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The use of *Phanerochaete chrysosporium* as an alternative bioremediation tool of heavy metal, chemical oxygen demand and phosphate from landfill leachate

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ABSTRACT

In this study, in order to investigate the bioremediation efficiency of *Phanerochaete chrysosporium*, chemical oxygen demand (COD) reduction, total phosphate levels and heavy metal (Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}) removal were determined in five different experimental groups (control, X, Y, Z, and T) under static culture conditions containing leachate at different incubation periods (5, 10, and 20 d). At the end of the 20th day of incubation, 84% Mn^{2+} and Zn^{2+} removal was achieved in X application group. In the same application group, 90.88% COD reduction was also obtained. The lowest total phosphate value was determined as 2.63 g L^{-1} in T group compared with control group (5.0 g L^{-1}). Similar improvements have been identified in other application groups in terms of metal removal, total phosphate levels and COD reduction. As a result, the findings obtained from our study show that white rot fungus *P. chrysosporium* can be used as an alternative biological resource in the studies for improving landfill leachate in terms of metal removal efficacy, COD and total phosphate level reduction capacity.

Keywords: Phanerochaete chrysosporium; Leachate; Chemical oxygen demand reduction; Metal removal; Bioremediation

1. Introduction

The leachate created by the leakage of rainwater falling into the landfills of the solid wastes through the waste heap is the wastewater that has a highly diverse pollution load, quantitatively and qualitatively. As it can contain high concentrations of organic, inorganic and heavy metal types, it has a more concentrated pollution load than many domestic and industrial wastewaters. Therefore, the amount of solid waste leachate in a region may adversely affect the quality of groundwater and surface water in that area. Transport of these dissolved substances in leachate is an important hazard for groundwater and surface water [1,2].

Leachate since it affects the water ecosystem, environment and public health; it must be treated before it is discharged to receiving environments. Various methods have been developed for the treatment of leachate. The methods to perform this treatment are physical, chemical, biological and advanced treatment methods [3].

Due to the complex nature of leachate, these methods do not allow effective treatment efficiency to be achieved by using these methods alone. Therefore, a combination of biological, physical-chemical and chemical processes is generally recommended for the treatment of leachate [3,4]. Various biological treatment methods are used in the treatment of leachate, as an example of this treatment method;

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sequential batch reactors, long ventilated systems and ventilated lagoons [5]. Among advanced treatment methods, membrane technologies and adsorption are used. Its common use is limited due to the high cost and the complexity of its management. It also does not allow re-use of valuable energy and nutrients in water. Considering all these, cost-effective treatment methods have been investigated [6]. There are many studies conducted for biotechnological purposes using white-rot fungi. Some of these studies, the use of white-rot fungi in the treatment of alcohol factory wastewater and olive oil plant wastewater [7], removal of color in textile wastewater [8], biological adsorption of heavy metals [9,10].

In this study, it was aimed to determine the bioremediation potential of the white rot fungus *Phanerochaete chrysosporium* from the leachate obtained from the Bingol sanitary landfill using heavy metal, chemical oxygen demand (COD) and total phosphate removal values.

2. Material and methods

2.1. Chemicals

Chemical materials used in this study is as follows; Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB) (Sigma-Aldrich, Germany), ethanol 96% (Merck, Germany), COD Kit (Hach, Germany), dipotassium phosphate (Sigma-Aldrich, Germany), hydrochloric acid (Sigma-Aldrich, Germany). Fe, Mn, Zn and Cu stock solutions (1,000 mg L^{-1}) were obtained from Merck (Germany). Other standard solutions were prepared by dilution of these stock solutions and standard solutions were used for calibration graphs.

2.2. Instruments

The elemental compositions of mediums were measured using a PerkinElmer AAnalyst™ 800 model (USA) with deuterium background correction. A hollow cathode lamp was used in the analysis of Fe, Mn, Zn and Cu measurements using air acetylene flame were carried out.

2.3. Leachate samples

The leachate samples used in the study were taken with 2.5 L plastic opaque bottles, in accordance with the principles of water and wastewater sampling from the solid waste landfill site in Bingol Province. It was brought to Munzur University, Environmental Engineering Laboratories within 4 h by means of cooler carrying bags and characterized by standard methods used in water and wastewater analysis. This leachate was preserved to be tested in the refrigerator at +4°C.

2.4. Fungus

In this study, *P. chrysosporium* (ME446), which is present in the culture collection of Munzur University Environmental Microbiology Laboratory, was used. *P. chrysosporium* main stock culture in Petri dishes in the 2% SDA medium was passaged with monthly periods and kept in the refrigerator at +4°C until use.

2.5. Experimental protocol

In the study, five different groups (control, X, Y, Z, and T) were designed (Table 1). In all experimental groups, SDB was added in medium as a nutrient and cotton stalk was added in medium as an inducer of growth and enzymes deal with degradation. Experimental studies were carried out in three replicates in each application groups. Cotton stalk used as supplementary material for Z and T groups. The cotton stalks were dried sufficiently at room temperature. The dried cotton stalk was ground in a Fritsch brand SK 90 LH/4 model grinder and turned into 1–2 cm sized pieces. It was passed through 425–250 mesh sieves and separated from small particles on it and then used in the studies. The all prepared application media were autoclaved for sterilization.

Medium cooled to approximately room temperature was transferred to the sterile cabinet. *P. chrysosporium*, which was previously taken from the refrigerator and left in the Gemo brand DT107 model incubator for 2 h at 27°C, was taken from the stock culture with 2 plugs of 1 cm diameter in a sterile condition and transferred into the application mediums. The mediums were taken into a static incubator at 27°C for 20 d. At end of the 5, 10 and 20 d of incubation, the mediums were filtered by vacuum filtration using a Buchner funnel. These filtrates were stored in the refrigerator at +5°C until used for heavy metal, COD and total phosphate analysis.

2.6. Analytical procedure for element analysis

The concentration of elements (Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}) in filtrates taken from the end of 20 d incubation were measured using a PerkinElmer AAnalyst™ 800 model. Under the optimum spectrometric conditions that aforementioned, standard working solutions at five different concentrations were injected to atomic absorption system.

Table 1
Experimental groups

Groups	Mediums
Control	Leachate 1/10 diluted with tap water without fungus (negative control)
X	Leachate 1/10 diluted with tap water + 2 g SDB + *P. chrysosporium*
Y	Leachate 1/10 diluted with tap water + *P. chrysosporium*
Z	Leachate 1/10 diluted with tap water + 2 g SDB + 2% (v/w) cotton stalk + *P. chrysosporium*
T	Leachate 1/10 diluted with tap water + 2% (v/w) cotton stalk + *P. chrysosporium*

The calibration curves were constructed by plotting the absorption (y) against the concentration of each element (x). The limit of detection (LOD), defined as a S/N of 3:1 and the limit of quantification (LOQ), defined as a S/N of 10:1 were determined by analyzing progressively lower analytic concentrations until the respective S/N ratios were obtained. According to signal to noise ratio (S/N), the LOD and LOQ were calculated. To evaluate the accuracy of the using method a standard addition method was applied.

2.7. COD and total phosphate analysis

COD values were determined with a colorimeter (Hach Lange DR890) at end of the 20 d of incubation. Total phosphates values were determined according to standard method 4500-PC at the end of 5, 10 and 20 d of incubation. The COD and phosphate analysis need heating, for this purpose, a thermo-reactor (Hach Lange DRB-200) was used.

2.8. Statistical analysis

SPSS version 24 (SPSS Inc., Chicago, USA) was performed for analysis of all data. ANOVA was carried out to analysis of data and when $p < 0.05$, results was considered significantly different. Differences between means were analyzed using Duncan's multiple range test for post hoc multiple comparisons. Values were presented as the mean ±standard error.

3. Results and discussion

Physicochemical parameters of leachate 1/10 diluted with tap water without fungus (negative control) are shown in Table 2.

Total phosphate values recorded in sampling periods in each groups are illustrated in Fig. 1. While the total phosphate value was found as 3.1, 3.13, 3.33 and 3.33 g L^{-1} in X, Y, Z and T group respectively at 20th day (Fig. 1), the value of control group was 5 g L^{-1} (Table 2). The total phosphate values between the groups for each application period, except for the 20th day, were found to be statistically significant ($p < 0.05$). Depending on the incubation period, especially in X groups, total phosphate value decreased according to control group. In the Z group with cotton stalk, a decrease firstly determined and then an increase was found. Adelani-Akande et al. [11] used *Aspergillus niger* as a fungus in a study they conducted and found that this fungus provides different levels of phosphate reduction in the development medium. In our study, low rates of phosphate decreases were observed in application medium. Similarly, Price et al. [12] in their study with *A. niger*, emphasized that this fungus can be used for the removal of nitrogen, phosphate and metal from domestic wastewater. Momba and Clote [13] mixed in their study reported that phosphate uptake from the medium can be increased by mix bacterial culture (*Pseudomonas fluorescens*, *Escherichia coli* and *Acinetobacter radioresistens*).

Table 2
Physicochemical parameters of control group

Parameters	Values
Total phosphate (g L^{-1})	5.0
COD (mg L^{-1})	187.0
Fe^{2+} (mg L^{-1})	2.09
Mn^{2+} (mg L^{-1})	0.18
Zn^{2+} (mg L^{-1})	0.9
Cu^{2+} (mg L^{-1})	0.13

The concentration of elements including Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} were calculated based on calibration curve. The calibration curves were constructed by plotting the absorption (y) against the concentration of each element (x). Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} standard solutions were prepared in the range from 0.5 to 5 mg L^{-1}, 0.25 to 3 mg L^{-1}, 0.1 to 1 mg L^{-1} and 0.5 to 5 mg L^{-1}, respectively for calibration plot. Obtained and used calibration graphs were linear between these ranges. Obtained liner equations, R^2 values, LOD and LOQ values are presented in Table 3.

In present study, metal removal efficiency was determined by *P. chrysosporium* from application media containing leachate during incubation at static liquid culture conditions. The Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} removal rates obtained at the end of the 20th day are shown in Table 4. It was determined that the highest Fe^{2+} removal rate was in the Z group as 79.28%, and the lowest removal rate was 58.17% in the Y group. The highest Mn^{2+} removal rate was achieved as 84.38% in X group and the lowest removal rate was found as 35.47% in Z group. While the highest Zn^{2+} removal rate was determined as 84.33% in X group, the lowest rate was 65.90% in Z group. The highest removal rate was found as 31.49% in Z group and the lowest removal value was 16.66% in Y group for Cu^{2+}. In the T group, the removal values could not be determined due to the difference between the level of leachate metal before the process and the metal values detected at the end of the 20th day. It was found that the removal rate differences in all metals for each application period were statistically significant ($p < 0.05$).

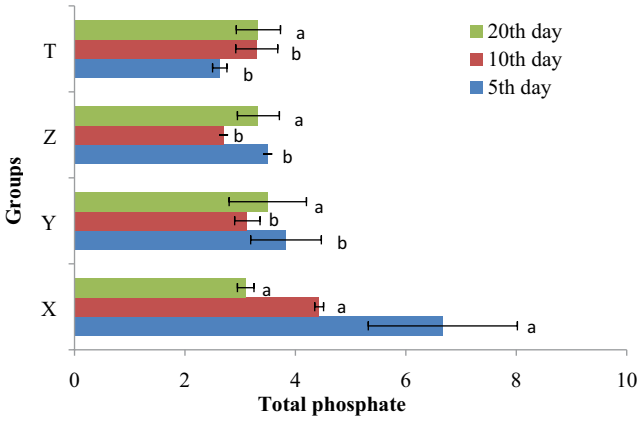

Fig. 1. Total phosphate (g L^{-1}) values in sampling periods in application groups. Total phosphate: 5 g L^{-1} in control group. Mean ± standard error, n = 3, different letters on the bars (^{abc}) indicate the statistical differences ($p < 0.05$) between the application groups according to Duncan's multiple range test.

Table 3
Metal analysis data

	Fe^{2+}	Mn^{2+}	Zn^{2+}	Cu^{2+}
Equation	$y = 15.1x + 1.6$	$y = 29.6x + 2.7$	$y = 143.6x - 0.4$	$y = 10.5x + 0.6$
R^2 values	0.9993	0.9993	0.9991	0.9990
LOD (mg L^{-1})	0.04	0.03	0.01	0.04
LOQ (mg L^{-1})	0.12	0.10	0.03	0.11

Table 4
Metal removal rates (%) at the end of the 20th day incubation

Groups	Heavy metal removal rates (%)			
	Fe^{2+}	Mn^{2+}	Zn^{2+}	Cu^{2+}
X	70.74 ± 2.3^{ab}	84.38 ± 3.19^{a}	84.33 ± 1.33^{a}	30.30 ± 3.02^{a}
Y	58.17 ± 5.31^{b}	52.43 ± 7.99^{b}	83.32 ± 1.92^{a}	16.66 ± 3.66^{a}
Z	79.28 ± 6.02^{a}	35.47 ± 2.07^{b}	65.90 ± 4.04^{b}	31.49 ± 3.70^{a}
T	ENR*	ENR	ENR	ENR

Mean ± standard error, $n = 3$, different letters on the means (^{abc}) indicate the statistical differences ($p < 0.05$) between the application groups according to Duncan's multiple range test;
*ENR: Element could not be removed.

Due to the fact that the fungus is alive in application lines; it is estimated that metal removal can be both by adhering to the surface with the biosorption mechanism and by taking it into the cell with the bioaccumulation mechanism. Some researchers have reported some important information on the biosorption mechanism listed below. Heavy metal biosorption to fungus occurs as a result of ionic interactions and complex formation between the functional groups on the fungal cell surface and metal ions. Functional groups related to heavy metal biosorption are phosphate, carboxyl, amine and amide groups [14–16]. Kapoor and Viraraghavan [17] suggested that amine and carboxyl groups are important functional groups in the biosorption of heavy metals. Gupta et al. [18] emphasized that the fungal cell wall among the microorganisms showed excellent metal biosorption property due to its feature. It can be considered that the organism we use in our study has a great contribution in obtaining significant removal percentages especially in terms of Mn and Zn. Jianlong [19] used saccharomyces cerevisiae biomass for Cu(II) biosorption and determined an effective biosorption capacity of 6 mg g^{-1}. However, in terms of Cu removal, the fungus *P. chrysosporium* we use has been found to provide relatively less removal compared to other metals. The reason for this can be interpreted as both the different surfactant component compositions and the metal accumulation abilities due to the different types of fungi.

COD removal was measured in all experimental groups as one of the best indicators of leachate remediation. The COD reductions obtained at the end of the 20 d in each application groups are illustrated in Fig. 2. It is seen that the highest COD reduction was determined as 90.88% in the X group. The lowest COD reduction was determined in the Y group as 8.24%. All differences in COD removal

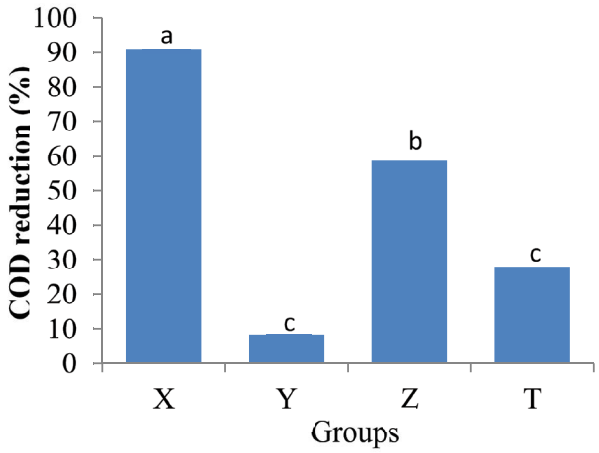

Fig. 2. COD reduction rates (%) at the end of the 20th day in groups. COD: 187 mg L^{-1} in control group. Mean ± standard error, $n = 3$, different letters on the means (abc) indicate the statistical differences ($p < 0.05$) between the application groups according to Duncan's multiple range test.

in all experimental groups were found to be statistically significant ($p < 0.05$).

There are many studies that are carried out to reduce COD from various synthetic pollutants or wastewater by fungi. In the literature, it has been found that the improvement in terms of COD reduction in the removal of dyes, especially in textile wastewater or textile industry, micro-pollutants such as bisphenol A, some antibiotics or pesticides [20–22]. Kalcikova et al. [23] reported that the fungus uses organic pollutants in the mature leachate as a carbon source and determined 60% COD decrease in the

application process with the white rot fungus *Dichomitus squalens*. In our study, it can be said that *P. crysosporium* used organic matarials in leachate intensively as a carbon source, especially in the X application group, and thus created a 90.88% reduction in COD. Noorlidah et al. [24] suggested that white rot fungus *G. australe* is a species with very high potential for leachate treatment. In our study, it can be state that *P. crysosporium* has a high efficiency in the improvement of leachate especially in X application group conditions. However, it was concluded that the presence of cotton stalk does not contribute to decrease COD values in Z and T groups that contains cotton stalks.

4. Conclusions

As a result of this study, the effectiveness of the white rot fungus *P. chrysosporium* has been proven in the bioremediation of leachate that has many environmental risks. Especially the metal removal and COD reduction rates obtained with *P. chrysosporium* revealed that this fungus was effective in the leachate bioremediation. It has also been observed how the addition of some lignocellulosic compounds, such as cotton straw, which can stimulate the enzyme system, to the removal medium may affect bioremediation.

This study demonstrated that both efficient and cost-effective leachate bioremediation process can be designed using *P. chrysosporium*. As a result used in present study *P. chrysosporium* can be used in the future for the biological treatment of leachate as it can tolerate an environment with a high level of toxicity, such as leachate, which is easily produced with agricultural residual materials. *P. chrysosporium* (ME446) fungus strain used in this study is of great importance since it is used for the first time in bioremediation of leachate. In this sense can be a resource for similar studies that can be done.

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