Global NEST Journal, Vol 20, No 3, pp 529-533
Copyright© 2018 Global NEST
Printed in Greece. All rights reserved

The chemical and biochemical oxygen demand reduction by *Armillaria tabescens* in malathion supplemented culture medium

Yildirim N.[1], Erguven G.O.[1*] and Adar E.[2]
[1]Munzur University, Faculty of Engineering, Department of Environmental Engineering, 62100, Tunceli, Turkey
[2]Yildiz Technical University, Faculty of Civil Engineering, Department of Environmental Engineering, 34220, Istanbul, Turkey
Received: 01/03/2018, Accepted: 26/09/2018, Available online: 27/09/2018
*to whom all correspondence should be addressed: e-mail: gokhanondererguven@gmail.com
https://doi.org/10.30955/gnj.002660

Abstract

The extensive use of pesticides for controlling insects has been widely used in agricultural activities. However, the indiscriminate use of the pesticides has inflicted serious harmful problems to humans in the ecosystem. Our study aims to evaluating the capacity of *Armillaria tabescens* to remove the chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) at different concentration of malathion insecticide (50, 100 and 150 ppm) under agitated (130 rpm) submerged culture conditions at 27 °C after 8 and 15 days. According to our results, *A. tabescens* has achieved COD reduction as 52.39, 27.24, and 38.30% at concentration of 50, 100 and 150 ppm respectively on 15 days. The BOD_5 reduction rates were found as 78.2, 74.76 and 81.26% at 50, 100 and 150 ppm concentration respectively at the end of the 15th days. At the end of this time period, the dried biomass of *A. tabescens* was weighted and we have suggested that malathion in medium reduced the biomass production compared the control group (Sabouraud dextrose broth + *A. tabescens*). Our experiments have focused that; *A. tabescens* could be an alternative and useful fungus for bioremediation of wastewater containing malathion insecticide.

Keywords: *A. tabescens*, malathion, chemical oxygen demand, biochemical oxygen demand.

1. Introduction

During the last half decade, pesticides have been the major concern of the agricultural activities. To be helpful for increasing the quality and efficiency of production, pesticides industry became obvious. (Oliveira-Silva, 2001).

Today, organophosphorus insecticides are the largest classes of pesticides and they are identified with their high degradation rates. The biodegradation of the malathion is more feebleness due to its eco-friendly, low investment and also low application costs (Khan *et al.,* 2016). In the environment, pesticides are permanent organic compounds and they withstand to chemical and biological degradation (Buccini, 2003).

One of the organophosphate insecticides is malathion and this insecticide can use for control of field crops from insects, pests, animal parasites and flies. The liquid form of malathion insecticide can be degraded biologically (Barlas, 1996).

The process that for remove the pesticides from the receiving environment consists of some technologies correlated with conventional treatment methods and these methods are not enough for eliminate the contaminants clearly (Ikehata and El-Din, 2006).

In the last decades, ecofriendly methods like using different microorganisms have preferred for remediate contaminated receiving environments. With this advance, bioremediation methods are generally preferred compared to other physicochemical methods (Kidd *et al.,* 2009). Biodegradation is a basic factor for eliminating the adverse effects of organophosphorus pesticides in the environment. Many scientists have focused on the microbial strains have an increasing degradation efficiency of the organophosphorus pesticides (Sorensen *et al.,* 2008). Researches about biodegradation are useful for eliminate the toxic effects of these insecticides by microorganisms. Various receiving environments polluted from pesticides includes microorganisms that capable of biodegrade them (Brajesh KS, Walker, 2006)

Armillaria root rot, which can be caused by several members of the basidiomycete genus *Armillaria*, occurs worldwide on a wide variety of hardwood and softwood plants *A. tabescens* belonging to white-rot fungi is a non-toxic, edible fungus (Hood *et al.,*1991).

In this study, we focused on evaluate the removal performance of *A. tabescens* on suggested concentrations of malathion insecticide (50, 100 and 150 ppm) for local farmers in their agricultural activities. We also try to determine the removal efficiency of this fungus on malathion insecticide with most important environment parameters such as COD and BOD_5.

2. Materials and methods

2.1. Chemicals

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

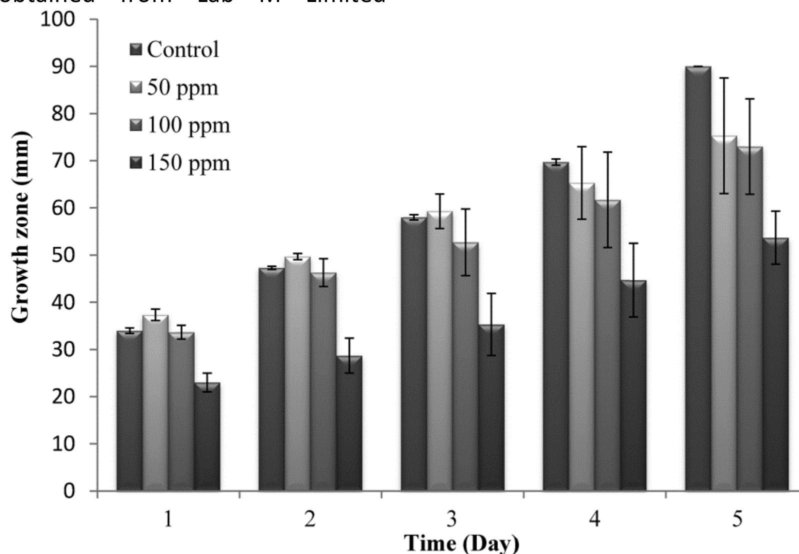

Figure 1. Agar plate screening of growth in SDA media supplemented with malathion (5 days)

2.2. Fungus

The fungal strains of *A. tabescens* are in the current station in our stock culture collections. The strains were sustained on SDA slants at 4°C in refrigerator.

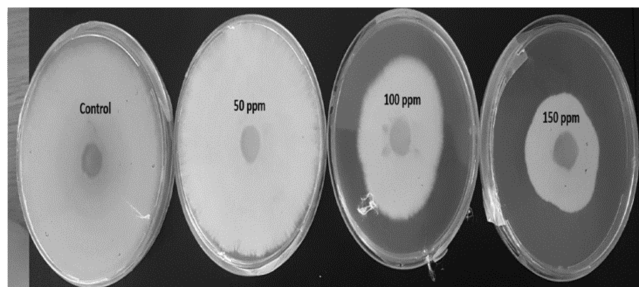

Figure 2. Growth of fungus in different concantration of malathion after 5 day

2.3. Monitoring the growing rate of A. tabescens in SDA media

For SDA plate monitoring the growing rate of *A. tabescens*, mycelial plugs (5 mm diameter) were placed into the center of Petri dishes (90 mm diameter) containing 50, 100 and 150 ppm of malathion insecticide. The plates were incubated at 27 ºC in the dark until they were exactly colonized with the fungus or after maximum period of 7 days. The diameters (mm) of the growing zones were defined in two diagonal directions of the agar plate. Plates containing the fungal mycelial plug but not supplemented with malathion used as blank media. All experiments were performed in triplicate.

2.4. Agitated culture conditions preparation

Submerged culture medium and inoculum of *A. tabescens* were cultured at 27˚C on SDA slants in glass tube. After incubation period of seven days, conidial suspensions were prepared and these were used for the preparation of inoculum. 10 ml of the suspension solutions were transferred into a 250 ml flask containing SDB and agitated on a rotary shaker at 130 rpm for periods of 10 days at 27˚C. At the end of the 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, SDB+50, SDB+100, and SDB+150 ppm of malathion insecticide on an agitated incubator for 8 and 15 days at 27 ˚C in triplicate. After incubation, all flasks filtered for removing fungal biomass and then filtrate was used in COD and BOD5 reduction experimental studies.

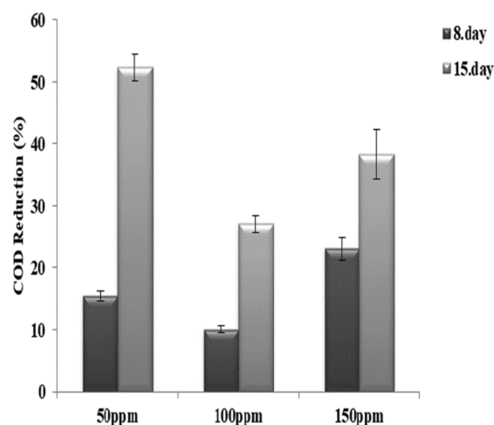

Figure 3. COD reduction in submerged culture medium supplemented with malathion

2.5. COD and BOD_5 reduction experiments

Closed reflux titrimetric method were used for the COD experiments according to the Standard Method 5220C while BOD_5 test was performed by the line of Standard Method 5210B (5 day BOD_5) test (APHA, 1998). All experiments were performed triplicate.

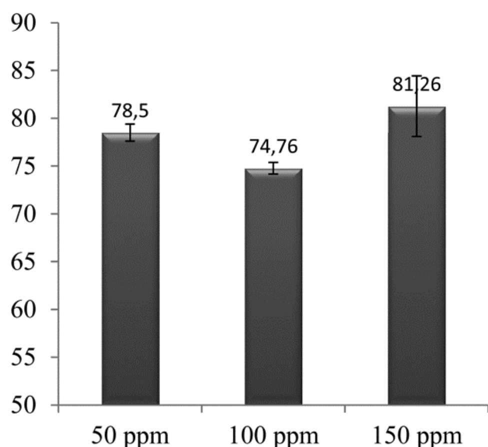

Figure 4. BOD reduction in Submerged Culture Medium supplemented with malathion

2.6. Dry biomass assays

Dry matter of *A. tabescens*, was determined by drying fungal biomass, taken from the filtration of flasks at end of the 15 days in 105°C for 48 hours to a constant weight.

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 dry biomass, growth zone, COD and BOD_5 reduction in media, the data were analyzed by analysis of variance (ANOVA).

3. Results and discussion

3.1. Screening of growth zone in solid media

All growth zones of *A. tabescens* in agar media were demonstrated in Figure 1. In the SDA medium, the diameter of the *A. tabescens* colony decreased with the increase in the malathion residuals in the medium. *A. tabescens* showed a reduction of over 40% in the colony growth diameter in the lowest concentration of malathion in comparison with the control experiment at 5 days. At higher concentrations of malathion, growing of *A. tabescens* decrease.

After the 5 days period, growth zones in control group and malathion supplemented groups were given in Figure 2. Malathion could to be little toxic effect to the *A. tabescens* used in this study for malathion supplemented groups, since slightly inhibited the fungal growth. Similar results were found in previous studies. The screening of fungal growth in media with malathion indicated that the *A. tabescens* is metabolized malathion.

3.2. Removal of COD and BOD_5

Reduction rates of COD and BOD_5 in the media supplemented with malathion have showed different results depend on the difference in concentrations and incubation period in the submerged culture medium. According to the results of COD assays; in medium with 50 ppm malathion, after 8 day, reduction was seen about 15.49 ± 0.82% and after 15 day, reduction increased to 52.39±2.13%. In 100 ppm, reduction value of 10.12%±0.52 increased to 27.14±1.37% and finally at 150 ppm medium with malathion, reduction rates were increased from 8 day to 15 day from 23.12±1.88% to 38.30± 4.01% respectively (Figure 3). According to these results, best reduction performance on COD occurred in 50 ppm concentrations after 15th day. For BOD_5 assays, the best removal performance seen on 150 ppm concentrations of malathion as 81.3% after 15 days (Figure 4). (Mawgoud, 2005) studied the biodegradation efficacy of *P. putida* and revealed that this bacterium has a great potential for malathion degradation. As a result, degradation rate of malathion was occurred about 72% at a concentration of 125 mgL^{-1}. Some studies about biodegradation of malathion has been investigated in Republic of Korea (Kim *et al.,* 2005) and Egyptian soils (Mawgoud, 2005). Zhongli *et al.* (2001) suggested microbial growth on different concentrations of organophosphorus pesticides as sources of carbon have been previously reported. Yonten *et al.* (2017) studied the biodegradation capacity of *P. eryngii var. ferulae* for COD and they found *P. eryngii var. ferulae* was a suitable species for bioremediation of pesticides. In a previous research on biodegradation of chlorsulfuron, COD removal efficiencies were observed between 70% and 93% on *B. simplex*, *B. muralis*, *M. luteus*, *M. yunnanensis* and *C. tetani* bacterial species (Erguven and Yildirim, 2016). Erguven (2018) observed that removal rates of herbicide acetochlor in agitated culture media obtained by *T. geodes, C. cicadae, M. owariensis, M. cylindrosporae* and *V. chlamydosporium* were 90, 90, 74, 61, and 52% as COD and 80, 76, 76, 54 and 50% as BOD_5 respectively. According on these reduction rates, it could be concluded that *T. geodes* has the highest removal rate for both parameters.

3.3. Dry biomass

The amount of the dry fungal biomass was measured for control, 50, 100 and 150 ppm medium as 1.17, 092, 0.86 and 0.49 g respectively (Figure 5). According to these results; in the submerged culture medium, 150 ppm concentration of malathion insecticide reduced the growth of fungus clearly. Similarly, in some another studies, fungal biomass production in the agitated culture medium in the presence of some pesticides (cypermethrin + chlorpyrifos and triazamate etc.), by the observation of inhibitory zones, was inhibit as 82% with the results occurred (Slavikova and Vadkertiova, 2003). Buck and Burpee (2002) reported significant reduction of yeast phylloplane population by the other ergosterol biosynthesis inhibitor propiconazole. Erguven *et al.* (2017) studied about biodegradation of insecticide malathion

with *P. chrysosporium* and they found 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. According to these results, it is revealed that especially 100 ppm and 150 ppm concentration of malathion clearly reduced the growth of fungus in submerged culture medium.

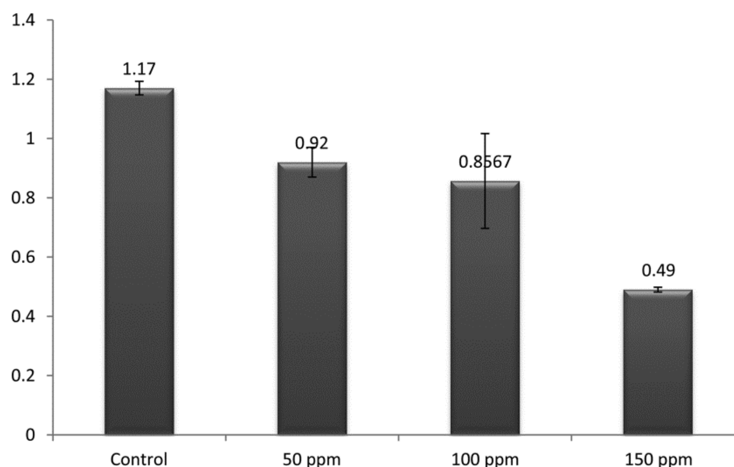

Figure 5. Dry fungal biomass in submerged culture medium supplemented with malathion after 15 days

4. Conclusions

According to the results of the experiments, *A. tabescens* might be used in malathion bioremediation with a significant COD and BOD_5 reduction in submerged culture conditions supplemented with malathion. *A. tabescens* may be also used in bioremediation of some other pesticides. In addition, some studies with other insecticides and herbicides with microorganisms are needed to confirm its clear mechanism of bioremediation process with other important environmental parameters such as active material, total organic carbon, etc. The data reported in this study indicate that *A. tabescens* can survive by degrading this kind of insecticide. Most microorganisms live in agricultural soil, enable to degrade insecticides. Additionally, since using other physical and chemical methods to degrade insecticides is very difficult and also expensive, most of researchers suggested the use of these cultures for easily degradation. In recent years, several studies on bioremediation/biodegradation of pesticides from agricultural fields were conducted. In this study, we found that *A. tabescens* fungi might be used in bioremediation studies in aquatic ecosystems polluted by malathion. *A. tabescens* also can be use for removal pesticide pollution from agricultural fields and receiving environments. COD and BOD_5 parameters can give us valuable information for understanding the biodegradation efficiency.

References

Abdel Mawgoud Y. (2005), Molecular characterization of malathion biode-grading enzymes extracted from Egyptian bacterial isolates. *New Egyptian Journal of Microbiology,* **10**, 226-231.

APHA (1998), *Standard Methods for the Examination of Water and Wastewater*, twentieth Edition, American Public Health Association, Washington, DC.

Barlas N.E. (1996), Toxicological assessment of biodegraded malathion in albino mice. *The Bulletin of Environmental Contamination and Toxicology*, **57**, 705-712.

Brajesh K.S. and Walker A. (2006), Microbial degradation of organophosphorus compounds. *FEMS Microbiology Reviews*, **30**, 428-471.

Buccini J. (2003), *The Development of a Global Treaty on Persistent Organic Pollutants (POPs) Persistent Organic Pollutants,* Springer, pp. 13-30.

Buck J.W. and Burpee L.L. (2002), The effects of fungicides on the phylloplane yeast populations of creeping bentgrass. *Canadian Journal of Microbiology,* **48**, 522-529.

Erguven G.O. (2018). Comparison of Some Soil Fungi in Bioremediation of Herbicide Acetochlor Under Agitated Culture Media. *The Bulletin of Environmental Contamination and Toxicology*, **100,** 570-575.

Erguven G.O. and Yildirim N. (2016). Efficiency of some soil bacteria for chemical oxygen demand reduction of synthetic chlorsulfuron solutions under agiated culture conditions. *Cellular and Molecular Biology*, **62,** 92-96.

Erguven, G.O., Yildirim N. and Adar E. (2017). The ability of Phanerochaete chrysosporium (ME446) on chemical oxygen demand remediation in submerged culture medium supplemented with malathion insecticide. *Desalination and Water Treatment*, **94,** 231-235.

Hood I.A., Redfern D.B. and Kile G.A. (1991), *Armillaria* in planted hosts. In: Shaw, III, C.G.

Ikehata K. and El-Din M.G. (2006), Aqueous pesticide degradation by hydrogen peroxide/ultraviolet irradiation and Fenton-type advanced oxidation processes: a review. *Journal of Environmental Engineering and Science,* **5**, 81e135.

Khan S., Zaffar H., Irshad U., Ahmad R., Khan A.R., Shah M.M., Bilal M., Iqbal M. and Naqvi T. (2016), Biodegradation of

malathion by Bacillus licheniformis strain ML-1, *Archives of Biological Sciences*, Belgrade, **68**, 51-59.

Kidd H. and James D.R. (1991), *The Agrochemical Handbook* 3rd ed. The Royal Society of Chemistry, Cambridge.

Kile, G.A. (Eds.), *Armillaria Root Disease*, Agric. Handbook No. 691. USDA Forest Service, Washington, DC, pp. 122-149.

Kim Y.H., Ahn J.Y., Moon S.H. and Lee J. (2005), Biodegradation and detoxification of organophosphate insecticide, malathion by Fusarium oxysporumf. sp. pisicutinase. *Chemosphere*, **60**, 1349-1355.

Luo X., Xu X., Yu M., Yang Z. and Zheng L. (2008), Characterisation and immunostimulatory activity of an α-(1→6)-d-glucan from the cultured *Armillariella* tabescens mycelia. *Food Chemistry,* **111,** 357-363.

Oliveira-Silva J.J., Alves S.R., Meyer A., *et al.* (2001), Influence of socioeconomic factors on the pesticides poisoning, Brazil. *Revista de Saúde Pública April*, **35**, 130-135.

Perucci P., Dumontet S., Bufo S.A., Mazzatura A. and Casucci C. (2000), Effects of organic amendment and bentgrass. *Canadian Journal of Microbiology* **48**, 522-529.

Slavikova E. and Vadkertiova R. (2003), Effects of Pesticides on Yeasts Isolated from Agricultural Soil, *Zeitschrift fur Naturforschung*., **58c**, 855-859.

Sorensen S.R., Albers C.N. and Aamand J. (2008), Rapid mineralization of the phenylurea herbicide diuron by Variovorax sp. strain SRS16 in pure culture and within a two-member consortium. *Applied and Environmental Microbiology*, **74**, 2332-2340.

Yonten V., Alp H., Yildirim N., Yildirim N.C. and Ogedey A. (2017). Investigation of optimum conditions for efficient COD reduction in synthetic sulfamethazine solutions by Pleurotus eryngii var. ferulae using response surface methodology. *Journal of the Taiwan Institute of Chemical Engineers,* **80**, 349-355.

Zhongli C., Shunpeng L. and Guoping F. (2001), Isolation of methyl parathion-degrading strain M6 and cloning of the methyl parathion hydrolase gene. *Applied and Environmental Microbiology,* **67,** 4922-4925.