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Comparing Decolorization of Dye by White Rot Fungi, Free Enzyme and Immobilized Enzyme

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Abstract: Four strains of white rot fungi (WRF) had been used for laccase and MnP production. These WRF were *Pleurotus ostratus, Pleurotus sajor-caju, Phanerochaete chrysosporium* (24725) and *P. chrysosporium 34541*. For enzyme production three different media were used namely I, II and III. The results revealed that *P. sajor-caju* produced the highest laccase and MnP on the three media except medium III. *P. sajor caju* produced 0.468 and 0.612 U/ml laccase on medium I and II respectively. Also *P. sajor-caju* produced 0.720, 0.594 and 0.540 U/ml MnP on medium I, II and III respectively. *P. chrysosporium* 34541 and 24725 produced MnP on the three media, but failed to produce any laccase on these media. *P. chrysosporium* 24725 produced more MnP than *P. chrysosporium* 34541. The ability of the four WRF to decolorized methylene blue (MB) and maxilon (Max.) have been reported after 7 and 10 days. *P. ostratus* decolorized MB with percentage range from 85.2% to 91.0% at concentration ranged from 25 mg/L to 700 mg/L M.B. *P. sajor-caju* was able to remove 26.1% of 25 mg/L MB after 7 days, this removal was increased to be 100% after 10 days. The range of removal was 83.6% to 90.3% for a range of 100 mg/L to 500 mg/L M.B. *P. chrysosporium* removed 88.4% to 97.2% of 25 mg/L to 700 mg/L MB *P. ostreatus* removed 100% of Max. till 150 mg/L after 10 days. However *P. sajor-caju* removed 100% of 150 mg/L Max. after 7 days. Free enzyme(s) of *P. sajor-caju* was able to decolorized MB and Max. completely after 12 hours, till 200 mg/L. Immobilized enzyme(s) of *P. sajor-caju* removed a range of 20-46.6% of 25-75 mg/L Max. after 2 hours. The growth results showed that growth of *P. sajor-caju* decreased gradually as dose of gamma radiation increased. Dose (0.25 KGy) enhanced MnP activity.

Key words: WRF · Laccase · MnP · Gamma radiation · Dye removal

INTRODUCTION

White rot fungi (WRF) are the most efficient degraders of lignin in nature. They are able to degrade a broad spectrum of structurally diverse organopollutants and produce several types of oxidative enzymes, which are useful for remediation of environmental pollutants [1-6]. *Phanerocheate chrysosporium,* a model organism for lignin and xenobiotics biodegradation studies produces a family of lip and MnP isoenzymes [7-10]. In addition, *P. chrysosporium* is also capable of degrading various azo, heterocyclic and polymeric dyes [11, 12]. This fungus produces extracellular ligninolytic enzymes such as lignin peroxidase (Lip) and manganese peroxidase (MnP) [6]. All *Pleurotus ostreatus* strains exhibited high laccase and manganese peroxidase activity [14]. Effluents generated by textile industry are of environmental concern because of the presence of dyes with complex molecular structure, which confer them recalcitrant characteristics. Biodegradation of textile effluent by *Pleurotus sajor-caju* have been studied [13]. Textile wastewater is rated as the most polluting among all industrial sectors. Important pollutant in textile effluents are mainly recalcitrant organics, colours, toxicants and inhibitory compounds, surfactants, chlorinated compounds and salts [15]. It has been proven that some of the dyes and/or products are carcinogenic and mutagenic [16-18]. Therefore, textile wastewater containing dyes must be treated before their discharge into the environment [19, 20]. Textile industry account for two thirds of the total dye stuff market [21,22]. White rot fungi in particular produce lignin peroxidase (Lip), manganese peroxidase and laccase that degrade

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many aromatic compounds due to their non specific enzyme systems [23-31]. Laccas enzymatic treatments have potential in a variety of industrial fields including textile industry. These systems are advantageous over physical and chemical treatments as enzymes are biodegradable catalyst and specific in action and enzymatic reactions are carried out in mild conditions [32]. Enzyme immobilization is advantageous as it simplifies enzyme recovery and therefore recyclibility for applications such as biocatalysis [33,34]. Immobilization has been shown to significantly improve the mechanical and chemical stability of enzymes [33-36]. It is though that these improvements are acquired through multipoint attachment of the enzyme during immobilization, which enhances the rigidity of the enzyme by stabilizing the tertiary structure of the protein [36]. Several studies on immobilization of laccase for potential applications such as decolourization have been published [37-39]. So, the aim of this study is comparing the abilities of white rot fungi, their free enzymes and their immobilized enzymes in decolourization of two dyes (methylene blue and maxilon), to be used in bioremediation technology of dyes.

MATERIALS AND METHODS

Microorganisms:

- *Pleurotus ostreatus* CBS 411-71
- *Pleurotus sajor-caju*
- *Phanerochaete chrysosporium* 1767 (ATCC 24725)
- *Phanerochaete chrysosporium* American Type Culture Collection (ATCC 34541)

White Rot Fungi (WRF) Maintenance: The four WRF were maintained on PDA Potato-Dextrose agar medium [40] slants and plates. The slants were stored at 4°C till use. The WRF strains were cultivated on Malt agar plates [41] for 14 days at 30°C. Mycelium agar plugs (10 mm in diameter) cut along the edge of an active growing colonies were used as inoculants.

Production of Ligninolytic Enzymes on Different Media: The four WRF basidomycetes were used to inoculate 50 ml in 250 ml conical flask of the three different production media: Basal medium I [41], N-limited medium II [42] and Defined medium III [43]. Two discs (10 mm in diameter) were used to inoculate each flask after sterilization of the media. Three replicates were used for each strain in each medium. For laccase production, the media was supplemented with 600 μM $CuSO_4$. $5H_2O$. While, for MnP production the media was supplemented with 1000 μM $MnSO_4.H_2O$. The flask for laccase production were incubated in dark without shaking, while that for MnP was incubated on shaking (150 rpm) incubator and both of them at 30°C for 14 days. Five ml from each flask was centrifuged at 8000 rpm for 5 minutes and the supernatant was used for enzyme assay.

Enzyme Assay: Laccase assay [44]. Laccase activity was carried out in 1 cm quartez cuvette. Reaction mixture of 1 ml contained 2 mM ABTS (2,2\, azino-bis (3-methyl benzo-thiazoline-6-sulphonic acid) diammonium salt, Sigma-Aldrich, USA in Mcllvaine buffer (pH 5.0). To the assay mixture 100 μl of centrifuged supernatant was added. The enzymatic activity was estimated in IU by monitoring the adsorbance change at 420 nm (ABTS), ε420 = 36 mM^{-1} cm^{-1} by spectrophotometer at 30°C. Manganese peroxidase (MnP) assay[44, 45]. MnP activity was carried out also in 1 cm quartez cuvette. The reaction mixture of 1 ml contained 2 mM ABTS and 1 mM of $MnSO_4$ in Mcllvaine buffer (pH 5.0). To the assay mixture, 100 μl of the centrifuged supernatant was added. The peroxidase activity was then initiated by the addition of 0.4 mM H_2O_2. The enzymatic activity was estimated in IU by monitoring the adsorbance change at 420 nm for ABTS ε420 = 36 mM^{-1} cm^{-1} by spectrophotometer at 30°C.

Decolorization of Dye: The four WRF were used to inoculate 50 ml of medium (I) in 250 ml conical flasks. Two discs were used for each flask. The flasks were supplemented with different concentrations (25, 50, 75, 100, 150, 200, 300, 500 and 700 mg/L) of methylene blue (M.B) basic dye (C.I 52015, λmax 678) or maxilon blue (Max.) basic dye (C.I 41, λ max 654), Mumbai, India. Three replicates were used for each strain in each concentration for each dye. The flasks were incubated in shaking (150 rpm) incubator at 30°C. The decolorization ratio has been determined after 7 and 10 days.

Production of Lignolytic Enzymes by Solid State Fermentation: According to Abo-State *et al.* [46] five grams of wheat bran (WB) or rice straw (RS) were moisited with 20 ml of moisting agent in 250 ml conical flask and sterilized by autocleaving at 121°C for 30 minutes. The flasks were inoculated with two discs (10 mm) of two of the WRF. Three replicates were used to each strain. The flasks were incubated for 14 days at 30°C. After incubation period, the content of the flasks were extracted

by 50 ml of distilled water. After shaking for 60 minutes, all the content was filtered in clean dry flask through muslin cloth on glass funnel. The filtrates were centrifuged at 8000 rpm for 10 minutes by cooling centrifuge. The supernatant crude enzymes were used for enzyme assay and decolorization of dyes.

Decolorization of Dyes by Free Enzymes of *Pleurotus sajor-caju* Produced on Solid State Fermentation: According to Lu *et al.* [38] and Deveci *et al.* [47] the crude enzymes produced by *P. sajor-caju* on (WB) by solid state fermentation were used to decolorize dyes. In a clean dry tubes a mixture of 300 µl of the crude enzymes and 2.7ml of the dyed in Mcllvaine buffer (pH 5.0) were incubated for zero, 10, 30, 60, 90, 120, 180 and 720 minutes. Three replicates were used for each dye in each concentration for each incubation period. In case of control, 300 µl of distilled water was used instead of the crude enzymes.

Decolorization of Dyes by Immobilized Enzymes: Crude enzymes were immobilized on controlled pore silica beads according to Robinson *et al.* [48], Paszczynski *et al.* [49], Germain and Crichton [50] and Van Aken *et al.* [51]. One gram activated CPC (A) was mixed with 5 ml or 10 ml crude enzymes (~ WB, WS, RS, 3 U/ml) resulted from production on WB, RS, WB + WS or WS by solid state fermentation (B). The activated immobilized enzymes resulted from mixing A with B were washed three times with Mcllvine buffer pH 5.0.These immobilized enzymes were mixed with 5 ml of maxilon dye in a test tubes with three concentrations (25, 50 and 75 mg/L). For each concentration and each enzyme, three replicates were used. Control tubes contained 5 ml of Maxilon dye without adding activated immobilized enzymes. All tubes were incubated in a water bath at 40°C for 2, 4 and 12 hours. The intensity of each tube was measured at 654 nm spectrophotometrically.

Effect of Gamma Irradiation on Growth and Enzymes Production by *P. sajor-caju*.

Growth: The well grown *P. sajor-caju* on malt agar plates (7 days) were sealed with parafilm and exposed to increasing doses of gamma radiation (0.25,0.50, 0.75, 1.0, 1.05,2.0 and 3.0 Kgy). Three replicates for each dose. From each plate, a disc (10 mm diameter) was used to inoculate a uniform malt medium in the centers of the plate. The inoculated plates were incubated at 30°C. Growth diameter was measured after 6 days.

Enzyme Production: The well grown mycelium colonies on malt agar plates were plugged (10 mm diameter) from the edge of the colonies of each dose and used to inoculate enzyme production medium I supplemented with 600 µM $CuSO_4$ or 1000 µM $MnSO_4$ for production of Laccase or MnP. Three replicates were used for each dose. The inoculated flasks for laccase were incubated at 30°C in dark without shaking, while that for MnP were incubated at 30°C in a shaking incubator (150 rpm) for 14 days. Laccase and MnP activities were determined as previously mentioned.

RESULTS AND DISCUSSION

White rot fungi (WRF) are the most efficient degraders of lignin in nature. Typical property of the WRF is their ability to degrade lignin and other aromatic compound with non specific extracellualr enzymes. Production of ligninolytic enzymes [Laccase and manganese peroxidase (MnP)] in different liquid media (submerged "SM") have been indicated in Table 1. Four strains of white rot fungi had been used for laccase and MnP production. These WRF were *Pleurotus ostratus*, *Pleurotus sajor-caju, Phanerochaete chrysosporium* (34541) and *Phanerochaete chrysosporium* (24725). For enzyme production three different liquid media were used namely I, II and III. The results revealed that

Table 1: Production of ligninolytic enzymes on different media by different white rot fungi (WRF)

	Laccase (U/ml)			Manganese peroxidase (U/ml)		
White rot fungi (WRF)	Media I	Media II	Media III	Media I	Media II	MediaIII
P. ostratus	0.165	0.190	0.165	0.360	0.309	0.288
P. sajor-caju	0.468	0.612	-ve	0.720	0.594	0.540
P. chrysosporium 34541	-ve	-ve	-ve	0.370	0.262	0.298
P. chrysosporium 24725	-ve	-ve	-ve	0.432	0.489	0.345

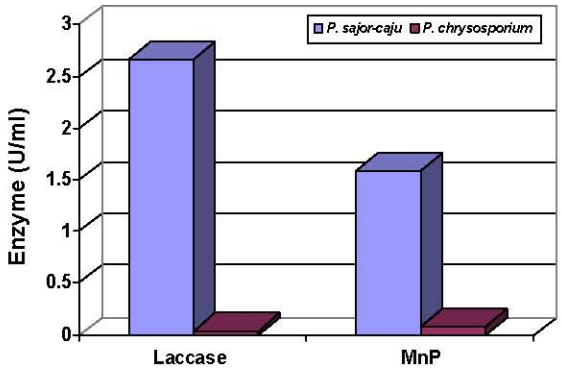

Fig. 1: Production of ligninolytic enzymes by solid state fermentation on wheat bran (WB) by *P. sajor-caju* and *P. chrysosporium*.

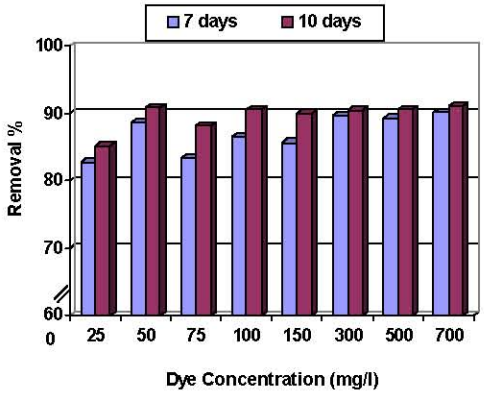

Fig. 2: Removal of methylene blue by *P. ostratus* after 7 and 10 days incubation period.

Pleurotus sajor-caju produced the highest laccase and MnP activities on the three media, except on medium III. Medium III have not support laccase production by *P. sajor-caju*. *P. sajor-caju* produced 0.468 and 0.612 u/ml laccase on medium I and II respectively. Also, *P. sajor-caju* produced 0.720, 0.594 and 0.540 u/ml MnP on medium I, II and III respectively. *P. ostratus* produced less ligninolytic enzymes than *P. sajor-caju* on the three media I, II and III. *P. chrysosporium* 34541 and 24725 produced MnP on the three media, but failed to produce any laccase on these media. *P. chrysosporium* 24725 produced more MnP than *P. chrysosporium* 34541.

Comparing, the three media used to support enzyme production, the result revealed that medium I gave the highest MnP (0.360, 0.720 and 0.370 U/ml) productivity for *P. ostratus, P. sajor-caju and P. chrysosporium* 34541, respectively. However, the highest MnP (0.489 u/ml) had been recorded on medium II by *P. chrysosporium* 24725. Medium II also gave the highest laccase activities (0.190 and 0.612 U/ml) by *P. ostratus* and *P. sajor-caju* respectively. From the above results it is clear that medium I and II can be used for ligninolyitc enzymes production and the best WRF was *P. sajor-caju*. The above results were confirmed by other investigators. *P. chrysosporium* is known to lack laccases in normal conditions but can produce them when the fungus is grown on high nitrogen cellulose medium [52]. *Ischnoderma resinosum* produced extracellular ligninolyitc enzymes laccase and MnP. The activity of laccase achieved the maximum on day 10 (29.4 U L^{-1}), the MnP on day 14 (34.5 UL^{-1}) [53]. *Pleurotus* species were reported to belong to a group of fungi that produces MnP and laccase but not lignin peroxidase [54].

The major components of the lignin-degrading enzyme system of *P. chrysosporium* are LiP and MnP with the expression during the secondary phase of fungal growth. This biochemical event is triggered by nitrogen or carbon limitation of the growth medium and the presence and activity of each of these two extracellular enzymes are highly dependent on culture conditions and composition of the growth medium [55]. Nitrogen-limited culture medium was used in the present study and the results obtained are in agreement with the studies that growth media containing low nitrogen concentrations generally provide the best nutrient conditions for the expressing ligninolytic enzymes by the fungus. Literature surveys show that the concentration of Mn^{2+} in the growth medium of *P. chrysosporium* affects the formation of LiP and MnP differently [56]. The formation of MnP is dependent on Mn^{2+} which has enhancing effect on transcription of MnP [57]. From the above results, *P. sajor-caju* and *P. chrysosporium* 24725 were used to investigate their abilities to produce ligninolyitc enzymes on wheat bran by solid state fermentation (SSF) as indicated in Fig. 1. *P. sajor-caju* produced 2.664 U/ml laccase and 1.584 U/ml MnP; while *P. chrysosporium* 24725 produced 0.036 U/ml laccase and 0.072 U/ml MnP. *G. lucidum* KMK2 strain produced high levels of laccase and very low levels of MnP during the 21 days incubation. High level of laccase production could be attributed due to the presence of ferulic acid in wheat bran [58].

The ability of the WRF to decolorize dyes had been studied. The ability of *P. ostratus* to decolorize different concentrations of methylene blue (MB) has been indicated in Fig. 2. The decolorization (removal) percentage was measured after 7 and 10 days. The result showed that as the incubation period increased the

decolorization percentage increased. However the percentage of decolorization (removal %) was ranged from 85.2% to 91.0% at concentrations ranged from 25 mg/L to 700 mg/L MB. This means that as, the concentration increased, the ability of *P. ostratus* to decolorize MB also increased, so the removal % increased for a wide range of concentrations (25-700 mg/L) MB. This may be attributed to the increasing in production of ligninolytic enzymes as the concentration of MB increased due to their stress on the mycelial cells of *Pleurotus ostratus*. The above results were confirmed by the work of other investigators. The white-rot fugus *Pleurotus pulmonarius* has been recently described as a good producer of lacase and able to decolourize textile dyes in submerged cultures [59]. Tychanowicz *et al.* [60] propose a solid state system to grow and produce laccase by *P. pulmonarius* as well as to study the capability of the fugus to decolourize several industrial dyes when cultivated in solid state cultures.

Amido black, congo red, trypan blue, methyl green, remazol brilliant blue R (RBB), methyl violet, ethyl violet and brilliant cresyl blue were completely decoloruized after 6 days of cultivation, while methylene blue and Roly R-478 were partially decolourized. The capability of fungal culture to decolourize industrial dye appears to be due to high titers of laccase (480 U/ml) produced in response to the presence of soluble phenolics [60]. Shaking has variable effects on laccase production. *Rhizoctonia practicola* gives similar lacase production in shaking and stationary cultures. In *P. cinnabarinus,* shaking increases laccase production whereas in many other white rot fungi, it is suppressed [61]. Extracellular lignin peroxidase (Lip) was not detected during decoloration of azo dye, Amaranth by *Trametes versicolor*. Approximately twice as much laccase and manganese peroxidase (MnP) was produced by decolorizing cultures compared to when no dye was added. At a low Mn^{2+} concentrations (3μM), N-limited (1.2mM NH^{+4}) cultures decolorized eight successive additions of Amaranth with no visible sorption to the mycelial biomass. At higher Mn^{2+} concentrations (200 μM), production of Mnp increased and that of laccase decreased, but the rate or number of successive Amaranth decolorizations was unaffected. There was always a 6-8 h lag prior to decoloration of the first aliquot of Amaranth, regardless of Mnp and laccase concentrations. Although nitrogen rich (12 mM NH^{4+}) cultures at an initial concentration of 200 μM Mn^{2+} produced high laccase and Mnp levels. The highest values for LiP (75376.34 IU L^{-1}), MnP (4484.30 IU L^{-1}) and laccase (898.15 UI L^{-1}) were obtained with the marine fungus *M. racemosus* CBMAI 847 and it is the first report concerning ligninolytic enzymes production by a zygomycete from this genus [53]. The ability of *P. sajor-caju* to decolorize M.B. was shown in Table 2. The results indicated that as the incubation period increased, the removal percentage increased. *P. sajor-caju* was able to remove 26.1% of 25 mg/L M.B after 7 days; this removal % was increased to be 100% of the same concentration after 10 days. *P. sajor-caju* was able to remove 100% of 75 mg/L M.B after 10 days. As, the concentrations of M.B increased more, the removal % decreased. The range of removal was 83.6% to 90.3% for a range of 100 mg/L to 500 mg/L M.B. So, as the concentrations of MB increased, *P. sajor-caju* ability to decolourize the dye slightly decreased. Biodegradation of textile effluents by *Pleurotus sajor-caju,* are of environmental concern because of the presence of dyes with complex molecular structure, which confer them recalcitrant characteristics. Indigo is one of the most widely used dyes within the textile sector and studies have suggested that *P. sajor-caju* may be capable of its biodegradation. The decolorization being evaluated after 14 days, when the process was observed. Enzymatic activities of laccase, peroxidase and manganese peroxidase were determined, the production of these ligninolytic enzymes being evident and a synergism among them being likely in the decolorizing process [13].

Table 2: Decolourization of methylene blue (MB) by *P. sajor-caju*

	Residual dye (mg/L)		% Removal	
Dye conc. mg/L	7 days	10 days	7 days	10 days
25	18.46	0.00	26.16	100
50	39.06	0.00	21.88	100
75	42.90	0.00	32.13	100
100	46.87	16.35	53.13	83.65
150	57.52	15.615	61.65	89.59
200	98.02	25.4	50.99	87.30
300	135.65	39.3	54.78	86.90
500	193.31	48.45	60.93	90.31

Table 3: Decolorization of methylene blue (M.B) by *P. chrysosporium* (34541)

	Residual of Dye (mg/L)		% Removal	
Dye conc. mg/L	7 days	10 days	7 days	10 days
25	6.4	3.4	74.4	86.4
50	7.8	3.9	84.4	92.2
75	8.7	3.6	88.4	95.2
100	8.6	4.9	91.4	95.1
150	15.4	7.3	89.7	95.1
300	29.0	10.7	90.3	96.4
500	37.8	12.7	92.4	97.4
700	38.6	32.6	94.4	93.3

Table 4: Decolourization of methylene blue (MB) by *P. chrysosporium* (24725)

	Residual of Dye (mg/L)		% Removal	
Dye conc. mg/L	7 days	10 days	7 days	10 days
25	3.9	2.9	84.4	88.4
50	4.2	3.0	91.6	94.0
75	5.5	2.1	92.6	97.2
100	7.0	5.3	93.0	94.7
150	9.0	4.3	94.0	97.1
300	25.5	15.4	91.5	94.8
500	40.6	32.8	91.8	93.4
700	47.9	41.8	93.1	94.0

Methylene blue (MB) removal by *P. chrysosporium* 34541 had been indicated in Table 3. As the incubation period increased, the removal percentage increased. Decolorization of MB was ranged from 74.4% to 94.4% at range of 25 mg/L to 700 mg/L after 7 days incubation by *P. chrysosporium* 34541. This means that *P. chrysosporium* was able to remove 671.4 mg/L from 700 mg/L of MB after 7 days. Decolorization of M.B was ranged from 86.4% to 97.4% at range of 25 mg/L to 700 mg/L after 10 days. As the concentration increased, the removal % increased. The ability of *P. chrysosporium* 24725 to decolorized M.B was shown in Table 4. Like the other WRF studied before, as the incubation period increased, the removal % increased. Decolorization of MB was ranged from 84.4% to 94.0% at a range of 25 mg/L to 700 mg/L of MB after 7 days incubation. The highest removal % was recorded at 150 mg/L of MB for both of the incubation periods. *P. chrysosporium* removed 141 mg/L and 145.7 mg/L of MB from 150 mg/L after 7 and 10 days respectively. The removal % was ranged from 88.4% to 97.2% at concentrations of M.B ranged from 25 mg/L to 700 mg/L after 10 days incubation.

The above results were in agreement with those obtained by Venkatadri and Irvine [62], they achieved 89-91% colour removal within 24 hours incubation when the concentration of the dye Vermelho Reanil P8B ranged between 16.0 and 20.0 mg/l, which resemble the range of concentrations found in industrial dye effluents. *Schizophyllum commune* also decolorized wastewater. The decolourization of three polymeric dyes polymeric B-411, polymeric R-481 and polymeric 4-606 (Sigma) by *P. chrysosporium* was confirmed by Glenn and Gold [1]. Their results suggested that the decolorization was a secondary metabolic activity linked to the fungus ligninolytic degradation activity. The process, however, was slow and optimum decolorization needed up to 8 days [2]. *Phanerochaete chrysosporium* was also shown to biodegrade the azo-and heterocyclic dyes orange II, tropaeofin O, Congo Red and Azune B [2]. The extent of colour removal varied depending on the dye complexity, nitrogen availability in the media and ligninolytic activity in the culture. At low nitrogen concentrations 90% of the colour was removed within the initial 6 h, while when excess nitrogen was provided, up to 5 days were required to achieve 63-93% decolorization of the above-mentioned dyes [2]. Capalash and Sharma [63] tested the biodegradation of 18 azo dyes using *P. chrysosporium* and only eight were degraded, with 40-70% colour removal. Kirby [64] has shown that *P. chrysosporium* had the ability to decolourize artificial textile effluent by up to 99% within 7 days. The value of residual dye for MB used at 60 ppm was 6% within 8 days of the incubation of the

nitrogen limited culture under the shaken conditions. Production of (Mnp) by *P. chrysosporium* occurred simultaneously in nitrogen limited culture medium with the added $MnSO_4$ at 100 ppm. The MnP activity was at relatively high level (170 U/I), the effect of Mn^{2+} added to the growth medium at different initial concentration was tested. At 10 and 30 ppm $MnSO_4$, the decolourization process was significant and the MB remaining after 6 days of the culture incubation was negligible [65].

Despite the findings of Kling and Neto [66] which showed MnP had no measurable role in the MB degradation by *P. chrysosporium,* interestingly they were able to detect manganese-dependent activity during the methylene blue decolourization process. The level of the decolourization of methylene blue for Kissiris-immobilized fungal cells did not change after 6 days of the incubation under shaking conditions. While 24% residual dye for the free cells after 6 days of the incubation gradually changed and a significant decrease in residual dye was observed by day 9 of the incubation. Culture agitation improved the decolourization ability of Kissiris-immobilized fungal cells considerably, although the repressive effect of culture agitation on the ligninase activity and lignin degradation by *P. chrysosporium* has been reported by Jager *et al.* [67] and Venkatadri and Irvine [68]. Decoloration of the synthetic textile dye reactive black 5 by the cultivated fungi, *Bjerkandera adusta, Irpex lacteus* and *Hypoxylon fragiforme* was studied. The most effective fungus in shaken flask experiments was *B. adusta*, which was able to decolorize the dye from black-blue to a yellow color in less than 10 days. The result showed that initially lignin peroxidases and subsequently manganese dependent peroxidases are presumably responsible for the decoloration of the diazo dye reactive black 5 by *B. adusta* [69].

The activity of MnP produced by the Kissiris-immobilized *P. chrysosporium* was highest at 7 days of the incubation (174 U/l). In a recent work the ability of *Lentinula edodes*, a white-rot fungus, to decolorize several synthetic dyes including methylene blue was studied using a solid-state culture system [70]. Their results show dependence of the decolourization rate on the concentration of methylene blue: the decolourization rate increased along with an increase in initial concentration of methylene blue until it reached a maximum value of 3.6 ppm day/1 at about 45 ppm, above which the rate decreased slightly. This decline was less noticeable when the cultures were incubated at the stationary mode. The initial concentration of methylene blue used in the present study was 10 ppm: the decolourization rate though was lower as compared to that of the higher initial concentration (40 ppm) but disturbance in the colour measurement assays was lower because less residual dye remained at the low initial concentration of the dye. To determine the influence of agitation on the decolourization process, the experiment with free mycelium and Kissirisimmobilized fungal cells was performed in static and shaken mode.

Irpex lacteus is a white rot fungus known to decolorize various synthetic dyes. Decolorization of other azo dye reactive orange 16 by immobilized cultures of *I. lacteus* was compared in three different reactor systems of laboratory size: small and large trickle bed reactors and a rotating disc reactor. The highest dye decolorization efficiency (90% in 3 days) was observed by *I. lacteus* in the small trickle bed reactor. A repeated batch performance test demonstrated the potential of immobilized fungal cultures to decolorize synthetic dyes over long time periods [71]. Methylene blue (MB) oxidation by Lip of *P. chrysosporium* has been reported by Kling and Neto [66] and Ferreira-Leitao *et al.* [72]. Despite the findings of Kling and Neto [66] which showed MnP had no measurable role in the M.B degradation by *P. chrysosporium*, Gandolfi-Boer *et al.* [70] concluded that MnP was the main agent responsible for the decolorization capacity of *L. edodes.*

The results shows dependence of the decolorization rate on the concentration of methelene blue (M.B). The decolorization rate increased along with an increase in initial concentrations until it reached a maximum value of 3.6 ppm day/L at about 45 ppm, above which the rate decreased slightly [65]. Decolorization of maxilon (Max.) dye by *P. ostratus* had been indicated in Table 5. As the incubation period increased the removal % increased. Meanwhile, as the concentration of maxilon increased, the removal % decreased. At the lower concentration still 150 mg/L Max., the removal % was 100% after 10 days. More increase in concentrations means more decrease in removal %. *P. ostratus* removed 35.4% and 62.8% of 700 mg/L Max. after 7 and 10 days, respectively. This means that *P. ostratus* was able to remove 248 mg/L and 440.2 mg/L of Max. from 700 mg/L after 7 and 10 days, respectively. Decolorization of Max. dye by *P. sajro-caju* had been shown in Table 6. *P. sajor-caju* was able to decolourize 100% of Max till 150 mg/L after 7 days incubation. As, the concentration increased, the removal % decreased. So, *P. sajor-caju* was more efficient in decolorization of maxilon than *P. ostratus* and reduced the time of incubation. *P. sajor-caju* removed 85.2% of 500 mg/L Max., while *P. ostratus* removed 73.6% of

Table 5: Decolourization of maxilon (Max) by *P. ostratus*

	Residual dye (mg/L)		% Removal	
Dye conc. mg/L	7 days	10 days	7 days	10 days
25	0.5	0.0	98.0	100
50	3.4	0.0	93.2	100
75	7.8	0.0	89.6	100
100	8.4	0.0	91.6	100
150	16.1	0.0	89.2	100
300	56.0	23.0	81.3	92.3
500	209.1	132.0	58.1	73.6
700	452.0	259.8	35.4	62.8

the same concentration after 10 days. *P. sajor-caju* removed 332.5 mg/L and 426 mg/L of Max from 500 mg/L after 7 and 10 days, respectively. Manganese peroxidase (Mnp) activity was detected during the decolouriuzation process. The dye could be decolorized by purified Mnp of *P. sordida* in the presence of Mn (II) and Tween 80. With shaking, the dye could be decolorized without the addition of hydrogen peroxide. The decolorization did not occur under anaerobic conditions, suggesting that dye decolorization by Mnp is influenced by dissolved oxygen [73]. When 200, 500, 750 and 1000 mg/l of dye were also treated for 7d, *P. sordida* decreased ΣOD by 90.7%, 89.4%, 85.6% and 75.9%. Adsorption of dye to mycelia after incubation for 7 d. was not found on all condition [73]. Biological decolorization of textile dyestuff basic blue 22 (C.I. 61512), aphthalocyanine type reactive dyestuff, by the white rot fungus *Phanerochate sordida* ATCC 90872 was studied. The highest decolorization efficiency was obtained with a rotational speed of 40 rpm. The minimum glucose concentrations for 78% decolorization efficiency by *P. sordida* was 59/l. TOC removal efficiency was around 80% for 50-200 mg/l initial dyestuff concentrations and decreased to 52% for 400 mg dye stuff/l [74].

White-rot fungus *Dichomitus squalens* has been found to easily decolorize synthetic dyes even at relatively high concentrations. For that the fungus could be promising for further biotechnological exploitation [75, 76]. It is known to secrete laccase and Mnp in submerged liquid cultures) [77, 78]. Both enzymes have been purified and characterized [78,79]. Four different white-rot fungi strains *Phanerochaete chyrsosporium*, *T. versicolor, Pleurotus ostreatus* and *Pleurotus sajor-caju* were used to investigate their capability to degrade the two reactive blue 4 and reactive red 2 dyes. Real textile waste water from a textile industry in Tanzania was furthermore treated by *Pleurotus flabellatus*, a fungus which was isolated from the environment in Tanzania [80]. The study focuses

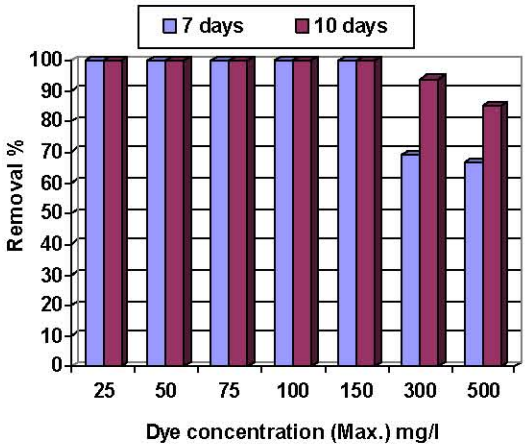

Fig. 3: Removal of maxilon (Max.) by *P. sajor-caju* after 7 and 10 days incubation period.

on the production of ligninolytic enzymes and dye degradation capacity of *Dichomitus squalens* immobilized on polyurethane foam (PUF) or pine wood (PW) in a fixed bed reactor at a laboratory scale. Immobilization of fungal cultures on pine wood improved eminently laccase production in comparison to the liquid cultures. Immobilized *D. squalens* was able to decolorize an anthraquinone dye remazol brilliant blue R and an azo dye reactive orange 16. The involvement of a laccase activity in dye decolorization was suggested by Susla *et al.* [81].

The decolorization of 12 different azo, diazo and anthraquinone dyes was carried out using a new isolated white rot fungus, strain L-25. A decolorization efficiency of 84.9-99.6% was achieved by cultivation in 14 days using an initial dye concentration of 40 mgL^{-1}. The strain L-25 produces manganese peroxidaes (Mnp) as its major ligninolytic enzyme. The adsorption of dye by cells was observed during the decolorization at the beginning of the process. However, this color disappeared when Mnp was released by the strain L-25 [82].

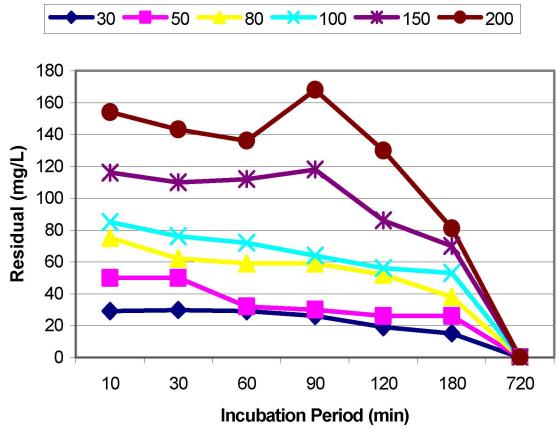

Fig. 4: Residual of maxilon after treatment withy free enzyme of *P. sajor-caju* on WB by SSF.

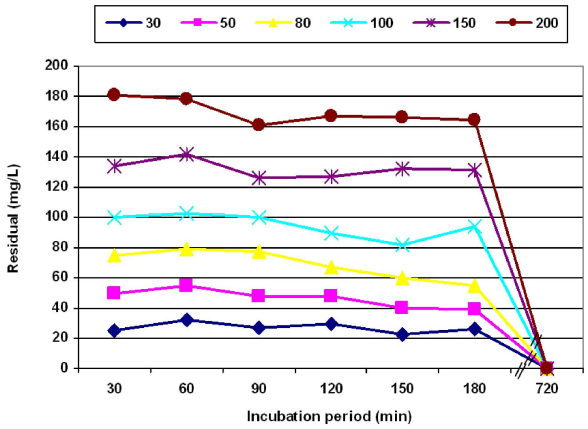

Fig. 6: Residual of methylene blue (M.B) after treatment with free enzyme of *P. sajor-caju* on WB by SSF.

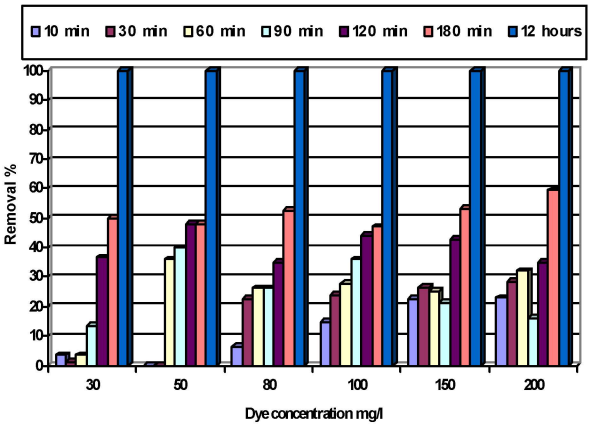

Fig. 5: Removal of maxilon (Max.) by free enzyme produced by *P. sajor-caju* on WB by SSF.

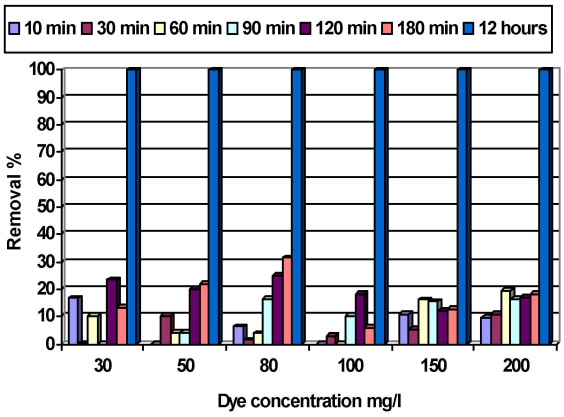

Fig. 7: Removal of methylene blue by free enzymes produced by *P. sajor-caju* opn WB by SSF.

The ability of free enzyme(s) produced by *P. sajor-caju* on wheat bran (WB) by SSF to decolorize Max. was shown in Fig. 4. The results revealed that as the incubation period increase the residual of Max. dye decreased as in Fig. 4. Three hours removed 50% of 30 mg and 59.5% of 200 mg/L Max. Twelve hours removed completely all concentrations of Max. as indicated in Fig. 5. The results also revealed that as the incubation period increased, the removal% increased. The ability of free enzyme(s) produced by *P. sajor-caju* on wheat bran (WB) by SSF to decolorize M.B had been indicated in Figs 6 and 7. The free enzyme(s) was incubated with different concentrations of MB for 10, 30, 60, 90, 120, 180 and 720 minutes. The residual of the MB dye was indicated in Fig. 6. Free enzyme (s) of *P. sajor-caju* was able to decolorize MB completely after 12 hours till 200 mg/L of M.B. After 3 hours, *P. sajor-caju* free enzyme(s) removed from 6.0 to 31.0% of a range of 30-200 mg/L M.B as indicated in Fig. 7. *P. sajor-caju* free enzyme(s) removed 36 mg/L of 200 mg/L MB after 3 hours incubation. However, after 12 hours, incubation of *P. sajor-caju* free enzyme(s) with different MB concentration led to complete decolorization (colourless) at all the different concentrations i.e., 100% removal.

Comparing decolorization of 200 mg/L MB by *P. sajor-caju* as an organism and the free enzyme(s) of the same organism, the results indicated that 12 hours completely removed 200 mg/L MB in case of using free enzyme(s), while the microorganism removed 87.3% of MB after 10 days incubation period. Also comparing decolorization of 150 mg/L Max. by *P. sajor-caju*s as organism and the free enzyme(s) of the same organism, the results showed that complete decolorization was achieved after 7 days incubation period in case of using the organism. However, complete decolorization of Max. was achieved after 12 hours in case of using free

enzyme(s).The above results revealed that free enzymes were more efficient in bioremediation of dyes than microorganisms. The results of the *in vitro* decolourization (i.e., crude cell free extract with or without $MnSO_4$ and H_2O_2) showed methylene blue decolourization when $MnSO_4$ (1 mM) and H_2O_2 (0.1 mM) were both present. Significant reduction in the decolourization was observed when $MnSO_4$ or H_2O_2 were omitted from the decolourization medium. The authors concluded that MnP was the main agent responsible for the decolorizing capacity of *L. edodes* [70].

Mn-peroxidase was found in some SOP-feedstuff mixtures, its activity being highest on barley with *P. pulmonarius* (2.4 IU g^{-1}) and on flour shorts with *P. pulmonarius* and *P. ostreatus* into all SOP feedstuff mixtures and highest activity levels were observed on wheat bran (12.5±4.0 IU g^{-1}) and wheat flour shorts (13.5±4.0 IU g^{-1}), respectively. Although both fungi are reported to produce the H_2O_2 producing enzyme aryl alcohol oxidase, an ancillary component of their lignin degrading systems [51]. Dye decolorizing potential of the white rot fungus *Ganoderma lucidum* KMK2 was demonstrated for recalcitrant textile dyes. *G. lucidum* produced laccase as the dominant lignolytic enzyme during solid state fermentation (SSF) of wheat bran (WB), a natural lignocellulosic substrate. Crude laccase produced by *G. lucidum* KMK2 enzyme shows excellent decolorization activity to anthraquinone dye Remazol Brilliant Blue R (RBBR) without redox mediator whereas diazo dye Remazol Black-5 (RB-5) requires a redox mediator [83]. RB-5 (50mgl^{-1}) was decolorized by 62% and 77.4% within 1 and 2h, respectively by the crude laccase (25Uml^{-1}). RBBR (50mgl^{-1}) was decolorized by 90% within 20h, however, it was more efficient in presence of HBT showing 92% decolorization within 2h. Crude laccase showed high thermostability and maximum decolorization activity at 60°C and pH 4.0. The decolorization was completely inhibited by the laccase inhibitor sodium azide (0.5mM). Enzyme inactivation method is a good method which averts the undesirable color formation in the reaction mixture after decolorization. High thermostability and efficient decolorization suggest that this crude enzyme could be effectively used to decolorize the synthetic dyes from effluents [83]. Similar results had been reported by Murugesan *et al.* [83]. They found that Dye decolorization potential of white rot fungus *Gandoderma lucidum* KMK2 was demonstrated for recalcitrant textile dyes. *G. lucidum* produced laccase as the dominant lignolytic enzyme during solid state fermentation (SSF) of wheat bran (WB), a natural lignocellulosic substrate. Crude enzyme shows excellent decolorization activity to anthraquinone dye Remazol brilliant blue R (RBBR) without redox-mediator, whereas diazo dye remazol black-5 (RB-5) requires redox mediator. RB-5 (50 mg/L) was decolorized by 62% and 77.4% within 1 and 2 hours, respectively by crude laccase (25 U/ml). RBB (50 mg/L) was decolorized by 90% within 20 h, however, it was more efficient in presence of HBT showing 92% decolorization within 2 hours. Also Rodriguez *et al.* [14] confirmed the above results. All *Pleurotus ostreatus* strains exhibited high laccase and manganese peroxidase activity, but highest laccase volumetric activity was found in *Tremetes hispida* [14]. Only laccase activity correlated with the decolorization activity of the crude extracts. Two laccase isoenzymes from *Trametes hispida* were purified and their decolorization activity was characterized [14]. The treatment of dyes with the culture liquid from *I. resinosum* cultures containing the same total amount of ligninolytic enzymes (25 mU/mL laccase and 115 mU/mL MnP) resulted generally in higher decolorization than the treatment using individual enzymes. Interestingly, this was independent on the presence of Mn and H_2O_2 indicating the insignificant involvement of MnP in the decolorization by total culture liquid. The decolorizaton was significantly improved by the addition of laccase synthetic mediators violuric acid and hydroxybenzotriazole. These compounds increased the decolorization to 80-90% after a 24 h treatment. In the case of RK and RY decolorization around 80% was already achieved after 1 h incubation.

The activity of Mnp in the cultures was over 1.0 U/ml at the end of cultivation. Meanwhile, Mnp produced by strain L-25 was used for the enzymatic decolorization of the dyes thus confirming the capability of the enzyme for this purpose [82]. Both enzyme forms catalyzed not only the conversion of typical peroxidase substrates such as 2, 6-dimethoxyphenol and 2, 2'-azino-bis (3-ethylthiazoline-6-sulfonate) but also the decolorization of the high-redox potential dyes Reactive Blue 5 and Reactive Black 5, whereas manganese (II) ions (Mn^{2+}) were not oxidized [84]. The potential of coconut flesh as a raw material for the production of extracellular laccase by the white rot fungus *Trametes hirsute* was investigated. For this, decolourizatoin of the textile dye lissamie green B was carried out both *in vitro* and *in vivo*. The former led to a decolourization percentage between 42% and 66% in 12 h depending on the culture age, whereas the latter showed a percentage higher than 96%

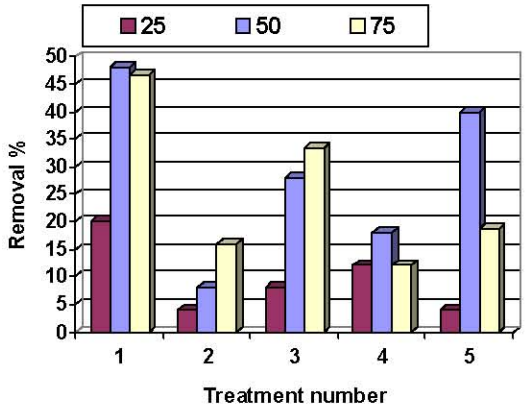

Fig. 8: Decolourization of maxilon (Max.) by immobilized enzymes (after two hours)

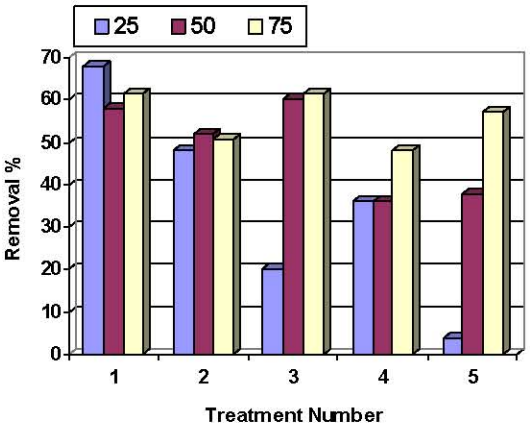

Fig. 9: Decolourization of maxilon (Max.) by immobilized enzymes (after four hours)

in 2-5h [85]. Decolorization of Maxilon dye by immobilized enzyme(s) was indicated in Figs 8 and 9. The treatment no. 1 to 5 revealed that the immobilized enzyme(s) resulted from cultivation of *P. sajor-caju* on WB, WS, RS and WB/WS by SSF and the resulted free enzyme(s) was immobilized on CPC activated carrier in a ratio (35 ml enzyme: 3 gram CPC) to give treatment No. 1. Treatment No. 2 represented the ratio (15 ml enzyme: 3 gram of CPC. Treatment No. 3, 4 and 5 was the result of free enzyme produced by *P. sajor-caju* on WS, RS and WB/WS with ratio (35 ml enzyme: 3 gram CPC), respectively.

The results showed that, as the incubation period increased, the decolorization of Max. increased. Immobilized enzyme(s) of *P. sajor-caju* removed a range of 20-46.6% of 25-75 mg/L Max. after 2 hours incubation. As the incubation period increased to be 4 hours, the removal % range increased at a range of 61.3% to 68.0% at the same concentrations of Max. The highest removal % had been recorded in case treatment No. "1". This means that, the highest ligninolyitc enzymes produced by *P. Sajor-caju* on WB followed by WS. The production of Ligninolytic enzymes on the agriculture wastes was in the following order WB > WS > WB/WS > RS. Also, the ratio of enzyme: activated carrier (CPC) was another factor influencing the immobilized enzyme(s). As the ratio of enzyme(s): CPC increased, the efficiency in decolorizing Max. increased. Comparing, the decolorization percentage by free and immobilized enzymes of the same organism (*P. sajor-caju*) at nearly the same concentrations, the results revealed that free enzyme produced on WB removed 35%-48% of 30-80 mg/L Max. after 2 hours, while immobilized enzyme(s) removed from 20% to 46.6% of 25-75 mg/L Max. at the same time. The results of immobilized enzymes also revealed that all immobilized enzymes treatments were able to decolorize the different concentrations of dye completely after 12 hours. When comparing the results of free enzyme(s) with that of immobilized enzyme(s) especially in the field of bioremediation of pollutants, immobilized enzyme(s) was superior, because immobilized enzyme(s) can be easily recovered and reused more than one time while free enzyme used once and lost. So the recent research in the field of biotechnology preferred to use immobilized enzyme in waste treatment. The results of this study were confirmed by a number of other investigators. They explain why immobilized enzyme was favorable than free enzyme. Immobilization of enzymes makes these biocatalysts reusable and this turns the enzyme based process into a more economically viable approach [85, 86]. Quite frequently, the immobilized enzyme is more stable towards harsh conditions like high temperatures [86]. This allows the bioctatalyst to survive longer during process conditions and allows one to operate the process at higher temperature so that arrhenious factor enhances reaction rates [87].

The use of immobilized laccase might be a suitable method to overcome such legal barriers as in this form it may be classified as technological aid. So laccase could find application in preparation of must and wine and in fruit juice stabilization [88, 89]. Although white-rot fungi have been shown to decolorize dyes in liquid fermentations, enzyme production has also been shown to be unreliable to the unfamiliar environment of liquid fermentations. The ability to utilize these fungi in their natural environment means that they are more likely to be more effective in solid state fermentation (SSF) [24]. Immobilized horseradish peroxidase enzyme (HRP), was investigated to degrade orange II (azo dye) within a two

compartment packed bed flow to evaluate the electroenzymatic degradation of orange II, electrolytic experiments were carried out with 0.42U/ml HRP at-0.5 Volt. It was found that removal of orange II was partly due to its adsorption to the graphit felt. The overall application of the electroenzymatic led to a greater degradation rate than the use of electrolysis alone [90]. Acid azo dye (Acid black 10 BX) dye removal by plant based peroxidase catalyzed reaction was investigated in both free and immobilized form. Mohan *et al.* [91] revealed that dye (substrate) concentration, aqueous phase pH, enzyme and H_2O_2 dose play significant role on the overall enzyme mediated reaction. Acrylsamide gel immobilized HRP showed effective performance compared to free HRP and alginate entrapped HRP. Alginate entrapped HRP showed inferior performance over the free enzyme due to the consequence of non availability of the enzyme to the dye molecule due to polymeric immobilization standard plating studies performed with *Pseudomonas putida* showed enhanced degradation of HRP catalyzed dye compared to control [91]. Three level Box-Behnken factorial design with three factors (pH, temperature and enzyme concentration) combined with response surface methodology (RSM) was applied to optimize the dye degradation of reactive red 239 (RR239), reactive yellow 15 (RY 15) and reactive blue 114 (RB 114) dyes by commercial laccase [92].

The decolourization of different synthetic dyes (indigo carmine, bromophenol blue, methyl orange and poly R-478) by the white-rot fugus *Tremetes hirsute* at bioreactor scale under solid state conditions, operating with ground orange peelings as a support substrate. Dye decolourization was performed in both batch and continuous mode. Batch cultivation led to high decolourization percentages in a short time (100% for Indigo carmine in 3 h and 85% for bromo phenol blue in 7h). As for continuous cultivation, different hydraulic retention times (HRT) where studied (0.8, 1, 1.5 and 3d). The highest decolourization percentages were obtained operating at a HRT of 3d especially for the dyes methyl orange and poly-R-478 (81.4% and 46.9%, respectively). This is a very interesting result, since there are few studies dealing with the continuous decolorization of dyes at bioreactor scale by fungal laccases [93]. The laccase family of the basidiomycete fungus *Pleurotus ostreatus* represents a variegated group of enzymes [94] having relevant roles in biotechnological applications of this fungus [95, 96]. Laccase was successfully self-immobilized into particles using spherezyme methodology, with retention of 53.9% of original enzyme activity.

Table 6: Effect of gamma radiation on growth of *Pleurotus sajor-caju*

Dose kGy	Growth diameter (mm)
Control	75
0.25	61
0.50	46
0.75	31
1.0	22
1.5	24
2.0	2
3.0	1

Table 7: Effect of gamma radiation on ligninolyitc enzyme production

Dose kGy	Laccase (u/ml)	MnP (u/ml)
Control	1.800	0.270
0.25	0.828	0.345
0.50	0.450	0.273
0.75	0.406	0.190
1.0	0.442	0.129
1.5	0.360	0.126
2.0	0.453	0.090
3.0	0.561	0.036

The immobilised preparation displayed enhanced stability during temperature incubation at 60-70°C with an improvement of 2.04-and 4.03-fold, respectively for ethanolamine post-treated spherezymes and polyethyleneimine coated spherezymes. Furthermore, enhanced stability at acidic pH was observed, with the most pronounced enhancement of 2.49-fold obtained at pH 2 and 1.69 and 1.38 at pH 3 and 4, respectively [97]. Effect of gamma radiation on growth and ligninolyitc enzyme(s) production of *P. sajor-caju* was indicated in Tables 6 and 7. The results showed that growth of *P. sajor-caju* decreased gradually as the doses of gamma radiation increased as indicated in Table 6 and Fig. 10. Dose 4 kGy reduced *P. sajor-caju* growth completely. Gamma radiation reduced the ability of *P. sajor-caju* to produce laccase at all doses used as indicated in Table 7 and Fig. 11. Laccase activities of the different dose were lower than that of the control. But surprisingly, dose 3 kGy which had a little or nearly no growth gave a detect laccase activity (0.561 u/ml). However, gamma radiation affected MnP activities with increasing the dose. Low doses (0.25 and 0.5 kGy) enhanced or nearly equal in MnP activities to the control. However, as the dose increased a MnP activity decreased. The reduction of growth and influence of enzymes production by gamma radiation had been recorded by other studies. These studies confirmed the findings of the present study as follows: Radiation reduced the viable count of bacteria and fungi.

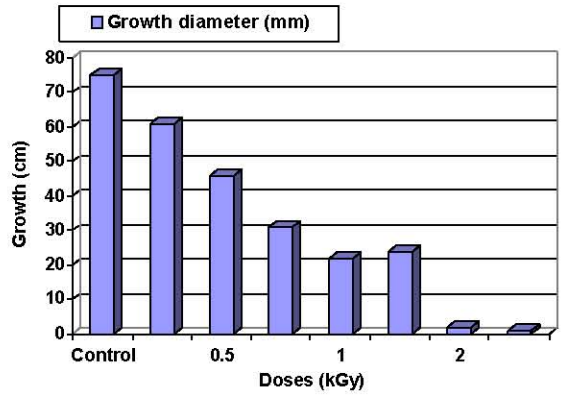

Fig. 10: Effect of gamma radiation on growth of *P. sajor-caju.*

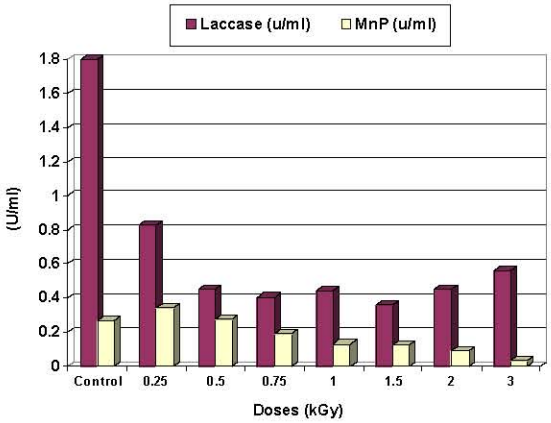

Fig. 11: Effect of gamma radiation on production of ligninolytic enzymes production by *P. sajor-caju.*

As the dose increased the viable count decreased gradually [98-101]. Aziz and Mahrous [102] recorded that the dose required for complete inhibition of fungi ranged from 4.0 to 6.0 kGy. El-Batal and Abo-State [103] found enhanced productivity in CMCase, FPase, Avicelase, xylanase, pectinase, α-amylase and protease by gamma-irradiation at dose 1.0 kGy with percent increase 8, 20, 10, 4, 31, 22 and 34%, respectively as compared with un-irradiated control. Also, the highest CMCase activity was recorded for *Fusarium neoceras* mutant No. 1"1 and No. "6" which exposed to 1 min UV-radiation. While, the highest CMCase of *F. oxysporum* was mutant No. "4" which exposed to 4 min. UV-radiation [100]. Mutant No. "36" which exposed to 10 kGy produced the highest extracellualr protein and xylanase activity (700µg/ml and 9993 U/g).This hyper producer mutant which exposed to 4 min UV-irradiation produced 10.350 U/g xylanase compared with the parent strain which produced 9.651 U/g [101]. Rajoka [104] reported 1.6 fold enhanced productivity of extracellualr endoglucanase of the mutant over the parent. After the optimization, the FPase in *T. reesei* MCG77 mutant was increased by 2.5 folds compared to that of *T. reesei* QM9414 mutant [105]. Also, Abo-State *et al.* [106,107] found that the most potent strains *Aspergillus terreus* MAM-F23 and *Aspergillus flavus* MAM-F35 for cellulases production were exposed to increasing doses of gamma radiation to determine their dose response curve. Gamma radiation reduced, the viable count of *Aspergillus* MAM-F23 and MAM-F35 gradually decreased as the dose increased. Doses 5.0 and 4.0 kGy reduced the viable count of *Aspergillus* MAM-F23 and MAM-F35 completely. Mutant No. "4" of *Aspergillus* MAM-F23 which exposed to 0.5 kGy produced higher cellulases (MCMase, 372 u/ml, FPase, 64 u/ml and Avicellase 39 u/ml) than the parent strain (MCMase, 305 u/ml, FPase, 48 u/ml and Avicellase 29 u/ml).

REFERENCES

1. Glenn, J.K. and M.H. Gold, 1983. Decolorization of several polymeric dyes by the lignin-degrading basidomycete *Phanerochaete chrysosporium.* Appl. Environ. Microbiol., 45: 1741-1747.
2. Cripps, C., J.A. Bumpus and S.D. Aust, 1990. Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium* Appl. Environ. Microbiol., 56: 1114-1118.
3. Dietrich, D., W.J. Hickey and R. Lamar, 1995. Degradation of 4, 4'-dichlorobiphenyl, 3, 3', 4, 4'-tetrchlorobiphenyl and 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl by the white rot fungus *Phanerochaete chrysosporium.* Appl. Environ. Microbiol., 61: 3904-3909.
4. Colombo, J.C., M. Cabello and A.M. Arambarri, 1996. Biodegradation of aliphatic and aromatic hydrocarbons by natural soil microflora and pure cultures of imperfect and lignolytic fungi. Environ. Poll., 94: 355-362.
5. Call, H.P. and I. Mucke, 1997. History, overview and applications of mediated lignolytic systems, especially laccase-mediator systems (Lyanozime-process). J. Biotechnol., 53: 163-202.
6. Hatakka, A., 2001. Biodegradation of lignin. In: M. Hofrichter A. Steinbuchel, (Eds.). Biopolymers. Vol. 1: lignin, humic substance and coal. Weinheim, Germany: Wiley-VCH, pp: 129-80.
7. Tien, M. and T.K. Kirk, 1988. Lignin peroxidase of *Phanerochaete chrysosporium.* Methods Enzymol., 161: 238-249.

8. Holzbaur, E., A. Andrawis and M. Tien, 1991. Molecular biology of lignin peroxidases from *Phanerochaete chrysosporium*. In: S.A. Leong and R.M. Berka, (Eds), Molecular industrial Mycology. Systems and Applications for Filamentous Fungi. Marcel Dekker, New York, pp: 197-223.
9. Chao, W.L. and S.L. Lee, 1994. Decoloration of azo dyes by three white-rot fungi: Influence of carbon source. World J. Microbiol., 10: 556-559.
10. Reddy, C.A., 1995. The potential for white-rot fungi in the treatment of pollutants. Curr. Opp. Biotechnol., 6: 320-328.
11. Ollikka, P., K. Alhonmaki, V.M. Leppanen, T. Glumoff, T. Raijola and I. Suominen, 1993a. Decolorization of azo, triphenyl methane, heterocyclic and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. Biotechnol., 53: 249-254.
12. Spadaro, J.T. and V. Renganathan, 1994. Peroxidase-catalyzed oxidation of azo dyes mechanism disperse yellow 3 degradation. Arch. Biochem. Biophys., 312: 301-307.
13. Kamida, H.M., L.R. Durrant, R.T.R. Monteiro and E.D.D. Armas, 2005. Biodegradation of textile effluents by *Pleurotus sajor-caju*. Quim. Nova [Online]., 28: 629-632.
14. Rodriguez, E., M.A. Pickard and R. Vazquez-Duhalt, 1999. Industrial dye decolorization by laccases from ligninolytic fungi. Curr. Microbiol., 38: 27-32.
15. Sen, S. and G.N. Demirer, 2003. Anaerobic treatment of real textile wastewater with a fluidized bed reactor. Water Res., 37: 1868-1878.
16. Manu, B. and S. Chaudhari, 2003. Decolourization of indigo and azo dyes in semi continuous reactors with long hydraulic retention time, Proc. Biochem., 38: 1213-1221.
17. Kornaros, M. and G. Lyberators, 2006. Biological treatment of wastewater from a dye manufacturing company using a trickling filter, J. Hazard. Mater., 136: 95-102.
18. Sudarjanto, G., B.K. Lehmann and J. Keller, 2006. Optimization of integrated chemical-biological degradation of a reactive azo dye using response surface methodology. J. Hazard. Mater., B138: 160-168.
19. Kim, T.H., Y. Lee, J. Yang, B. Lee, C. Park and S. Kim, 2004. Decolourization of dye solutions by a membrane bioreactor (MBR) using white rot fungi. Desalination, Strategies in South Mediterranean Countries, 168: 287-293.
20. Tantak, N.P. and S. Chaudhari, 2006. Degradation of azo dyes by sequential Fenton's oxidation and aerobic biological treatment. J. Hazard. Mater., B136: 698-705.
21. Fang, H., H. Wenrong and L. Yuezhong, 2004. Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium. Chemosphere, 57: 293-301.
22. Asad, S., M.A. Amoo Zegar, A.A. Pourbabaee, M.N. Sarbolouki and S.M.M. Dastgheib, 2007. Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Biores. Techn., 98: 2082-2088.
23. Stolz, A., 2001. Basic and applied aspects in the microbial degradation of azo dyes. Appl. Microbiol. Biotechnol., 56: 69-80.
24. Robinson, T., B. Chandran and P. Nigam, 2001a. Studies on the production of enzymes by white rot fungi for the decolorization of textile dyes. Enzyme Microbial. Technol., 29: 575-579.
25. McMullan, G., C. Meehan, A. Conneely, N. Kirby, T. Robinson, P. Nigam, I.M. Banat, R. Marchant and W.F. Snyth, 2001. Microbial decolourization and degradation of textile dyes. Appl. Microbiol. Biotechnol., 56: 81-87.
26. Toh, Y.C., J.J.L. Yen, J.P. Obbard and Y.P. Ting, 2003. Decolourization of azo dyes by white rot fungi (WRF) isolated in Singapore. Enzyme Microbial. Technol., 33: 569-575.
27. Wesenberg, D., I. Kyriakides and S.N. Agathos, 2003. White-rot fungi and their enzyme for the treatment of industrial dye effluents. Biotechnol. Adv., 22: 161-187.
28. Forgacs, E., T. Cserhati and G. Oros, 2004. Removal of synthetic dyes from wastewaters: A Review. Environ. Int., 30: 953-971.
29. Pazarlioglu, N.K., R.O. Urek and F. Ergun, 2005. Biodecolourization of direct blue 15by immobilized *Phanerochaete chrysosporium*. Process Biochemistry, 40: 1923-1929.
30. Mohorcic, M., S. Teodorovic, V. Golob and J. Friedrich, 2006. Fungal and enzymatic decolourization of artificial textile dye baths. Chemosphere, 63: 1709-1717.
31. Madhavi, S., S. Revankar and S. Lele, 2007. Synthetic dye decolorization by white rot fungus, *Ganoderma sp.* WR-1 Biores. Technol., 98: 775-780.
32. Arora, D.S. and R.K. Sharma, 2010. Ligninolytic fungal laccase and their biotechnological application. Appl. Biochem. Biotechnol., 160: 1760-1788.

33. Foresti, M.L. and M.L. Ferreira, 2007. Chitosan-immobilized lipases for the catalysis of fatty acid esterifications. Enzyme Microbial. Technol., 40: 769-77.
34. Hara, P., U. Hanefeld and L.T. Kanerva, 2008. Sol-gels and cross-linked aggregates of lipase PS from *Burkholderia cepacia* and their application in dry organic solvents. J. Mol. Catal. B: Enzymatic., 50: 80-86.
35. Chao, L., L. Van Langen and R.A. Sheldon, 2003. Immobilized enzymes: carrier-bound or carrier-free? Curr. Opin. Biotechnol., 14: 387-94.
36. Rodriguez, D.S., A.A. Mendes, W.S. Adriano, L.R.B. Goncalves and R.L.D. Giordano, 2008. Multipoint covalent immobilization of microbial lipase on chitosan and agarose activated by different methods. J. Mol. Catal. B. Enzymatic., 51: 100-9.
37. Zille, A., T. Tzanov, G.M. Gubitz and A.C. Avaco-Paulo, 2003. Immobilization laccase for decolourization of reactive black 5 dying effluent. Biotechnol. Lett., 5: 1473-1477.
38. Lu, L., M. Zhao and Y. Wang, 2007. Immobilization of laccase by alginate chitosan microcapsules and its use in dye decolorization. World J. Microbiol. Biotechnol., 23: 159-166.
39. Kunamneni, A., I. Ghazi, S. Camarero, A. Ballesteros, F.J. Plou and M. Alcalde, 2008. Decolorization of synthetic dyes by laccase immobilized on epoxy-activated carriers. Process Biochem., 43: 169-178.
40. Oxoid, 1982. Manual of culture media, Ingredients and other laboratory services. Published by Oxoid limited, Wade Road, Basing stoke, Hampshire RG240PW. UK.
41. Zouari-Mechichi, H., T. Mechichi, A. Dhouib, S. Sayadi, T.A. Martinez and M.J. Martinez, 2006. Laccase purification and characterization from *Trametes trogii* isolated in Tunisia: decolorization of textile dyes by the purified enzyme. Enzyme and Microbial Technol., 39: 141-148.
42. Rogalski, J., J. Szczodrak and G. Janusz, 2006. Manganese peroxidase production in submerged cultures by free and immobilized mycelia of *Nematoloma frowardii*. Biores. Technol., 97: 469-476.
43. Mansur, M., T. Suarez, J.B. Fernandez-Larrea, M.A. Brizuela and A.E. Gonzalez, 1997. Identification of laccase gene family in the new lignin degrading basidomycete CECT 20197. Appl. Environ. Microbiol., 63: 2637-2647.
44. Grazillo, A.M., M.C. Colao, V. Buonocore, R. Oliva, L. Falciqno, M. Saviano, A.M. Santoro, R. Zappala, R.P. Bonomo, C. Bianco, P. Giardina, G. Palmieri and G. Sannia, 2001. Structural and kinetic characterization of native laccases from *Pleurotus ostreatus*, *Rigidoporus lignosus* and *Trametes trogii*. J. Protein Chem., 20(3): 191-201.
45. Field, J.A., R.H. Vledder, J.G. Van Zelst and W.H. Rulkens, 1996. The tolerance of lignin peroxidase and manganese-dependent peroxidase to miscible solvents and *in vitro* oxidation of anthracene in solvent: water mixtures. Enzyme and Microbial Technology, 18: 300-308.
46. Abo-State, M.A.M., M.A.M. Hammad, M. Swelim and R.B. Gannam, 2010. Enhanced production of cellulose (S) by *Aspergillus spp*. Isolated from Agriculture Wastes by Solid State Fermentation. American-Eurasian J. Agric. Environ. Sci., 8(4): 402-410.
47. Deveci, T., A. Unyayar and M.A. Mazmanci, 2004. Production of remazol brilliant blue R decolorizing oxygenase from culture filtrate of *Funalia trogii*. J. Mol. Catalysis. B. Enzymatic, 30: 25-32.
48. Robinson, P.J., P. Dunnil and M.D. Lally, 1971. Porous glass as a solid support for immobilization nor affinity chromatography of enzymes. Biochem. Biophys. Acta., 242: 659-661.
49. Paszczynski, A., V.B. Huynh and R. Crawford, 1986. Comparison of ligninase-I and peroxidase-M2 from the white-rot fungus *Phanerochaete chrysosporium*. Arch. Biochem. Biophys., 244: 750-765.
50. Germain, P. and R.R. Crichton, 1988. Characterization of chemically modified β-amylase immobilized on porous silica. J. Chem. Tech. Biotechnol., 41: 297-315.
51. Van Aken, B., P. Ledent, H. Naveau and N.S. Agathos, 2000. Co-immobilization of manganese peroxidase from *Phlebia radiate* and glucose oxidase from *Aspergillus niger* on Porous silica beads. Biotechnol. Lett., 22: 641-646.
52. Srinivasan, C., T.M. D'souza, K. Boominathan and C.A. Reddy, 1995. Demonstration of laccase in the white rot basidiomycete *Phanerochaete chrysosporium* BKM-F-1767. Appl. Environ. Microbiol., 61: 4274-4277.
53. Bonugli-Santos, R.C., L.R. Durrant, M. Da Silva and L.D. Sette, 2010. Production of laccase, manganese peroxidase and lignin peroxidase by Brazilian marine-derived fungi. Enzyme and Microbial. Technology, 46: 32-37.

54. Cohen, R., L. Persky and Y. Hadar, 2002. Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. Appl. Microbiol. Biotechnol., 58: 582-594.
55. Kirk, K., E. Schultz, W. Connors, L. Lorez and J. Zeikus, 1978. Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. Arch. Microbiol., 117: 277-285.
56. Gold, M. and M. Alic, 1993. Molecular biology of the lignin-degrading basidomycete *Phanerochaete chrysosporium*. Microbiol. Rev., 57: 605-622.
57. Perez, J. and T.W. Jafferies, 1993. Role of organic acid chelators in manganese regulation of lignin degradation by *Phanerochaete chrysosporium*. Appl. Biochem. Biotechnol., 39: 227-238.
58. Hegde, S., S. Kavitha, M.C. Varadaraj and G. Muralkikrishna, 2006. Degradation of cereal bran polysaccharide-phenolic acid complexes by *Aspergillus niger* CFR, 1105. Food Chem., 6: 14-9.
59. Nigam, P., G. Armour, I.M. Banat, D. Singh and R. Marchant, 2000. Physical removal of textile dyes from effluents and solid-state fermentation of dye-adsorbed agricultural residues. Biores. Technol., 72: 219-226.
60. Tychanowicz, G.K., A. Zilly, C.G.M. De Souza and R.M. Peralta, 2004. Decolourization of industrial dyes by solid state cultures of *Pleurotus pulmonarius*. Process. Biochem., 39: 855-859.
61. Eggert, C., U. Temp and K.E.L. Eriksson, 1996. The ligninolytic system of the white rot fungus *Pycnoporus cinnabarinus:* Purification and characterization of laccase. Appl. Environ. Microbiol., 62: 1151-1158.
62. Corso, C.R., D.F. De Angelis, J.E. De Oliveira and C. Kiyan, 1981. Interaction between the diazo dye, *Vermelho reamil* P8B and *Neurospora crossa* strain 74A. Eur. J. Appl. Microbiol. Biotechnol., 13: 64-66.
63. Capalash, N. and P. Sharma, 1992. Biodegradation of textile azo dyes by phanerochaete chrysosporium for potential application in azo dye degradation and decolorization in wastewater. World J. Microbiol. Biotechnol., 8: 309-312.
64. Kirby, N., 1999. Bioremediation of textile industry wastewater by white rot fungi. Ph.D. Thesis. University of Ulster, Coleraine. UK.
65. Karimi, A., F. Vahabzadeh and B. Bonakdarpour, 2006. Use of *Phanerochaete chrysosporium* immobilized on kissiris for synthetic dye decolourization: involvement of manganese peroxidase. World J. Microbiol. Biotechnol., 22: 1251-1257.
66. Kling, S.H. and J.S. Neto, 1991. Oxidation of methylene blue by crude lignin peroxidase from *Phanerochaete chrysosporium*. J. Biotechnol., 21: 295-300.
67. Jager, A., S. Croan and K. Kirk, 1985. Production of ligninases and degradation of lignin in agitated submerged cultures of *Phanerochaete chrysosporium*. Appl. Environ. Microbiol., 50: 1274-1278.
68. Venkatadri, R. and R. Irvine, 1990. Effect of agitation on ligninase activity and ligninase production by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol., 56: 2684-2691.
69. Mohorcic, M., J. Friedrich and A. Pavko, 2004. Decoloration of the diazo dye reactive black 5 by immobilized *Bjerkandera adusta* in a stirred tank bioreactor. Acta. Chem. Solv., 51: 619-628.
70. Gandolfi-Boer, C., L. Obici, C. Souza and R. Perelta, 2004. Decolorization of synthetic dyes by solid state cultures of *Lentinula (Lentinus), edodes* producing manganese peroxidase as the main ligninolytic enzyme. Biores. Technol., 94: 107-112.
71. Tavcar, M., K.J. Svobodova, Kuplenk, C. Novotny and A. Pavko, 2006. Biodegradation of azo dye R016 in different reactors by immobilized *Irpex lacteus*. Acta Chim. Slov., 53: 338-343.
72. Ferreira-Leitao, V.S., J.G. Da Silva and E. Bon, 2003. Methylene blue and azure B oxidation by horseradish peroxidase: a comparative of class II and class III peroxidases. Appl. Catal. B. Environ., 42: 213-221.
73. Harazono, K., Y. Watanabe and K. Nakamura, 2003. Decolorization of azo dye by the white-rot basidomycete *Phanerochaete sordida* and by its manganese peroxidase. J. Biosci. Bioeng., 95: 455-459.
74. Ge, Y., L. Yan and K. Qinge, 2004. Effect of environment factors on dye decolorization by *P. sordida* ATCC 90872 in an aerated reactor. Proc. Biotechen., 39: 1401-1405.
75. Eichlerova, I., L. Homolka, L. Lisa and F. Nerud, 2005. G. Orange and Remazol Brilliant Blue R. decolorization by white rot fungi *Dichomitus squalens. Ischnoderma resinosum* and *Pleurotus calyptratus*. Chemosphere, 60: 398-404.

76. Chander, M. and D.S. Arora, 2007. Evaluation of some white rot fungi for their potential to decolorize industrial dyes. Dyes and Pigments, 72: 192-198.
77. Perie, F.H. and M.H. Gold, 1991. Manganese regulation of manganese peroxidase expression and lignin degradation by the white rot fungus *Dichomitus squalens*. Appl. Environ. Microbiol., 57: 2240-2245.
78. Perie, F.H., G.V.B. Reddy, N.J. Blackburn and M.H. Gold, 1998. Purification and characterization of laccases from the white-rot basidomycete *Dichomitus squalens*. Arch. Biochem. Biophys., 353: 349-355.
79. Perie, F.H., D. Sheng and M.H. Gold, 1996. Purification and characterization of two manganese peroxidase isoenzymes from the white-rot basidomycete *Dichomitus squalens*. Biochem. Biophys. Acta, 1297: 139-148.
80. Nilsson, I., A. Moller, B. Mattiasson, M.S.T. Rubindamayugi and U. Welander, 2006. Decolorization of synthetic and real textile wastewater by the use of white-rot fungi. Enzyme Microbial. Technol., 38: 94-100.
81. Susla, M., C. Novotny and K. Svobodova, 2007. The implication of *Dichomitus squalens* laccase isoenzymes in dye decolorization by immobilized fungal cultures. Biores. Technol., 98: 2109-2115.
82. Hamedaani, H.R.K., A. Sakurai and M. Sakakibara, 2007. Decolorization of synthetic dyes by a new manganese peroxidase-producing white rot fungus. Dyes and Pigments, 72: 157-162.
83. Murugesan, K., A. Dhamija, I.H. Nam, Y.M. Kim and Y.S. Chang, 2007. Decolourization of reactive black 5 by laccase optimization by response surface methodology. Dyes Pigments, 75: 176-184.
84. Lievs, C., C. Bobeth, M. Pecyna, R. Ullrich and M. Hofrichter, 2010. DYP-like peroxidases of the Jelly fungus *Auricularia auricular*-Judae oxidized non phenolic lignin model compounds and high redox potential dyes. Appl. Microbiol. Biotechnol., 85: 1869-1879.
85. Katchalski-Katzir, E., 1993. Immobilized enzymes-learning from past successes and failures. Trends Biotechnol., 11: 471-478.
85. Couto, S.R., M.A. Sanroman, D. Hofer and G.M. Giubitz, 2005. Stainless steel sponge: A novel carrier for the immobilization of the white-rot fungus *Trametes hirsute* for decolourization of textile dyes. Biores. Technol., 95: 67-72.
86. Tischer, W. and V. Kasche, 1999. Immobilized enzymes: Crystals or Carrier's? Trends in Biotechnology, 17: 326-335.
87. Gupta, M.N., 1993. Cross-linking techniques: application to enzyme and protein stabilization and bioconjugate preparation, pp: 307-324. In M.E. Himmel and G. Georgiou (Eds), Biocatalyst Design for Stability and specificity. American Chemical Society, Washington DC.
88. Giovanelli, G. and G. Ravasini, 1993. Apple Juice stabilization by combined enzyme membrane filtration process. *Lebensmittel-Wissenschaft + Technologie*, 26: 1-7. Doi: 10.1006/fstl.1993.1001.
89. Minussi, R.C., G.M. Pastore and N. Duran, 2002. Potential applications of laccase in the food industry. Trends I Food Science and Technology, 13: 205-216 doi: 10.1016/s0924-2244(02)00155-3.
90. Kim, T.H., Y. Lee, J. Yang, B. Lee, C. Park and S. Kim, 2004. Decolourization of dye solutions by a membrane bioreactor (MBR) using white rot fungi. Desalination, Strategies in South Mediterranean Countries, 168: 287-293.
91. Mohan, S., K. Prasad, N. Rao and P. Sarma, 2005. Acid azo dye degradation by free and immobilized horseradish peroxidase (HRP) catalyzed Process. Chemo. Sphere, 58: 1097-1105.
92. Tavares, A.P.M., R.O. Cristovao, J.M. Loureiro, R.A.R. Boaventura and E.A. Macedo, 2009. Application of statistical experimental methodology to optimize reactive dye decolourization by commercial laccase. J. Hazard. Mater., 162: 1255-1260.
93. Couto, R.S., E. Rosales and M.A. Saunroman, 2006. Decolourization of synthetic dyes by *Trametes hirsute* in expanded-bed reactors. Chemosphere, 62: 1558-1563.
94. Pezzella, C., F. Autore, P. Giardina, A. Piscitelli, G. Sannia and V. Faraco, 2008. The *Pleurotus ostreatus* laccase multi-gene family: Isolation and heterologous expression of new family members. Curr. Genet., 55: 45-57.
95. Palmieri, G., G. Cennamo and G. Sannia, 2005a. Remazol brilliant blue R decolourization by the fungus *Pleurotus ostreatus* and its oxidative enzymatic system. Enzyme Microbial. Technol., 36: 17-24.
96. Faraco, V., C. Pezzella, A. Miele, P. Giardina and G. Sannia, 2009a. Bioremediation of colored industrial wastewaters by the white-rot fungi *Phanerochaete chrysosporium* and *Pleurotus ostreatus* and their enzymes. Biodegradation, 20: 209-220.

97. Jordaan, J., S. Mathye, C. Simpson and D. Brady, 2009. Improved chemical and physical stability of laccase after spherezyme immobilization. Enzyme and Microbial Technol., 45: 432-435.
98. Abo-State, M.A.M., 1991. Control of *Bacillus cereus* isolated from certain foods. M.Sc. Thesis, Fac. Sci., Cairo Univ.
99. Abo State, M.A.M., 1996. Study of genetic background and effect of radiation on toxin production by *Bacillus cereus*. Ph.D. Thesis, Fac. Sci., Cairo Univ.
100. Abo-State, M.A.M., 2003. Production of carboxymethyl cellulase by *Fusarium oxysporium* and *Fusarium neoceros* from gamma-pretreated lignocellulosic wastes. Egypt. J. Biotechnol., 15: 151-168.
101. Abo-State, M.A.M., 2004. High level xylanase production by radio resistant, thermophilic *Bacillus megaterium* and its mutants in solid state fermentation. Egypt. J. Biotechnol., 17: 119-137.
102. Aziz, N.H. and S.R. Mahrous, 2004. Effect of gamma irradiation on aflatoxin B_1 production by *A. flavus* and chemical composition of three crop seeds. Nahrung-Food, 48: 243-238.
103. El-Batal, A.I. and M.A. Abo-State, 2006. Production of cellulase, xylanase, pectinase, amylase and protease enzyme cocktail by *Bacillus* spp. and their mixed cultures with *Candida tropicalis* and *Rhodotorula glutinis* under solid strate fermentation. Egypt. J. Rad. Sci. Applic., 19: 139-156.
104. Rajoka, M.I., 2005. Double mutants of *Cellulomonas biazotea* for production of cellulose and hemicellulases following growth on straw of a perennial grass. World J. Microbiol. Biotechnol., 21: 1063-1066.
105. Latifian, M., Z. Hamidin-Esfahani and M. Barzegar, 2007. Evaluation of culture conditions of recellulase production by two trichoderma reesei mutants under solid strate fermentation conditions. Biores. Technol., 98: 1-4.
106. Abo-State, M.A.M., M.A.M. Hammad, M. Swelim and R.B. Gannam, 2010a. Enhanced production of cellulose (S) by *Aspergillus* spp. Isolated from Agriculture Wastes by Solid Sate Fermentation. American-Eurasian J. Agric. Environ. Sci., 8(4): 402-410.
107. Abo-State, M.A.M., M.A.M. Hammad, M. Swelim and R.B. Gannam, 2010b. Some critical factors Affect in Cellulase (S) Production by *Aspergillus terreus* Mam-F23 and *Aspergillus flavus* MAM-F35 under solid-strate fermentation of wheat straw. World Appl. Sci. J., 9(10): 1171-1179.