THE ONSET OF LIGNIN-MODIFYING ENZYMES, DECREASE OF AOX AND COLOR REMOVAL BY WHITE-ROT FUNGI GROWN ON BLEACH PLANT EFFLUENTS

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ABSTRACT

Decrease of adsorbable organic chlorine (AOX) is becoming the most important criterion for the efficiency of pulp mill effluent treatment in the 1990s. Two methods, designated MYCOR and MYCOPOR which utilize the white-rot fungus Phanerochaete chrysosporium have earlier been developed for the color removal of pulp mill effluents, but the processes have also a capacity to decrease the amount of chlorinated organic compounds. Lignin peroxidases (ligninases) produced by P. chrysosporium may dechlorinate chlorinated phenols. In this work possibilities to use selected white-rot fungi in the treatment of E-stage bleach plant effluent were studied. Phlebia radiata, Phanerochaete chrysosporium and Merulius (Phlebia) tremellosus were compared in shake flasks for their ability to produce laccase, lignin peroxidase(s) and manganese-dependent peroxidase(s) and to remove color from a medium containing effluent. Softwood bleaching effluents were treated by carrier-immobilized P. radiata in 2 l bioreactors and a 10 l Biostafl® fermentor. Dechlorination was followed using Cl" ion and AOX determinations. All fungi removed the color of the effluent. In P. radiata cultivations AOX decrease was ca. 4 mg l\(^{-1}\) in one day. Apparent lignin peroxidase activities as determined by veratryl alcohol oxidation method were negligible or zero in a medium with AOX content of ca. 60 mg l\(^{-1}\), prepared using about 20 % (v/v) of softwood effluent. However, the purification of extracellular enzymes implied that large amounts of lignin peroxidases were present in the medium and, after the purification, in active form. Enzyme proteins were separated using anion exchange chromatography, and they were further characterized by electrophoresis (SDS-PAGE) to reveal the kind of enzymes that were present during AOX decrease and color removal. The most characteristic lignin peroxidase isoenzymes in effluent media were LiP2 and LiP3.

KEYWORDS

White-rot fungi; Phlebia radiata; pulp bleach plant effluent; decolorization; dechlorination; lignin peroxidase; laccase; manganese-dependent peroxidase; protein purification.

INTRODUCTION

Chlorinated effluents from pulp bleaching plants are an increasingly alarming threat to the environment, especially in Scandinavian countries where softwood pulping is very intense. For example, according to planned legislation in Finland (Anonymous, 1989) the amount of organically
bound chlorine measured as AOX (adsorbable organic halogens) should be decreased by the year 1995 - either by modification of the process or by efficient wastewater treatment - to 1.4 kg per ton pulp, and probably later virtually zero. This low level of AOX is difficult to achieve even by the newest bleaching methods. In activated sludge plants, which have become common in Finland, the decrease of AOX is 48-65%, provided the plants are modern and well-operating, but only 23-32% in aerated lagoons which still are the most common in many other countries (Gerkov et al., 1988). However, it is not known how much of the high molecular weight material (MW > 1000) is truly mineralized. Even when the required decrease of organically bound chlorine has been achieved, there will be problems caused by the color of effluent water because it is not removed by bacteria in activated sludge.

The ability of white-rot fungi to decolorize bleach plant effluents from kraft pulp mills (Fukuzumi et al., 1977) and to degrade 14C-labelled chlorolignins (Lundquist et al., 1977) was published over 10 years ago. Since then two methods which utilize the white-rot fungus Phanerochaete chrysosporium have been developed for the treatment of pulp mill effluents and patented (Chang et al., 1987; Messner et al., 1988; for more references, see Eriksson and Kirk, 1985; Pellinen et al., 1988a,b; Messner et al., 1989). The processes have been designated as MYCOR and MYCOPOR. Later it was found that the treatment of bleach plant effluent by MYCOR also markedly decreases total organic chlorine (TOCl) (Matsumoto et al., 1985). Several studies have shown the ability of P. chrysosporium to degrade many kinds of chlorinated aromatic compounds and other organopollutants (Hammel, 1989).

A study focusing on dechlorination and decolorization of bleach plant effluents was undertaken using Phlebia radiata and other white-rot fungi. The studies on the enzymology, i.e. the onset as well as the profile of various enzymes under the conditions used, have not been reported so far. In our earlier paper (Hatakka et al., in press) we reported that apparent lignin peroxidase activities were very low or negligible in medium containing high AOX. However, since P. chrysosporium lignin peroxidases are known to dechlorinate many compounds (Hammel and Tardone, 1988) we continued investigations and studied more thoroughly extracellular lignin-modifying enzymes by purifying them.

MATERIALS AND METHODS

Fungi. Phlebia radiata 79 (ATCC 64658) was isolated at the Department of Microbiology, University of Helsinki, Finland (Hatakka and Uusi-Rauva, 1983). Phanerochaete chrysosporium BKM-F-1767 (ATCC 24725) was obtained from Dr. M. Leisola, ETH, Zürich, Switzerland. Merulius (Phlebia) tremellosus 2845 was obtained from Dr. Ian Reid, National Research Council of Canada, Plant Biotechnology Institute, Saskatoon, Canada. Fungi were maintained on 2% malt agar slants. Conidial inoculum was prepared from P. chrysosporium (Hatakka and Uusi-Rauva, 1983) grown on malt agar plates. Inoculum of the other fungi was grown in standing flasks on LN-ADMS medium containing 2 mM nitrogen as asparagine and NH4NO3 as nitrogen source and glucose (56 mM) as a carbon source (Hatakka and Uusi-Rauva, 1983). Fully grown mycelium was broken in a Waring Blender and used as an inoculum (2-4% (v/v) in shake flasks and 10% (v/v) in bioreactors and in a fermentor.

Cultivation medium. Cultivation medium was low nitrogen (2 mM-N) glucose (56 mM) medium LNADMS buffered with 2,2-dimethylsuccinate at pH 4.5 (Hatakka and Uusi-Rauva, 1983). In bioreactor cultivations 56 or 29 mM glucose was used as a carbon source. Veratryl alcohol (0.2 mM) and Tween 80 (0.05% (w/v)) were added in cultures to stimulate the production of ligninolytic enzymes (Hatakka et al., 1987; Kantelinen et al., 1989).

Effluents. E1-stage bleach plant effluents from hardwood (HWE) or softwood treatment (SWE) were obtained from two commercial kraft pulp mills. Effluents were stored at -20°C and prior to use their pH was adjusted to 4.5 with 10% (v/v) CH3COOH (SWE) and with 2 M H3PO4 (HWE). The effluents were sterilized by autoclaving for 15 min at 121°C.
Effect of white-rot fungi

Shake flask cultivations. Fungi were cultivated in 250 ml conical flasks containing 50 ml medium on a rotary shaker (New Brunswick) 180 rpm under an air atmosphere at 28°C. P. chrysosporium was grown at 37°C. Hardwood effluent was used to replace part (20-60 % (v/v)) of the water in medium.

Bioreactor and fermentor experiments. P. radiata was cultivated immobilized on plastic carriers (Hatakka et al., 1987; Kantelinen et al., 1989; Niku-Paavola et al., 1990) both in bioreactors and in a fermentor. Bioreactors were glass bottles with working volume of 2 l. They were aerated ca. 1.5 l min⁻¹ by sterile air flow through perforated plastic tube. The temperature was maintained at 28°C. A Biostat E fermenter (working volume 8 l) was filled with plastic carrier which was wrapped around the temperature control loops. Impeller agitation speed was 100 rpm, aeration 1.65 l min⁻¹ and temperature 28°C. pH was kept at pH 4.5 using 2 M H₂PO₄ or 2 M NaOH. Foaming was controlled by Struktol or Deconex 31 (Borer Chemie).

Enzyme activities. All samples were filtered through Whatman 4 filter paper before analysis. Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) was determined at 25°C using ABTS (2,2-azinodi-3-ethyl-benzothiazoline-6-sulphuric acid) as a substrate at pH 3.0 ("oxidase") (Niku-Paavola et al., 1988) or syringaldazine as a substrate at pH 5.0 ("laccase") (Ander and Eriksson, 1976). Lignin peroxidase (LiP, ligninase) activity was determined at 37°C by veratryl alcohol oxidation method (Kirk et al., 1986) at pH 3.0. In effluent-containing samples manganese-dependent peroxidase (MnP) was assayed at 30°C by phenol-red oxidation in the presence of Mn²⁺ (Glenn and Gold, 1985) using 10 min reaction time and the activity was expressed as ΔA₅₆₅ nm ml⁻¹. Peroxidase activity was assayed without Mn²⁺. In purified fractions MnP was assayed by the same phenol-red oxidation method but modified by omitting NaOH supplement and measuring the absorbance change at 520 nm for 3 min at 30°C (ΔA₃₄₀nm min⁻¹ ml⁻¹). In both MnP assays reaction mixtures (1 ml) contained 25 mM lactate, 0.1 mM MnSO₄, bovine serum albumin (1 mg ml⁻¹), phenol-red (0.1 mg ml⁻¹) and 0.5 ml culture filtrate or purified fraction in 20 mM sodium succinate buffer, pH 4.5. The reaction was initiated by adding 0.1 mM H₂O₂.

Protein purification. All protein purifications were carried out from frozen samples (-20°C). Extracellular enzymes were fractionated with anion exchange chromatography by using Sepharose-Q (Pharmacia) column and FPLC apparatus (Pharmacia) as described by Niku-Paavola et al. (1988). Culture liquids were equilibrated with 25 mM sodium acetate buffer pH 5.5. A linear gradient of 0.05-0.3 M NaCl in 300 ml of 25 mM sodium acetate buffer, pH 5.5 was used to elute LiPs, MnP and laccase. Fractions containing LiP, MnP and laccase activity were pooled and identified by SDS-polyacrylamide gel electrophoresis (Laemmli, 1970) with silver staining. Low molecular weight standard (Pharmacia) was used as Mᵣ-reference.

Analytical methods. Protein was determined by using Bio-Rad reagent (Bradford, 1976) and glucose by the hexokinase method (Boehringer-Mannheim). Cl⁻ ion was determined with a model 9417SC ion-selective electrode (Orion Research Inc., Boston, MA, USA). Color was determined spectrophotometrically at 465 nm. For that, pH of the SWE containing samples was adjusted to pH 7.6.

Organically bound chlorine (AOX) was determined by a modified method of Gerkov et al. (1988) using an Euroglas apparatus where 2 hours shaking of the sample was included as a pretreatment. The analyses were carried out at the Finnish Pulp and Paper Research Institute, Espoo, Finland.

RESULTS

Treatment of bleach plant effluents by various white-rot fungi

Comparison of white-rot fungi for the treatment of bleach plant effluents was carried out in shake flask cultivations. The fungi Phlebia radiata, Phanerochaete chrysosporium and Merulius (Phlebia) tremellosus were studied for their ability to produce laccase, lignin peroxidases (LiPs, ligninases) and...
manganese-dependent peroxidases (MnPs) and to remove color from effluent-containing medium. *P. radiata* produced laccase, oxidase, LiP and MnP, *M. tremellosus* produced laccase, oxidase and MnP but not LiP, and *P. chrysosporium* produced LiP and MnP but not laccase or oxidase. Fig. 1 shows the removal of color from hardwood effluents during the growth. The color of the medium at first increased by laccase-producing fungi but especially *P. radiata* abruptly on day four started to decolorize the medium to the same level as *P. chrysosporium*. LiP, as determined by veratryl alcohol oxidation method, was not detected in any of the fungal cultures during the sharp decrease of the color. However in these cases LiP may have been in inactive form as shown in bioreactor and fermentor cultivations. Among laccase-producing fungi, *M. tremellosus* decolorized the medium less than *P. radiata*. It did not produce (active) LiP.

Fig. 1. Removal of color from 40 % (v/v) hardwood effluent containing medium. (■) *Phlebia radiata*, (△) *Phanerochaete chrysosporium*, (●) *Merulius* (*Phlebia*) *tremellosus*

Fig. 2. Removal of color (■) and AOX (vertical bars) during semicontinuous cultivation of immobilized *Phlebia radiata* in a 2 l bioreactor in a medium containing 20 % (v/v) softwood effluent. Original color 5400 C.U. (C.U. = mgPt l⁻¹).

**Bioreactor experiments by Phlebia radiata**

Carrier-immobilized *P. radiata* was semicontinuously cultivated in 2 l bioreactors. The softwood effluent (20 % (v/v)) was added in the beginning of the cultivations. The mycelium rapidly removed
color from the effluent as can be seen in Fig. 2. In the beginning of the cultivation the color even increased, which may reflect the activity of laccase: laccase oxidizes phenols to quinones which may increase color. During the first day after the replacement of the effluent, the color removal was even more rapid, ca. 33 %. The AOX removal was less rapid than color removal (Fig. 2), and apparently showed different kinetics. When the effluent was added in the beginning of the cultivation at the same time as the medium and inoculum, the growing mycelium removed 27 % of AOX in 6 days, but the pregrown mycelium removed 40 % of the applied AOX in 5 days (Fig. 2). Cl⁻ ion concentration increased while AOX decreased but, especially during the second cycle, not synchronously. When the amount of glucose in the medium was lower, 29 mM instead of 56 mM, no clear difference in the color or AOX removal could be seen compared to the 56 mM glucose containing medium (data not shown). Glucose consumption accelerated during the second cycle of the cultivation. However, even 29 mM glucose was unnecessarily high amount, since a lot of glucose was still left in the medium.

In Fig. 3 the apparent extracellular enzyme activities in bioreactor cultivations are shown. Oxidase assay using ABTS as a substrate was more sensitive but less specific than syringaldazine for laccase activity. The kinetics of oxidase activity indicated that oxidizing activity other than laccase activity may have been present during the days 8-10 (Fig. 3a.). Also, peroxidase activity where phenol red was used as a substrate may have been the same as LiP activity (Fig. 3b.). Phenol-red method used with or without Mn²⁺ is not very specific for only MnP, since also LiP may oxidize phenol red. LiP as determined using veratryl alcohol as a substrate, however, showed only a little activity during the first cultivation cycle. In spite of this, enzyme purifications at the end of the first cycle revealed that the culture liquors did contain large amounts of LiP and SDS-PAGE made from culture liquors on days 6-10 showed strong bands which indicated the presence of LiP2 and LiP3. In other words, in the presence of the effluent, LiP apparently could not oxidize veratraldehyde.

P. radiata was cultivated also in a Biostat®-fermentor with working volume of 8 l. In a typical cultivation the mycelium was pregrown for 7 days on LN-ADMS medium before the addition of the effluent (21.3 % (v/v)). On day 13 ca. 13 % of the volume was replaced by new effluent. In two days, 11 % of AOX was removed, and in 6 days 40 %, to the level of 34.9 mg l⁻¹. During the next cycle the color removal was more rapid in the same way as in the bioreactor cultivations. The amount of plastic carrier and thus the amount of immobilized mycelium was smaller in the fermentor, which probably explains the lower rate of AOX removal.

The production of extracellular enzyme activities in fermentor cultivations is shown in Fig. 4. When no effluent was added LiP activity reached 2000 nkat l⁻¹ in 4 days. After the replacement of 21.3 %
(v/v) of the medium with effluent, the apparent LiP activity was zero throughout the entire residual cultivation. Oxidase and laccase activity rapidly started to increase one day after the change of the medium, and reached almost 2000 nkat l⁻¹ ("laccase") in 5 days during the first cycle with the effluent. Extracellular protein curve followed the excretion of enzymes. However, the presence of the effluent may have given too high values in the protein assay, due to the strongly colored culture filtrates.

![Graph](image)

**Fig. 4.** Production of lignin peroxidase (●), oxidase (▲), laccase (●) and extracellular protein (+) during the cultivation of immobilized *Phlebia radiata* in a 8 l Biostat fermentor. 21.3 % (v/v) of the medium was replaced with softwood effluent on day 7 and 13 % (v/v) on day 13. Arrows show the days of enzyme purifications (Fig. 5).

![Graph](image)

**Fig. 5.** Purification of extracellular proteins from *Phlebia radiata* culture liquor by anion exchange chromatography on Sepharose-Q. Cultivation conditions as in Fig. 4. Growth time 7 days without the effluent (- - - - -) or 7 days without effluent plus 2 days with effluent (- - - -). A_{280} = - - - - -; A_{405} = - - - - -; h1 - h3, heme proteins.
Extracellular enzyme profiles during effluent treatment by Phlebia radiata

Extracellular enzyme proteins from the fermentor cultivation were purified by anion exchange chromatography (Fig. 5). The hemeproteins as monitored at 405 nm (symbol h in Fig. 5) were separated and the fractions were studied using SDS-PAGE. The enzymes in the separated fractions were two Mn-peroxidases (h1 = MnPX, h2 = MnP, see Niku-Paavola et al. (1990), for designation of hemeproteins) and a large hemeprotein peak which consisted mainly of LiP complex LiP2 and LiP3. After the purification LiPs were in active form. Two days after 21.3 % of the medium was replaced by softwood effluent the apparent lignin peroxidase activity with veratryl alcohol oxidation method was zero. However, calculated from purified fractions the level of the true LiP activity in the medium was ca. 400 nkat l⁻¹. The profile of extracellular enzymes showed that MnPX (h1) had disappeared, MnP (h2) was present as well as the major peak LiP2-3 (h3). When purification was carried out from the liquor of the day 19 (cultivation for 7 days without effluent, 21.3 % effluent on day 7 and 13 % on day 13), the enzyme profile had become more versatile: there were at least MnPX, MnP, LiP1, LiP2 and LiP3 present and the level of the total true LiP activity in the medium was approximately 3600 nkat l⁻¹ (sum of LiP1, LiP2 and LiP3 containing fractions).

DISCUSSION

The treatment of bleach plant effluent by the white-rot fungus Phanerochaete chrysosporium has originally been optimized for color removal (Eaton et al., 1980; Sundman and Kirk, 1981; Eriksson and Kirk, 1985). Later it was found that the treatment of bleaching effluent with P. chrysosporium also decreases total organic chlorine (TOCl) by ca. 45 % per day (Matsumoto et al., 1985) and the organic chlorine content of chlorolignin decreases about 50 % in one day, when the starting level has been about 3.7 mM (Pellinen et al., 1988a). Adsorption to the mycelium was not significant, and the increase of Cl⁻ concentration indicated true dechlorination. Waste waters are usually treated by P. chrysosporium by rotating biological contactors (Chang et al., 1987; MYCOR process), while the use of trickling filter type bioreactor where the fungus is immobilized on foam carrier has been adopted in the MYCOPOR system (Messner et al., 1989). Since control and asepsis in sensor- and computer-connected fermentors are much easier to maintain than in rotating biological contactors, in this work carrier-immobilized P. radiata was cultivated in a 10 l Biostat® E-fermentor. Smaller bioreactors were also used.

In P. radiata cultivations with pregrown mycelium the decrease of AOX was 11 % in two days and 32 % during the next four days, which means approximately 4 mg AOX decrease per litre per day. This is lower amount than those reported in the MYCOR process (Matsumoto et al., 1985; Pellinen et al., 1988a) where more concentrated effluents were used. In contrast to the MYCOR process which utilizes oxygen, air was used in our cultivations which may have influenced the treatment rate. The amount of mycelium was presumably much lower in our bioreactor and fermentor cultivations than with P. chrysosporium growing on the rotating disc contactor. The mycelium-effluent ratio seems to be important, as well as the precultivation of the mycelium which may involve acclimation of the mycelium to the effluent. The amount of AOX adsorbed on the fungal mycelium was not determined. P. radiata does not grow vigorously, and under the same conditions the amount of mycelium of P. radiata is much less than that of P. chrysosporium although the efficiency of lignin degradation is similar or even higher in P. radiata (Hatakka et al., 1983). In the effluent treatment a large amount of mycelium may be beneficial, but on the other hand, more nutrients (glucose, nitrogen, phosphorus) are certainly needed to produce a thick mycelial mat.

It has been assumed that the efficiency of the MYCOR process is based on the lignin-degrading ability of the fungus, and thus lignin peroxidases should play a central role. Results of Eriksson and Kolar (1985) and Eriksson et al. (1985) support the concept that the lignin-degrading machinery of the fungus is involved. These authors studied the degradation of high relative molecular mass fraction of both chlorination stage (C) and alkali stage (E) using ¹⁴C-labelled chlorolignins. They found that the fungus Sporotrichum pulverulentum (an anamorph of P. chrysosporium) degraded 35 % of the C-
stage material and 45% of the E-stage material to $^{14}$CO$_2$ in 1-2 months whereas bacterial consortia did not degrade these materials. For efficient lignin degradation P. chrysosporium (Kirk and Farrell, 1987) and P. radiata (Hatakka et al., 1983; Hatakka and Uusii-Rauva, 1983) require low nutrient nitrogen, high oxygen partial pressure and a cosubstrate. As the treatment of bleach plant effluents probably includes an attack on chlorinated lignins (Eriksson et al., 1985; Eriksson and Kolar, 1985; Pellinen et al., 1988a) it is advisable to use conditions which favor lignin degradation and the appearance of ligninolytic enzymes. The production of lignin peroxidases does not necessarily correlate with the degradation of lignin polymer (Kirk and Farrell, 1987) and presumably not with degradation of chlorolignins. Recently, however, LiPs have been shown to be able to dechlorinate chlorinated phenols (Hammel and Tardone, 1988) and pentachlorophenol (Mileski et al., 1988). It has been reported that P. chrysosporium also utilizes methylation, oxidation and reduction for the degradation of chlorolignins. Recently, however, LiPs have been shown to be able to dechlorinate chlorinated phenols (Hammel and Tardone, 1988) and pentachlorophenol (Mileski et al., 1988). It has been reported that P. chrysosporium also utilizes methylation, oxidation and reduction for the degradation of small molecular weight material (Huynh et al., 1985). The presence of specific intracellular dechlorinating enzymes resembling those described in bacteria (Håggblom et al., 1988) also in fungi has not been ruled out.

The production, the activity, and the stability of ligninolytic enzymes on media containing bleach plant effluents has not previously been investigated. P. radiata produces LiPs in bioreactors and fermenters under an air atmosphere provided that the mycelium is immobilized on a carrier and the surfactant Tween 80 is added (Hatakka et al., 1987). P. radiata also efficiently degrades $^{14}$C-(RING)-DHP to $^{14}$CO$_2$ under an oxygen atmosphere in shake flasks in the presence of veratryl alcohol and Tween 80 (Hatakka et al., 1986). Thus, the lignin-degrading machinery of the fungus was expected to be active in this work. The production of relatively high amounts of LiPs and MnPs can also be seen in the case where effluent is omitted from the medium. However, it was found that the addition of the effluent to the growth medium disturbed the normal measurement of LiP activity which is based on oxidation of veratryl alcohol to veratraldehyde in the presence of H$_2$O$_2$. Purification of extracellular enzymes revealed that the fungus produced large amounts of LiPs during decolorization of the softwood, and maybe also in shake flasks with hardwood effluent (purifications were not done), and after the purification LiPs were active. The degradation products or phenolic residues maybe prevented the normal reaction cycle of LiP (cf. Harvey et al., 1989). In a mixture containing a dimeric nonphenolic lignin model compound 1-(4-ethoxy-3-methoxyphenyl)-1-(2-methoxyphenoxy) propane-1,3-diol and veratryl alcohol, P. radiata LiP does not oxidize veratryl alcohol to veratraldehyde although the dimer is cleaved at C$_6$ - C$_7$ (Hatakka and Lundell, 1989). Part of the inhibition can also be caused by chloride. Miki et al. (1987) found that 32 mM chloride caused 50% inhibition of lignin peroxidase I (from P. chrysosporium) catalyzed veratryl alcohol dehydrogenation reaction. In our experiments Cl$^-$ ion concentration varied from 500 to 650 ppm (14-18 mM).

Although lignin peroxidase I could oxidize bromide it could not oxidize chloride, suggesting that the redox potential of lignin peroxidase I was lower than that of chloroperoxidase.

The profile of extracellular enzymes did not show any indication for enzymes specific for dechlorination. The results from the medium containing softwood effluent and thus a rather large amount of AOX - up to 60 mg l$^{-1}$ - showed that under these conditions P. radiata apparently started to produce laccase. Isozyme LiP3 was almost always present in purified fractions. LiP3 was most often found in the presence of insoluble lignocellulose (Niku-Paavola et al., 1990). In both cases the medium contained polymeric lignin or chlorolignin.

Although Pellinen et al. (1988a) suggest that in MYCOR process the dechlorination, decolorization and degradation of chlorolignin are metabolically connected, the different kinetics of color and AOX removal indicated that different enzyme systems could have been operating during decolorization and dechlorination by P. radiata. Recent results of Momohara et al. (1989) indicate that the decolorization of E$_1$-effluent by P. chrysosporium is not catalyzed by lignin peroxidases. The fungal decolorization cannot be explained by adsorption or precipitation. In our work all fungi readily removed color although their lignin-modifying enzymes had different profiles. Laccase-producing fungi P. radiata and M. tremellosus increased color in the beginning of cultivation. This could be attributed to the oxidation of phenolic structures to quinones especially by laccase or peroxidases (LiPs and MnPs) (cf. Buswell and Odier, 1987). With P. chrysosporium Pellinen et al. (1988a) also found that polymerization of chlorolignin took place.
Preliminary experiments to optimize culture conditions of _P. radiata_ for dechlorination and interdependence of selected variables, such as the concentration of glucose and E₄-effluent, were carried out in shake flasks using response surface methodology. However, as shown above, the validity of veratryl alcohol oxidation method to show LiP activity in effluent-containing media was poor. Thus, of enzyme activities, only laccase activity could be considered reliable. The studies of the relationships between above-mentioned parameters and various true enzyme activities as well as decrease of AOX are in progress in our laboratory.

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REFERENCES


