Short Communication

Bioremediation of Endosulfan under Solid-State and Submerged Fermentation of *Pleurotus ostreatus* and its Correlation with Lignolytic Enzyme Activities

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Abstract

　　During the past few decades, organochlorine pesticides (OCPs) have emerged as global pollutants. Despite their ban, Endosulfan, one of the most persistent and toxic pesticides of the OCP group, was most commonly used in agriculture and other sectors. Endosulfan bioremediation experiments were conducted using *Pleurotus ostreatus*. The Endosulfan spiked wheat straw was inoculated with *Pleurotus ostreatus* and incubated under solid state and submerged fermentation conditions. The enzyme production and activities and degradation of Endosulfan isomers (α- and β-isomers) was monitored periodically for 40 days. Degradation rate and half-life (DT50) of both the isomers was calculated using a simple first-order kinetics model. Under both conditions, the addition of Endosulfan showed stimulating effects on MnP and laccase enzyme activities. Activities of both the enzymes at each time interval were higher in solid-state fermentation than submerged fermentation. The degradation of α- isomers was higher under solid-state fermentation than submerged fermentation, while the inverse was true for β-isomer. The calculated DT_{50} of α- and β-Endosulfan under solid-state fermentation was 3.99 and 44 days, respectively, with 73.69±3.43 mg kg^{-1} Endosulfan sulfate accumulation. While under submerged fermentation conditions, DT_{50} of α- and β-Endosulfan was 10 and 20 days, respectively. The formation of Endosulfan sulfate, under submerged fermentation conditions, was maximum at day 10 (39.67±5.73 mgL^{-1}) and declined to 8.71±3.24 mgL^{-1} until the end of incubation. A poor correlation between degradation and activities of both enzymes was observed. Rapid reduction in Endosulfan sulfate under submerged fermentation revealed its ascendancy in degradation over solid-state fermentation.

Keywords: bioremediation, submerged fermentation, Solid State Fermentation, endosulfan, Lignolytic enzymes

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Introduction

Endosulfan, a persistent and toxic organochlorine (OC) pesticide, has been used during the past few decades to kill various insects/pests for maximum crop yield. However, due to reported detrimental effects on various components of the ecosystem other than targeted insects/pests [1, 2], a ban was imposed on the use of this pesticide. Despite the ban, it is still used as an agricultural pesticide in turf, sugarcane, cotton and many other crops. Its traces can be detected easily in various environmental components like soil, surface water and air due to its high persistence [3]. Pakistan and a few other countries shared the major market of Endosulfan [4]. The point and non-point contamination of the pesticide need appropriate techniques for remediation and management. Bioremediation is a cost-effective and ecofriendly technique compared to other conventional remediation techniques used for Endosulfan [5].

Among the bioremedials, fungi are very well-known for bioremediation of pesticide-contaminated soils as they have substantial potential to degrade organic and inorganic pollutants; white-rot fungi (WRF) are the most effective and prominent among them [6]. Among others, the use of *Pleurotus ostreatus* for this purpose has been studied widely in the past under different growth conditions [7] like soil, slurry and culture media [8]. Many researchers have connected the bioremediation ability of WRF with the production of lignolytic enzymes [9] that degrade toxic compounds superficially as part of secondary metabolism. *Pleurotus* spp. has been studied extensively for the production of Laccase (Lac) and Manganese peroxidase (MnP) [10, 11]. Growth conditions of *Pleurotus* play a key role in the production and expression of lignolytic enzymes [12]. Two growth conditions, i.e., solid-state fermentation (SSF) and enriched submerged fermentation (SMF), are generally used for the growth of fungi. The SSF is a traditional technique in which this fungus is grown on lignocellulosic agricultural byproducts (for example, wheat straws and corn cobs), in the absence/small quantity of free water. Wheat straw, a lignocellulosic agricultural byproduct, is easily available and contains a significant amount of soluble carbohydrates and other inducers of enzyme synthesis. These materials help in the efficient production of lignolytic enzymes [13]. Contrarily, SMF (harvesting of fungus biomass by growing on enriched liquid broth medium) has also received increasing attention and emerged as a promising alternative for efficient production of fungal biomass and enzymes [14]. The bioremediation potential of WRF for different toxic compounds has been tested successfully under both conditions, SSF and SMF, in the past [15].

Broadly, two mechanics of fungal bioremediation are narrated in the literature, i.e., toxic compound degradation due to lignolytic enzymes [16] and adsorption of toxic compounds by mycelial biomass or associated microflora [17]. This study was aimed to: i) assess the Endosulfan bio-remediation potential of *P. ostreatus* by growing under SSF and SMF conditions and ii) develop a relationship between the degradation of Endosulfan and activities of two extracellular lignolytic enzymes, i.e., laccase and MnP to determine the role of lignolytic enzymes.

Materials and Methods

Reagents and Chemicals

The analytical-grade Endosulfan (99.9%) used in this study was shipped from sigma Aldrich (St. Louis, Missouri). All the organic solvents, such as acetonitrile, acetone and n-hexane, used for preparing stock/working solution and extraction of samples were of pestanal grade and were obtained from VWR (Radnor, PA). The helium gas used during GC-MS/MS analysis was 99.999% pure. Growth and maintenance of *Pleurotus ostreatus* was done using yeast extract, malt extract, peptone, glucose and agar, which were purchased from Merck & Co. All other chemicals used in enzymes activities analysis, i.e., hydrogen peroxide (H_2O_2), O-dianisidine (DNS), manganese sulfate, sodium tartrate and ABTS (2,2'-azino-di-[3-ethyl-benzo-thiazolin-sulphonate), were also of analytical grade. For method validation and chromatographic performance evaluation, OCPs Mix AB#1 (Cat # 32291 Restek, Bellefonte, PA), internal standard mix (Cat # 33267 Restek, Bellefonte, PA) and pesticide surrogate mix (Cat # 32000 Restek, Bellefonte, PA) were used.

Multiplication and Maintenance of *P. ostreatus*

Yeast-malt-peptone-glucose (YMPG) agar was used for growing pure cultures of *P. ostreatus* (WC-814), which were obtained from a mushroom research program at Pennsylvania State University, USA. YMPG media was prepared using a recipe described by Nasim and Co. (2001) [18] using yeast extract, malt extract, peptone, agar and glucose that were added to deionized double-distilled water (pH 5.5). After cooling, the media was inoculated with fungal cultures and grown fungal was preserved at 4ºC for further use during the study.

Bioremediation Microcosms

Solid-State Fermentation

Air-dried and ground wheat straws using Ball Mill (Hills Technologies, LLC) were placed in Pyrex flasks and moistened with water to ≈77% in order to promote the activities of MnP and laccase [15, 19]. The flasks were plugged with a cotton swab to avoid anaerobic conditions and autoclaved at 121ºC and 15psi for 30 min. On cooling, straws were inoculated with cultures of the

Table 1. Summary of quant ion of used internal standard (I.S) and selected organochlorine pesticides (n = 5).

Compound	Quant Ion	Quant Ion	Quant Ion
TMX(I.S)*	244	246	242
PCB 18(I.S)	256	260	258
PCB 28(I.S)	256	260	258
Triphenylmethane(I.S)	165	167	166
Endosulfan I	195	197	193
Tris-(1,3-dichloroisopropyl) phosphate(I.S)	325	327	326
Endosulfan II	195	197	193
Endosulfan sulfate	387	389	385
DecaPCB	498	500	496

*Internal Standard

P. ostreatus and subsequently spiked with Endosulfan @ 55 mg kg^{-1}. There were two Controls: i) without fungus but spiked with Endosulfan and ii) with the fungus but not spiked with Endosulfan. Control 2 was used to calculate the effect of Endosulfan on enzymatic activities. Degradation of Endosulfan was monitored by sampling flasks on days 4, 10, 16, 22, 28, 34 and 40. The sampled mixtures were analyzed for Endosulfan immediately.

Enriched Submerged Fermentation (SMF)

SMF cultures were prepared by pouring YMPG broth medium into Erlenmeyer flasks (rubber-stoppered) and then inoculating with mycelial plugs. Subsequently, the SMF liquid cultures were spiked with a mixture of Endosulfan isomers (α and β 1:1) @ 55 mg L^{-1}. After inoculating the flasks, 100% oxygen was flushed on a daily basis in order to evade any anaerobic consequence. The spiked SMF cultures were grown at 22ºC by agitating @125 rpm using an incubator shaker under dark conditions. In addition to the above treatment, two controls were processed in similarly. Control – 1: only YMPG broth medium was spiked with the same rate of pesticides (without mycelial) and Control – 2: mycelial culture of *P. ostreatus* without Endosulfan spiking. Contents of all the flasks were sampled at day 4, 10, 16, 22, 28, 34 and 40 days of incubation. The control was used to assess the effect of produced lignolytic enzymes on pesticide reduction and their activities.

Extraction and Analysis

The QuEChERS (quick, easy, cheap, effective, rugged and safe) method [20] was used for extraction of Endosulfan. After harvesting, samples were placed in a centrifuge tube (Restek, Bellefonte, PA) and mixed with an un-buffered Restek salts kit (containing anhydrous magnesium sulfate and sodium chloride). Then acetonitrile was added as extraction solvents with some water for better phase separation. Six components of internal standard (ISTD) mix and the suspensions were shaken vigorously. The acetonitrile upper layer was removed and analyzed using GC-MS/MS for monitoring the reduction of Endosulfan under both fermentation conditions.

For GC-MS/MS analysis, 60 m × 0.18 mm × 0.10 μm Rtx Dioxin-2 (Restek, Bellefonte, PA) column was used. Helium was used as carrier gas and the GC was operated with a 10:1 split ratio at a flow rate of 1.2 mL min^{-1}, while the injector was maintained at 290ºC. Samples were injected at a final concentration of 1.0 μL using an auto sampler. The GC oven temperature program was as follows: initial oven temperature was 100ºC and hold for 1.0 min. Then the temperature was ramped @ 10ºC min^{-1} to 300ºC and hold for 7.0 min. The instrument was simultaneously operated in SIM (selected ion monitoring) mode and full-scan mode with electron ionization energy of 70eV and source temperature of 300ºC. SIM masses used for quantification of Endosulfan and internal standards are given in Table 1.

Quality control (QC) and quality assurance (QA) procedures were followed in order to determine and maintain the quality of analytical data [21]. For calibration, matrix-matched blank extracts (1mL) were fortified with 400μl of ISTD solution (20 ng μL^{-1}) and with OCP mix in order to get 10 μl kg^{-1} and 15 μl kg^{-1} of final concentrations. Accuracy of the method was monitored in terms of recovery (%) of Endosulfan (Table 2). The results showed that mean recoveries of analytes were between 70-100%, with RSD values at or below 20% for all analytes [21]. The sensitivity of instruments was measured by calculating the limit of quantitation (LoQ) and limit of detection (LoD) (Table 3).

Lignolytic Enzyme Assays

At each time series, enzymes under both the conditions were extracted and activities were measured using already-tested protocols [22]. Enzyme extraction

Table 2. Chromatographic performance of GC triple quadrupole mass spectrometer (n = 5).

Compounds	R$_t$ (Minutes)	r^2	Linearity Range	LoD (μg kg^{-1})	LoQ (μg kg^{-1})
α-Endosulfan	19.52	0.999	10pg-10ng	0.001	0.01
β-Endosulfan	20.96	0.998	10pg-10ng	0.001	0.01

Table 3. Recovery data of Endosulfan using the proposed procedure under solid-state. fermentation and enriched submerged fermentation (n = 5).

Compounds	SSF		SMF	
	Recovery(%)	RSD (%)*	Recovery(%)	RSD (%)*
α-Endosulfan	83.33	5.41	88.33	3.46
β-Endosulfan	84.33	1.81	86	1.16

*Peak Area

was carried out by adding sodium citrate buffer (50 mM, pH 5.3) to the sample. The suspension was mechanically shaken for 30 minutes at 4ºC on an incubator shaker and squeezed manually using Mira cloth (quick filtration material). Extract was centrifuged twice at 4ºC (first for 25 minutes at 30,000 x g and then for 15 minutes at 10,000 x g) using the method described by [23] with slight modifications. Lignolytic enzyme activities were measured in supernatant using a Spectra Max 250 Microplate Reader at different wavelengths. MnP activities were determined by oxidizing o-dianisidine (DNS) and read at 460 nm (molar extinction coefficient = 29,400 M^{-1}cm^{-1}) for 5 minutes using reaction mixture. Laccase was also measured using ABTS (2,2'-azino-di-[3-ethyl-benzo-thiazolin-sulphonate) as a substrate. For this purpose, ABTS, tartaric acid(L+), water and enzyme extract were measured for calorimetric changes at a wavelength of 436 nm for 5 minutes [22]. Enzyme activities were calculated by the following equation:

$$\frac{\Delta C}{t} = \frac{\Delta A}{\varepsilon \Delta t.L} \tag{1}$$

…where C is the concentration of enzymes, A is the absorbance, ε extinction coefficient (Mm^{-1}cm^{-1}) and L is path length equal to 0.49. Activity was expressed in U L^{-1}, where 1 U = 1 µM min^{-1}.

Kinetic Studies

Simple first-order kinetics (SFO) fit the observed degradation data with time well. The parameters were calculated using computer program R (version 3.0.3) with active kin fit software. A degradation DT$_{50}$ (Half-Life) was calculated by Equation 3 used in line with FOCUS guidelines for each set of laboratory conditions to which samples were exposed [24]. The following linear equation was used [25]:

$$\ln (C) = a + K_1 t \tag{2}$$

…where Ln(C) is natural logarithm of concentration, t is time given to the compound for degradation, K$_1$ is the first-order rate constant (slop) and a is the empirical constant equal to intercept.

DT$_{50}$) was calculated by the following equation

$$DT_{50} = t_{1/2} = \frac{0.693}{k1} \tag{3}$$

…where 0.693 is the constant.

Statistical Analysis

Pearson Correlation between the enzymatic activity and percentage relative reduction of Endosulfan was obtained using statistix (8.1) software.

Results and Discussion

The purpose of this work was to assess the role of *P. ostreatus* in the degradation of Endosulfan under two growth conditions, i.e., SSF and SMF. The fermentation of this fungus under conditions affect the formation of lignolytic enzyme, e.g., Laccase and MnP [26]. It was considered that these enzymes play a role in bioremediation of different types of pollutants [27]. Therefore, bioremediation of Endosulfan under two fermentation conditions and activities of Laccase and MnP were monitored simultaneously, and data was recoded periodically during the 40-day incubation.

Bioremediation under Solid State Fermentation

Initially, when biomass production of *Pleurotus ostraetus* in wheat-straw-mediums was very low, about half of the applied α-Endosulfan (29 mg kg^{-1}) from the mixture was recovered. This showed that 47% reduction in applied α-Endosulfan occurred in the first 4 days. However, in its associated control, most of this isomer (45 mg kg^{-1} out of 55 mg/kg^{-1}) was recovered on the 4th day. This showed that out of 47% reduction during the first 4 days, 30% degradation was most probably due to the inoculated fungus and the remaining 18% was due to other factors. Significantly less DT$_{50}$ (3.99 days) of α-Endosulfan than control (60.55 days) under SSF conditions indicated the important role of fungal biomass in reducing the amount of α-Endosulfan. The recovery of 8 mg kg^{-1} of this isomer in day 10 indicated that 85% of the applied isomer was reduced (Fig. 1). The remaining 15% of this

isomer was degraded in the next 6 days of incubation. Since α-Endosulfan is more bio accumulative and about three times more toxic than β-Endosulfan [28], complete disappearance before 16 days showed that SSF of *P. ostreatus* is the best method to degrade α-Endosulfan. Under control conditions, more than half (32 mgkg⁻¹) was recovered even after the incubation period. The complete disappearance of α- isomer, during the first 16 days by fungus inoculation, was in agreement with the earlier findings, where complete mineralization of Endosulfan was observed after 12 days of incubation with fungal (*Aspergillus niger*) innoculation [29]. A possible mechanism of the Endosulfan removal could be adsorption by mycelial biomasses of *P. ostreatus* [7].

Under fungal inoculated conditions, the estimated DT_{50} of β- isomer of Endosulfan was 44.12 days, while under associate control it was 341.35 days (Fig. 1). Significantly higher applied β-Endosulfan was recovered (47 mgkg⁻¹) on day 4 and at the end of incubation (31.4 mgkg⁻¹) than that of α-Endosulfan. The results showed that after day 16, the disappearance rate of this isomer declined and complete mineralization was not achieved even after the termination of the incubation experiment. In associate control, insignificant degradation of β-isomer was observed till day 10 (Fig. 1). The decline in rate of β-isomer mineralization was observed soon after day 16, when Endosulfan sulfate started to appear in the system. On day 4, 45.7 mg kg⁻¹ of Endosulfan sulfate was observed and was increased to 66.7 mg kg⁻¹ at day 10. The formation of Endosulfan sulfate was continued till the termination of the experiment (73.7 mgkg⁻¹). Surprisingly, no Endosulfan sulfate formation was detected in control. The results were analogous to the fact that the microbial activity and certain fugal enzymes favor oxidative pathways during bioremediation of Endosulfan in soil, resulting in the formation of Endosulfan sulfate [30]. A decline in reduction of both the isomers of Endosulfan with time can be attributed to the accumulation of Endosulfan sulfate, which is considered more toxic and persistent
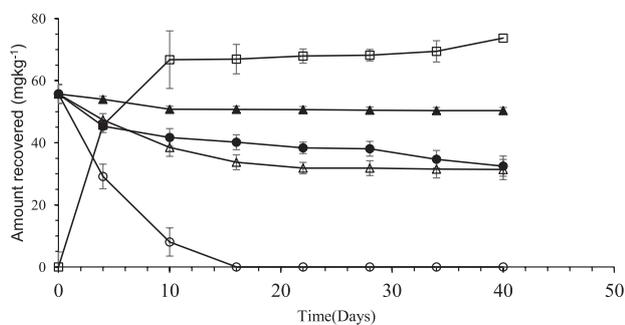
than its parent compound. Hence, its accumulation during the bioremediation process is a serious concern [31]. However, no other metabolites were detected during the samples analysis, even at a very high injector temperature (290ºC). The formation of Endosulfan sulfate as a major metabolite using WRF, and the corresponding reduction of the parent compound is in line with earlier studies [31]. This formation showed that oxidation was the main cause of degradation of Endosulfan because other hydrolytic metabolic products do not appear during remediation [32]. In this type of study, interconversion of isomers or conversion to metabolites could be another possible phenomenon.

Bioremediation under Submerged Fermentation

Under SMF conditions, complete degradation/mineralization of α-Endosulfan took 34 days as compared to SSF, where it took only 16 days (Fig. 2). An entirely different trend in reduction and metabolite formation under SMF conditions was observed than that of SSF. Total α-Endosulfan recovered, under associated control, was 40.36 mg L⁻¹ (equivalent to 73% applied dose), and only 15.64 mg L⁻¹ (equivalent to 27% applied dose) was degraded during the experimental period. On the other hand, total reduction of β-Endosulfan in associated control under SMF conditions was higher (87.89%) than that under SSF (42%) conditions (Fig. 2). Under SMF conditions, DT_{50} of α-Endosulfan was 9.52 days in fungal inoculated treatment and in associated control, and the DT_{50} was 106.52 days (Table 4). About 2 times higher DT_{50} (20.08 days) of β-Endosulfan was observed than α-Endosulfan under the same fermentation conditions, while DT50 of β-Endosulfan in its associated control was 70 days. Other kinetic perimeters are given in Table 4. Results revealed that that SMF conditions were a good way for lowering the persistence of β-isomer of Endosulfan. Endosulfan sulfate was detected at day 10 of incubation (39.67 mg L⁻¹) in this case. However, after that it started declining and within 40 days, only 8.71 mg L⁻¹ of this

Fig. 1. Recoveries of α and β isomers of Endosulfan and the formation of Endosulfan sulfate (mgkg⁻¹) under solid-state fermentation (n = 3), where α-Endosulfan (○), α-Endosulfan (Control) (●), β-Endosulfan (Δ), β-Endosulfan (Control) (▲) and Endosulfan sulfate (□)isomer.
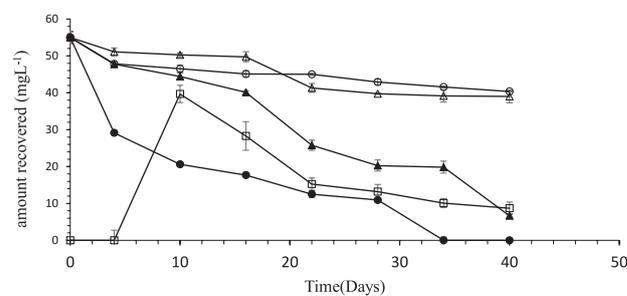

Fig. 2. Recoveries of α and β isomers of Endosulfan and the formation of Endosulfan sulfate (mg kg⁻¹) under submerged fermentation (n = 3), where α-Endosulfan Control (○), α-Endosulfan (●), β-Endosulfan Control (Δ), β-Endosulfan (▲) and Endosulfan sulfate (□).

Table 4. Kinetic performance perimeter calculated by single simple first-order equation under SSF and SMF of *Pleurotus* against Endosulfan.

Kinetic Perimeters Under Solid State Fermentation and enriched submerged fermentation								
Compounds	DT_{50}		DT_{90}		r^2		k	
	SSF	SMF	SSF	SMF	SSF	SMF	SSF	SMF
α-Endosulfan	3.99	9.52	13.24	31.69	0.99	0.89	0.017389	0.0728
α-Endosulfan(non-Fungal Control)	60.55	106.52	201.14	353.8561	0.86	0.8412	0.01144	0.0065
β-Endosulfan	44.12	20.0864	146.56	66.7255	0.8	0.926797	0.0157	0.0345
β-Endosulfan(non-Fungal Control)	341.35	70.79	1139	235.17	0.7	0.89	0.002059	0.00979

metabolite was left. This accumulation and subsequent reduction showed that SMF is a better method to remediate Endosulfan sulfate.

Effect of Endosulfan on Activities of Lignolytic Enzymes

Screening of bio remedial agents for tolerance of pesticides is very important because sometimes microbes are sensitive to lethal effects of pesticide and their growth can be severely affected. Before starting the remediation experiment, using microbes as bio-remedial, it is important to test their tolerance to a particular dose of selected pesticides [33]. Some fungi are more tolerant and can grow up to 400 mg L^{-1} concentrations of Endosulfan [29].

Under SMF conditions, the activity of Laccase was 6.56 U g^{-1}±0.23 in control 2 at day 4, while in Endosulfan-spiked flasks, the activity was slightly high (7.08 U g^{-1}±0.15). The same trend was observed until the end of incubation, i.e., lower activity of Laccase in the associated controls than under Endosulfan-spiked conditions. Similarly, under SSF, Laccase higher activity was higher (11 U g^{-1}±0.42) under Endosulfan-spiked conditions as compared to associated control (10.23 U g^{-1}). This showed that Endosulfan pesticide had no detrimental effect on the production of Laccase activities, rather it wasslightly stimulated under both fermentation conditions. A similar pattern was also observed for another lignolytic enzyme, MnP. It is clear from Fig. 3(a, b) that activities of both the enzymes were slightly higher in Endosulfan-spiked samples than control, showing that the addition of Endosulfan has no adverse effects on their activities. This can be concluded from results that lignolytic enzymes are tolerant to selected concentrations (55 mg kg^{-1}) of Endosulfan. *P. ostreatus* is considered the most tolerant fungus to many pollutants [34] and the increase in Laccase and MnP activity during the incubation under SMF and SSF conditions due to Endosulfan spiking is in accordance with the earlier observations [34] that observed the higher activity of Laccase in the presence of atrazine than its associated control. This seems unusual because generally the addition of pollutants decreases lignolytic enzyme activities.
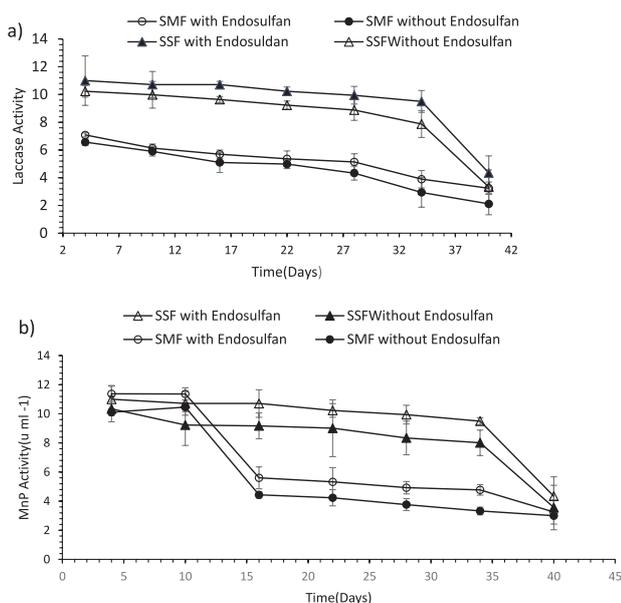

Fig. 3. Effect of Endosulfan on the activity of lignolytic enzymes during solid-state fermentation (U g^{-1}) and submerged fermentation conditions (Uml^{-1}) a) laccase b) manganese peroxidase (n = 3).

Table 5. Pearson correlation between biodegradation of Endosulfan and lignolytic enzyme activity of Pleurotus ostreatus under solid-state fermentation and submerged fermentation (WC-814) (α = 0.05).

Conditions	Biodegradation	Correlation with Lignolytic enzymes	
		Laccase	MnP
SSF	α-Endosulfan	0.53[NS]	0.28[NS]
	β-Endosulfan	0.39[NS]	0.28[NS]
SMF	α-Endosulfan	0.14[NS]	0.49[NS]
	β-Endosulfan	-0.25[NS]	0.1[NS]

NS = Not Significant

Correlation between Lignolytic Enzymes Activities and Reduction in Endosulfan

The majority of *Pleurotus* strains have an incredible capability to secrete laccase and MnP under SSF conditions, whereas under SMF conditions they secrete better hydrolytic enzymes [35]. Laccase appeared to be a major extracellular lignolytic enzyme produced by *P. ostreatus* in the past [36], with negligible detection of other lignin peroxidases [12]. Bioremediation of OCPs and other pollutants using WRF cultures was generally correlated with lignolytic enzyme activities in the past [17, 37, 38], while some studies have stated their non-involvement in the bioremediation process [7, 22]. However, only a limited number of researchers have studied the relationship between the extracellular lignolytic enzyme activities and reduction rates of OCPs during fermentation. During this study, a Pearson correlation between reduction (%) of Endosulfan isomers and activities of laccase and MnP was calculated periodically. Results clearly demonstrated that there was no significant correlation between the activities of these enzymes and the rate of reduction of Endosulfan (Table 5). These results are contradictory to a few earlier findings and support the non-involvement of extracellular peroxidases in the metabolism of Endosulfan [27]. In a similar study using WRF (*Trametes versicolor*), DDT degradation had no correlation with the activity of laccase and MnP. However, LiP played some role in bioremediation [39]. Since in this case mineralization of Endosulfan was observed despite the absence of lignin peroxidase (LiP) and hence, the possibly significant role of LiP in the remediation process could be ruled out.

Conclusions

The growth conditions of any *Pleurotus* species significantly affect lignolytic enzyme production. During this study, *Pleurotus ostreatus* was grown under two different conditions: SSF and SMF. Activities of lignolytic enzymes, Laccase and Manganese peroxidase were monitored periodically during the incubation. Simultaneously, Endosulfan mineralization rate was also measured. Despite the low growth of mycelium under both fermentation conditions, degradation of Endosulfan was higher in the initial stage and subsequently declined gradually. Complete mineralization of α-Endosulfan was achieved in 16 days under SSF conditions, while under SMF conditions, it took 10 days. Although complete degradation of β-Endosulfan took a long time under both fermentation conditions, SMF proved efficient in biodegradation of this β-isomer. Endosulfan sulfate continued to accumulate and a total of 73.69 mg kg^{-1} was observed at the termination of the incubation experiment. However, under SMF the trend was different and after accumulation of 28.28±2.35 mg L^{-1} until day 16, it started declining to 8.71 mg L^{-1} until

the end of incubation. Activity of both the enzymes, Laccase and MnP, was higher under SSF than SMF. There was no significant correlation between degradation of both the isomers and enzyme activity.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

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