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Optimization of Enzymatic Chlorine Removal from Kraft Pulp

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Kraft pulping is conventionally used for production of good quality cellulose pulp from woods and plant materials for subsequent production of paper and paperboard by pulp and paper industry. During Kraft pulping most of the ligning cementing the cellulose fibers in raw materials (wood and plant materials) is removed through its solubilization in alkaline cooking solution. The lignin compounds still remaining bound to cellolose fibers after the application of alkaline cooking gives brownish colour to pulp and their removal is technically carried out by the treatment of pulp with chlorine and chlorine compounds. Due to high solubilization ability of chlorinated lignin derivatives formed particularly during chlorine treatment are easily removed from cellulose pulp. However, while highly bleached and brightened pulp for production of white paper is obtained, discharge water of this treatment process becomes very rich with respect to chlorinated organic compounds.

There are some research studies reporting that these chlorinated organics dissolved in bleach effluent of Kraft mill have toxic and mutagenic effects (Douglas et al. 1983; Holmbom et al. 1984; Salem et al. 1994).

Therefore, in the last decade substantial efforts have been spent which were devoted to find techniques for dechlorination of bleaching effluents and also to develope chlorine free methods for bleaching of Kraft pulps. On the other hand, possibility of degradation of some toxic chlorinated organics by using lignin degrading white-rot fungal strains has been shown by some laboratories (Lin and Wang 1990; Bumpus and Aust 1995).

Ozonization, oxygenation and hydrogenperoxide treatment were tried as alternative chemical bleaching processes to chlorine-based bleaching (Senior at al. 1992). Most recently bleaching of Kraft pulp by its treatment with xylanase enzyme was developed as a solution to the environmental problem which is faced during chlorine bleaching (Yang et al. 1993; Daneault et al. 1994; Suurnakki et al. 1994). Despite those efforts for seeking non chlorine based bleaching processes treatment of pulp with chlorine and chlorine compounds are preferably used by most of pulp producing plants over the world due to high efficiency.

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Depending on the consideration that using of chlorine consisting paper and products cause some health problems, there is an increasing demand for totaly chlorine free paper and paper products such as baby diapers and food packaging (Shoham et el. 1992).

Removal of chlorine from chlorine bleached pulps will result in production of chlorine free pulp from which chlorine free paper products can be obtained. Previously chlorine removal from some chlorinated aromatics with laccase enzyme has been demonstrated (Arcand and Archibald 1991; Iimura et al. 1996).

In this study, the possibility of chlorine removal from chlorine bleached Kraft pulp was investigated by using laccase produced from white-rot fungus, *Trametes (Coriolus) versicolor.*

MATERIALS AND METHODS

Seven white-rot fungi, *Phanerochaete chrysosporium* ME446, *Pleurotus sajor-caju, Pleurotus sapidus, Pleurotus eryngii, Pleurotus osteratus, Pleurotus florida and Trametes* (*Coriolus*) *versicolor* were screened to choose the efficient laccase source. *Phanerochaete chryosporium* ME446 was kindly supplied by Dr. T.K. Kirk (U.S. Dept. of Forest Products Agriculture Lab., Madison, Wisconsion 53705, U.S.A.). All the *Pleurotus* strains were obtained from Dr. I. F. Zadrazil (Weisdrangveg 4, 3300 Braunschweing, Federal Republic of Germany). *Trametes (Coriolus) versicolor* was provided from Biology department of Inonu University in Turkey.

Stock cultures were maintained on malt agar-slants by periodic subculture. Basal medium for the production of laccase was prepared by dissolving (gram per liter) 0.2 KH_2PO_4, 0.5 $MgSO_4$, 0.5 $NH_4H_2PO_4$, 0.1 Yeast-Extract (Difco) and 10.0 Glucose in distilled water and dispensed into 500 mL conical flasks as 300 mL medium. After autoclave sterilization 3 mL of separately sterilized trace metal solution consisting of (gram per liter) 1.4 $ZnSO_4.7H_2O$ and 1 $FeSO_4.7H_20$ was added into each flask. And then flasks were innoculated with 10 mL of mycelial suspension of *T. versicolor* stock cultures.

Culture was carried out by shaking the flasks in incubator (Psycrotherm Incubator Shaker, New Brunswick Co.) at 150 r.p.m and at 30 °C for 12 days. On the 5th days of culture 2,5 xylidine (2,5 dimethylaniline) at final concentration of $2x10^{-3}$ M was added into the culture medium. After the incubation liquid phase of the medium was separated from the microorganisms by centrifugation. The culture supernatants so obtained were used as crude enzyme source during enzymatic dechlorination studies.

Laccase activity in culture supernatant was measured according the method suggested by Coll et al. (1993). For measuring the activity, 0.1 mL of enzyme

solution was mixed with 50 mM Na-Acetate buffer (pH 4.5) consisting of 1 mM Guaiacol as substrate to make final reaction volume 5 mL. Blank tube was only containing 5 mL of acetate buffer. Tubes were allowed for incubation at 37 °C for 15 minutes. Enzyme activity was determined by reading optical density in spectrophotometer (Jenway 6105 U.V./VIS Spectrophotometer) adjusted to 465 nm wave length. One unit lactase activity was defined as amount of enzyme which caused 0.1 unit increase in the optical density of reaction mixture in one minute.

In enzymatic dechlorination studies the chlorine-based bleached Kraft pulp provided from SEKA-Mugla/Dalaman pulp plant was used after it was washed in 0.1 M KNO_3 solution to remove it from free chlorine content according the procedure suggested by Parker et al. (1993). After washing and drying residual organicaly bound-chlorine (ROX) content pulp samples were measured by the aid of organic halogen analysing apparatus (Euroglass Analytical Instrument).

Enzymatic dechlorination experiments to search the optimum substrate concentration was performed by adding the pulp at warying concentrations between 50 and 200 mg into tubes each containing 4.5 mL of 50 mM citrate buffer and 0.5 mL enzyme source of having 0.4845 U/mL enzyme activity. For optimizing enzyme activity, 0.5 mL enzyme source of varying activities was added into tubes containing buffer and 50 mg substrate.

Blank tubes were only containing citrate buffer. Unless otherwise stated, test and blank tubes were incubated at 37 °C for 15 minutes. Amounts of chlorine ion released in reaction tubes during the enzymatic dechlorination were measured by using mercury thiocyanate procedure (Vogel 1961).

RESULTS AND DISCUSSION

T. versicolor has been offered as a good laccase source by various investigators. (Arcand and Archibald 1991; Iimura at al. 1996).

Data obtained during our study to select the potent laccase source has also shown that *T. versicolor* is the best laccase source among the white-rot fungal strains examined (Figure 1). When laccase activities in supernatants of xylidine induced fungal cultures were experimentaly compared *T. versicolor was* found to have higher activity than that of the others.

Therefore culture supernatant of *T. versicolor* was used as crude laccase source in dechlorination studies. During the studies carried out for optimizing dechlorination, incubation temperatures between 37 °C and 40 °C were seemed to be effective for highest chlorine removal from chlorine-based bleached Kraft pulp (Figure2).

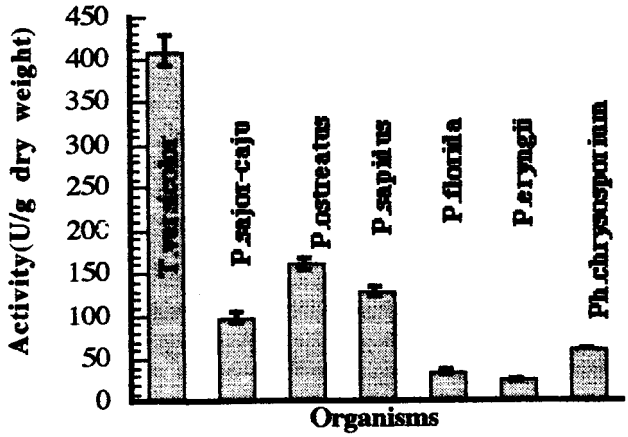

Figure 1. Laccase activities of white-rot fungi. The values are means of the least 3 experiments. (Bars: Standard deviation).

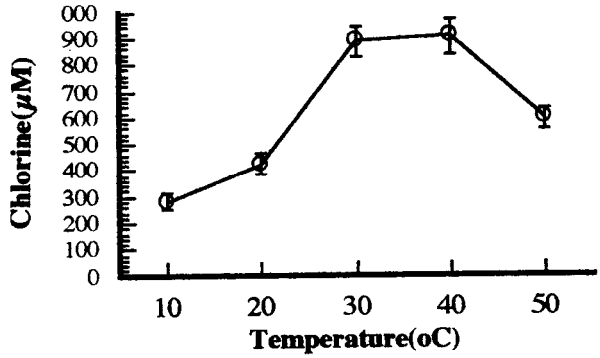

Figure **2.** The effect of incubation temperature on enzymatic dechlorination. 50 mg kraft pulp (ROX value= 8.05 mg Cl^-/g pulp) was treated with culture fluid of having 0.4845 U/mL laccase activity. The values are means of at least 3 experiments. (Bars: Standard deviation).

In the experiments performed to find out the effect of incubation pH on the enzymatic dechlorination, it was observed that there was a slight increase in chlorine removal up to pH 5.0. The pH values more than pH 6.0 of the reaction medium resulted in releasing of less chlorine ion from the substrate (Figure 3). Previously it was reported that pH 5.0 and 25 °C were the optimum reaction conditions for laccase enzyme isolated from *T. versicolor* cultures (Arcand and Archibald 1991). Therefore, it can be easily concluded that laccase activity of the culture supernatant of *T. versicolor* is mainly responsible for the dechlorination of the chlorine bleached Kraft pulp during our experiments. The extent of dechlorination was found to be related with laccase activity of culture supernatant. Release of chlorine ion from the substrate linearly inclined depending on the increase of laccase activity up to 0.968 U/mL (Figure 4).

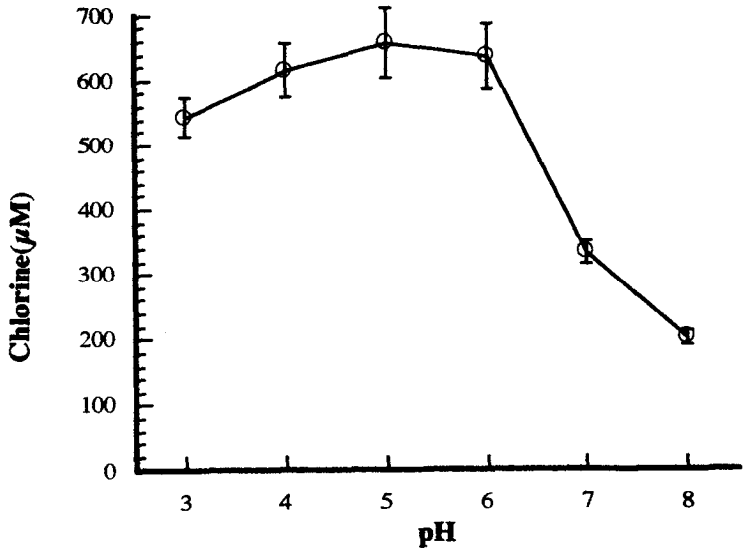

Figure 3. The effect of pH on enzymatic dechlorination. 50 mg kraft pulp (ROX value= 8.05 mg Cl^-/g pulp) was treated with culture fluid of having 0.4845 U/mL laccase activity. The values are means of at least 3 experiments. (Bars: Standard deviation).

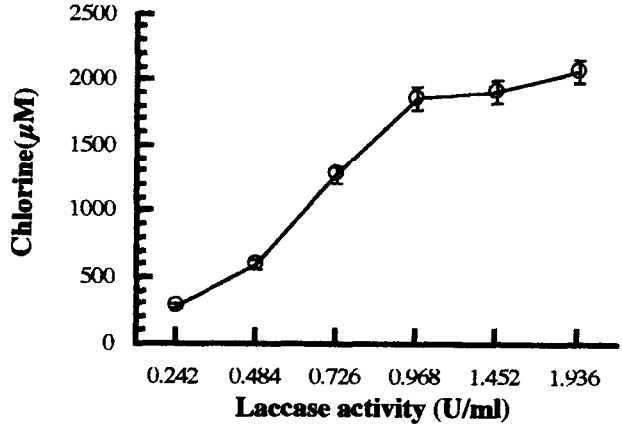

Figure 4. The effect of enzyme activity on dechlorination. 50 mg kraft pulp (ROX value= 8.05 mg Cl^-/g pulp) was used. The values are means of at least 3 experiments. (Bars: Standard deviation).

As it is seen in Figure 5, level of chlorine ion increased being dependent on the increase in substrate concentration up to 100 mg in reaction mixture.

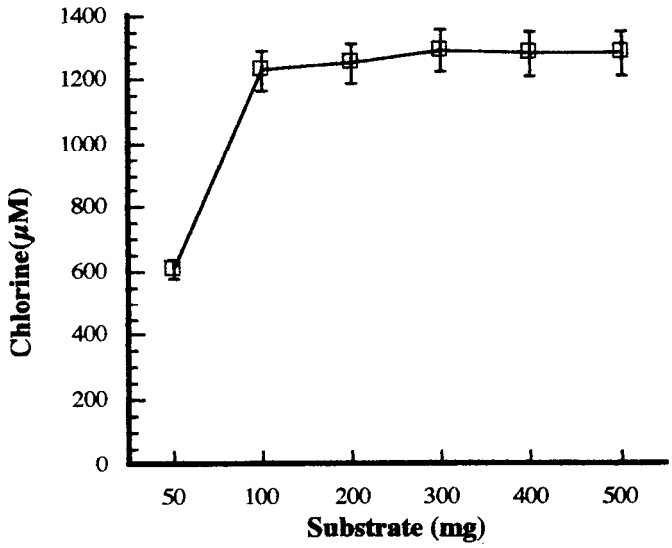

Figure 5. The effect of substrate concentration on enzymatic dechlorination. Kraft pulp (ROX value= 8.05 mg Cl^-/g pulp) was treated with culture fluid of having 0.4845 U/mL laccase activity. The values are means of at least 3 experiments. (Bars: Standard deviation).

The substrate concentrations more than 100 mg had not substantial effect on the level of chlorine ion relesed due to laccase activity.

According to the data obtained in this study, the enzymatic chlorine removal from chlorine-based bleached kraft pulp by using laccase at optimized reaction conditions seems quite probable and very promising. Consequently this using of laccase enzyme can be suggested as a novel biotechnological method for dechlorination of any items which may consist toxic organicaly bound chlorine compounds.

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