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Research Article

Comparison the Performance of Some Soil Fungi on Ethalfluralin Biodegradation with Chemical Oxygen Demand and Turbidity

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Abstract

The aim of this study is investigate the efficiency of some soil fungi on ethalfluralin biodegradation with chemical oxygen demand (COD) and reveal the population dynamics of these fungi during biodegradation under agitated culture conditions with turbidity. As a result the highest and lowest COD removal efficiency of was determined by *Cordyceps cicadaeas* as 91% and *Metacordyceps owariensis* as 67%, respectively at the end of the 120th hour. According to the results COD removal efficiency showed some differences depend on the fungal species. It was also observed that *Cordyceps* cicadaeas had the highest removal efficiency on COD and it was a suitable fungus species for bioremediation contaminated waters by ethalfluralin.

Keywords: *Ethalfluralin; Biodegradation; Chemical Oxygen Demand; Fungi; Turbidity*

Introduction

One of the most potential environmental hazard from pesticides is raising concerns for the public and regulatory agencies [1]. Pesticides degregate after application and concern has been expressed for the possible effects of them and final products on environment and human health [2]. Biodegradation by microorganisms is an environment friendly treatment approach for detoxification of pesticides, compared to conventional methods. Furthermore, certain biotechnological applications could be given as examples of biodegradation [3]. Fungi are both responsible for biodegradation of PAHs [4]. Microorganisms have high biodegradation capacity for organic materials [5].

Microbial degradation of pesticides is not only an important mechanism for controlling pesticides, it is also an environmentally friendly method [6]. Biological degradation is the most frequently used method for the remediation of pesticides in water. According to the results of experiments abut biodegradation have shown that biodegradation depend on the removal of pesticide residuals. Additionally, biodegradation is a low cost alternative process that does not result in toxic final products [7].

The ability of organisms to degregate pesticides is mainly based on their biodegradation activity. Bioremediation has been firstly achieved using fungi can be used [8].

In the environment, the rapid increase in population has resulted in accumulation of chemicals [9]. The upcoming technology which utilizes the ability of microorganisms to remove pollution from the environment is economical and versatile [10]. The extensive use of pesticides has resulted in serious environmental problems [11].

In this study, the performance of some soil fungi isolated from agricultural field investigated for ethalfluralin biodegradation under agitated culture media with turbidity parameter.

Material and Methods

Isolation of fungi and molecular characterization

For isolation fungi, soil samples were obtained at 0 - 20 cm soil depth in Thrace region in Turkey. Then these samples was placed in sterile glass jars initially and stored at +4 C0 in plastic containers placed in ice bags. Approximately 10g of the soil sample was diluted in 0.8% sodium chloride isotonic water up to 10^{-4}. To isolate the fungi; some selective agar medium were prepared according to manufacturer's instructions. 0.1 ml of this solution was poured into plates under sterile conditions [12]. After inoculation, petri dishes were taken into a 20^0C incubator for storage and the fungi completed their development in five days. Fungi colonies developed in petri dishes were taken separately into the enrichment media (malt extract) and reproduced under 20^0C incubation. They were selected visually and separated. Then, they were coded for fungi as F1 - F6.

Fungi prepared for molecular studies were taken into malt extract agar. For PCR processes, mycycler thermal cycler system, electrophoresis device, gel imaging system (ORTE) and genetic analysis system (Beckman Coulter CEQ 8000) were used. Wizard Genomic DNA Purification kit manual was used for characterization studies. "Isolating Genomic DNA from Yeast", [13] method was used for fungi. Initially, nucleic acid extraction was conducted on the samples. Obtained DNA were stored at -20°C. 16S rRNA genes in these DNA mixtures were multiplied with Thermal Cycler device using PCR method and then methanogen specific diversity was determined with DGGE cycle and DNA analysis.

Characterization of fungi: Fungi were streak plated in petri dishes with PDA to enable single spore reproduction. Fungi were grown in room temperature and fungus colonies grown from one spore were taken into another petri dish with PDA and grown under room temperature. Grown fungi were scraped from the petri dish using a scalpel and pulverized in a mortar with liquid nitrogen. DNA isolation was conducted on pulverized hypha using Promega Wizard® Genomic DNA Purification Kit method (3.E. Isolating Genomic DNA from Plant Tissue).

As a result of this PCR, only M1 (*Metacordyceps owariensis*), M4 (*Verticilum chlamydosporium*) and M5 (*Cordyceps cicadae*) fungi produced expected strip lengths in agarose electrophoresis.

Fungi species, isolated and identified in agricultural soil are presented in Table 1.

Fungi Code and Approximate species	Identity	Accession no
Metacordyceps owariensis	88%	HQ165699.1
Metarhizium cylindrosporae	88%	HQ165693.1
Tolypocladium geodes	88%	FJ973059.1
Verticilum chlamydosporium	99%	AJ291804.1
Cordyceps cicadae	88%	AJ536574.1

Table 1. *Types of the identified fungi.*

Preparation of the ethalfluralin

Ethalfluralin was supplied by an agricultural products store under the trade name "Izolan". pH of ethalfluralin was 7.0 and studied temperature was 20^0C. This herbicide contains 333 gr l^{-1} of ethalfluralin active ingredient.

Monitoring microbial biodegradation studies with cod and turbidity

For determine the capacity of ethalfluralin biodegradation, five different soil fungi species, (*Metacordyceps owariensis, Metarhizium cylindrosporae, Tolypocladium geodes, Verticilum chlamydosporium* and *Cordyceps cicadae*) (approximately 2 x 10^7 CFU/ ml each) were incubated under liquid culture conditions with ethalfluralin.

To prepare the liquid media, 1 ml of izolan and 1 ml of enriched culture were added to 98 ml 0.8 % isotonic saline water. The ethalfluralin was prepared from izolan in the concentration of 1500 µg/l that is actually used in the field.

Solutions were monitored at 12-hour intervals for turbidity and COD levels. COD was measured by standard 522^{0}C closed reflux titrimetric method [14]. Turbidity measurements were taken from ethalfluralin media at 650 nm (Photolab 6600 UV-VIS Spectrophotometer) according to [15].

Results and Discussion

COD reduction in liquid media

The results of COD reduction in ethalfluralin solution by five different fungi species are presented in Figure 1. In the media with ethalfluralin, COD reduction rates have showed different results depend on differences in fungi species. The COD reduction efficiencies of *Metacordyceps owariensis, Metarhizium cylindrosporae, Tolypocladium geodes, Verticilum chlamydosporium* and *Cordyceps cicadae* species were 67, 87, 87, 89 and 91%, respectively (Figure 1-5). At the end of the 5. day, there were negligible changes. According to these results, the highest COD removal level was achieved by *Cordyceps cicadae*. At the end of 5 days, approximately 16350 mg/l COD of ethalfluralin was reduced to 1720 mg/l. The lowest COD removal efficiency was seen by *Metacordyceps owariensis* as 67%.

Previous studies on microbial degradation of certain herbicides revealed that relatively few culture species were actually able to degrade pesticides. In another study, bacteria species were isolated in agricultural soil contaminated with trifluralin to decompose the herbicide in a liquid medium [8]. In a previous study about biodegradation of chlorsulfuron, COD removal rates were observed between 70% and 94% [16].

Monitoring microbial activity in ethalfluralin through turbidity

The results of the turbidity study conducted with *Metacordyceps owariensis, Metarhizium cylindrosporae, Tolypocladium geodes, Verticilum chlamydosporium* and *Cordyceps cicadae* species in ethalfluralin media are given in Figures 1, 2, 3, 4 and 5, respectively.

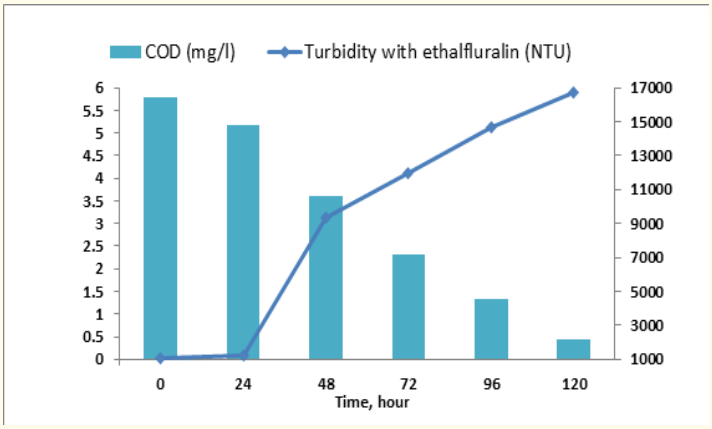

Figure 1: *COD reduction of ethalfluralin related with turbidity by Metacordyceps owariensis.*

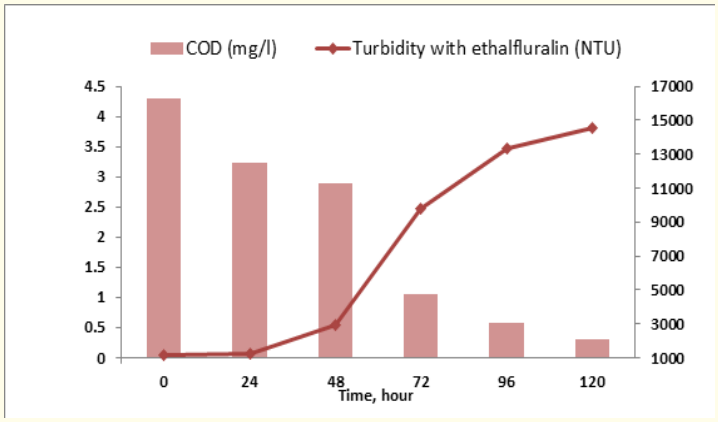

Figure 2: *COD reduction of ethalfluralin related with turbidity by Metarhizium cylindrosporae.*

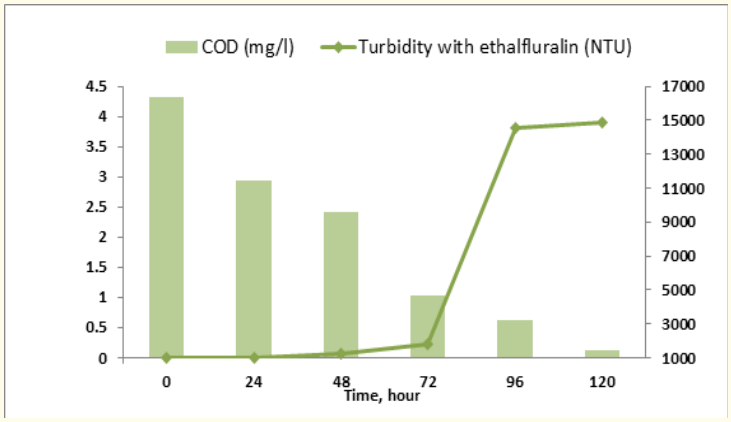

Figure 3: *COD reduction of ethalfluralin related with turbidity by Tolypocladium geodes.*

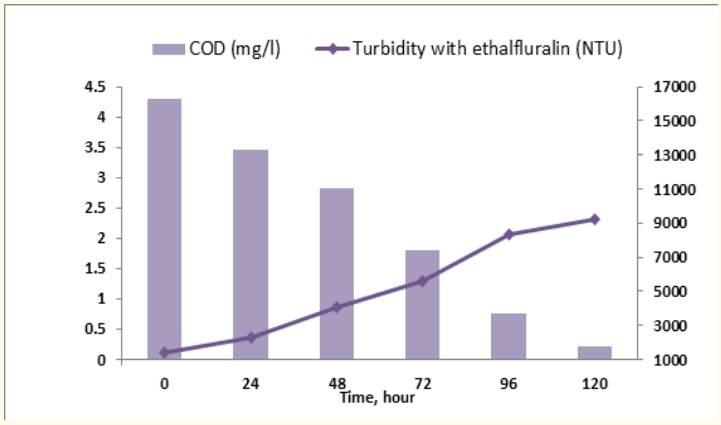

Figure 4: *COD reduction of ethalfluralin related with turbidity by Verticilum chlamydosporium.*

According to the results of the experiments, it was found that COD re¬moval rates obtained by *Metacordyceps owariensis, Metarhizium cylindrosporae, Tolypocladium geodes, Verticilum chlamydosporium* and *Cordyceps cicadae* species were 67, 87, 87, 89 and 91%, respectively. Based on these results, the highest removal rate achieved by *Cordyceps cicadae.*

Experimental results on monitoring microbial activ¬ity in ethalfluralin medium showed a slight in¬crease in turbidity, particularly after from the 24th hour on *Metacordyceps owariensis* (Figure 1), from the 48th on *Cordyceps cicadae* (Figure 5) and after the 72th hour on Tolypocladium geodes (Figure 3). The distinct increase in turbidity occurred after from the 48th hour, which demonstrated that the best COD re¬moval in medium with ethalfluralin was observed with *Cordyceps cicadae* (Figure 5). This means, *Cordyceps cicadae* was a suitable fungi species for bioremediation of ethalfluralin contaminated medium.

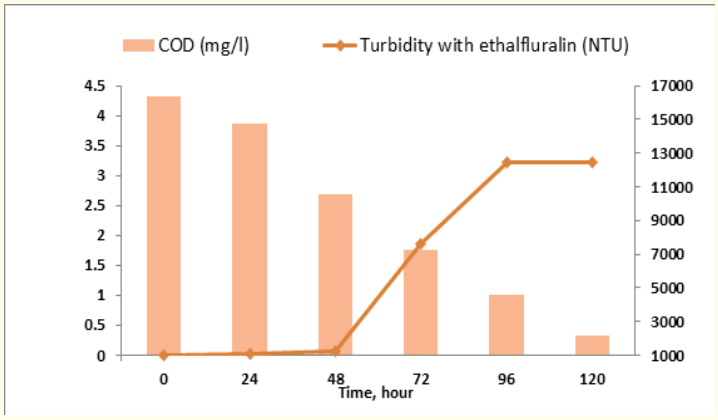

Figure 5: *COD reduction of ethalfluralin related with turbidity by Cordyceps cicadae.*

Conclusion

The soils includes microorganisms that can remove pesticides from the environment. Enzymatic degradation of pesticides from polluted environment represents most important strategy for removal and degradation of persistent chemical substances [17]. Pesticide persistence in environment is caused by either their physico-chemical properties of organisms able to degrade them [18]. This also means variety of microorganisms has been involved to degrade pesticides with successful results [19].

The minor structural changes that fungi does to degrade pesticides is susceptible to further degradation. Pesticide pollution is a serious environmental problem and remediation of pesticides are necessary [17].

The biodegradation of pesticides by a fungi mixed culture, especially that of ethalfluralin requires more studies which is a community of microorganisms, are effective in degrading other organic compounds in the environment [20].

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