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PRODUCTION AND APPLICATION OF LACCASE ENZYME IN PULP AND PAPER INDUSTRY

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ABSTRACT

Laccase is an enzyme that has potential ability of oxidation. There are diverse sources of laccase producing organism like fungi, plants and microorganism. The possibility of using crude laccase in the dechlorination of chlorine-based bleached kraft hard wood pulp was investigated. The present work comprises the laccase enzyme production by isolated ligninolytic fungal strain SL_2 and SL_4 and its industrial application. Experiment was conducted on bleaching of kraft hard wood pulp with laccase enzyme (122.33 IU/ml) produced by ligninolytic fungal strain SL_4 which shows brightness, whiteness and improvement along with CLO_2 reduction. The addition of laccase inducer Cuso4 in to the culture medium led to an increase dechlorination activity.

KEYWORDS: Kraft Pulp, Laccase, Dechlorination, Biobleaching

INTRODUCTION

Laccases are the oldest and most studied enzymatic systems. Yoshida first described laccase in 1883 when he extracted it from the exudates of the Japanese lacquer tree, *Rhus vernicifera* and its characteristics as a metalcontaining oxidase was discovered by Bertrand in 1985. In 1896, laccase was demonstrated to be present in fungi for the first time by both Bertrand and Laborde.

Fungal laccases have higher redox potential than bacterial or plant laccases (up to +800 mV). Thus, fungal laccases are involved in the degradation of lignin or in the removal of potentially toxic phenols arising during lignin degradation. Laccase is generally found in higher plants & fungi such as basidiomycetes, white root fungi and ascomycetes. The white rot basidiomycetes are the most efficient degraders of lignin and enzymes which are implicated in lignin degradation are lignin peroxidase, manganese-dependent peroxidase and laccase. In plants laccases play an important role in lignification whereas in fungi laccases have been implicated in many cellular process including delignification, sporulation, pigment production, fruiting body formation and plant pathogenesis.

Laccases are able to depolymerize lignin and delignify wood pulps & kraft pulp fibers in the biopulping process (Bourbonnais et al. 1997; Lund and Ragauskas, 2001; Chandra and Ragauskas, 2002; Camarero et al. 2004; Rodríguez and Toca, 2006; Vikineswary et al. 2006). One of the most studied applications in the industry is the laccases-mediator bleaching of kraft pulp and the efficiency of which has been proven in mill-scale trials (Strebotnik and Hammel, 2000). Lignin peroxidases (LiP) compared with laccase, are the biocatalysts of choice for bleaching (Bajpai, 2004; Sigoillot et al. 2005). LiP and MnP were reported to be effective in decolourizing kraft pulp mill effluents (Ferrer et al. 1991; Michel et al. 1991; Moreira et al. 2003).

In the present study attempt has been made to reduce (25%) the use of chlorine (ClO2) during bleaching of kraft pulp by the use of laccase enzyme produced by lignolytic fungal strains SL4 & it was studied that 1.8 unit brightness & 25% saving in ClO2 demand was observed.

MATERIALS AND METHODS

For the isolation of lignolytic fungi, samples of rotted wood & garden soil samples were collected from CPPRI Campus, Saharanpur and industrial soil sample was collected from Star Paper Mill, Saharanpur & Kraft hardwood pulp was collected from star paper mill, Saharanpur. The culture media used for growth and estimation of mycelium were Malt Extract Agar (MEA) & Malt Extract Broth (MEB). The diameter of fungal colony & dry weight of mycelium was measured according to Aneja, 2006.

Isolation of lignolutic strains was done by removing upper surface of rootted wood with sterilized forcep & by using piece of rotted wood and placed on MEA plates. Incubate the inoculated MEA plates at 35 ^{0}C in incubator for 2-3 days.

Purification & Screning of isolated strains for lignolytic enzyme complex is done. During screening Gallic acid (0.01%), Phenol red (0.01%), ABTS (2μM) (2.2'-azino-bis-(3-ethylthiazoline-6-sulfonate) reagents were used. Malt Extract Medium was used for screening medium was supplemented with Gallic acid (0.01%w/v) to test for Polyphenol oxidase enzyme, Phenol red (0.01%w/v) for Manganese Peroxidase enzyme, ABTS (2uM) for Laccase enzyme. A piece of 5mm diameter agar disk of 3-4 days old fungus is inoculated on each selective medium and incubated at 35^{0}C in dark for three days. The Ligninolytic activity is selected by development of colour around the mycelium. Enzyme Polyphenol oxidase show brown, Manganese Peroxidase show Yellow, Laccase show Green colour. Determine the optimum temperature by method of (Farnet et al. 2000) & optimum pH by method suggested by Heinzkill *et al.*, 1998. $CuSO_4$ Suspension (500ul) was added to the culture medium to induce laccase synthesis.

Laccase activity was assayed according to the procedure suggested by Aroa & Sandhu (1985). For measuring laccase activity source enzyme was added to sodium citrate buffer solution and Guaiacol as substrate. The optical density of the reaction tubes was measured against reagent blank in spectrophotometer at 470nm wavelengh. The activity of laccase enzyme was determined as IU/ ml.

Kraft Hardwood pulp sample was taken from Star Paper Mill, Saharnpur. Bleaching of Pulp was done in four successive stages. L- Stage in which pulp was treated by lacasse enzyme and after washing pulp pad was prepared, In Do- Stage ClO_2 treatment was given to the enzyme treated pulp with higher and lesser doses & measure against control, In Ep- Stage ClO_2 treated pulp was treated with 2.5% 0f NaOH and 1.0% of H_2O_2, In D_1- Stage - alkali extracted pulp was treated with ClO_2 and pulp was washed and pulp pad was prepared.

RESULTS AND DISCUSSIONS

In the present study, results of screening of Ligninolytic activity of selected fungal strains revealed that four cultures (SL_1, SL_2, SL_3, SL_4) out of 9 cultures showed positive results for ligninoolytic activity. Culture (SL_2, SL_5, SL_6, SL_7) showed positive activity for Manganese Peroxidase and culture SL_2, SL_4 showed positive activity for all three Ligninolytic enzymes (Poly phenol Peroxidase, Manganese Peroxidase & Laccase). SL_9 culture showed negative results for all three Ligninolytic enzymes.

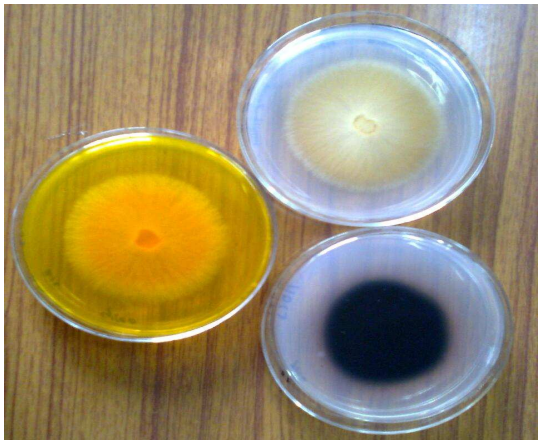

Figure 1: Lignolytic Activity of the Fungus SL_4

Results in Table 1 & figure 1 indicated that optimum temperature for the maximum growth of fungal strain SL_2 is 34^0C & SL_4 is 30^0 C whereas Table 2 & figure 2 indicated that optimum pH for maximum Laccase activity and biomass production is 4.5 pH after 8 days of incubation by selected fungal strains SL_2 & SL_4.

Table 1: Comparative Growth of Selected (SL_2, SL_4) Fungal Strains after 24 Hours of Incubation at Different Temperatures

Temp. (^{0}C)	Diameter of the Colony (cm)	
	SL_2	SL_4
28	2.9	4.8
34	2.1	5.6
38	3.1	5.2
40	1.6	4.2

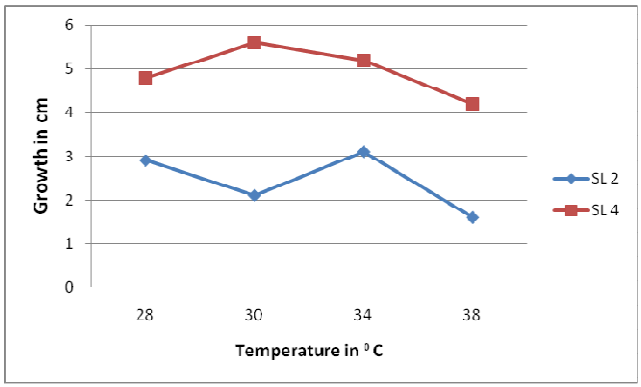

Figure 2: Graphical Analysis of Comparative Growth of Selected (SL_2, SL_4) Fungal Strains at Different Temperatures

Table 2: Effect of pH on Biomass and Laccase Production by Selected (SL_2, SL_4) Fungal Strains after 8 Days Incubation

Initial pH		Final pH		Biomass Production (gm)		Laccase Activity (IU/ml)	
SL_2	SL_4	SL_2	SL_4	SL_2	SL_4	SL_2	SL_4
4.0	4.0	3.9	3.4	0.118	0.240	0.06	59.63
4.5	4.5	4.4	3.8	0.166	0.269	2.16	61.66
5.0	5.0	4.3	3.5	0.136	0.236	0.43	29.12
5.5	5.5	4.6	5.1	0..132	0.271	2.46	29.33
6.0	6.0	4.4	4.6	0.122	0.312	1.66	16.34

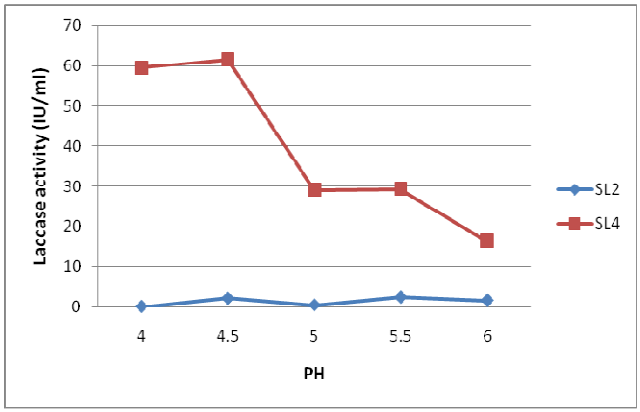

Figure 3: Graphical Analysis of Effect of pH on Laccase Production by Selected (SL_4) Fungal Strains after 8 Days Incubation

SL_2 did not showed measurable laccase activity so further study was progressed with SL_4 fungus which showed appreciable amount of laccase activity i.e 60.13 IU/ml at 4.5 pH. Table 3 & figure 3 showed that $CuSO_4$ supplemented medium shown highest laccase activity i.e. 122.33 IU/ml, when compared with the maximum enzyme activity of medium without inducer, i.e. 60.13 IU/ml respectively. $CuSO_4$ increased the laccase activity by 2 fold when compared with the medium without inducer.

Table 3: Effect of Inducers on Biomass Production and Laccase Production by Selected SL_4 Fungal Strain

	Incubation Period (in Days)	Final pH	Biomass (in gm)	Laccase Activity (IU/ml)
Without Inducer	4	4.3	0.252	24.07
	6	4.7	0.404	52.24
	8	4.5	0.499	60.13
	10	4.8	0.495	58.33
	12	4.6	0.459	47.33
	14	4.7	0.480	28.33
	16	4.6	0.527	22.44
With $CuSO_4$	4	4.1	0.231	46.60
	6	4.7	0.256	59.00
	8	4.7	0.349	69.40
	10	4.8	0.401	98.33
	12	4.8	0.493	122.33
	14	4.5	0.466	71.33
	16	4.7	0.515	44.66

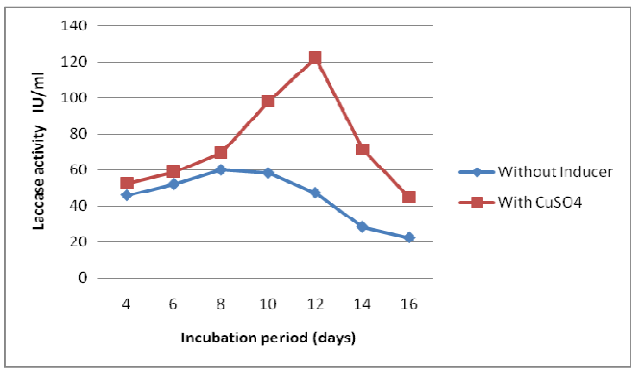

Figure 4: Graphical Analysis of Effect of Inducer on Laccase Enzyme Production by Selected SL_4 Fugal Strain

After enzyme pretreatment, the enzyme treated pulp was divided into 3 equal halves. The first part of the enzyme treated pulp was treated with same dose as that of control and second part of enzyme treated pulp was treated with 25% less chlorine dioxide dose & third part of enzyme treated pulp was treated with 35% less chlorine dioxide dose. After chlorination the pulp was passed through alkali extraction and brightness of pulp was measured. Results of Bleaching of both control and enzyme treated pulps are shown in table 4. Data indicated that 1.8 unit brightness and 25% saving in ClO_2 demand was observed in case of enzyme treated pulps when compared with control.

Table 4: D_0, E(p), D_1, D_2 Bleaching of Enzyme Treated and Untreated Pulps

Particulars	Control	Enzyme Treated		
		Same Dose	Lessdose (25%)	Lessdose (35%)
ClO_2 Stage (Do)				
Applied chlorine, %				
Brightness, % ISO	52.32	54.10	48.00	46.20
Yellowness, %ISO	27.76	26.80	30.40	32.16
Whiteness %ISO	0.00	0.00	0.00	0.00
Alkali Extraction (Ep) Stage				
Applied NaOH, %	2.5	2.5	2.5	2.5
Applied Peroxide, %	1.0	1.0	1.0	1.0
Brightness, % ISO	65.65	68.45	58.70	54.80
Yellowness, %ISO	19.70	17.60	24.50	25.70
Whiteness %ISO	29.55	32.12	9.70	4.75
ClO_2 stage (D1)				
Applied chlorine, %	3.5	3.5	3.5	3.5
Brightness, % ISO	84.10	85.00	79.20	76.20
Yellowness, %ISO	7.90	7.80	11.57	13.00
Whiteness %ISO	69.82	69.92	56.87	51.50
Brightness Improvement, Unit	-	**0.90**	-	-

Keeping the observations in mind it is concluded that the laccase base biobleaching process offers an environmentally benign way to improve pulp and paper production.

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