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The ability of *Phanerochaete chrysosporium* (ME446) on chemical oxygen demand remediation in submerged culture medium supplemented with malathion insecticide

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ABSTRACT

This present study aims to evaluating the efficiency of *P. chrysosporium (P.c)* to degrade the different concentrations of malathion (MLT) (50, 100 and 150 ppm) under agitated (130 rpm) submerged culture conditions via reduction of chemical oxygen demand (COD). We have previously screening the growth of fungus in sabouraud dextrose agar (SDA) medium for 6 days. We have also determined the laccase activity during degradation process for 15 days. According to our results the fungus has achieved 99.6, 98.8 and 98.7 % COD reduction at concentration of 50, 100 and 150 ppm respectively the end of the 15 days. The highest laccase activity (180 ± 5.69 U/L) was measured at concentration of 50 ppm the end of the 15 days and the lowest activity (36 ± 3.26) was determined at concentration of 150 ppm the end of the 15 days. The dried biomass of fungus was weighted at end of the 15 days and we have determined that malathion in culture medium reduced the biomass production compared the control group (SDB + *P.c*). Our results showed that, *P. chrysosporium* could be an effective bioremediation tool for treatment of malathion containing wastewater.

Key words: *P. chrysosporium*; Malathion; COD reduction; Laccase activity

1. Introduction

For agricultural activities, pesticides are used on a large scale. The adverse effects of them on both environment and human health are a matter of society concern. Thus both the residue levels of pesticides in agricultural products and actual state should be monitored extensively. Insecticides are one of the most commonly used class of pesticides which are used for treatment and prevention of several infections from insects [1].

Today, with their high degradation rates, organophosphorus pesticides (OPs) constitute one of the largest classes of agricultural insecticides used in the global world. In insects, insecticides act as pesticides because of their property of inhibiting acetilcholinesterase (AchE). Although these insecticides show preferred toxicity to insects, they are also toxic to mammals for human exposure existing regulatory limits based on inhibition of AchE either in humans or in animals used in experimental studies [2]. Malathion, diethy [(dimethoxythiophosphinothioyl) thio] butanedioate is a non-systemic insecticide which has a cholinesterase enzyme inhibitor. This insecticide acts by the inhibition of this enzyme in the insects body [1]. The most serious problem on the contamination of the agricultural fields and water bodies are pharmaceutical compounds, petroleum products, polycyclic aromatic hydrocarbons, chloro-and nitrophenols organic dyes, heavy metals and pesticides [3–9].

Bioremediation is non-invasive and eco-friendly methods and it is a consistent solution that can end with degradation or transformation of environmental contaminants into less toxic or harmless forms [10,11]. Biodegra-

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dation involves the biological reactions that modify the chemical structure of the compound, so, this implies a decrease in toxicity [12]. Microorganisms can degrade pesticides as a source of energy and carbon, or by cometabolism [13].

Phanerochaete chrysosporium is a commonly studied white rot fungus to mineralize and degrade wide variety of agricultural and industrial pollutants [14,15]. The ability to degrade pesticides has generally been attributed to the lignin-degrading enzyme system of this organism. Under nutrient-deficient culture conditions; the major components of the lignin degrading enzyme system include manganese-dependent peroxidases (MnP), H_2O_2-generating system and lignin peroxidases (LiP), which are induced during secondary metabolism. This enzyme system has been shown to catalyze the premier oxidation of many persistent xenobiotics including chloroaromatic compounds, polycyclic aromatic hydrocarbons and dioxins [16–19]. These enzymes produced during different metabolic pathways in microbes present in soil are the key for bioremediation of pesticides. Optimum environmental conditions support fast rate of removal of toxic intermediates [20].

This present study aims to evaluating the efficiency of *P. chrysosporium* to remediate the different concentration of malathion (suggested for agricultural activities as 50, 100 and 150 ppm) under agitated (130 rpm) submerged culture conditions via remediation of chemical oxygen demand (COD), as one of the alternative and important environmental parameter for evaluating the removal rate of pesticides. We have also aimed to find out the role of the laccase activity on the bioremediation process.

2. Materials and methods

2.1. Fungus

White rot fungus *P. chrysosporium* (ME446) used in this study. These fungal strains are currently available in our culture collections. The strains were maintained on sabouraud dextrose agar (SDA) slants at 4°C in refrigerator.

2.2. Chemicals

Malathion was obtained from sigma-aldrich (Germany) with a CAS number of 121-75-5. SDA and sabouraud dextrose broth (SDB) were purchased from Lab M Limited (United Kingdom) with a lot number of 143118.

2.3. Screening the growth capacities of fungus in solid media

For agar plate screening the growth capacities of fungus, mycelial plugs (5 mm diameter) were inoculated into the center of Petri dishes (90 mm diameter) containing 50, 100 and 150 ppm of malathion, in triplicate. The plates were incubated at 27°C in the dark until they were completely colonized with the fungus or for a maximum period of 5 d. The diameters (mm) of the growth halos were determined in two perpendicular directions of the plate. Plates containing the malathion but not inoculated served as control.

2.4. Preparation of submerged culture conditions

For preparation of inoculum and submerged culture medium, *P. chrysosporium* were cultured at 27°C on SDA slants in glass tube. After one week of incubation, conidial suspensions were prepared and used for the preparation of inoculum. 10 ml of the suspension was transferred into a 250 ml flask containing SDB and agitated on a rotary shaker at 130 rpm for 15 d at 27°C. After incubation, flasks homogenized and then these homogenized mycelial cultures were used as inoculum for studies under submerged culture medium. 10 ml homogenized mycelial culture was transferred into 250 ml flasks containing 150 ml SDB and 50, 100 and 150 ppm of malathion on an agitated incubator for 5 and 15 d at 27ºC in triplicate. After incubation, all flasks filtered for removing fungal biomass and then filtrate was used in laccase activity and COD reduction assays.

Dry matter of fungal biomass was determined by drying *P. chrysosporium* biomass, taken from the filtration at 105°C for 48 h to a constant weight.

2.5. Laccase activity assays

Laccase (E.C. 1.10.3.2) activity was determined spectrophotometrically by monitoring the increase in absorbance at 420 nm. One unit was defined as the amount of enzyme that oxidized 1 µmol of ABTS (2,2_-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) per minute [21].

2.6. COD reduction assays

The COD experiments were performed in line with the closed reflux titrimetric method identified in the Standard Method 5220C [22] and decreasing of the substrate followed. According to this method, and 3.5 ml of 0.0176M Ag_2SO_4 solution and 1.5 ml of standard potassium dichromate digestion solution ($K_2Cr_2O_7$) were added to a 2.5 ml sample. Later on, these samples were heated in a Velp WTW CR3200 thermoreactor for 2 h at 150°C. After the samples were cooled, they were taken to Erlenmeyer flasks and 3–4 drops of ferroin indicator ($FeSO_4{\cdot}7H_2O$) were added to the samples. After that, samples were titrated with 0.25 M standard ferrous ammonium sulfate (FAS) titrant and COD results were calculated.

2.7. Statistical analyses

All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA). The data presented are the averages of the results of three replicates with a standard error (SE). To compare the Laccase activity and COD reduction in media, the data were analyzed by analysis of variance (ANOVA).

3. Results and discussion

3.1. Agar plate screening of growth in solid media

All growth zones of fungus in solid media are illustrated in Fig. 1. In the SDA medium, the diameter of the fungal colony decreased with the increase in the MLT amounts in the

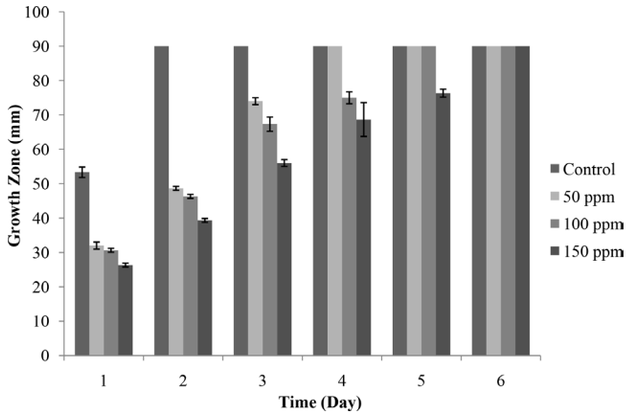

Fig. 1. Agar plate screening of growth in SDA media supplemented with MLT.

medium. The fungus showed a reduction of over 20% in the colony growth diameter in the lowest concentration of MLT in comparison with the control experiment at 3 d. At higher concentrations of MLT there was a greater growth decrease.

At the end of the 6 d, growth zones in control and MLT supplemented media is given in Fig. 2. MLT proved to be little toxic to the *P. chrysosporium* used in this study for 50 and 100 ppm concentration of MLT, since slightly inhibited the fungal growth. Similar results were found in earlier studies, it was observed a reduction in the population of bacteria in a soil portion after it passes by treatment with organophosphate pesticides cyolan, malathion and chlorpyrifos [23]. It was found that methyl parathion have so toxic to some marine-derived fungal strains in solid media. The screening of fungal growth in media with MLT indicated that the *P. chrysosporium* is metabolized this pesticide [24].

3.2. Laccase activity

These enzymes are used for pulp delignification, pesticide degradation and organic synthesis [25]. It is already known that the laccase enzymes are produced for pesticide degradation in order to provide nutrients for the growing organism. In this current study, enzyme activities were monitored within different concentrations of MLT insecticide inoculated with *P. chrysosporium*. When the laccase activities were increased at 50 and 100 ppm concentration of MLT supplemented media, in media supplemented with 150 ppm MLT was decreased. The highest laccase activity was measured as 180 U/L in 50 ppm after 15 days and the lowest activity (36 U/L) in 150 ppm after 15 d (Fig. 3.). These results indicated that presence of MLT in low and medium concentration (50 and 100 ppm) stimulated the laccase activities. Because pesticide degradation was achieved by lignocellulolitic enzymes such as laccase, increasing in laccase activity provided high levels of COD reduction after 15 d. Similarly in another study, *T. versicolour* is used for the bioremediation of atrazine in soil with low moisture and organic contents that are normally found in semiarid and Mediterranean-like ecosystems. The removal of polycyclic aromatic hydrocarbons (PAHs) in soil by fungal laccase, which increased with the increase of laccase dosage [26]. In another study, featuring *P. chrysosporium* in liquid culture has reported biotransformation of the insecticide lindane independently of the production of ligninolytic enzymes [27]. We have also observed the same relation between laccase enzyme activity and removal efficiency of *P. chrysosporium* in 100 ppm supplemented media.

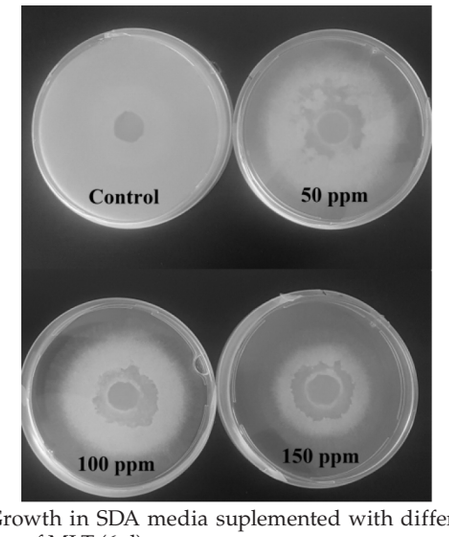

Fig. 2. Growth in SDA media suplemented with different concentration of MLT (6 d).

3.3. COD reduction

COD reduction rates in the media supplemented with MLT have showed different results depend on the concentration differences and time in the submerged culture medium. According to the results of COD reduction; in medium with 50 ppm MLT, after 5th day, reduction was seen about 32.5 % and after 15th day, reduction increased to 94%. In 100 ppm and 150 ppm medium with MLT, reduction rates were 76% and 50% respectively in 5 days. After the 15th day, this rate is same at 100 ppm and 150 ppm concentrations as 98% (Fig. 4). This obtained COD removal results are related with both biosorption and bioremoval.

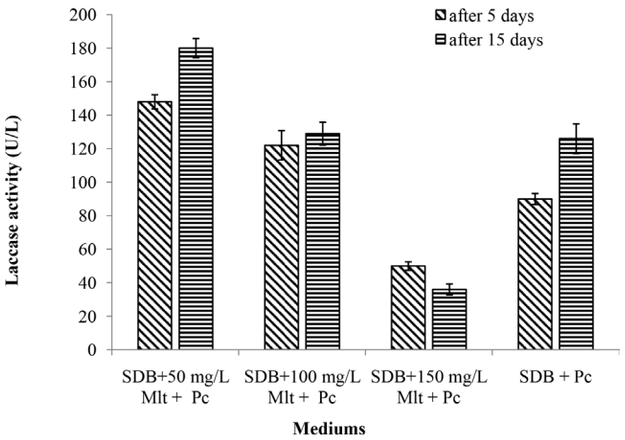

Fig. 3. Laccase activity in submerged culture medium supplemented with MLT.

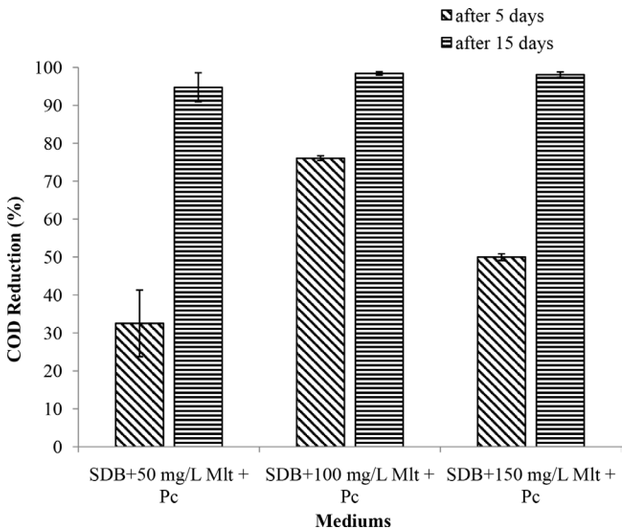

Fig. 4. COD reduction in submerged culture medium supplemented with MLT.

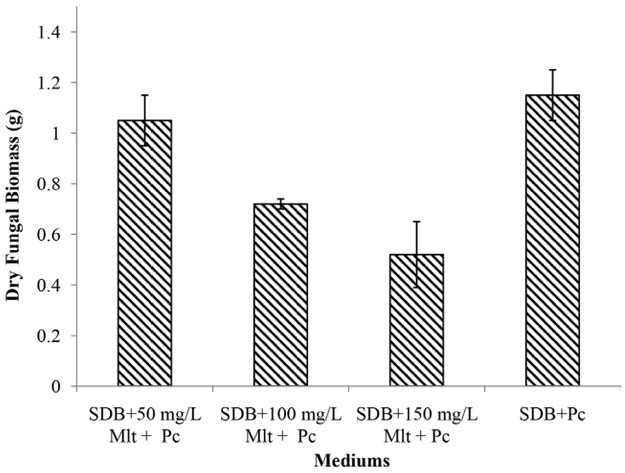

Fig. 5. Dry fungal biomass in submerged culture medium supplemented with MLT after 15 d.

Biodegradation of herbicide Aclonifen invesigated and COD reduction efficiencies of *B. simplex, B. muralis, M. yunnanensis, M. luteus and C. tetani* species found as 94, 78, 79, 70 and 74%, respectively at the end of 108th h [28]. The reduction of herbicide Aclonifen investigated with some different concentrations of soil bacteria and fungi mixtures in filtrate water taken from soil test units. According to the results; the best remediation performance was seen nearly 98% for COD in 10 mL mixed cultures. In COD tests, remediation performance in 5 mL mixed cultures was also similar to 97%. At the end of the fifth week, COD remediation was 69% in the blank media. Additionally, the nutrients in the blank media, and the 1 mL, 2 mL, 5 mL, and 10 mL mixed cultures increased proportionally, the COD values also increased [29]. One of the other studies conducted on media with aclonifen; the removal rate of COD seen between 91% and 53%. *Metacordyceps chlamydosporia* showed the best removal performance. 15600 ppm of COD decreased to 1040 mg/l after the end of day five. The lowest removal performance was seen with *Penicillum talaromyces* from 15,600 ppm to 7330 ppm Results for the *penicillium thrichoderma, penicillium siplicissimum, stachybotrys chartarum* and *alternaria alternata* occured between these values [30]. Biodegradation of trifluralin performed in liquid media with 6 different types of identified fungi cultures in agitated culture media. The COD-removal efficiency varied according to the microbial differences. The removal efficiency by *Penicillium thrichoderma, Penicillium simplicissimum, Penicillium talaromyces, Metacordyceps chlamydosporia, Stachybotrys chartarum* and *Alternia alternata* species, were 71, 59, 64, 80, 70 and 74% respectively in five days [31].

3.4. Dry biomass

The amount of the dry fungal biomass was measured as 1.05, 0.72, 0.52 and 1.15 g for 50, 100, 150 ppm and control medium respectively (Fig. 5). These results revealed that especially 100 ppm and 150 ppm concentration of MLT clearly reduced the growth of fungus in submerged culture medium. Similarly in another study, It was demonstrated that yeast biomass production in the liquid medium in the presence of some pesticides (cypermethrin + chlorpyrifos and triazamate etc.), was inhibit as 82% with the results ascertained by the observation of inhibitory zones [32].

4. Conclusion

In conclusion, the data reported in this study indicate that *P. chrysosporium* might be used in MLT bioremediation with a significant COD reduction in submerged culture conditions supplemented with MLT. This fungus may be also used in bioremediation of some other insecticides. Nevertheless, further studies with other pesticides and fungi are needed to confirm its clear mechanism of bioremediation action by other environmental parameters.

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