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Dechlorination of Bleached Kraft Pulp by Laccase Enzyme Produced from Some White-Rot Fungi

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Abstract : The possibility of using crude laccase in the dechlorination of chlorine-based bleached kraft pulp was investigated. Culture supernatants of seven white-rot fungal strains and kraft-pulp samples taken from E1 (Alkaline Extraction-1), E2 (Alkaline Extraction-2) and D2 (Chlorinedioxide treatment) stages of chlorine-based bleaching processes were used as the laccase source and substrates respectively during the studies. The dechlorination activity of *Trametes versicolor* was found to be more than that of the other fungi examined. The addition of a laccase inducer, xylidine, into the culture medium of *T. versicolor* led to an increase in dechlorination activity. In the experiment performed to find out the role of laccase activity in dechlorination reactions, a considerable reduction in the dissolved oxygen concentration due to laccase-dependent dechlorination activity was observed. Stepwise enzyme treatment was found to be necessary for increasing the yield of dechlorination.

Key Words: Biodechlorination, Kraft pulp, Laccase, *Trametes (Coriolus) versicolor*.

Introduction

The cooking and subsequent alkaline extractions are the main operational processes used in the production of kraft pulp from woody and other plant materials. During the application of the processes lignin bound to cellulose fibers is removed due to its high solubility in hot alkaline solution. However, lignin residues remain on the cellulose fibers and are responsible for the characteristic brownish colour of the kraft pulp. Bleaching of kraft pulp, which is necessary for the production of white paper products, is based on the removal of residual lignin from cellulose fibers. Since it has no adverse effect on cellulose fiber quality, the bleaching of kraft pulp by its treatment with chlorine and chlorine compounds such as hypochloride and chlorinedioxide has become preferable by most pulp mills. However, chlorine-based bleaching is known to cause serious environmental problems (1,2). Discharging of wastewaters containing chlorinated aromatics formed during chlorine-based bleaching into receiving waters has been proved to have cytotoxic and cytomutagenic effects on various living organisms (3-5). Therefore, studies based on biological degradation and dechlorination of chlorinated lignin derivatives in waste

water discharges of kraft pulp plants using various lignolytic microorganisms have been studied in some laboratories (6,7).

On the other hand, because of health reasons, there is increasing demand for chlorine-free paper products all over the world. Therefore, since pulps bleached with chlorine-based processes may contain residual chlorinated organic compounds, their dechlorination is necessary in paper production. Dechlorination of the kraft pulp containing bound chlorine compounds with crude laccase enzyme that are produced by various white-rot fungi was first reported in this paper.

Materials and Methods

Seven white-rot fungal strains were used as laccase sources during the study. Those strains of *Pleurotus* group, *P. sajor-caju, P. florida, P. sapidus, P. osteratus* and *P. eryngii* were kindly supplied by Dr. I. F. Zadrazil (Weisdrangveg 4, 3300 Braunschweing, Federal Republic of Germany). *Phanerochaete chrysosporium* ME446 was provided by Dr. T.K. Kirk (U.S. Dept. of Forest Products Agriculture Lab., Madison, Wisconsion 53705, U.S.A). *Trametes (Coriolus) versicolor* was our original isolate. Stock cultures of the organisms were maintained on malt agar (Difco) slants. The culture medium used for laccase production was prepared by supplementing the stock basal medium which consisted of (all in g/L) 0.2 KH_2PO_4, 0.5 $MgSO_4$, 0.5 $NH_4H_2PO_4$, 0.1 Yeast-Extract (Difco) and 10.0 glucose with 0.1% stock mineral medium containing (all in g/L)1.4 $ZnSO_4.7H_2O$ and 1 $FeSO_4.7H_2O$. The medium was sterilized with a Millipore filter. Then 250 mL Erlenmayer flasks containing 50 mL culture medium were innoculated with 3 mL mycelial or conidial suspension prepared by suspending mycelia grown on agar slants of particular microorganisms in 10 mL sterile distilled water. Cultures were left to grow in a shaking incubator (Physcrotherm Incubator Shaker, New Brunswick Co.) rotating at 150 r.p.m at 30 °C for 12 days. After 4 days of growth period, $2x10^{-3}$ M 2,5 Xylidine (Sigma Co.) was added to the culture flask to induce the laccase synthesis. Supernatants of 12 day old fungal cultures were used as crude laccase sources during the experiments.

Laccase activity was assayed according to the procedure suggested by Coll et al. (8). For measuring laccase activity, source enzyme was added to 50 mM Na-acetate buffer (pH 4.5) containing 1mM guaiacol (Sigma Co.) as substrate, to make a final volume of 5mL. The tubes were incubated at 37°C for 15 minutes. The blank contained substrate and the source enzyme that was inactivated by boiling. The optical density of the reaction tubes was measured against reagent blank in a Jenway 6105 spectrophotometer at 465 nm wavelength. One unit relative enzyme activity was described as the amount of enzyme causing a 0.1 unit increase in the optical density of the reaction mixture under the experimental conditions.

Chlorine-based bleached kraft pulp samples were provided from the Dalaman pulp and paper plant near Muğla, Turkey. The bleaching process used in this plant was a combination of

6 successive operational stages: chlorine treatment (C), alkaline extraction-1 (E1), hypochloride treatment (H), chlorinedioxide treatment (D1), alkaline extraction-2 (E2) and chloredioxide treatment (D2). Samples taken from E1, E2 and D2 stages were used as substrates in the enzymatic dechlorination studies. In order to remove free chlorine from the pulps, these were initially rinsed with 0.5 M KNO_3 solution and then with distilled water. After overnight drying in air, 50 mg pulp was weighed and suspended in reaction tubes containing 5 mL of 0.5 M sodium-citrate buffer, pH 5.0. The dechlorination reaction was started by adding 0.5 mL enzyme solution to the reaction tubes and then the tubes were left for incubation at 37°C for 15 minutes. The amount of chloride ion formed due to the enzyme activity in the reaction tubes was measured by the mercury thiocyanate procedure (9).

The variations in dissolved oxygen concentration during the dechlorination reactions were detected with an oxygen meter (YSI Model). The residual organic chlorine (ROX) content of the pulps was measured by AOX (Adsorbe Organic Halogen) analyser (Euroglas, Analytical Instruments Method Development by KIWA-EBI).

Results and Discussion

As depicted in Figure 1, *T. versicolor* culture supernatant seemed to be more promising than that of the other white-rot fungi examined with respect to dechlorination of bleached kraft pulp taken from the D2 stage of the bleaching process.

In a previous study the culture supernatant of *T. versicolor* was shown to have higher laccase activity than other white-rot fungal strains (10). As seen in Figure 1, the addition of laccase inducer 2,5-xylidine resulted in an increase in the extent of the chloride ion released by

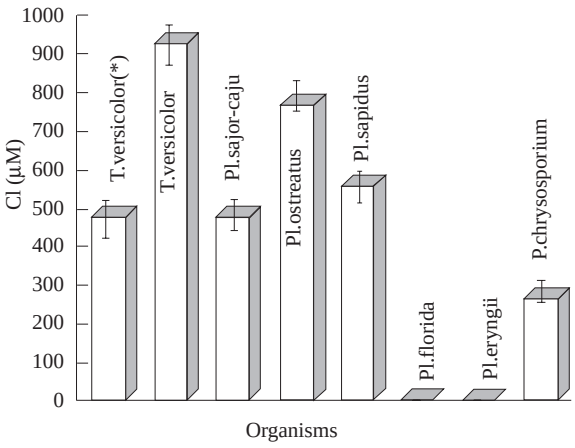

Figure 1. Removal of chlorine from the kraft pulp (from D2 stage of bleaching process) with culture supernatant of white-rot fungi. Values are means of at least 3 experiments. (Bars: Standard deviation). (*) marks that the supernatant of *T. versicolor* culture not induced with xylidine.

culture supernatants of *T. versicolor*. Laccase is known to use oxygen as an electron acceptor during the laccase-dependent oxidation reactions (11,12). There seemed to be a negative correlation between the oxygen concentration and the dechlorination activity catalyzed by the culture supernatant of *T. versicolor* (Figure 2). The data also suggest that the dechlorination reaction is catalyzed by the laccase activity of the culture supernatants.

The ROX content of kraft pulp taken from different stages of bleaching decreased to some extent after laccase treatment as seen in Figure 3.

The efficiency of dechlorination was higher for the kraft pulp taken from the D2 stage than from the E1 and E2 stages of the bleaching process. The high ROX content of the pulp samples

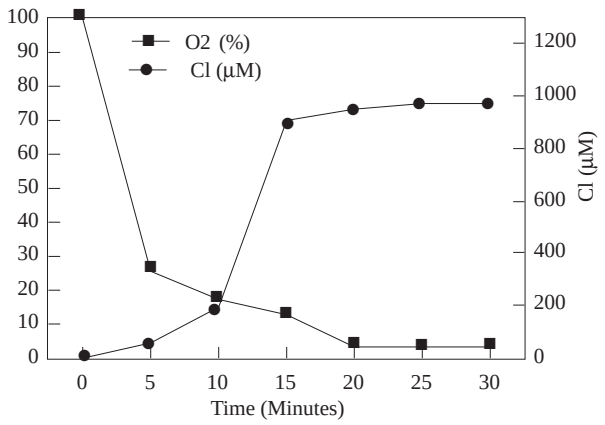

Figure 2. Oxygen consumption during the dechlorination of kraft pulp (from D2 stage of bleaching process) with the culture supernatant of *T. versicolor*.

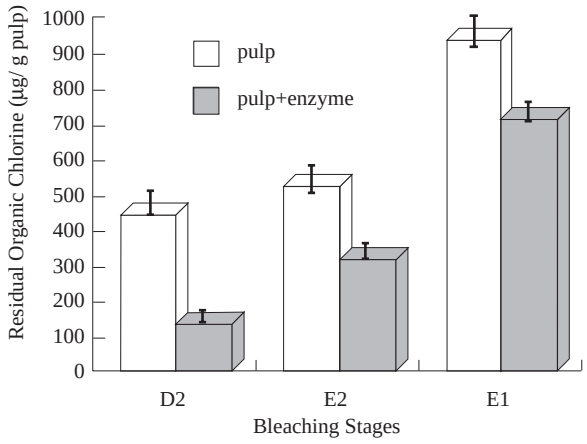

Figure 3. Reduction in residual chlorine content of kraft pulp samples (from different stages of bleaching process) due to the treatment with the culture supernatant of *T. versicolor*. Values are means of at least 3 experiments. (Bars: Standard deviation).

seemed to have an adverse effect on the yield of laccase-dependent dechlorination. Stepwise enzyme treatment was also applied to remove chlorine ions from the pulp samples. At least 4 or 5 steps of enzyme treatment were found to be necessary in order to eliminate free chlorine ions below the detection limit in treatment solutions of the pulp samples from the D2 and E2 stages of the bleaching process as shown in Figure 4.

The dechlorination of some polychlorinated aromatics by using laccase enzyme has previously been reported (13,14). The results of this study are promising in the sense that laccase may be used for the removal of chlorine from other chlorinated-organic compounds for detoxification.

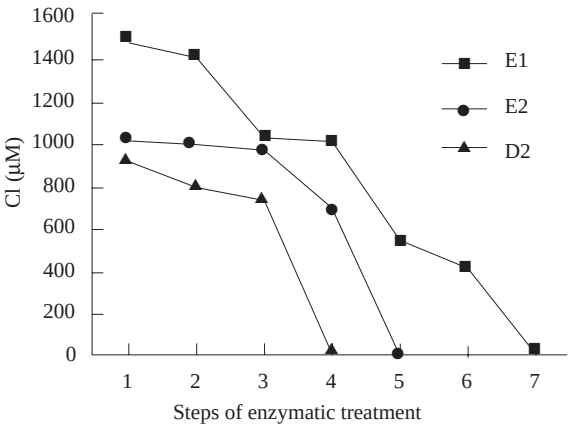

Figure 4. Removal of chlorine from kraft pulp samples (taken from E1, E2 and D2 stages of bleaching process) with stepwise enzyme treatment. Culture supernatant of *T. versicolor* was used as the enzyme source during the experiments.

References

1. Samadni, G., The race for safer methods. Chem. Eng., 98: 37-43, 1991.
2. Shoham, Y., Schwartz, Z., Khasin, A., Gat, O., Zosim, Z., Rosenberg, E., Delignification of wood pulp by a thermostable xylanase from *Bacillus stearothermophilus* strain T-6. Biodegradation, 3: 207-218, 1992.
3. Walden, C.C., Howard, T.E., Toxicity of pulp and paper mill effluents. Tappi, 60: 122, 1997.
4. Kringstad, K.P., Lindstrom, K., Spent liquors from pulp bleaching. Environ. Sci. Teac., 18: 236A, 1984.
5. Curtis, W.B., Gary, L.A., Seasonal and in-mill aspects of organic halide removal by an aerated stabilization basin treating a kraft mill wastewater. Wat. Sci. Tech., 21: 231-239, 1989.
6. Haggblom, M.M., Apajalahti, H.A., Salkinoja-Salonen, M.S., Degradation of chlorinated phenolic compounds occurring in pulp mill effluents. Wat. Sci. Tech., 2: 205-208, 1988.
7. Berbauer, M., Eggert, C., Kraepelin, G., Degradation of chlorinated lignin compounds in a bleach plant effluent by the White-rot fungus *Trametes versicolor*. Appl. Microbiol. Biotechnol., 35: 105-109, 1991.

8. Coll, P.M., Fernandez-Abalos, J.M., Villanueva, J.R., Santamaria R, Perez, P., Purification and characterization of a phenoloxidase (Laccase) from the lignin-degrading Basidiomycete PMI (CECT 2971). Appl. and Environ. Microbiol., 59: 2607-2613, 1993.

9. Vogel, A.I.: Quantitative Inorganic Analysis Including Elementary Instrumental Analysis, London, 1961 Longmans, Green and Co. Ltd. 48 Grosvenor Street, 808-809.

10. Taşpınar, A., Kolankaya, N., Optimization of enzymatic chlorine removal from kraft pulp. Bull. of Environ. Contam. and Toxicol., 61: 15-21, 1998.

11. Kirk,T.K., Farrel, R.L. Enzymatic "Combustion": The microbial degradation of lignin. Ann. Rew. Microbiol., 41: 465-505, 1987.

12. Yarapolov, A.I., Skorobogatko, O.V., Vartanov, S.S., Varfolomeyev, S.D., Laccase properties, catalytic mechanism and applicability. Appl. Biochem. and Biotechnol. 49: 257-279, 1994.

13. Kawai, S., Umezava, T., Higuchi, T., Degradation mechanisms of phenolic β-1 lignin substructure model compounds by laccase of *Coriolus versicolor*. Arch. Biochem. Biophys., 262: 99-110, 1988.

14. Iimura, Y., Hartikainen, P., Tatsumi, K., Dechlorination of tetrachloroguaiacol by laccase of white-rot basidiomycete *Coriolus versicolor*. Appl. Microbiol. Biotechnol., 45: 434-439, 1996.