus, but not aspen pulp treated for longer periods of time. Alkaline extraction considerably increased the brightness of spruce pulp after

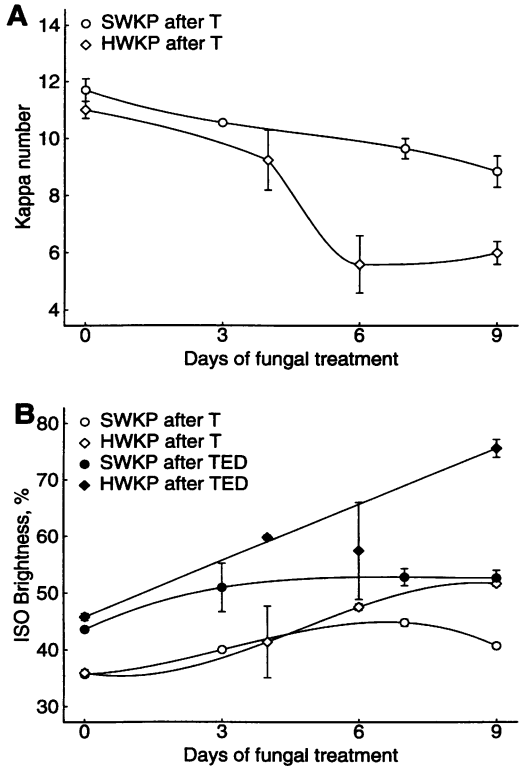

FIG. 1. Comparison between delignification of low-lignin-content SWKP and delignification of conventional HWKP by *T. versicolor* 52P. (A) Kappa number. (B) Brightness. T, treatment with *T. versicolor*; TED, treatment with *T. versicolor* followed by chemical brightening.

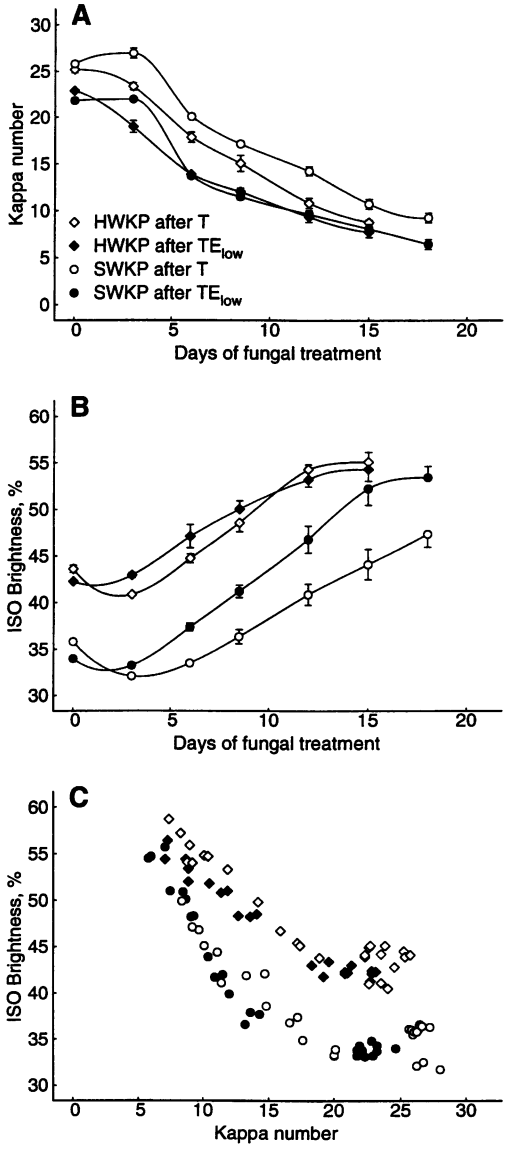

FIG. 2. Time course of biological bleaching of spruce and high-lignin-content aspen pulps by *T. versicolor*. (A) Residual lignin content. (B) Brightness. (C) Relationship between brightness and residual lignin content. T, treatment with *T. versicolor*; TE$_{low}$, treatment with *T. versicolor* followed by alkali extraction.

all fungal treatments longer than 3 days, and the extent of improvement increased with the length of fungal treatment. The maximum brightness of the spruce pulp, after fungal treatment for 18 days and alkaline extraction, was comparable to the maximum brightness observed with the aspen pulp.

As mentioned above, the initial brightness value of the aspen pulp was higher than that of the spruce pulp, even though the kappa numbers of the two pulps were the same. During fungal treatment, the spruce pulp had a lower brightness value at a given kappa number than the aspen pulp, although the brightness of the spruce pulp increased rapidly at kappa numbers below 10 (Fig. 2C). Control pulp samples that were incubated along with the experimental cultures but were not inoculated did not change in brightness or kappa number.

Chemical differences between residual lignins of softwood and hardwood pulps. The high-lignin-content aspen pulp (pulp B) and the conventional spruce pulp (pulp D) had higher total methoxyl contents than the normal HWKP (pulp A) (Table 2), which is consistent with the higher lignin contents of pulps B and D. The methoxyl content of the high-lignin-content aspen pulp was considerably higher than that of the spruce pulp with the same kappa number, reflecting the presence of syringyl groups (two methoxyls per aromatic ring) in the aspen residual lignin. The ratio of total methoxyl content to kappa number was lower in the normal HWKP (0.27) than in the high-lignin-content aspen pulp (0.37), indicating that the mild pulping conditions used to produce a high-kappa-number hardwood pulp left more of the syringyl units in the pulp. The softwood pulp had even fewer methoxyl groups per kappa number (0.22) than either the normal HWKP or the high-lignin-content HWKP. In both the normal HWKP and the spruce pulp, about 40% of the methoxyl groups were on phenolic rings; in the high-lignin-content aspen pulp, only 20% of the methoxyl groups were apparently adjacent to phenolic groups. This value

TABLE 2. Methoxyl contents of residual lignins in kraft pulps

Pulp	Total methoxyl content (mg/g)	Phenolic methoxyl content (mg/g)	Kappa no.
Normal HWKP, (pulp A)	3.2	1.27	12
High-lignin-content HWKP (pulp B)	9.6	1.90	26
Normal SWKP (pulp D)	5.6	2.30	26

TABLE 3. Biobleaching of six batches of SWKP by *T. versicolor* 52J

Pulp	Kappa no.			Brightness (%)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Before alkaline extraction						
D	25.5 ± 0.1	16.2 ± 0.7	9.3 ± 0.2	35.5 ± 0.2	38.0 ± 0.8	51.6 ± 0.5
E	30.4 ± 0.3	19.5 ± 0.1	12.2 ± 0.4	32.6 ± 0.1	33.1 ± 0.3	46.0 ± 0.3
F	30.1 ± 0.1	17.7 ± 1.1	10.1 ± 1.3	30.4 ± 0.1	34.4 ± 1.8	45.5 ± 4.8
G	26.9 ± 0.0	14.1 ± 0.1	7.2 ± 0.3	31.2 ± 0.3	39.5 ± 0.4	55.7 ± 0.4
H	32.1 ± 0.1	18.1 ± 0.9	11.8 ± 1.5	30.5 ± 0.4	33.0 ± 0.9	40.5 ± 4.1
I	24.3 ± 0.2	15.4 ± 0.7	9.9 ± 0.7	33.8 ± 0.2	35.1 ± 2.7	42.9 ± 3.3
After alkaline extraction						
D	22.8 ± 0.2	9.5 ± 0.6	5.9 ± 0.1	34.4 ± 0.2	44.8 ± 1.5	59.9 ± 0.8
E	27.1 ± 0.3	11.4 ± 0.6	6.2 ± 0.2	31.3 ± 0.2	38.8 ± 1.0	56.1 ± 0.3
F	27.3 ± 0.1	11.2 ± 1.6	6.4 ± 1.5	29.4 ± 0.1	41.0 ± 3.2	55.8 ± 5.9
G	24.2 ± 0.2	7.7 ± 0.1	3.9 ± 0.0	31.0 ± 0.1	47.4 ± 0.4	64.4 ± 0.3
H	29.1 ± 0.1	11.9 ± 1.0	8.1 ± 1.3	29.0 ± 0.1	39.9 ± 1.9	48.5 ± 4.6
I	21.7 ± 0.1	10.9 ± 1.2	7.1 ± 1.2	33.0 ± 0.1	40.4 ± 3.7	50.3 ± 4.4

may be an underestimate; recovery of methoxyl from syringyl rings is less than quantitative (2). The data do suggest, however, that the residual lignin in the high-lignin-content aspen pulp had more remaining aryl ether linkages than the lignins in the other pulps.

Biobleaching of various SWKPs. The brightening of the spruce pulp in the experiments described above was considerably greater than the brightening obtained in previous experiments performed with softwood pulp (25). To check the possibility that the spruce pulp was atypically susceptible to fungal delignification, we tested the responses of six softwood pulps with kappa numbers ranging from 24.3 to 32.1, which were obtained from the Paprican pilot plant and commercial mills, to treatment with *T. versicolor*. The sources of these pulps are shown in Table 1 (pulps D through I). *T. versicolor* was able to lower the kappa number and increase the brightness of each of these pulps, although there were significant variations in the rate of delignification (Table 3). Thus, the behavior of the pilot plant spruce pulp (pulp D) was typical of SWKPs. The most responsive pulp (pulp G) reached a brightness value of 64% (kappa number 4) after 14 days of fungal treatment and alkaline extraction; the least responsive pulp (pulp H) had a final brightness value of 48.5% (kappa number 8).

To further investigate the effect of residual lignin content on SWKP delignification and brightening by *T. versicolor*, we used pulps that had lower-than-normal lignin contents without extensive lignin condensation; these pulps were prepared by EMCC (17) (pulp L) or oxygen delignification (pulp K). *T. versicolor* 52J delignified these pulps at the same initial rate that it delignified normal SWKP (pulp J) (Fig. 3A). When the residual lignin content became low, the rate of delignification slowed; this was especially noticeable in the O_2-delignified pulp. The EMCC pulp, and even more so the O_2-delignified pulp, reached substantially higher brightness values than the normal SWKP during the fungal treatment (Fig. 3B), which was consistent with the lower residual lignin contents of these pulps. For pulps with kappa numbers less than 18 and brightness values less than 55%, there was a linear relationship between brightness and kappa number, and the slopes for the three pulp types were the same. A covariance analysis showed that the brightness at a given kappa number was significantly ($P > 0.995$) less for the EMCC pulp than for either the normal SWKP or the O_2-delignified SWKP (Fig. 3B).

In an additional experiment, a series of softwood pulps that were O_2 delignified to different extents (pulps N, O, Q, S, and T) were incubated with *T. versicolor* for up to 8 days. The O_2-delignified pulps with initial kappa numbers higher than 12 (pulps O and Q) were delignified by the fungus at the same rate as the normal SWKP (pulp M) (Fig. 4). However, the fungus removed lignin more slowly from the more extensively delignified pulps (pulps S and T). There seemed to be a basal kappa number of about 6.5 below which the fungus could not take the pulp in the first 6 days of treatment. Between days 6 and 8 the rate of delignification in these pulps increased, and

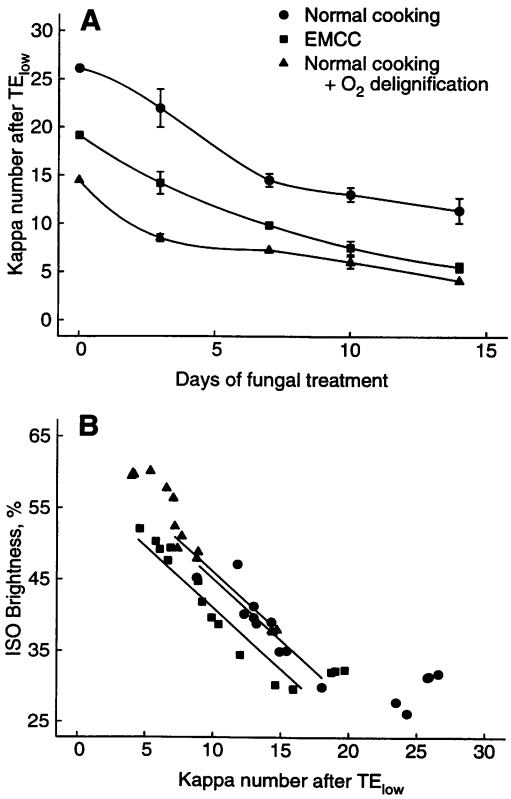

FIG. 3. Delignification of normal (●), EMCC (■), and O_2-delignified (▲) SWKP by *T. versicolor* 52J. (A) Residual lignin content. (B) Relationship between brightness and residual lignin content. TE_{low}, treatment with *T. versicolor* followed by alkali extraction.

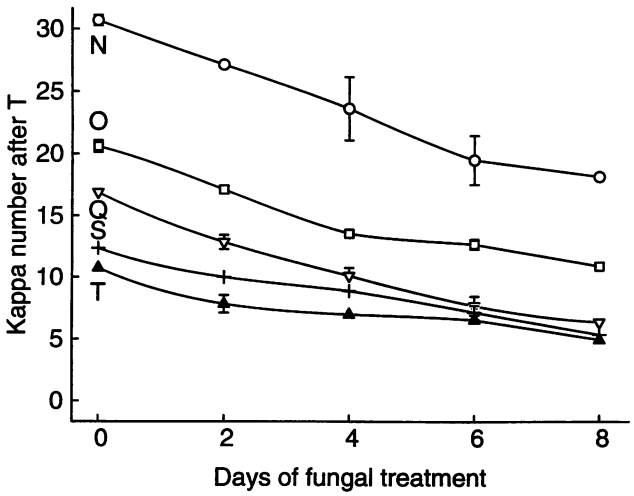

FIG. 4. Kinetics of delignification by *T. versicolor* of SWKP after various extents of O_2 delignification. The points are means of three replicates; the bars indicate standard errors. The letters on the graph are pulp designations. T, treatment with *T. versicolor*.

pulp T reached a kappa number of 5. Parallel cultures of *T. versicolor* containing fully bleached pulp (Whatman no. 1 filter paper) yielded handsheets with kappa numbers of 2.35 ± 0.13. The brightness values of the pulps in this experiment were related to their kappa numbers in the way that was observed previously (Fig. 3B).

DISCUSSION

SWKP which had been pulped to an unusually low residual lignin content (kappa number 12) was difficult for *T. versicolor* to delignify. This difficulty was probably caused by the highly condensed nature of the lignin resulting from prolonged cooking. During kraft cooking, the proportion of noncondensed phenyl nuclei in the residual lignin progressively decreases, as the noncondensed units are preferentially solubilized, and new bonds of the condensed type are formed (7). The diphenylmethane bridges and other alkali-stable linkages which hold together the residual lignin in overcooked pulps may be more difficult for the fungus to cleave than the linkages typical of native lignin are.

HWKP which had been lightly cooked to leave a high residual lignin content was delignified by *T. versicolor* at the same rate as SWKP having the same kappa number. During kraft pulping of hardwoods, syringyl units are removed faster than guaiacyl units (8), and the residual lignin of a normal HWKP may contain very few syringyl units. We expected, nevertheless, that our high-lignin-content aspen pulp would retain some residual syringyl groups, and the high methoxyl content of the pulp confirmed this expectation. The presence of syringyl groups in this residual lignin, however, did not significantly increase its susceptibility to solubilization by *T. versicolor*. Apparently, *T. versicolor* is able to degrade guaiacyl residual lignin as quickly as it degrades guaiacyl-syringyl residual lignins. This finding apparently contradicts the finding of Faix et al. (10) that synthetic syringyl-guaiacyl lignins are depolymerized more rapidly than guaiacyl lignins by *Phanerochaete chrysosporium*. The possible explanations for this discrepancy are discussed below. (i) Lignin depolymerization might not be a rate-limiting step in delignification of kraft pulps. (ii) The syringyl content of the residual lignin, even in high-lignin-content aspen pulp, might be too low to have a detectable effect on the biodegradability of the lignin. The syringyl-guaiacyl lignin studied by Faix et al. (10) and by Wariishi et al. (26) had a syringyl content of about 70%. The proportion of syringyl groups in our aspen pulp is not known, but it was probably much lower. (iii) Because it is immobilized and dispersed in a polysaccharide matrix, residual lignin might behave differently during biodegradation than soluble lignins do. In particular, residual lignin may be less susceptible to the radical coupling reactions which probably counteract the depolymerization of soluble guaiacyl lignins. (iv) Delignification of kraft pulps by *T. versicolor* might involve fundamentally different mechanisms than degradation of synthetic lignins by *P. chrysosporium*. One of the major enzymes of *P. chrysosporium*, lignin peroxidase, does not participate in pulp delignification by *T. versicolor* (4). However, another enzyme, manganese peroxidase, seems to play an important role in lignin degradation by both fungi (23). In vitro, syringyl and syringyl-guaiacyl lignins are more readily depolymerized and less readily repolymerized by manganese peroxidase than guaiacyl lignins are (26).

During kraft pulping, chromophoric groups are introduced into the lignin by side reactions (13), which decreases the pulp brightness. The relatively high initial brightness value of the high-lignin-content aspen pulp probably resulted from the decreased opportunity for these side reactions to occur during the brief cooking which produced it. During the initial attack by the fungus, the brightness values of both the aspen and spruce pulps decreased, probably as a result of formation of quinones and conjugated carbonyls by fungal oxidation of the lignin. At kappa numbers below 20, there is a clear negative correlation between brightness and the residual lignin contents of the pulps during fungal delignification, both before and after alkaline extraction (Fig. 2C and 3B). Fujita et al. (11, 12) have found a similar correlation between brightness and residual lignin content in HWKP (beech) and SWKP (Japanese red pine) during delignification with fungus strain IZU-154. This correlation suggests that the chromophoric structures were being removed at the same rate as the bulk of the residual lignin by the action of the fungus.

The SWKP brightness values reached after 14 days of treatment with *T. versicolor* in this study were generally higher than those found previously (25). In part this reflects the lower residual lignin contents observed. The reason for the more extensive delignification and greater brightening observed in our experiments is probably a better inoculum. The inoculum was grown in a synthetic medium instead of the dilute mycological broth used previously (25), and a higher inoculum volume, which was optimal for biobleaching HWKP (16), was used. Monokaryotic strain 52J has a greater ability to bleach HWKP than dikaryotic strain 52P, which was used in previous studies, but in direct comparisons strains 52J and 52P were equally effective at bleaching SWKP (1). The fact that six different batches of SWKP were all substantially brightened by *T. versicolor* shows that the improved bleaching was not the result of using an especially susceptible pulp. There were significant variations in the responses of the different pulps; generally, those with relatively low initial lignin contents had higher brightness values after fungal treatment, although the correlation was imperfect. The same trend was quite apparent in the responses of the EMCC and O_2-delignified pulps to delignification and brightening by *T. versicolor*. This trend is consistent with the hypothesis that *T. versicolor* removes lignin from the pulp at an approximately constant rate that is independent of the amount or type of residual lignin.

O_2-delignified softwood pulps were further delignified by *T.*

versicolor as readily as regular SWKP was if their kappa numbers were greater than 12. The more extensively O_2-delignified pulps were less susceptible to fungal delignification, and there appeared to be a lower kappa number limit of 6.5. Permanganate consumption by the fungal mycelium in the pulp (18) could contribute about 2.4 U of apparent kappa number to this basal level, as shown by the kappa number analysis of lignin-free cellulose fibers incubated with *T. versicolor*. The other 4 U of kappa number could consist of particular lignin structures that are difficult for the fungus to degrade. O_2 delignification preferentially leaves condensed lignin units linked by diphenylmethane bridges in the pulp (20); these units may be resistant to attack by the fungus.

The longer time taken by *T. versicolor* to brighten SWKP compared with HWKP results from the higher residual lignin content of the SWKP; differences in the structures of the residual lignins are unimportant, unless the lignin is highly condensed because of overcooking or extensive O_2 delignification. The lignins remaining after modified continuous cooking and moderate O_2 delignification are as susceptible to degradation by *T. versicolor* as normal residual lignin is; fungal bleaching is compatible with these developments in pulping and bleaching technology.

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