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Evaluation of Some Fungal Nano-Particles for Removing of Oil Pollution

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Abstract. Petroleum pollution is a global disaster and bioremediation is a cost effective method that was interested in recent years. In a field study, four fungal strains, *Acromonium sp., Alternaria sp., Aspergillus terreus* and *Penicillium sp.*, were collected determined from an oil contaminated sites of Arak refinery (Iran) and their growth ability was checked in the PDA media containing 0-10% petroleum. Results showed that the all fungi were able to growth in the subjected concentrations. The nano-particles prepared from the fungi were added to the petroleum polluted media and remained oils were extracted and determined after 30 days and compared with its amounts in the beginning of experiments. Results showed that the studied fungal particles were able to decrease petroleum pollution. The highest removing efficiency of *Aspergillus terreus, Penicillium sp., Alternaria sp.* and *Acromonium sp.* particles was in the media with 10%, 8%, 8% and 2% petroleum pollution respectively.

Keywords: Bioremediation, Petroleum pollution, *Acromonium sp., Alternaria sp., Aspergillus terreus, Penicillium sp.*, nano-particles.

1. Introduction

Petroleum pollution is a global disaster that is a common phenomenon in the oil-bearing and industrial regions [1]. There are several soil cleaning methods including burning, washing, chemical applying and bioremediation [2]. Bioremediation is using of plants and microorganisms to remove or detoxify environmental contaminants. Bioremediation has been intensively studied over the past two decades, driven by the need for a low-cost, *in*-situ alternative to more expensive engineering-based remediation technologies [1, 3]. In petroleum polluted conditions, plants or plant associated microflora can convert hydrocarbons (HCs) to non-toxic forms [4]. Bioremediation has been applied to remove crude oil [1, 4], motor oil [5], and diesel fuel [6] from soil but the removal efficiency is highly variable. Bioremediation of petroleum-contaminated soils is mainly based on biodegradation by the fungal strains that are present in the rhizospher of plants or in the soils of petroleum polluted soils [4].

The aim of this research was to find fungal strains from petroleum polluted soils of Arak refinery and evaluation of their nano-particles ability in removing of petroleum pollution in *In vitro* condition.

2. Selection of fungal strains

Arak oil refinery is located at 25 Km far away from Arak city. Regarding the oil refining activities in this region, a high degree of petroleum pollution was observed in some areas.

Plant root samples with 1 cm length were harvested, washed and dried. The samples were kept in sodium hypochloride 1% (3 min) and then ethanol 70% (3 min), for removing the peripherally attached

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microorganisms, and dried after washing with distilled water. The samples were kept in PDA media containing lactic acid. The Petri dishes were incubated in 25 ± 2 ˚C for 4 days. Then, different fungal colonies were isolated and cultured separately in PDA. Fungal specimens were examined under light microscope after preparations and were identified using morphological characters and taxonomical keys [7, 8].

3. Determination of fungal growth

The growth assay was used to find the resistant fungal species to petroleum contamination. The assays were conducted by comparing the growth rates of fungal strains, as colony diameter, on the oil contaminated and control Petri dishes. Test dishes were prepared by adding crude oil to warm PDA solution. In order to have a uniform concentration of oil in all plates, the solution was thoroughly mixed with a magnetic stirrer, right before it was added to the plates. Different concentrations of oil/PDA mixture (2, 4, 6, 8 and 10% v/v) were prepared. Pure PDA was used in control plates. All dishes were incubated with 2 mm diameter plugs of fungal mycelia taken from agar inoculums plate. The dishes were incubated at 25±2 ˚C in an incubator. Fungal mycelia extension on the plates (colony diameter) was measured using a measuring tape after 14 days and compared with the control plates.

4. Evaluation of petroleum removing

The fungi including *Acromonium* sp.*, Alternaria* sp.*, Aspergillus terreus, Penicillium* sp. were chosen for this study. Ninety six Petri dishes were selected for this study. The dishes were divided in to 4 groups; each group containing 24 dishes and used for each fungal strain. The experimental groups were as following: Groups A, B, C and D including nano-particles of the fungus *Acromonium* sp., *Alternaria* sp.*, Aspergillus terreus* and *Penicillium* sp. respectively and sub-groups are the media containing different concentrations (0, 2, 4, 6, 8 and 10%) of crude oil.

They were incubated in the same conditions with the temperature of 25±2 ˚C. Concentrations of crude oil were determined and compared in the media of experimental and control Petri dishes.

5. Determination of total oil (TOG)

The samples from experimental and control media were collected separately. Each sample was homogenized and stored at 4 °C until further processing. Total oil was analyzed according to the EPA method 9071 A and EPA Method 3540 B [9]. Five gram of the PDA in two replicates were acidified with hydrochloric acid to pH=2 and dehydrated with magnesium sulphate monohydrate. After 15 min, samples were transferred into paper extraction thimbles and placed into a Soxhlet type apparatus. TOG was extracted with dichloromethane for 8 h. The extract was filtered through filter paper (Whatman No. 4) with 1g sodium sulphate. The solvent was evaporated with a rotary evaporator and the weight of dry extract was determined. Percentage of TOG decreasing was calculated based on media weight and compared in the experimental and control plates.

6. Fungal growth ability

The growth activity of the isolated fungal strains was carried out under different concentrations of crude oil and was expressed as the diameter of the colony (Figure 1). The results showed that all the studied fungi were more or less resistant to petroleum pollution and they made a sufficient colony in 1% crude oil concentration; meanwhile, only some of them save their growth rates in 10% petroleum pollution. Among the studied fungi, *Alternaria* sp. had the highest resistance to 10% petroleum pollution (with 47.50 mm diameter of colony after 14 days growth) and *Aspergilus terreus* (31.25 mm), *Penicilium* sp. (23 mm), and *Acromonium* sp. (20.50 mm) were relatively sensitive ones.

7. Results of bioremediation

After 14 days of incubation of fungal nano-particles in petroleum-contained media, concentration of petroleum was determined in the experimental Petri dishes and compared with the beginning of experiment. The obtained data showed that the concentration of petroleum was decreased considerably in the all

experimental Petri (Figure 2). For *Aspergillus terreus*, the most decreasing of petroleum was evaluated in the group containing 10% petroleum (63%) and the lowest decrease was in the group containing 2% petroleum (20%). *Acromonium* sp. was also reduced amount of crude oil in the growing media. The highest removal ability was in the media containing 2% petroleum (68%) and the lowest one was in the media with 10% petroleum (27%) (Figure 2). For *Alternaria* sp., the highest decrease (80%) was in the media with 8% oil and the lowest one (56%) is in media with 2% oil. Finally, for *Penicillium* sp., the highest decreasing of oil was in the group with 8% petroleum (82%) and the lowest decrease was in the group with 2% petroleum (45%) (Figure 2).

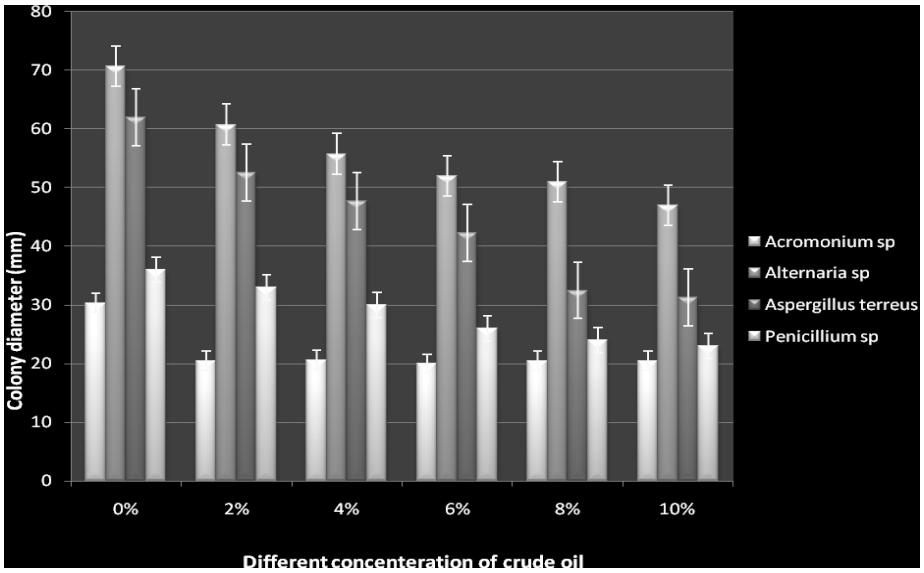

Fig. 1: The growth ability of the isolated fungal strains under different percentage of petroleum pollution. Results showed that Alternaria sp. has higher and Acromonium sp. lower growth ability.

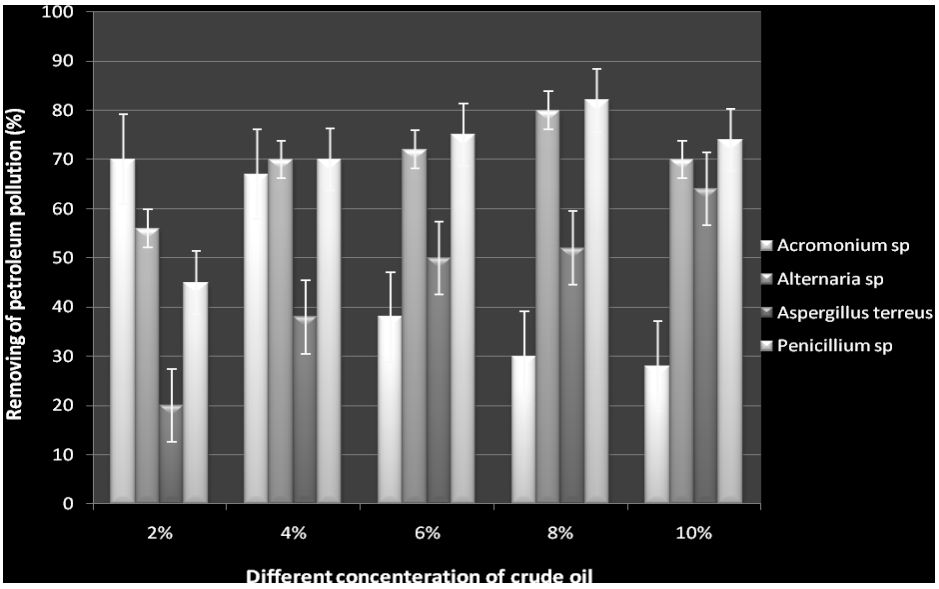

Fig. 2: Petroleum removing (%) by studied fungal strain nano-particles. Results showed that Penicillium sp. and Alternaria sp. are the more effective one in high petroleum polluted media.

8. Discussion

Study on fungal species showed that *Acromonium* sp., *Alternaria* sp., *Aspergillus terreus* and *Penicillium* sp. were the common fungi in petroleum polluted soils, with high frequency. It seems that the fungal species used oil compounds as nutrients . The similar results were reported by some researchers [4, 10].

The *In vitro* growth test of the isolated fungi showed a species-specific response. All of studied fungal strains were able to growth in 2% v/v oil pollution and therefore could be useful for the remediation of light soil pollution. Although the growth of fungal species were reduced by increasing oil concentrations (more than 4% v/v), but all of them were still able to growth in the high concentrations of petroleum. They were produced sufficient colony in the high polluted media with a lagging time. It seems they could be used also for oil degradation in the soils with high pollution effectively. Our results are accordance with the some finding of other researchers about other different fungal species [4].

Bioremediation of a petroleum-contaminated soil is mainly based on biodegradation by microorganisms [11] and fungi are one of the most important factors. The results of our study proposed the above mentioned fungal nano-particles for using in remediation of petroleum-polluted environments in a field study. It means that the data of this study indicated that isolated fungal nano-particles, *Acromonium* sp., *Alternaria* sp., *Aspergillus terreus* and *Penicillium* sp., may have the potential for bioremediation of soil, water and other environments in highly polluted conditions.

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