Biodegradation of petroleum hydrocarbons by fungi strains of Aspergillus sp.-17, Rhizopus sp.-81, Penicillium sp.-94 isolated from oil-contaminated soils of Azerbaijan

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Abstract. Environmental security and biocentric lifestyles are the approaches that today's man must follow and choose, which cause major environmental problems in modern times and threaten the existence of civilization in the future. The soil ecosystem, which is one of the polluted environments, is polluted naturally and anthropogenically because of oil extraction, transportation and accidents that occurred during this time. Undoubtedly, with the constant increase in the need for oil, land recultivation becomes more urgent than ever for the modern era. Although physical, chemical, and biological solutions are proposed for the recultivation of oil-contaminated soil from oil and oil products in modern times, bioremediation is a more favorable, less capital-intensive, environmentally friendly technology from an ecological and economic point of view. Thus, the main purpose of this study is to determine the ability of micromycetes to biodegrade petroleum hydrocarbons and to determine the advantages of bioremediation of lightly and moderately polluted soils with oil using Aspergillus sp.-17, Rhizopus sp.-81, Penicillium sp.-94 fungal strains with high lipolytic activity. It also mentioned tolerance of oil to different hardnesses.

1 Introduction

The problem of soil pollution with polycyclic aromatic hydrocarbons (oil), which is at the forefront of anthropogenic pollution in the world, remains relevant today [1]. There is still a need for new technologies and work in this field. This problem is not alien to the Republic
of Azerbaijan. Thus, it is known that more than 10,000 ha of oil-contaminated land in the Absheron peninsula, has a total area of 220,000 ha [2].

Oil pollution of soil not only harms human health [3], but also causes a decrease in soil biological indicators, biodegradation of existing vegetation, loss of biodiversity, and degradation of arable land [1, 4]. Physical, chemical, and biological (bioremediation) solutions are proposed to solve this problem. Although physical and chemical solutions are superior to bioremediation in terms of indicators such as solving the problem in a short time and precision, they lag bioremediation methods owing to greater capital investment, secondary pollution during their application, destruction of existing microbiota in the area, and resetting of the biological indicators of the soil [5, p.33-37].

Bioremediation is the process of converting harmful-toxic compounds in the environment into simpler harmless compounds for the environment and human health by using fungi, plant, and other microorganisms, and as the name suggests, it involves taking advantage of the appropriate properties of plants, fungi, and microorganisms [6-8].

Worldwide, patents related to the use of fungi in the bioremediation of oil-contaminated soils are mainly applied in countries such as the United States of America, the United Kingdom, China, and Brazil due to the wide range of uses of micromycetes belonging to the genera Aspergillus, Penicillium, Mucor, and Trichoderma. Bacteria are the first with 57% of the agents, enzymes are the second with 19%, and fungi are the third with 13% used in patents. The interesting fact is that patented technologies are used in the domestic and local market of the countries in question, and there is no information about their use in other countries. This is explained by the fact that these works and the characteristics of microorganisms are suitable for the ecological conditions of the country they belong to and do not show the same high efficiency in other countries [9-10].

Also, studying the effect of oil and petroleum hydrocarbons on the metabolic profile of the soil and the condition of the biota is of particular importance during the implementation of bioremediation works. Thus, comparing the metabolic activities of clean and oil-contaminated soils allows us to tell what the level of contamination is. The study of the long-term effect of oil on the synthesis of metabolites in microorganisms makes a great contribution to the evaluation of soils and the study of the level of microbiota activity there [11].

2 Materials and methods

2.1 Soil sampling

Soil samples were taken from the vicinity of oil wells located in Binagadi, Surakhani, Sabail, Balakhani districts of Baku and Absheron Peninsula and 129 micromycete strains were cultured from 24 soil samples. So, four samples were taken from each oil well, one of the samples was taken from the part where the oil was discharged, and the other three were taken from the depth of 0-20 cm, 1-1.5 m, 25 m, and 100 m away from the well. The reason for this was to determine the effect of the level of pollution on the ability of micromycetes to synthesize enzymes (Fig 1).
Fig 1. Research area and soil samples

Sampling of samples from the research area, separation of fungi and removal to pure culture were carried out according to classical methods accepted in microbiology and mycology [12]. Malt Extract Agar (MEA), Saburo agar, Czapek Dox Agar and Potato Dextrose Agar (PDA) nutrient media were used as nutrient media. The order of preparation of nutrient mediums, planting of the taken samples, inoculation and incubation were carried out according to appropriate methods [13]. Identification of isolated strains to the genus level was performed using known identification books based on morphological-cultural and physiological characteristics and microscopic appearance [14].

2.2 Screening due to the ability of fungi to biodegrade petroleum hydrocarbons.

Primary screening of micromycetes based on oil biodegradation was carried out according to Hanson's method [15]. For this, Bushnell-Haas (BH) broth nutrient medium: MgSO4 (0.2 g/l), CaCl2 (0.02 g/l), KH2PO4 (1 g/l), K2HPO4 (1 g/l), NH4NO3 (1 g/l) and FeCl2 (0.05 g/l), distilled water (1000 ml), final pH 7.0 were used for screening [16]. At this time, a solution is prepared by adding 50 ml of Bushnell-Haas nutrient medium, 1% crude oil and 0.016 mg/ml redox indicator 2.6-dichlorophenol indophenol (DCPIP) in a 250 ml flask. Then, fungal spores from the 5-day culture are inoculated there (for control, no fungus are planted in one flask) and all flasks are incubated for 2 weeks at 30°C in deep seeding conditions in a 110 rpm thermostat (Fig 2). The change of the indicator from blue to colorless in solution indicates the ability of fungi to biodegrade [17].
2.3 Petroleum hydrocarbons tolerance test.

Petroleum hydrocarbons tolerance of fungi was determined by adding agar to Bushnell-Hans nutrient medium. At this time, 1%, 5% and 10% crude oil were added to the nutrient medium, and then it was autoclaved for sterilization. After filtering the nutrient medium into 8 mm petri dishes, fungi were inoculated there. Each experiment was set up in four replicates, one petri dish without crude oil was added as a control and the growth of the fungus there was taken as a control. After inoculation, the samples were placed in a thermostat for development at 28°C for one week. After development, the percentage of growth of the control fungus was taken as 100%, and based on this, the percentage of growth of others was determined [18-19].

2.4 Fungal biomass gain via biodegradation

To measure the biomass of strains after 2 weeks of growth all the flasks were filtered using filter paper (Whatman No.1) with the aid of a funnel and dried at 105°C until constant weight was obtained. At this time, the filter paper was pre-weighed on an analytical balance and recorded. After the constant weight was obtained, the previous weight of the paper was subtracted from the obtained weight, and the remaining weight was determined as the biomass produced by the strains [20].

3 Results and discussion

When determining the ability of micromycetes to biodegrade petroleum hydrocarbons according to the Hanson method, three main indicators are considered: the first is the change of color from blue to colorless, the second is the decrease in the amount of oil in the flask, and the third is the growth of mycelium [17]. So, although one or more of these indicators were observed in all the strains used, all three indicators were quite high in *Aspergillus sp.*-17, *Rhizopus sp.*-81, *Penicillium sp.*-94 strains. Thus, these three strains showed better indicators than the others due to the decolorization of the indicator, the reduction of the amount of oil, and the growth of mycelium after 14-day incubation with crude oil (Tab. 1).
Table 1. Decolorization of 2,6-DCPIP and visual assessment of fungal growth.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Decolourization</th>
<th>Reduction of oil</th>
<th>Mycelial growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus sp.-17</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Rhizopus sp.-81</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Penicillium sp.-94</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*The indicator change color. -: no change in color, +: weak, ++: medium, +++: complete decolourization.*

*Mycelial growth. -: no visual growth, +: weak, ++: medium, +++: strong.*

*Reduction of oil. -: no reduction, +: weak, ++: medium, +++: complete reduction.*

As can be seen from the results, the greatest growth was observed at 1% concentration of oil, so all three strains showed growth between 50-75%. As the concentration of oil increased, the growth percentage of the strains decreased and finally, at 10% concentration of oil, the growth phase of micromycetes compared to the control samples was lower than 10%. It is also known from the literature that micromycetes can be active in soils contaminated with oil up to 5-7%, concentration above the specified limit seriously hinders the development of micromycetes and makes it impossible to carry out bioremediation works on these soils (Tab. 2).

Table 2. Test of tolerance of isolates to different concentrations of crude oil

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Concentrate of crude oil</th>
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<tr>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>Aspergillus sp.-17</td>
<td>+++</td>
</tr>
<tr>
<td>Rhizopus sp.-81</td>
<td>+++</td>
</tr>
<tr>
<td>Penicillium sp.-94</td>
<td>+++</td>
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</tbody>
</table>

(+ up to 10%, ++ from 10% to 25%, +++ from 25 to 50%, ++++ from 50 to 75%)

By measuring the biomass of the strains after 14 days of development, it was found that the highest biomass was produced by *Aspergillus sp.-17* strain (0.285 g/l), followed by *Rhizopus sp.-81* strain (0.250 g/l), *Penicillium sp.-94* strain and (0.215 g/l) produced biomass. It should be noted that when the experiment was conducted, crude oil was used as the only carbon source, which is directly proportional to the ability of the biomass of fungi to biodegrade crude oil. Thus, the biomass produced by micromycetes was obtained from the biodegradation of crude oil. That is, all three strains can be used for bioremediation in oil-contaminated soils due to their biodegradability. Oil tolerance of these strains should be considered during bioremediation. As you know, micromycetes retain the ability of bioremediation in soils contaminated with oil 5-7%.

As a conclusion of the research conducted, we can say that the strains of *Aspergillus sp.-17*, *Rhizopus sp.-81*, *Penicillium sp.-94* can biodegrade crude oil, show the highest activity at 1% concentration of oil, and can maintain their activity at 5% concentration. They are micromycetes that spread in the oil-contaminated soils of the Republic of Azerbaijan. In previous studies, the lipolytic activities of these strains were studied and it was found that all three strains have high lipolytic activity. Summarizing the results, all three strains can be used in the bioremediation of oil-contaminated soils by injecting them into the soil and...
obtaining preparat from them. The result from our side is that the joint use of both is more effective.

4 Conclusion

Bioremediation, one of the available solutions for the recultivation of oil-contaminated soils, is the demand of today as an environmentally friendly technology. The fulfillment of this demand falls on the scientists and researchers of each region. So, to carry out mycoremediation works in any region, the micro and microbiota of that region should be thoroughly studied. Later, the bioremediation works carried out at the expense of the local biological resources of that particular region become more effective. Thus, a microorganism belonging to a different region, a drug, does not show the same effectiveness in another region. Guided by this idea, we conducted our research in the direction of improving the ecological situation of our Republic at the expense of local bioresources. The results of the study of the ability of Aspergillus sp.-17, Rhizopus sp.-81, Penicillium sp.-94 to biodegradation oil, their survival in different concentrations of crude oil, and the analysis of the amount of biomass of all three strains with oil are low and moderate (5-7% up to) allows us to make a positive opinion about what can be used in the bioremediation of contaminated soils. It was also determined by us that these strains show high lipolytic activity in previous studies. Therefore, all three mentioned micromycete strains can be directly used in the bioremediation of oil-contaminated soils as fungal strains with high lipolytic activity, which can biodegrade oil and are effective at concentrations of oil up to 5-7%. On the other hand, the fact that these strains have high lipolytic activity means that the enzyme preparations obtained from them can also be used in bioremediation. We conclude that the combined use of direct fungal strains and enzyme preparations obtained from them can give better results.

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References


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