Preliminary

Structure and Microbial Decolorization of Xylanase-resistant Chromophoric Xylans Isolated from Unbleached Kraft Pulp*[1]

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One of the most characteristic features of kraft pulp is that uronic acid and arabinose side chains of xylans are cleaved during the cooking process, and the xylans are redeposited on the cellulose fibrils as the alkaline concentration of the cooking liquor decreases[1,2]. The redeposited xylans are known to be categorized into two types, (1) xylans extractable with solvents which are associated weakly with cellulose fibrils and resistant to a xylanase-digestion, and (2) alkali-resistant xylans co-crystallizing with cellulose[3-7]. In this study, a water-soluble xylanase-resitant chromophoric xylan fraction (LF-D) and a residual lignin fraction (HF-P) were separated from a hard wood unbleached kraft pulp (UKP) by a cellulase/xylanase-membrane bioreactor (Fig. 1). Chemical and spectroscopic analyses revealed that this chromophoric xylanase-resitant fraction (LF-D) contained a large amount of unremovable inorganic atoms including Si, Na and S, together with non-branched β-1, 4-linked xylan chain. Although storong UV absorption was observed in LF-D, a signal originating from methoxyl group of lignin nuclei was below the back ground noise level in its ^{13}C-NMR spectrum. A nucleus exchange reaction of LF-D also revealed that a part of lignin nuclei in LF-D had been demethylated during the course of kraft pulping because LF-D produced a large amount of catechol and pyrogallol in a nucleus exchange reaction at 110°C. A nitrobenzene oxidation of LF-D gave vanillic acid but yields of vanillin and syringaldehyde were very low, suggesting that quinoid structures are involved in LF-D. On the other hand, a higher molecular weight residual lignin fraction (HF-P), which was obtained from an impermeable part of the enzymatic digests, was found to contain much diphenylmethane structure based on the nitrobenzene oxidation and nucleus exchange reaction.

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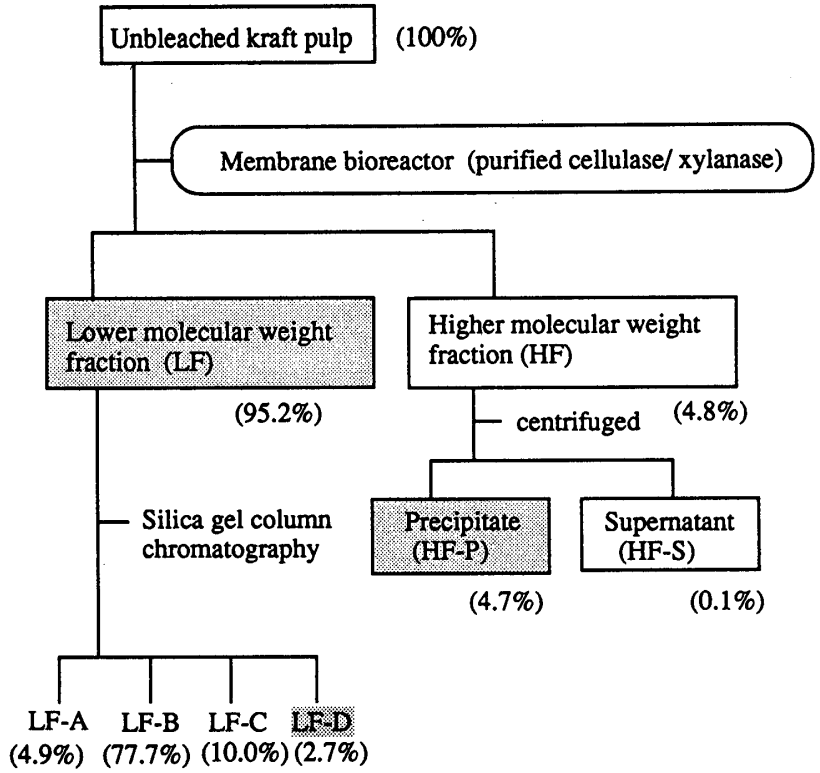
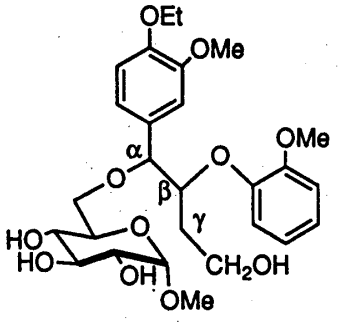


Fig. 1. Enzymatic degradation and fractionation of unbleached kraft pulp.

LF-D was more effectively decolorized by bacterial microflora from soil rather than selected white rot fungi including *Phanerochaete chrysosporium* and *Bjerkandera adusta* which can partly decolorize the original unbleached kraft pulp (UKP). However, *Coriolus versicolor* was found to decolorize not only the UKP but also the isolated xylanase-resistant chromophoric xylan fraction. However, activities of extracellular lignin peroxidase, Mn-peroxidase, laccase and xylanase in the culture filtrates of *C. versicolor* and the bacterial microflora were very low during the cultivation with the chromophoric xylans. In contrast with the oxidizing enzymes, reducing activity of sodium 2, 6-dichlorophenol-indophenol was detected in the cultures of the bacterial microflora. Thus, chromophores of the xylanase-resistant xylans are different from typical residual lignin in UKP, and their biochemical decolorization needs enzymes different from the extracellular ligninolytic enzymes. Although the xylanase-resitant xylans can be removed by D-E-D process from UKP[4], their microbial decolorization is important for environmentally-safe high yield bleaching[8].

Because *p*-etherified benzyl ether bonds between lignin and carbohydrates have been considered to be one of the major cause for bleaching resistance of UKP, screening of microorganisms which effectively cleave the benzyl ethers was studied. When a purified lignin peroxidase (H8) from *P. chrysosporium* was reacted with a *p*-ethylated β-*O*-4 LCC model bearing methyl β-glucoside at its α-position under the condition that veratryl alcohol



Scheme 1

was oxidized to veratraldehyde, methyl β-glucoside was not detected in the reaction products by HPLC[9]. Therefore, screening of microorganisms which effectivery split off the benzyl ether model compound was carried out[9]. As a result, several bacterial single colonies which emit fluorescence during the cultivation were isolated. Extensive studies on the screeneing and characterization of those benzyl ether-cleaving microorgansisms would also contribute to an environmentally safe biobleaching system of UKP.

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References

1) J.K. Hamilton, E.V. Partlow and N.S. Thompson: *Tappi,* **41**, 803–811 (1958).
2) S. Yllner and B. Enstrom: *Svensk Papperstidn.,* **59**, 229–232 (1956).
3) F. Mora, K. Ruel, J. Comtat and J.P. Joseleau: *Holzforschung,* **40**, 85–91 (1986).
4) S. Skjold-Jørgensen, N. Munk and L.S. Pederson: "Biotechnology in Pulp and Paper Industry" ed by Kuwahara, M. and Shimada, M., Uni Publishers, Tokyo, 93–99 (1992).
5) A. Ishizu: *Mokuzai Gakkaishi,* **39**, 1335–1340 (1993).
6) A. Kantelinen, B. Hortling, M. Ranua and L. Viikari: *Holzforschung,* **47**, 29–35 (1993).
7) A. Kantelinen, B. Hortling, J. Sundquist, M. Linko and L. Viikari: *Holzforschung,* **47**, 318–324 (1993).
8) T. Watanabe: *Trends in Glycosci. Glycotechol.,* **7**, 57–68 (1995).
9) K. Mikame, T. Watanabe, Y. Honda and M. Kuwahara: *Proc. of 39th Lignin Symp.,* 79–82 (1994).