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# PHYTOREMEDIATION OF AIR ORGANIC POLLUTION (PHENOL) USING HYDROPONIC SYSTEM

*Mohsen Shahriari Moghadam[1*], Fatemeh Kool[2], Mahnaz Nasrabadi[3]*

[1] Department of Environmental Sciences, Faculty of Natural Resources, University of Zabol, Zabol, Iran
[2] Department of Environment Management (HSE), Faculty of Engineering and Technology, Islamic Azad University, Zahedan Branch, Zahedan, Iran
[3] Department of Environment Management (HSE), Faculty of Engineering and Technology, Islamic Azad University, Zahedan Branch, Zahedan, Iran

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**CORRESPONDING AUTHOR:**

mohsen.shahriari @ uoz.ac.ir
Tel: (+98 54) 05432232600
Fax: (+98 54) 05432232600

**ABSTRACT:**

**Introduction:** Indoor air pollution plays important role on human health. People spend 90 ٪ of their time in closed spaces, which cause the interest of study about indoor air quality. The main objective of the present study was to design a hydroponic system for indoor air remediation by bacteria and mondo grass (*Ophiopogon japonicus*).

**Materials and methods:** Soil surrounding mondo grass roots was sampled and enriched in a mineral salt medium, and at the end enriched consortium was used for bioreactor inoculation. The following is a brief overview of the biofiltration system: Indoor air, sucked by an air compressor, was injected to the bioreactors and circulated to the pots. In order to study the efficiency of biofiltration system, phenol was added to the nutrient solution on a daily basis. After determining the optimum temperature, the system's ability to degrade various concentrations of phenol was measured.

**Results:** Results showed that enriched consortium had high degradation ability. Among the isolated strains, *Staphylococcus epidermis* and Pseudomonas sp. were found to be more efficient. Biodegradation rate was higher in 35°C and in all selected phenol concentrations (500 to 1500 mg/L) biofiltration system was able to degrade phenol but in high concentrations, system performance was somewhat reduced.

**Conclusions:** In a nutshell, the results revealed significant ability of hydroponic system for remediation of air by bacteria and plant. Obviously, the system may be seen as an important tool in air bioremediation.

## INTRODUCTION

People spend much as 90 % of their time in closed spaces, which represents the increasing interest in indoor air quality [1, 2]. Many activities that have been done to increase human welfare show adverse effects and led to the release of pollutants to the environment. These compounds have entered to the food chain and threaten human health and different ecosystems [3]. Today indoor air pollution plays important roles on human health. According to U.S. Environmental Protection Agency (EPA), some pollutant levels in indoor air may be 2 to 5 times greater than out-

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door environment and it is also worth noting that some pollutants can increase up to 100 times [4]. Discomfort, headaches, nausea, dizziness, sore throats, dry or itchy skin, sinus congestion, nose irritation or excessive fatigue are symptoms of the indoor air pollution [5]. Contaminants can enter through the outdoor or originating from building materials and home equipment [6]. Preventing the entry of contaminants into the indoor, removing them using ventilation systems and indoor air purification are methods for improving indoor air quality [7]. In recent decades, extensive studies have been conducted on the use of plants for bioremediation [8]. In phytoremediation, plants are used to remove contaminants or reduce their adverse effects [9]. Large number of houseplants have been used for the treatment of indoor pollutants that can be pointed to *Tillandsia usneoides* [10], *Hedera helix, Chrysanthemum morifolium, Dieffenbachia compacta* and *Epipremnum aureum* [11], *Zamioculcas zamiifolia* [12], *Chlorophytum comosum* [13], *Syngonium podophyllum* and *Epipremnum aureum* [14], *Chlorophytum comosum* [15]. Potted plants are able to absorb different pollutants such as volatile organic compounds from the indoor environment. For example, studies have shown that many plants are able to remove trichloroethylene, benzene and formaldehyde and other volatile organic compounds [16]. Common systems for indoor air purification generally have been developed to attract particulate matter and have little ability to absorb gaseous compounds. Common procedures to remove gaseous components such as absorption filters and ozone generators are expensive and have low efficiency [17]. Treatment of organic gaseous pollutants using biological systems is more effective than other methods. Biological methods are cheaper than traditional methods and they are important as they do not produce secondary pollutants [18]. As simultaneous use of plants and bacteria for biological treatment of pollutants is considered a new approach, the aim of this study is to design a hydroponic system for remediation of air by bacteria and plants.

## MATERIAL AND METHODS

### *Plant preparation*

Mondo grass (*Ophiopogon japonicus*) were purchased from the local market and transferred to the laboratory. Plants were gently removed from the soil and roots washed to remove adhering soil.

### *Enrichment of phenol – degrading consortium*

About 5 g of soil surrounding plant roots (*Ophiopogon japonicus*) was transferred to a 250 ml conical flask containing 50 ml mineral salt medium (MSM) with 1000 mg/L phenol as the sole sources of carbon and energy. MSM medium [19] was consisted of (g/L): $K_2HPO_4$, 0.5; $NH_4Cl$, 1; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.01; $FeSO_4.7H_2O$, 0.01; 1 mL trace mineral solution. After observing growth in the flask, 1 m/L of enriched medium was transferred to a new MSM medium and the process was repeated four times. Incubation was done on a rotary shaker at 130 rpm, initial pH 7 and 30 °C. At the end of the enrichment, *consortium* was used for bioreactor inoculation. All chemical reagents were purchased from Merck company (Germany).

### *The biofiltration system*

Biofiltration system was made up of three main parts: a pump for sucking air; a hydroponic bioreactor and hydroponic pot. The bioreactor (15 L) was composed of an automatic temperature control system, an air inlet valve and a pump to move nutrient solution from the bioreactor to the hydroponic pot. Hydroponic pot (10 * 10 * 50 cm aquarium covered by aluminum foil) was equipped with input and output valves of nutrient solution. Three grid containers were in the interior of each hydroponic pot which were filled with pumice granules (30 ± 4 g) to fix the plant. The following is a brief overview of biofiltration

system: indoor air, sucked by an air compressor, entered a terminal and then injected into the bioreactors by 50 L / min speed and then circulated to the pots (Figs. 1 and 2).

### *Hydroponic nutrient solution*

Hydroponic nutrient solution (Hogland nutrient solution) was consisted of: $KH_2PO_4$ 0.115 g / L; $KNO_3$ 0.606 g / L, $Ca(NO_3)_2.4H_2O$ 0.656 g / L; $MgSO_4.7\ H_2O$ 0.240 g / L; 1 mL micronutrient stocks and 2 ml of iron stock solution. Micronutrient stocks contained 2.86 g $H_3BO_3$; 1.81 g $MnCl_2\ .4H_2O$; 0.22 g $ZnSO_4.7\ H_2$; 0.08 g $CuSO_4.5H_2O$ and 0.02 g $H_2MoO_4.\ H_2O$ in 1 L distilled water [20].

### *Biodegradation of phenol in biofiltration system*

In order to check the efficiency of biofiltration system, it is important to control the exact amount of the pollutant entering the bioreactor. For this purpose phenol (pollutant) was added to the nutrient solution on a daily basis ( It is assumed that phenol will enter through the air ). The optimum temperature for phenol biodegradation in biofiltration system was determined in two temperatures (30 and 35 ˚C). Treatment were as follows: 1. temperature (35˚C), mondo grass (60 ± 4 g in each grid containers) and phenol (500 mg / L); 2. temperature (35˚C) and phenol (500 mg / L); 3. temperature (30˚C), mondo grass (60 ± 4 g in each grid containers) and phenol (500 mg / L) and 4. temperature (3˚C) and phenol (500 mg / L). During the experimental period phenol (500 mg / L) was added to the medium cultures on a daily basis. After determining the optimal temperature, in order to study the effect of different phenol concentration on biodegradation rate, phenol was added to nutrient solution with three different concentrations (500, 1000 and 1500 mg / L) on a daily basis in 6 treatment as follows: 1. temperature (35˚C), mondo grass (60 ± 4 g in each grid containers) and phenol (500 mg / L); 2. temperature (35˚C) and phenol (500 mg / L); 3. temperature (35˚C), mondo grass (60 ± 4 g in each grid containers) and phenol (1000 mg / L); 4. temperature (35˚C) and phenol (1000 mg / L); 5. temperature (35˚C), mondo grass (60 ± 4 g in each grid containers) and phenol (1500 mg / L); 2. temperature (35˚C) and phenol (1500 mg / L). In all treatments 9 L Hogland nutrient solution was used and indoor air was injected by 50 L / min speed. Enrichment consortium was used for bioreactor inoculation (final optical density of 0.1). Phenol biodegradation in different treatments and optical density ($OD_{600nm}$) was determined every 24 h. To determine a biotic phenol degradation biofiltration was done without plant and bacterial inoculation.

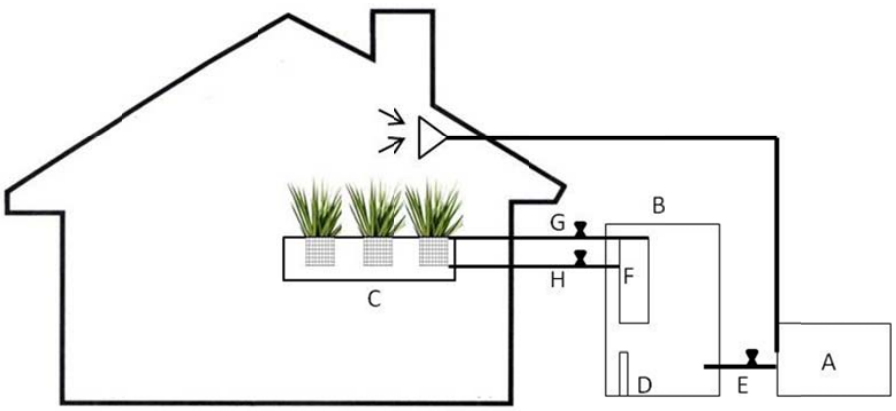


Fig. 1. A Schematic view of biofiltration system, A: sucking air pump; B: hydroponic bioreactor; C: hydroponic pot; D, E: automatic temperature control system; F: pump; G:input valve; H: output valve.

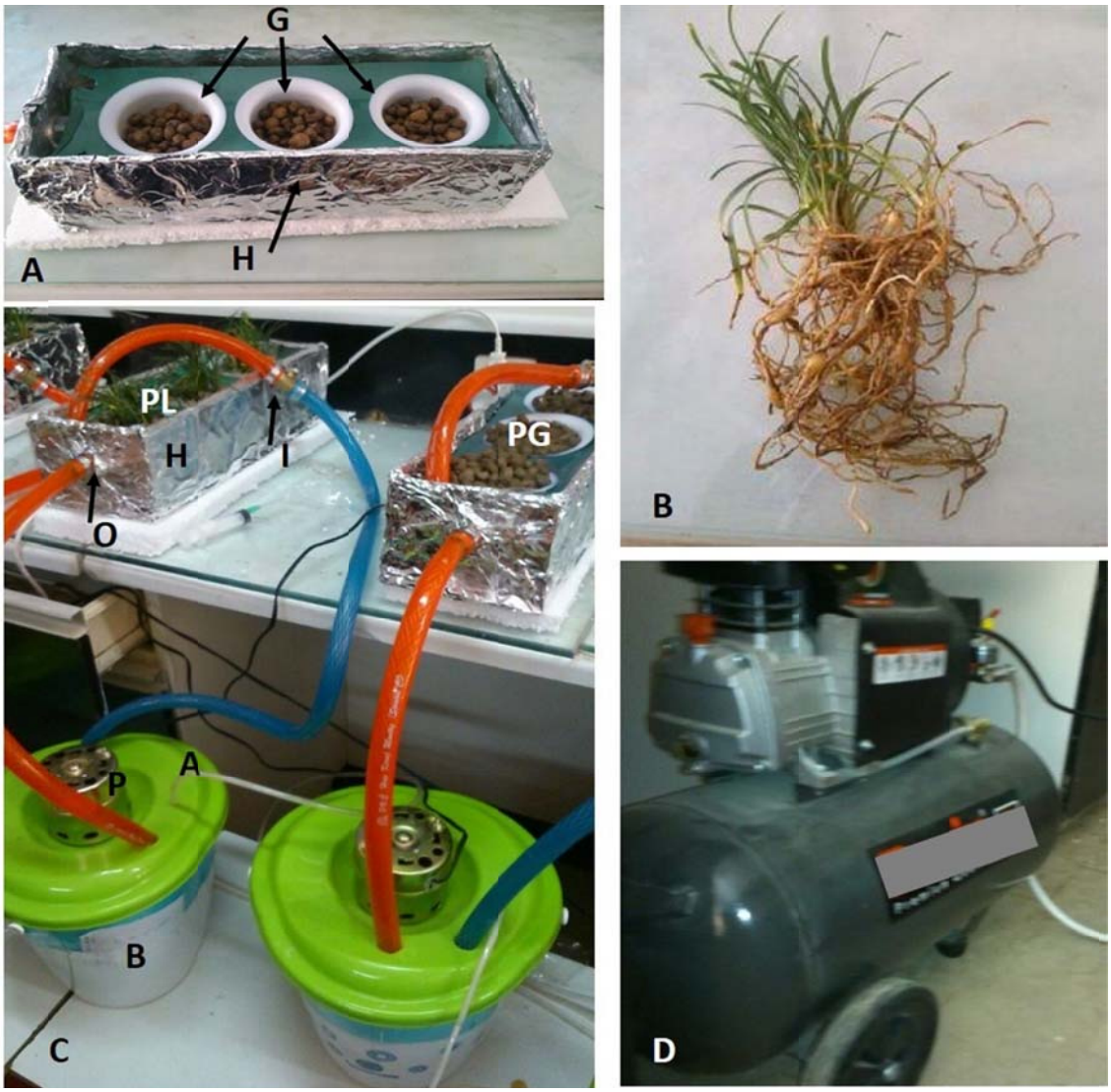


Fig. 2. The biofiltration system; A: Hydroponic pot (H) and grid containers (G) with pumice granules; B: *Ophiopogon japonicus*; C: Hydroponic pot (H), input (I) and output (O) valves of nutrient solution, bioreactor (B), air inlet (A) and pump (P); D: air compressor

### *Phenol determination and cell growth*

Colorimetric method was used to measure phenol concentration in samples. In this way employing 4 - aminoantipyrene followed by oxidation with potassium ferricyanide under alkaline conditions to give a red color product, which was measured at 500 nm. A calibration curve was used to calculate the phenol concentration [21]. Bacterial growth was measured using optical density (600 nm) against Hogland nutrient solution as reference.

### *Isolation and identification of bacterial strains*

In order to identify phenol degrading bacteria in bioreactors, 5 m / L of bioreactor medium was sampled at the end of the experiment and enrichment was done in the MSM with phenol as the carbon and energy source. Then, using the serial dilution on nutrient agar medium, bacteria were isolated and purified at 30 °C for 48 h. DNAs of the isolated bacteria were extracted using the DNA extraction kit (Roche - Germany). The by 16S rDNA gene sequence analysis after amplifi-

cation of the gene by PCR using the set of primers isolated strains were then identified 27F (5′- AGA GTT TGA TCC TGG CTC AG -3′) and reverse 1492R (5′- GGCTACCTTGTTACGACTT -3′). DNA sequences of the 16S rDNA fragments were compared using BLAST at http://blast.ncbi.nlm.nih.gov/Blast.cgi maintained by National Center of Biotechnology Information (NCBI).

### *Statistical analysis*

To compare the roles of temperature, pH, nitrogen and phosphate amounts on bacterial growth rates, one way ANOVA was used. Significant differences were determined using Tukey's test. All analyses were performed using SPSS 19.

## RESULTS AND DISCUSSION

Houseplants are practical, attractive and sustainable to improve indoor air quality. In fact, plants, roots and bacteria work together and volatile organic contaminants are removed. In the present study mondo grass (*Ophiopogon japonicus*) was used in the biofiltration system. Mondo grass is one of the plants that is used on the green wall. Although outdoor cultivation of this plant is more common, but it is also used in indoor spaces and requires little care. Mondo grass has been used in some studies for biological treatment among which we can refer to removing formaldehyde from the air [22]. Mondo grass has a dense leaf mass that can be used for trapping suspended solids in the air. In a research it was showed that the presence of plants in indoor environments can cause change in the amount of suspended particulate matters in the indoor space [23]. In addition, plants that have a rough surface or raised veins are more efficient in the deposition of suspended particulate matter than plants with smooth leaves. Since phenol widely exists in the nature, different bacteria have been isolated and identified with the ability to degrade phenol [24, 25].

In the present study, enriched consortium had high degradation percentages and approximately at the end of incubation (3 days) phenol was degraded completely. Two strains of bacteria were isolated and identified with the ability to degrade phenol in the bioreactors which were called FK1 and FK2. According to the obtained results, strain FK1 was most similar to *Staphylococcus epidermidis*, while strain FK2 was most similar to pseudomonas sp. sequences were deposited to Gen - bank (FK1: KY689640 and FK2: KY689639). One of the most common methods for bioremediation is using the Pseudomonas genus as mixed or pure [26]. Also this genus has been widely used in biotechnology [27]. The results of studies on *Staphylococcus epidermis* have shown that this strain is able to degrade the triphenylmethane [28]. The results of a study have also shown that *Staphylococcus epidermis* is able to degrade polyurethane [29].

Among the various factors, temperature is one of the most important factors in biodegradation of hydrocarbon pollutant [30]. Figs. 3 and 4 show the effect of temperature on the microorganism growth and phenol biodegradation in the presence and absence of *Ophiopogon japonicus*. The results showed that biodegradation rate was higher in 35 °C, while the presence and absence of plant did not show any significant effect. Based on the above results 35 °C was considered as optimum temperature. Temperature affects the microorganism metabolism and therefore influences the biodegradation rates [31]. In this study, the highest degradation was performed at 35 °C. Although microorganisms are able to degrade pollutants at different ranges of temperatures, biodegradation rates usually reduce by decreasing the temperature [32]. In the present study the biodegradation rates were lower in 30 °C compared to 35 °C which confirms the results of other researchers. Biodegradation rates are generally dependent on the type of microorganisms, as well as the concentration of pollutants [33].

Results of the biodegradation and cell biomass

curve at different phenol concentrations are shown in Figs. 5 and 6. Biofiltration system was able to degrade about 1000 mg / L phenol daily but at high concentrations of phenol some of the contaminants remain in the mediums. According to the results, presence and absence of *Ophiopogon japonicus* in different phenol concentrations did not have significant effect on microbial growth and phenol biodegradation. The effect of pollutant concentration on biodegradation rates is important because high pollutant concentrations reduce microbial growth [34]. For example, other studies have shown that the strain *Serratia plymuthim* when used in an environment of high concentration phenol, prolong the lag phase of microbial growth [35]. In another study, it was also showed that at high concentrations of phenol, phenol reducing microbial growth due to increased toxicity of pollutant. The results also showed that despite the biofiltering performance in phenol biodegradation, increasing concentrations of phenol reduced system performance. This could be due to an inhibitory role of phenol in the growth of microorganisms at high concentration [36].

## CONCLUSIONS

In general, the results showed that the simultaneous use of phytoremediation and microbial biodegradation is an effective method for the remediation of indoor air. Biofiltration system was able to degrade about 1000 mg / L phenol daily which represents the high performance of this system. The advantage of biofiltration system is that the contaminants eventually decompose and will not damage the environment. Also, the plant used in this system, in addition to partially absorbing particulate matter, produces oxygen to improve air quality. Considering the above mentioned items, this device is recommended for the biofiltration of closed spaces.

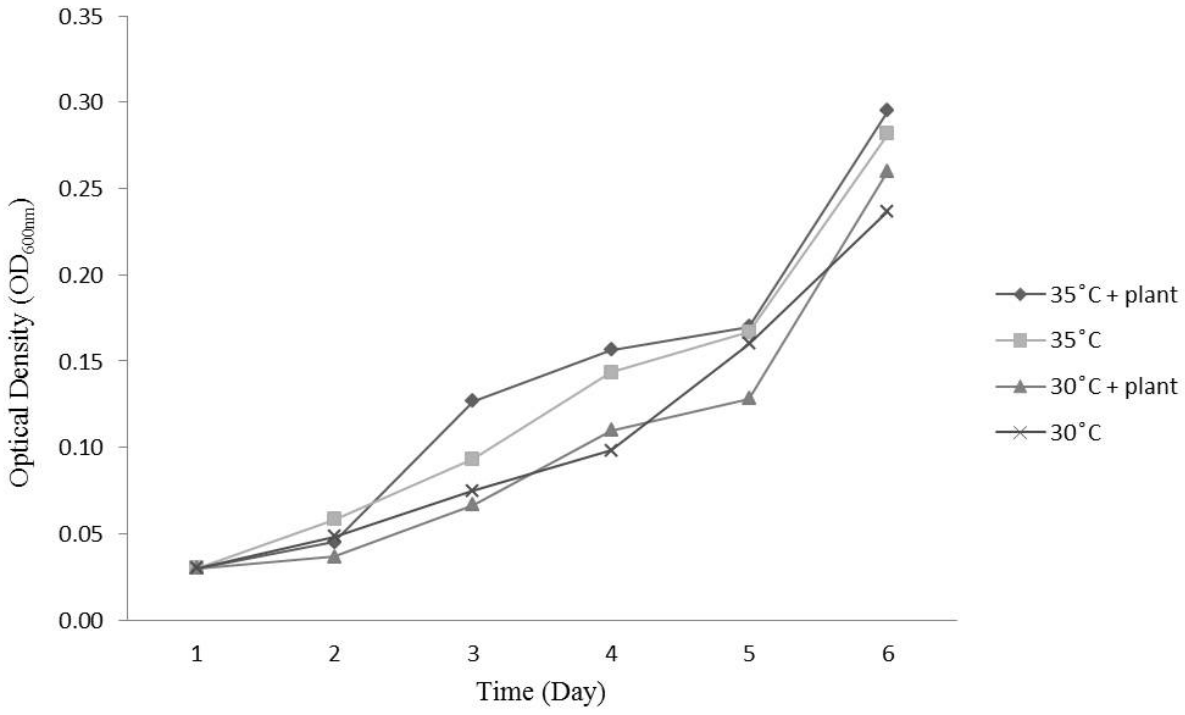


Fig. 3. The effect of temperature on the microbial growth in the presence and absence of *Ophiopogon japonicus*.

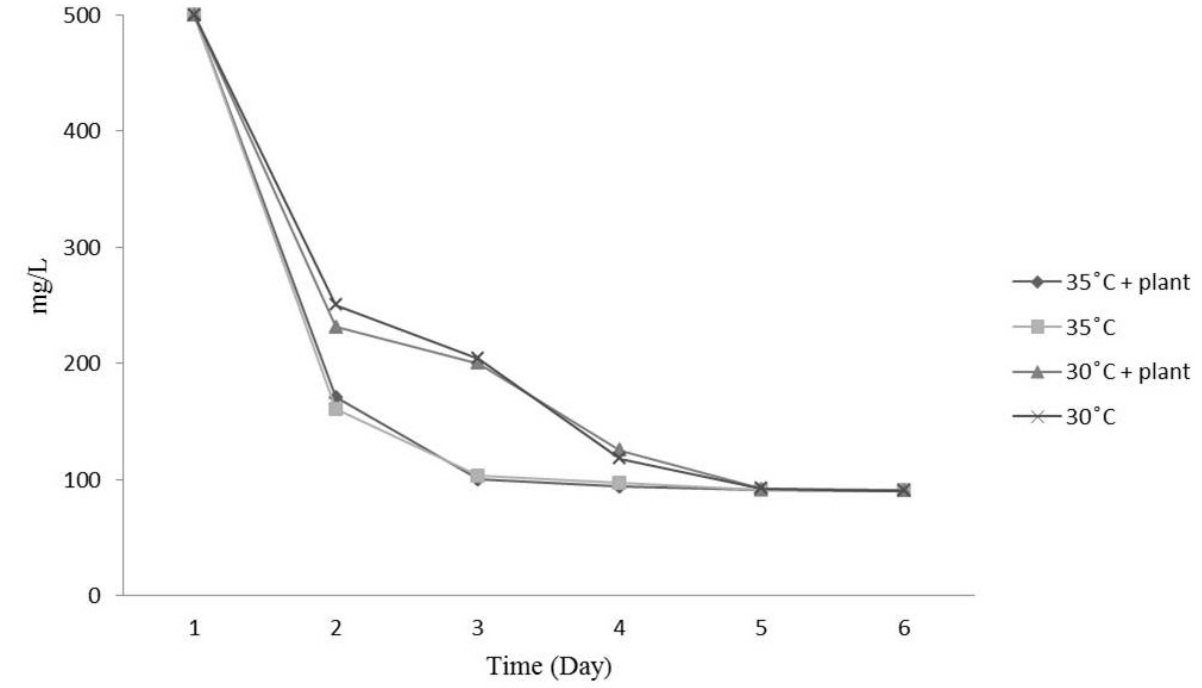


Fig. 4. The effect of temperature on phenol biodegradation in the presence and absence of *Ophiopogon japonicus*

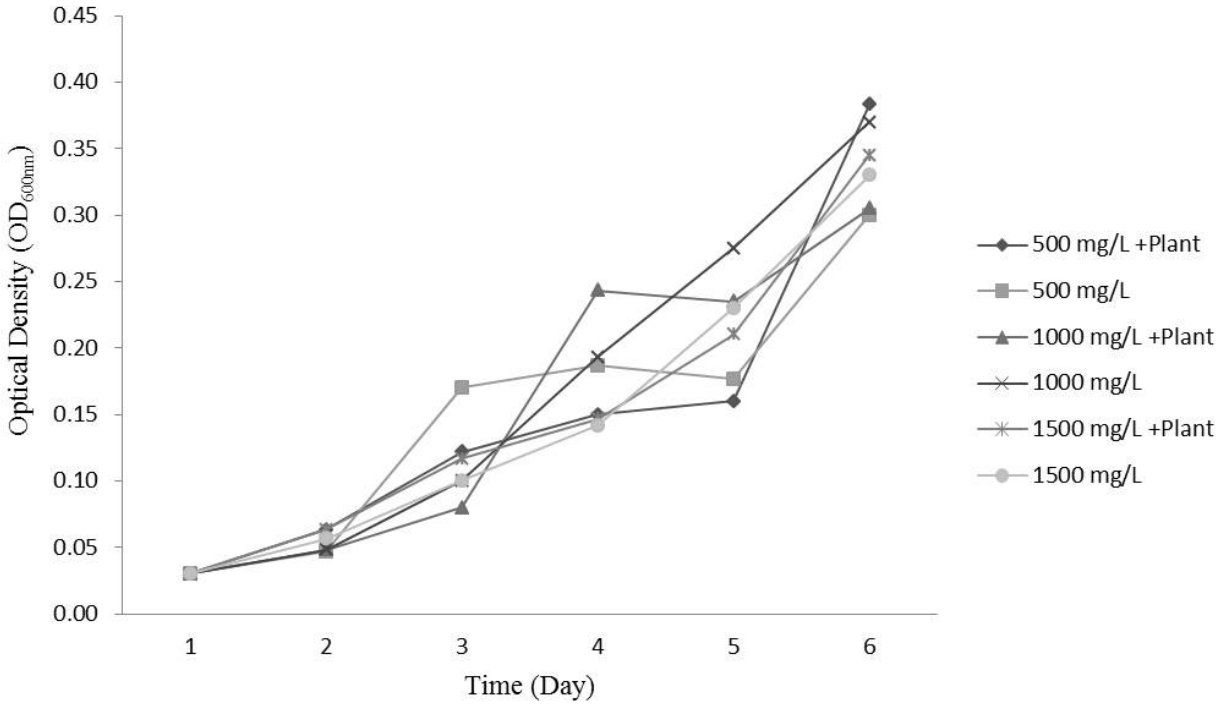


Fig. 5. The *effect* of phenol concentrations on the microbial growth in the presence and absence of *Ophiopogon japonicus*

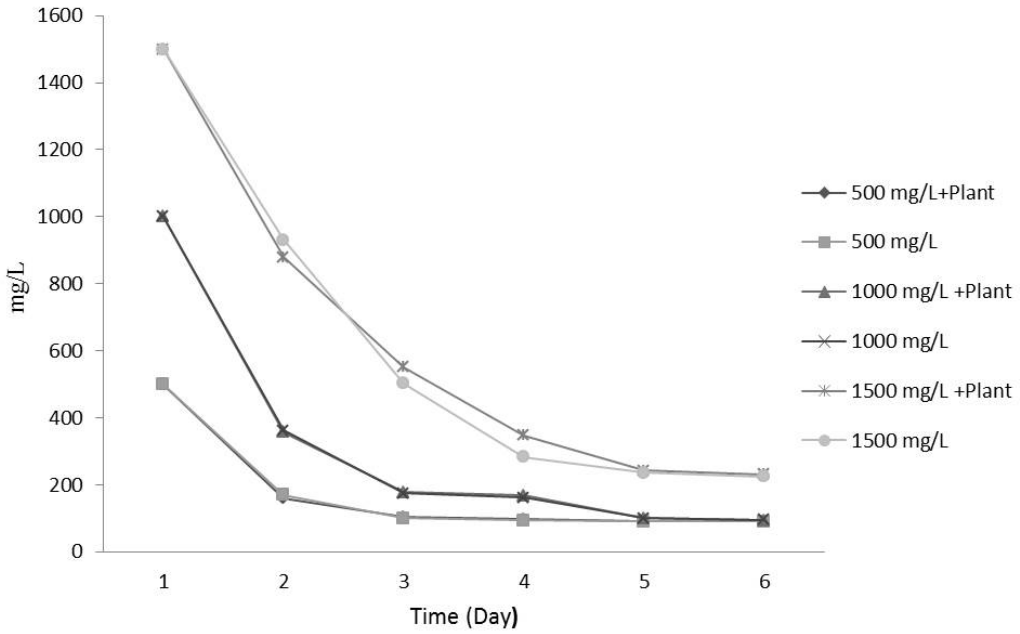


Fig. 6. The effect of phenol concentrations on the phenol biodegradation in the presence and absence of *Ophiopogon japonicus*.

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## COMPETING INTERESTS

The authors declare that they have no competing interests.

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## ETHICAL CONSIDERATIONS

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/ or submission, redundancy, etc) have been completely observed by the authors.

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