Original Research Article

Resistance and Application of the Biomass from *Fusarium solani* in the Removal of Pentaclorophenol in Aqueous Solution

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ABSTRACT

Recently, the removal and degradation capacity of different environmental contaminants such as heavy metals, organic solvents, oils, plastics, pesticides and herbicides, from sites contaminated by low-cost materials has been studied, with promising results. These adsorbents include agricultural and industrial waste, clay minerals, live and dead microorganismos, and others materials. The objective of this work was analize the resistance and removal capacity of pentaclorophenol by the environmental polluting fungus *Fusarium solani*, by UV-visible spectrophotometry method. The fungal strain grew on LMM supplemented with 80 mg/L of PCP about 6% of growth relative to control (118 mg of dry weight without of pesticide) was obtained. The highest adsorption was obtained at pH 5.0, at 28^{o}C after 24 hours of incubation with 1 g of fungal biomass, and 100 rpm. If we increase the PCP concentration, the removal decrease, and if the biomass concentration is increase, the removal is most efficient, and the laccase activity is increase in presence of 10 mg/L of PCP, with a degradation of 63% at 15 days of incubation.

Keywords: Resistance, Fusarium solani, removal, pentaclorophenol

1. INTRODUCTION

The indiscriminate and uncontrolled use of pesticides causes serious problems for ecosystems and their components such as deterioration of flora, fauna, and aquatic terrestrial, as well as contamination of soil and groundwater. On the other hand, uncontrolled use can generate microorganisms and pests resistant to pesticides, many of which can cause serious health problems for humans [1, 2]. Pesticides reaching water bodies by runoff, infiltration, and soil erosion in places where they have applied. They can also be mobilized by both atmospheric transport and by runoff during rainfall or agricultural irrigation and thus transported into bodies of water, both surface and groundwater to pollute water and sediments [1,2].

In the case of Mexico, the use of pesticides shows a behavior that differentiates it from the rest of the world, for example, the three subgroups of pesticides: insecticides, herbicides, fungicides, and bactericides represent practically 100 % of what is consumed in the country, in contrast to 52 % globally [3]. In 2018, in our country it registered a total consumption of pesticides of 53.1 thousand tons, of which 54 % corresponded to fungicides and bactericides, 34 % to insecticides and 22 % to herbicides, and between 1990 and 2018, the growth in Mexican demand for pesticides doubled, and the subgroup of insecticides grew by 122 %, fungicides at 104 %, and herbicides at 72 %. Note that, in Mexico, herbicides have less absolute importance and relative than in the rest of the world [3]. Their most frequency use is in the agriculture, and it is estimated that about 65% of domestic consumption of pesticides applied to combat pests and diseases and prevent crops in corn, sorghum, soybeans, sugar cane, rice, vegetables, and grasses [4].

The pentachlorophenol (PCP) is the third pesticide most used in USA, preceded only by the atrazine and alachlor: its main use is to control termites and is frequently used as a ester (laureate pentachlorophenyl), to protect the wood of the putrefaction by fungus. This is also used as a microbicide for the skin, indoor spraying of railcar shipments formulating

solutions insecticides [5]. This is present in the environment and has been detected in superficial and rainwater, drinking water, sediments in aquatic organisms, soil, and food [5], and it was identified as a priority organic pollutant by the Environmental Protection Agency of the USA [6], because it is very toxic because it uncouples oxidative phosphorylation and forms adducts with DNA, and acute exposure to PCP causes local irritation, systemic effects, allergic reactions, alterations immunological and gastrointestinal and in the case of fatal poisonings have been severe damage to organs such as liver and kidneys. Symptoms of acute poisoning are increased body temperature, profuse sweating, abdominal pain and nausea, damage to the nervous system center and, in extreme cases, cardiac arrest. Too, has been linked with anemia and aplastic leukemia, is neurotoxic, affects the reproductive functions and fetal development, and has found evidence that can bioaccumulate in the human organism [7, 8, and 9].

Pollution of surface waters by PCP is due to direct and nonpoint sources of water as agricultural returns, industrial, and municipal surplus leachate dumps, and landfills downloads. Chlorinated phenolic compounds are present in aqueous effluents of different industrial, such as polymeric resins oil production, oil refining, iron and steel, paints, solvents, pesticides, pharmaceuticals operations and chemical wood preservation [10, 11].

In the literature, there is a variety of adsorbents for the removal of PCP from contaminated niches, like: three strains of *Pseudomonas* (*P. putida* S121, *P. rhizophila* S211, and *P. fuscovagiceae* S115), as bioremediation agents in depletion and detoxification of PCP in soil microcosms [10], the fungus *Aspergillus sydowii* and the plant *Typha angustifolia,* the fungal strain was isolated and identified from agricultural soil irrigated for 20 years with wastewater [12], carbon nanotubes [13], agricultural and industrial wastes [14], Bi_2O_3/TiO_2–montmorillonite nanocomposites [15], activated carbon [16,17], peat [18], modified chitosan [19], the PCP tolerance and removal by *Rhizopus nigricans* [20, 21], the fungi *Byssochlamys nivea* and *Scopulariopsis brumptii* [22], fifteen fungi strains isolated from a marine invertebrate, the ascidian *Didemnun ligulum*, were evaluated according to their ability to grow in solid culture media (3% malt extract agar) in presence of different concentrations of PCP (10, 25, 30, 40, and 50 mg/L) [23], the surfactant efficiency on pentachlorophenol-contaminated wastewater enhanced by *P. putida* AJ 785569 [24], *T. angustifolia* concerning PCP tolerance and removal from wastewater [25], the use of dead biomass of fungi like *R. nigricans* and *Rhizopus oryzae* [26, 27],*Trichoderma and Cunninghamella* strains [28], *A. niger* [29], *Anthracophyllum discolor* [30], and *Pleurotus pulmonarius* [31].

Too, PCP is capable of being biodegradable by only a limited number of bacteria and fungi, like: *Acinetobacter* sp., [32], *Bacillus cereus* (DQ002384), *Serratia marcescens* (AY927692) and *S. marcescens* (DQ002385) [33], *Bacillus* sp., [34], the white rot fungi *Pleurotus chrysosporium*, *Trametes sp.,* and *Pleurotus sp.,* [35], *Trametes versicolor* [36], *Phanerochaete chrysosporium* [37], *R. nigricans* [38], *T. versicolor* [39], different fungi [40], *Pleurotus ostreatus* [41], different microorganisms [42], and a consortia of specialized microorganisms [43], which, involve the use of enzymes laccases [34, 35, 36, 37, 38, 39, 41, 42, 43]. The objective of this study was to analyze the biosorption of this pesticide by fungal biomass of *F. solani* resistant to PCP, which is of universal distribution, ubiquitous and of great economic importance since this is common phytopathogens [44]. Sometimes he causes infections in the normal patient (keratitis, onychomycosis, etc.) [45]. However, each time describe more serious infections in immunosuppressed patients, hence its importance has grown exponentially [46].

2. MATERIAL AND METHODS

2.1 Microorganism and culture conditions and PCP resistant test

We used the phytopathogenic fungus *F. solani*, isolated from a culture of tomato, located in the municipality of Villa de Arista, San Luis Potosí, México. The fungal isolate was routinely maintained in Potato Dextrose Agar (PDA) at 28°C. PCP-resistant tests of the strain, were performed on liquid Lee´s minimal medium (LMM) [47], containing the appropriate nutritional requirements and different concentrations of PCP, and determining the dry weight.

2.2 Obtain of the fungal biomass and Pentachlorophenol solutions

For his propagation, were used 1000 mL Erlenmeyer flasks containing 600 mL of thioglycolate broth (8 g/L). The prepared flasks were inoculated with 1 x 10^6 spores/mL, and were incubated at 28°C, with constant stirring (100 rpm). After 5 days of incubation, the cells were filtered in Whatman paper No. 1, washed twice with trideionized water, and then dried at 80°C for 12 h in an oven. Finally, the fungal biomass was milled and stored in an amber bottle in the refrigerator until their use. PCP solutions are prepared from a stock solution of 1 000 mg/L and diluting it with trideionized water. The standard solution of 1 000 mg/L was prepared by adding 1000 mg of PCP to a volumetric flask of 1 000 mL and carrying to 1 L with 0.1 M NaOH solution.

2.3 Determination of PCP

The concentration of PCP in aqueous solution was determined by UV-visible spectrophotometry. The absorbance was determined in a Spectrophotometer Thermo Scientific, Genesys 10 S Uv-Vis, and was measured at a wavelength of 250

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nm for concentrations of 0-10 mg/L and 320 nm for 10-100 mg/L. The spectrophotometer was calibrated to zero using as white, the solution that was used to prepare PCP solution. PCP analysis was performed in a sample by measuring the absorbance of the sample and calculating the concentration using a calibration curve [16]. Tree dependent experiments were carried out and the mean value was shown.

2.3 Determination of laccase activity

The prepared flasks with basic medium for laccase: 0.4 g/L KH_2PO_4, 0.2 g/L $MgSO_4.7H_2O$, 1.0 g/L $NH_4(NO)_3$, 0.01 g/L $FeSO_4$, 0.01 g/L $ZnSO_4$, 0.01 g/L $MnSO_4$, 0.2 g/L KCl, and 0.25% (w/v) saccharose [48], (with and without 10 mg/L of PCP), were inoculated with 1×10^6 spores/mL, and incubated at 28°C, with constant stirring (100 rpm). After 5 days of incubation, the cells were filtered at 4°C in Whatman paper No. 1, and the supernatant was used to determine the activity of laccase. Laccase activity was determined by the oxidation of ABTS (2,2-azino-bis (3-ethylbenzthia-zoline-6-sulfonic acid): the assay mixture contained 0.5 mM ABTS, 0.1 M Sodium acetate (pH 5.0), and 0.5 mL of enzyme (extracellular culture filtrate). The oxidation of ABTS was monitored by determining the increase in A_{420}. One unit of enzyme activity was equivalent to 1 µMol of the product formed per minute [49].

3. RESULTS AND DISCUSSION

The cells of the analyzed strain grew on LMM supplemented with 80 mg/L g/L of PCP about 6% of growth relative to control (118 mg of dry weight without metal) was obtained (Figure 1) and therefore probably is resistant to the PCP. Different microorganisms that are PCP resistant have been isolated from different contaminated sites: The bacterial *strains P. putida, P. rhizophila,* and *P. fuscovagiceae*, showed good capability to tolerate and degrade PCP [11], *R. nigricans* displayed a high tolerance to grow in the presence of PCP (up to 100 mg/L) [20]. Too, *Trichoderma harzianum* CBMAI 1677 showed optimal growth at 50 mg/L, showing toxicity resistance, and suggesting its potential for biodegradation of the pesticide [23], in mineral salt medium added with PCP (100 mg/L) by strain *P. putida* AJ 785569, showed a significant increase in the optical density value [24], a wild strain of *R. nigricans*, isolated from paper mill effluent, which grew 4.5 mg/mL with 12.5 and 25 mg PCP/mL, but decreased to 1.5 and 1.8 mg biomass/mL when 50 and 100 mg PCP/L was added [26], in *P. pulmonarius*, the addition of PCP inhibited considerably the growth of the fungus in the basal condition, and this caused only a slight inhibition of the fungal growth in the corn cob medium [50], *P. chrysosporium* grown in different culture mediums with 25 mg PCP/mL [37], *Bacillus* sp., tolerant to higher levels of PCP (500 mg/mL) [34], *E. coli*, *Pseudomonas aeruginosa,* and *Acinetobacter* sp., strains had higher growth on PCP [51], and 33 fungal isolates were isolated from the aging sawdust simple, which are grew with different concentrations of the same (5-100 mg/L) [48].

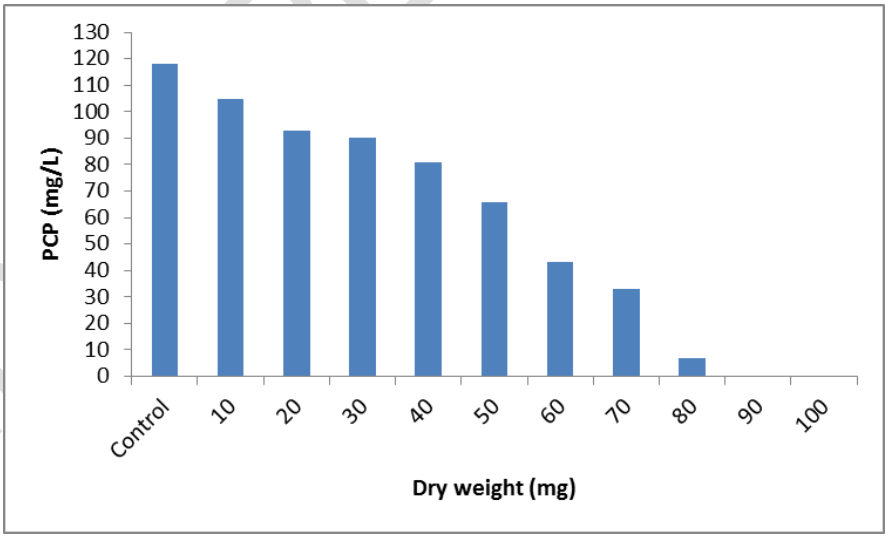

Fig.1. Growth in dry weight of *F. solani* with different concentrations of PCP, 1×10^6 spores/mL, 28°C, seven days of incubation, 100 rpm.

Figure 2 and 3 shows the effect of incubation time and pH on the biosorption of 10 mg/L of PCPC by the biomass of *F. solani.* It was found that a higher removal (90%) occurs at 24 hours a pH 5.0. With respect to incubation time, for three strains of *Pseudomonas* was reported a removal of 85% with 500 mg/kg of PCP and 55 days of incubation [11], *R. nigricans* degrades 60% in 24 h, at an initial concentration of 12.5 mg PCP/L [20], The fungi *B. nivea* and *S. Brumptii,*

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removal 60% and 62% during 28 days of incubation, respectively [22], a strain of *P. putida* AJ 785569 could adsorb around 30 mg/L and remove 600 mg/L of PCP within 168 h of incubation [24], the efficiency of *T. angustifolia* phytoremediation treatments of the different effluents artificially contaminated with 200 mg/L of PCP after 20 days [25], Mathialagan and Viraraghavan [29] report an incubation time of 6 hours, when working with biomass from *A. niger*, too, 8 hours for *R. oryzae* ENHE [27], 4h hours for *Bacillus* sp., [34], 3 days for *E. coli*, *P. aeruginosa,* and *Acinetobacter* sp., strains isolated of tannery effluents [51], and 15 days for *Trichoderma* and *Cunninghamella* strains isolated from sawdust [48]. The differences in the incubation time may be due to changes in the permeability of the cell wall of unknown origin, providing greater or lesser exposure of the functional groups of the cell wall of biomass analyzed [1].

We observe the effect of pH on PCP sorption, to seven different values of pH (2.0-8.0), and the most effective was 5.0 (90% of removal, 10 mg/L, 24 hours). In isolated microorganisms of tannery effluent, the percentage removal decreased with an increase in pH for all types of *A. niger* biomass, except for the conditioned *A. niger* biomasses. In the case of this treated biomass, almost 100% removal was achieved between pH 3 and10, but at pH 11, the removal slightly decreased to 95% [51], was reported a pH of 3.0 for a laccase mutant of *T. versicolor* [39], under strong alkaline conditions (pH 9–12) for *P. osteratus* [41], for *R. oryzae* ENHE, the optimum pH was 5.0 [27]. Viraraghavan and Slough (1999), reported that the removal of PCP at an initial concentration of 1 mg/L by a peat-bentonite mixture, decreased from approximately 96 to 79%, and 85 to 30% when the pH was increased from 2 to 8 [50], and for *Bacillus cereus* RMLAU1, the PCP degradation by live cells at the initial pH range of 3 to 8. As the pH increased from 3 to 5, there was a concomitant increase in PCP degradation from 2.5% (w/v) to 7.5% (w/v) followed by gradual decrease approaching 5.5% (w/v) at pH 8.0. Maximum PCP degradation of 7.5% (w/v) was noted at pH 5.0 [52]. The solution pH can also affect the surface characteristics (e.g., surface charge and potential) of the adsorbent i.e., the biomass [53]. Generally, a bacterium or fungi exhibits a net negative charge at neutral and basic pH conditions. At the same time, PCP exists entirely in the anionic form at those pH ranges. Therefore, electrostatic repulsion between the negatively charged biomass surface and the anionic PCP may lead to a lower biosorption. But when the pH is lowered, the net negative charge on the biomass decreases and PCP tends to exist in the molecular form [53].

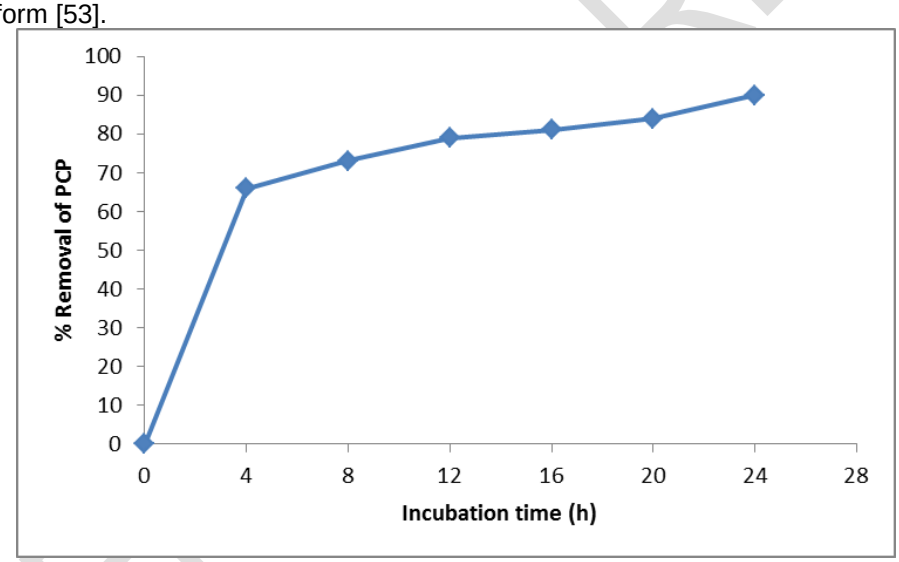

Fig.2. Effect of incubation time on PCP removal by *F. solani* of 10 mg/L PCP, 100 rpm, 28°C, pH 5.0, and 1.0 g of fungal biomass.

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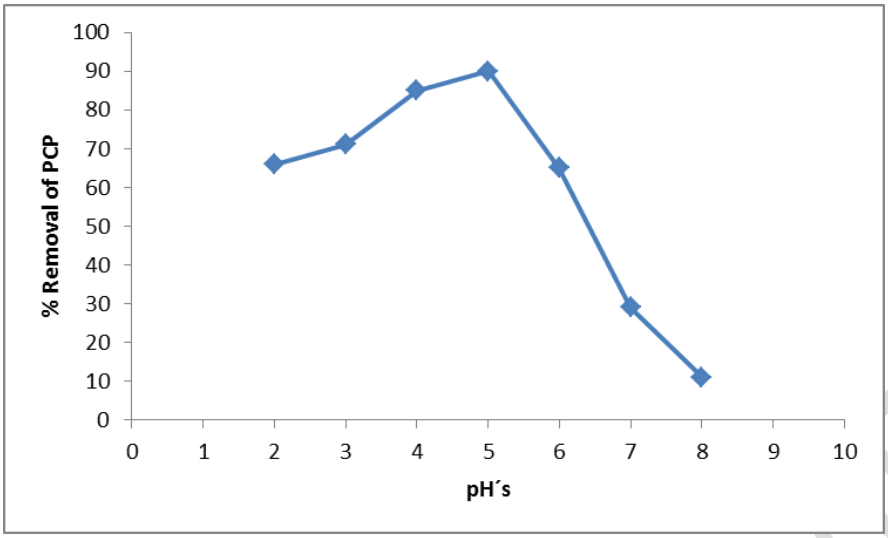

Fig.3. Effect of pH on PCP removal by *F. solani* of 10 mg/L PCP, 100 rpm, 28°C, 24 h, and 1.0 g of fungal biomass.

The temperature is a critical parameter in the removal of this pesticide (Figure 4), because we observe greater removal as 28^{o}C, 90% of the metal is removal at 24 hours. These results are similar to those reported for the conditioned *A. niger* biomass [51] and for *B. cereus* RMLAU1, the degradation of PCP to the extent of 3.5 to7.5% (w/v) was noted in the temperature range of 25°C to 35°C by live biomass only, which was reduced to more than half (3.0%, w/v) when temperature was further elevated to 40°C [32], and there are different for non-viable *R. oryzae* biomass, in which the capacity of removal was assayed at pH 5.0 and five temperatures (20, 25, 30, 35, and 40°C), founded that the values of removal increased from with an increase in temperature [27]. One of the reasons for the decreasing trend of adsorption with temperature may be mainly due to the weakening of adsorptive forces between the active sites of the biomass and PCP, and between the adjacent molecules of the adsorbed phase [34].

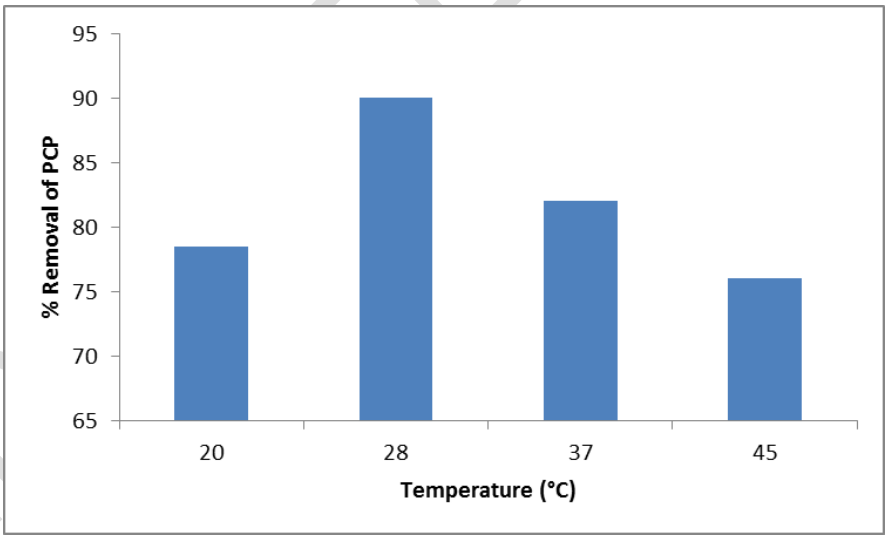

Fig.4. Effect of the temperature on PCP removal by *F. solani* of 10 mg/L PCP, 100 rpm, pH 5.0, 24 h, and 1.0 g of fungal biomass.

The results obtained for the removal, depend on the concentration of PCP, at lower concentration of the same, removal is faster, 10 mg/L are removal in an 90%, and 50 mg/L a 31%, at 24 hours, respectively, (Figure 5), which may be due to sorption at low concentrations (which is a very fast process) happens. These results are similar those with *A. sydowii* and *T. angustifolia,* which removal 200 mg/L of PCP in 30 days of incubation [12], for the removal of 0.127, 0.168, 0.214 and 0.296 (mg/g) for the sunflower seed hulls, milk thistle seeds, hazelnut shells and pine sawdust, respectively [14], *R. nigricans* degrades 60% at an initial concentration of 12.5 mg PCP/L [20]. Co-inoculation of *B. nivea* and *S. brumptii* showed a synergetic effect on PCP removal resulting in 95% and 80% decrease when initial concentrations were 12.5

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and 25 mg/kg, respectively [22], for *Trametes hirsuta* in PCP treated ammunition boxes (0, 5, 10, 15, 20 and 25 ppm of PCP) [35] and are different those of *R. nigricans* [20]. For other contaminants, indicated that the removal of this increases in direct proportion to the increasing concentration of the contaminant in solution [54, 55]. This may be due to the increased number of competing for the functional groups of the surface of the biomass ions [56].

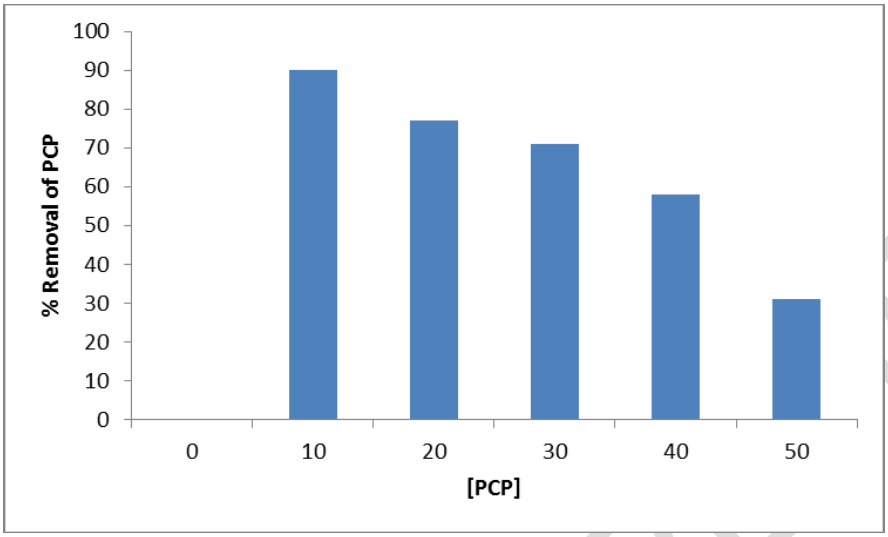

Fig.5. Effect of the initial PCP concentration on the removal of PCP by *F. solani.* 100 rpm. 28°C, pH 5.0, 24 h, and 1.0 g of fungal biomass.

To evaluate the removal of 10 mg/L of PCP with different concentrations of biomass, it was found that the higher concentration of the latter, the removal of this pesticide is greater and faster. 1 g of biomass with the maximum removal was observed at 24 hours, whereas 5 g removal time was 4 hours (Figure 6). These observations can be explained as the amount of added bioadsorbent determines the number of sites available for biosorption of PCP. This is different for the degradation by the bacterial strains, *P. putida, P. rhizophila*, and *P. fuscovagiceae*, showed good capability to degrade PCP with 108 UFC/g soil inoculum size of each strain [11], by *B. cereus* RMLAU, was not significantly affected by the concentration of biomass, and maximum remediation (7.5%, w/v) of initial 500 mg/L PCP was recorded by 2 g live biomass/L [52], and similar results have been reported for other contaminants [57, 58].

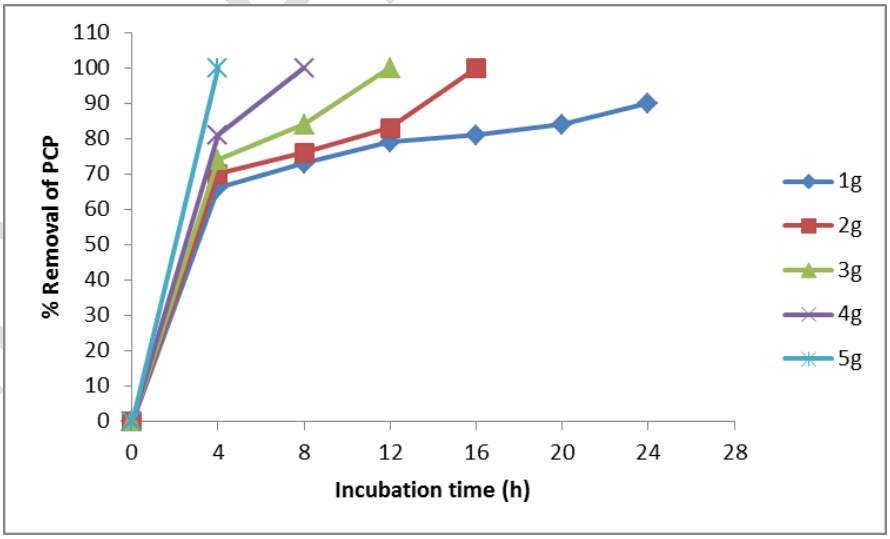

Fig.6. Effect of biomass concentration on PCP removal by *F. solani.* 10 mg/L PCP. 28°C, 24 h, and pH 5.0, 100 rpm.

The extracellular activity of *F. solani*, induced in basic medium for laccase [48], was tested. Oxidation of ABTS indicates the presence of laccase activity, and the maximum activity was 25 u/L, at 3 days, without PCP (Figure 7), and with 10 mg/L of PCP, the maximum laccase activity was 75 u/L on the 3rd day of incubation at which 15 % of the initial amount of

the pesticide was degraded (Figure 8). The degradation reached to the maximum level of 63% after 15 days of incubation. These data indicated that PCP was degraded under these conditions. *F. solani* and other *Fusarium* sp., can colonize, modify, and degrade lignin [59], and his physiological variability led us to examine the production of different extracellular ligninolytic enzymes by strains isolated from different sources [41], and the laccase activity was reported in some *Fusarium* species [59, 60, 61, 62, 63, 64, and 65]. On the other hand, the enzymes of the lignin degradation system of white rot fungi have been indirectly implicated in the degradation process in several organic compounds including PCP [66, 67].

Our results indicate that this fungus can biosorb and degrade PCP. There are some reports of different fungi can remove and degrade this, like *R. oryzae* [27], *Trichoderma* and *Cunninghamella* strains Isolated from sawdust [48], *Anthracophyllum discolor* [51], *P. Pulmonarius* [50], *B. cereus* (DQ002384), *S. marcescens* (AY927692) and *S. marcescens* (DQ002385) [53], *Bacillus* sp., [34], *P. chrysosporium* (IFO 31249), *Trametes sp.* (KFCC 10941), *Pleurotus sp*. (KFCC 10943) [35], *T. versicolor* [36] *P. chrysosporium* [37], *B. cereus* RMLAU1 [52], *P. chrysosporium* and *P. sordida* [35]. Finally, in has been reported that a strain of *Fusarium* was able to degrade PCP only if tyrosine was present in the culture medium [68]. Moreover, *in vitro* experiments, was showed phenol degradation by tyrosinase [69], and these fungi have an interesting potential to be used in processes for biodegradation of chlorophenols.

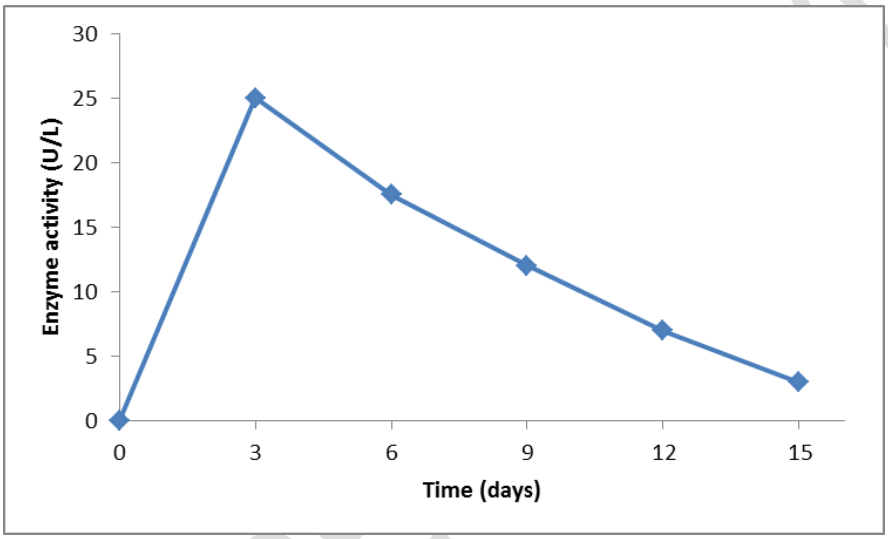

Fig.7. Laccase activity in *F. solani* cultures. 28°C, and pH 5.0, 100 rpm.

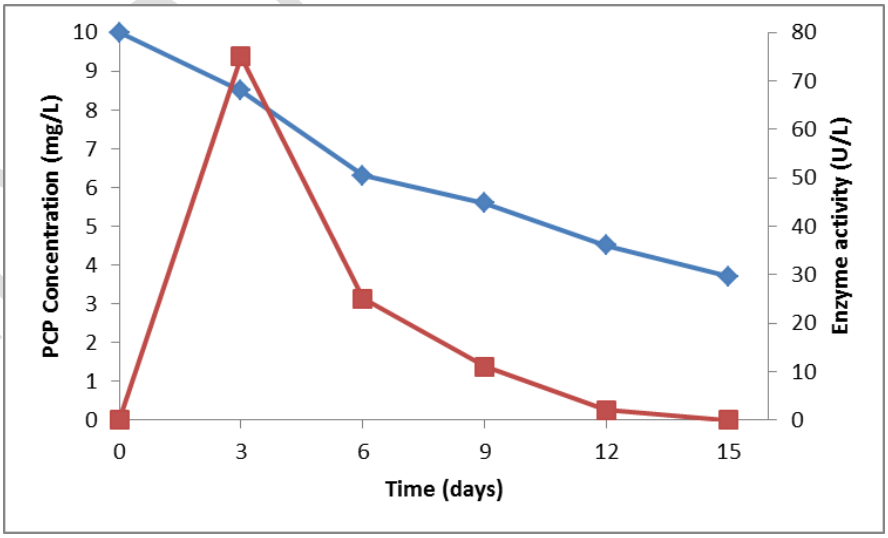

Fig.8. Laccase activity extracellular and biodegradation of 10 mg/L PCP in *F. solani* cultures. 28°C, and pH 5.0, 100 rpm.

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4. CONCLUSION

In this study, PCP uptake in vitro by fungal biomass of *F. solani* was investigated. The performance of the biosorbent was examined as a function of the operating conditions, in particular incubation time, pH and initial metal ion concentration, and fungal biomass. The experimental evidence shows a strong effect of the experimental conditions. Maximum biosorption capacity values showed that this biosorbent is very effective in removal PCP from aquatic systems. When the ease of production and economical parameters are concerned, it was observed that *F. solani* is a very promising biomaterial for removal of the pesticide. It was found that the biomass was very efficient in removal the pesticide in solution, determined by UV-visible spectrophotometry. The highest adsorption was obtained at pH 5.0, at 28^{o}C after 24 hours of incubation with 1 g of fungal biomass, and 100 rpm. If we increase the PCP concentration, the removal decrease, and if the biomass concentration is increase, the removal is most efficient. The laccase activity is increase in presence of 10 mg/L of PCP, with a degradation of 63% at 15 days of incubation.

REFERENCES

1. Franco Bernardes MF, Pazin M, Pereira LC, Junqueira Dorta D. Impact of Pesticides on Environmental and Human. In: Andreasa AC, Scola G. eds. Advances in Toxicology Studies-Cells, Drugs and Environment. 1st ed. Intechopen books series. Croatia. 2015. Chapter 8:195-233. DOI: 10.5772/59710

2. Mendarte-Alquisira C, Alarcón A, Ferrera-Cerrato R. Fitorremediación: Alternativa biotecnológica para recuperar suelos contaminados con DDT. Una revision. TIP Rev. Esp. en Cien. Quím. Biol. FEZ. UNAM. 2021;24:1-15. https://doi.org/10.22201/fesz.23958723e.2021.326: Español

3. Centro de Estudios para el Desarrollo Rural Sustentable y la Soberania Alimentaria. Impacto en el Uso de Plaguicidas en el Sector Agropecuario. 2020;1-44. http://www.cedrssa.gob.mx. Español.

4. Hernández-Antonio A, Hansen AM. Uso de plaguicidas en dos zonas agrícolas de México y evaluación de la contaminación de agua y sedimentos. Rev. Int. Cont. Amb. 2011;27(2):1-10. https://www.revistascca.unam.mx › rica › article › view. Español.

5. Agency for Toxic Substances and Disease Registry (ATSDR). 2021. Toxicological Profile for Pentachlorophenol (Draft for Public Comment). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. https://wwwn.cdc.gov › ToxProfiles

6. USEPA. Granular activated carbon treatment. EPA/540/2-91/024. U.S. Environmental Protection Agency. 2011. https://www.epa.ie

7. Castillo Morales I. Bárcenas Pazos C. Pentaclorofenol: toxicología y riesgos para el ambiente. Madera y Bosques. 1998;4(2):21-37. https://doi.org/10.21829/myb.1998.421357. Español.

8. Roberts HJ. Pentachlorophenol associated aplastic anemia, red cell aplasia, leukemia and other blood disorders. J. Florida Med. Assn. 1990;77(2):86-90. PMID: 2106570.

9. Werheni Ammeri R, Kraiemc K, Riahid K, Eturkib S, Hassene W, Mehria I, et. al. Removal of pentachlorophenol from contaminated wastewater using phytoremediation and bioaugmentation processes. Wat. Scien. Technol. 2021;84(10-11):3091-3103. doi: 10.2166/wst.2021.328

10. Martínez Valenzuela C, Romano Casas G, Cuadras Berelleza AA, Ortega Martínez D. Plaguicidas, impacto en salud y medio ambiente en sinaloa (México): implicaciones y retos en gobernanza Ambiental. TraHs Números especiales N°4. 2019:103-122. Políticas públicas: desafios nos contextos atuais http://www.unilim.fr/trahs. Español.

11. Hassen W, Cherif H, Werhani R, Raddadi N, Neifar M, Hassen A. et al. Exhaustion of pentachlorophenol in soil microcosms with three *Pseudomonas* species as detoxification agents. Arch. of Microbiol. 2021:1-13. https://doi.org/10.1007/s00203-021-02451-y

12. Werheni Ammeri R, Di Rauso Simeone G, Hassen W, Smiri Sadfi N, Hidri Y, Hassen A. *Aspergillus sydowii* and *Typha angustifolia* as useful tools for combined bio-processes of PCP removal in wastewater. Int. J. Environ. Scien. Technol. 2022:1-13. DOI:10.1007/s13762-021-03853-7

13.- Zhang X, Cui Ch, Zheng Q, Wang Y, Jing Chang J, Shuxian Wang S. Development of highly efficient and reusable magnetic nitrogen-doped carbon nanotubes for chlorophenol removal. Environ. Scien. Poll. Res. 2021;28:37424–37434. https://doi.org/10.1007/s11356-021-13302-0

14. Kuśmiereka K, Dąbekb L, Świątkowski A. Adsorptive removal of pentachlorophenol from water using agricultural and industrial wastes. Desal. and Water Treat. 2018;117:142-148. doi: 10.5004/dwt.2018.22178

15. Boumahdi N, Hadj-Ziane-Zafour A, Yaiche-Achour H, Khalaf H. Preparation of Bi_2O_3/TiO_2–Montmorillonite Nanocomposites and Their Applications to the Photodegradation of Pentachlorophenol. Bull. Chem. Reac. Engin. & Cat. 2022;17(1):78-87. https://bcrec.id

UNDER PEER REVIEW

16. Hernández Orta MMG, Mendoza Barrón J, Guerrero Coronado RM, Leyva Ramos R. Adsorción de Pentaclorofenol en solución acuosa sobre carbones activados comerciales. Rev. Int. Cont. Am. 2003;19(3):137-144. https://www2.revistascca.unam.mx › rica › article. Español.
17. Abdel-Ghani NT, El-Chaghaby GA, Zahran EM. Pentachlorophenol (PCP) adsorption from aqueous solution by activated carbons prepared from corn wastes. Int. J. Environ. Sci. Technol. 2015;12: 211–222. DOI 10.1007/s13762-013-0447-1.
[18] Tanjore S, Viraraghavan T. Effect of Oxygen on the Adsorption of Pentachlorophenol by Peat from Water. Water, Air, and Soil Pollution. 1997;100(1):151-162. DOI:10.1023/A:1018316211572
19. Shankar A, Kongot M, Kumar Saini V, Kumar A. Removal of pentachlorophenol pesticide from aqueous solutions using modified chitosan. Arab. J. Chem. 2020;13(1):1821-1830. https://doi.org/10.1016/j.arabjc.2018.01.016
20. Cortés D, Barrios-González J, Tomasini A. Pentachlorophenol tolerance and removal by *Rhizopus nigricans* in solid-state culture. Process Biochem. 2002;37 (8):881-884. https://doi.org/10.1016/S0032-9592(01)00295-3
21. Ruiz-Lara A, Fierro F, Carrasco U, Oria JA, Tomasini A. Proteomic analysis of the response of *Rhizopus oryzae* ENHE to pentachlorophenol: Understanding the mechanisms for tolerance and degradation of this toxic compound. Process Biochem. 2020;95:242-250. https://doi.org/10.1016/j.procbio.2020.02.016
22. Hechmi N, Bosso L, El-Bassi L, Scelza R, Testa A, Naceur Jedidi N, et. al. Depletion of pentachlorophenol in soil microcosms with *Byssochlamys nivea* and *Scopulariopsis brumptii* as detoxification agents. Chemosphere. 2016;165:547-554. https://doi.org/10.1016/j.chemosphere.2016.09.062
23. Vacondio B, Garcia Birolli W, Regali Seleghim MH, Gonçalves S, Pantaroto de Vasconcellos S, Porto AL. Screening of Marine-derived Fungi Isolated from the sponge *Didemnun ligulum* for Biodegradation of Pentachlorophenol. In: Shiomi N, editor. Advances in Bioremediation of Wastewater and Polluted Soil. 1st ed. Croatia. Intechopen books series. Croatia. 2015. Chapter 9:193-225. DOI: 10.5772/60777
24. Werheni Ammeri R, Hidri Y, Hassen W, Ines Mehri I, khlifi N, Hassen A. Surfactant efficiency on pentachlorophenol-contaminated wastewater enhanced by *Pseudomonas putida* AJ 785569. Arch. Microbiol. 2021;203:5141–5152. doi: 10.1007/s00203-021-02486-1
25. Werheni Ammeri R, Hassen W, Hidri Y, Di Rauso Simeone G, Hassen A. Macrophyte and indigenous bacterial coremediation process for pentachlorophenol removal from wastewater. Int. J. Phytorem. 2022;24(3):271-282. https://doi.org/10.1080/15226514.2021.1933897
26. Tomasini A, Flores V, Cortes D, Barrios-González J. An isolated of *Rhizopus nigricans* capable of tolerating and removing pentaclorophenol. World J. Microbiol. Biotechnol. 2001;17: 201-205. DOI: 10.1023/A:1016694720608
27. León-Santiesteban HH, Wrobel K, García LA, Revah S, Tomasini A. Pentachlorophenol Sorption by *Rhizopus oryzae* ENHE: pH and Temperature Effects. Water Air Soil Pollut. 2014;225:1-10. DOI:10.1007/s11270-014-1947-4
28. Sing NN, Zulkharnain A, Azman Roslan H, Assim Z, and Husaini A. Bioremediation of PCP by *Trichoderma* and *Cunninghamella* Strains Isolated from Sawdust. Braz. Arch. Biol. Technol. 2014;57(6):811-820. DOI:10.1590/S1516-8913201402852
29. Mathialagan T, Viraraghavan T. Biosorption of pentachlorophenol from aqueous solutions by a fungal biomass. Biores. Technol. 2009;100:549–558. doi: 10.1016/j.biortech.2008.06.054.
30. Rubilar O, Tortella GR, Cuevas R, Cea M, Rodríguez-Couto S, Diez MC. Adsorptive Removal of Pentachlorophenol by *Anthracophyllum discolor* in a Fixed-Bed Column Reactor. Water Air and Soil Pollution. 2011;223(5):2463-2472. DOI:10.1007/s11270-011-1039-7
31. de Souza DF, da Costa SC, Dacome AS, Marqués de Souza CG, Bracht A, Peralta RM. Pentachlorophenol Removal by *Pleurotus Pulmonarius* in Submerged Cultures. Braz. Arch. Biol. Technol. 2011;54(2):357-262. https://doi.org/10.1590/S1516-89132011000200018
32. Srivastava S, Ahmad AH, Thakur IS. Removal of chromium and pentachlorophenol from tannery effluents. Biores. Technol. 2006;98(5):1128-1132. doi:10.1016/j.biortech.2006.04.011.
33. Singh S, Singh BB, Chandra R, Patel DK, Rai R. Synergistic biodegradation of pentachlorophenol by *Bacillus cereus* (DQ002384), *Serratia marcescens* (AY927692) and *Serratia marcescens* (DQ002385). World J. Microbiol. Biotechnol. 2009;25(10):1821–1828. DOI:10.1007/s11274-009-0083-6
34. Tripathi M, Vikram S, Jain RK, Garg SK. Isolation and Growth Characteristics of Chromium(VI) and Pentachlorophenol Tolerant Bacterial Isolate from Treated Tannery Effluent for its Possible Use in Simultaneous Bioremediation. Indian J Microbiol. 2011;51(1):61–69. doi: 10.1007/s12088-011-0089-2
35. Ryu Ryu W, Hoon Shim S, Yup Jang M, Joong Jeon Y, Keun Oh K, Hwan Cho M. Biodegradation of Pentachlorophenol by White Rot Fungi under Ligninolytic and Nonligninolytic Conditions. Biotechnol. Bioproc. Eng. 2000;5:211-214. https://www.koreascience.or.kr › article › JAKO2.
36. Nguyen LN, Hai FI, Kang J, Leusch FDL, Roddick F, Magram SF, et al. Enhancement of trace organic contaminant degradation by crude enzyme extract from *Trametes versicolor* culture: Effect of mediator type and concentration. J. Taiwan Instit. Chem. Engin. 2014;45: 1855–1862. http://dx.doi.org/10.1016/j.jtice.2014.03.021
37. Aiken BS, Logan BE. Degradation of pentachlorophenol by the white rot fungus *Phanerochaete chrysosporium* grown in ammonium lignosulphate media. Biodegrad. 1996;7:175-182. https://doi.org/10.1007/BF00058177

UNDER PEER REVIEW

38. Mayera AM, and Staples RC. Laccase: new functions for an old enzyme. Phytochemistry. 2002;60(6):551-565. doi: 10.1016/s0031-9422(02)00171-1.
39. Chiadò A, Bosco F, Bardelli M, Simonelli L, Pedotti M, Marmo L, et al. Rational engineering of the lccb *T. versicolor* laccase for the mediator-less oxidation of large polycyclic aromatic hydrocarbons. Comp. Struc. Biotecnol. J. 2021;19:2213-2222. https://doi.org/10.1016/j.csbj.2021.03.017
40. Jasu A, Lahiri D, Nag M, Ray RR. Fungi in bioremediation of soil organic pollulants. In: Sharma VK, Shah MP, Kumar A. eds. Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nano-technology. Volume 2: Extremophilic Fungi and Myco-Mediated Environmental Management. 2021, Chap. 17:381-405. https://doi.org/10.1016/B978-0-12-821925-6.00017-4
41. Liu X, Deng W, Yang Y. Characterization of a Novel Laccase LAC-Yang1 from White-Rot Fungus *Pleurotus ostreatus* Strain Yang1 with a Strong Ability to Degrade and Detoxify Chlorophenols. Molecules. 2021;26(473):1-31. https://doi.org/10.3390/molecules26020473
42. Kumar M, Gupta A, Srivastava S. Degradation and Biotransformation of Pentachlorophenol by Microorganisms. In: Kaushik A., Kaushik C.P., Attri S.D. Eds. Climate Resilience and Environmental Sustainability Approaches. Springer, Singapore. 2021. Chap. 15:299-318. https://doi.org/10.1007/978-981-16-0902-2_16
43. Sonkara Vivek M, Prasum Kumar MP, Shad CB, Majumdare JK, Biswas JK, Dutt D, et al. Bioaugmentation with existing potent microorganisms to accelerate the treatment efficacy of paper industry wastewater pollutants. J. Environm. Chem. Eng. 2021;9(5):105913. https://doi.org/10.1016/j.jece.2021.105913
44. Forero-Reyes CM, Alvarado-Fernández AM, Ceballos-Rojas AM, González-Carmona LC, Linares-Linares MY, Castaneda-Salazar R, et al. Evaluación de la capacidad patogénica de *Fusarium* spp. en modelos vegetal y murino. Rev. Arg. Microbiol. 2018;50(1):90-96. https://doi.org/10.1016/j.ram.2016.11.009. Español.
45. García Lozano T, Aznar Oroval E. Fusariosis: infecciones por *Fusarium* spp. Una breve nota. Rev. Lab. Clin. 2013;6(4):185-189. https://www.elsevier.es. Español.
46. Maquera-Afaray J, Pérez-Lazo G, Rodriguez R, Illescas R, Rodríguez L, Hidalgo J, et al. Fusariosis invasiva: reporte de tres casos en Perú. Rev. Peru. Med. Exp. Salud Publica. 2018;35(3):523-526. doi: 10.17843/rpmesp.2018.353.3175. Español.
47 Lee KL, Buckley HR, Campbell CC. An aminoacid liquid synthetic medium for the development of mycelial and yeast forms of Candida albicans. Sabou. J. Med. Vet. Mycology. 1975;13(2):148-153. doi: 10.1080/00362177585190271.
48. Córdova D, Roncero M. Functional analyses of laccase genes from *Fusarium oxysporum*. Phytopathol. 2008;98:509-578. doi: 10.1094/PHYTO-98-5-0509.
49. Niku-Paavola ML, Raaska L, Itävaara M. Detection of white-rot fungi by a non-toxic stain. Mycol. Res. 1990;94(1):27-31. https://doi.org/10.1016/S0953-7562(09)81260-4
50. Viraraghavan T, Slough S. Sorption of pentachlorophenol on peat-bentonite mixtures. Chemosphere. 1999;39:1487–1496. doi: 10.1016/s0045-6535(99)00053-3.
51. Thakur IS, Srivastava S. Bioremediation and bioconversion of chromium and pentachlorophenol in tannery effluent by microorganisms. Int. J. Technol. 2011;2(3):224-233. DOI:https://doi.org/10.14716/ijtech.v2i3.70
52. Tripathi M, Mishra SS, Tripathi VR, Garg SK. Predictive approach for simultaneous biosorption of hexavalent chromium and pentachlorophenol degradation by *Bacillus cereus* RMLAU1. Afric. J. Biotechnol. 2011;10(32):6052-6061. DOI: 10.5897/AJB10.2490
53. Shimizu Y, Yamazaki S, Terashima Y. Sorption of anionic pentachlorophenol (PCP) in aquatic environments: the effect of pH. Water Science Technol. 1992;25(11):41-48. https://search.proquest.com › openv...
54. Lamar RT, Scholze RJ. White-Rot Fungi Biodegradation of PCP-Treated Ammunition Boxes. In: Proceedings of R&D 92 National research & Development Conference on the Control of Hazardous Materials; 1992 February 4-6; San Francisco, CA. Greenbelt, MD: Hazardous Materials Control Resources Institute. 1992;89-94. https://www.fpl.fs.fed.us › pdf1992 › lamar92a
55. Morales-Barrera L, Cristiani-Urbina E. Hexavalent chromium removal by a *Trichoderma inhamatum* fungal strain isolated from tannery effluent. Water, Air, Soil Pollut. 2008;187(1-4):327-336. https://doi.org/10.1007/s11270-007-9520-z
56. Tejada-Tovar C, Villabona-Ortiz A, Garcés-Jaraba L. Adsorción de metales pesados en aguas residuales usando materiales de origen biológico. Tecno Lógicas: 2015;18(34):109-123. DOI:10.22430/22565337.209. Español.
57. Acosta-Rodríguez I, Arevalo-Rangel D, Cárdenas-González JF, Moctezuma-Zárate MG, Martínez-Juárez VM. Hexavalent Chromium Removal by *Penicillium* sp. IA-01. In: Bioremediation of Wastewater and Polluted Soil. Edited by Shiomi, N. Intech. pp. 166-192. Croatia. 2015. DOI: 10.5772/60660
58. Xu A, Jian H, Liu Y, Zeng G, Gu Y, and Tan X. Removal of chromium (VI) from aqueous solution using mycelial pellets of *Penicillium simplicissimum* impregnated with powdered biochar. Biorem. J. 2015;19(4):259–268. https://doi.org/10.1080/10889868.2015.1066302
59. Rodríguez A, Perestelo F, Carnicero A, Regalado V, Pérez R, de la Fuente G, et al. Degradation of natural lignin and lignocellulosic substrates by soil-inhabiting fungi imperfect. FEMS Microbiol. Ecol. 1996;21:213-219. https://doi.org/10.1016/S0168-6496(96)00057-8

UNDER PEER REVIEW

60. Nazareno Saparrat MC, Martínez MJ, Tournier HA, Cabello MN, Arambarri AM. Production of ligninolytic enzymes by *Fusarium solani* strains isolated from different substrata. World J. Microbiol. Biotechnol. 2000;16:799-803. DOI:10.1023/A:1008923804859
61. Hernández Fernaud JR, Marina A, González K, Vázquez J, Falcón MA. Production, partial characterization and mass spectrometric studies of the extracellular laccase activity from *Fusarium proliferatum*. Appl. Microbiol. Biotechnol. 2006;70: 212–221. DOI 10.1007/s00253-005-0221-5
62. Anderson AJ, Kwon S, Carnicero A, Falcón MA. Two isolates of *Fusarium proliferatum* from different habitats and global locations have similar abilities to degrade lignin. FEMS Microbiol. Lett. 2005;249(1):149–155. https://doi.org/10.1016/j.femsle.2005.06.014
63. Regalado V, Perestelo F, Rodríguez A, Carnicero A, Sosa AFJ, de la Fuente G, et al., Activated oxygen species and two extracellular enzymes: laccase and aryl-alcohol oxidase, novel for the lignin-degrading fungus *Fusarium proliferatum*. Appl. Microbiol. Biotechnol. 1999;51(3):388-390. DOI:10.1007/s002530051407
64. Regalado V, Rodríguez A, Perestelo F, Carnicero A, de la Fuente G, Falcón MA. Lignin Degradation and Modification by the Soil-Inhabiting Fungus *Fusarium proliferatum.* Appl. Environ. Microbiol. 1997;63(9):3716-3718. doi: 10.1128/aem.63.9.3716-3718.1997.
65. Reyes-Medina MA, Macías-Sánchez KL. GTPase Rho1 regulates the expression of xyl3 and laccase genes in *Fusarium oxysporum*. Biotechnol. Lett. 2015;37(3):679-683. doi: 10.1007/s10529-014-1709-9.
66. Fenando T, Bunpus JA, Tien M, Wright MD, Aust SD. Biodegradation of TNT by *Phanerochaete chrysosporium.* Appl. Environ. Microbiol. 1990;56(6):1666-1671. doi:10.1128/aem.56.6.1666-1671.1990
67. Kennedy DW, Aust SD, Bunpus JA. Comparative biodegradation of alkyl halide insecticides by the white rot fungus, *Phanerochaete chrysosporium* (BKM-F-1767). Appl. Environ. Microbiol. 1990;56:2347-2353. doi: 10.1128/aem.56.8.2347-2353.1990.
68. Kwasniewska K. The degradation of pentachlorophenol by fungus of *Fusarium* sp. In: Trace Subs. Environ. Health. Proceedings of University of Missouri's. Annual Conference. 1981;15:392-398. Jglobal.jst.go.st
69. Leyva R, Serrato LE, Guerrero RM, Mendoza J. Descomposición enzimática del fenol en solución acuosa usando tirosinasa. Avan. Ing. Quím. 1996;6:41-45. https://amidiq.com › avances-en-ingenieria-quimica. Español.

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