Influence of phosphorous fertilizers on fungial growth and oil hydrocarbon degradation

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Pure fungal cultures – active utilizers of oil products were selected. The ability of *Trichoderma harzianum* VNB-16 to decompose mazut, diesel fuel and naphthalene was shown. The obtained results indicate that NPK fertilizers Kemira Horti-2 used as a source of nitrogen and phosphorus can significantly increase the rate of oil product degradation by fungi.

Keywords: fungi, hydrocarbon biodegradation, phosphorous fertilizers

INTRODUCTION

The bioremediation of contaminated soil is based on the ability of microorganisms to grow in polluted surroundings and degrade corresponding pollutants. For acceleration of this process, the activity of indigenous microorganisms may be stimulated or active hydrocarbon-oxidising microorganisms may be introduced into the contaminated substrata [1]. Bacteria are playing the leading role in the biodegradation of oil products. Nevertheless, fungi have some advantages: the mycelial growth allows rapid colonization of substrates, the extracellular degradative enzymes allow to tolerate high concentrations of toxins, many of fungal enzymes are nonspecific [2]. The fungal species able to grow on oil products in most cases belong to the genera *Cuninghamella, Rhizopus, Fusarium, Aspergillus, Penicillium, Trichoderma* [1, 3–4]. Fungi, contrary to bacteria, are able to degrade polyaromatic high molecular weight hydrocarbons [4].

The shortage of the hydrocarbon-oxidising microorganisms and available N and P sources in contaminated soil are the main limiting factors for soil self-bioremediation. Populations of hydrocarbon-degrading microorganisms normally constitute less than 1% and in polluted soils – about 10% of the total microbial communities [5]. Enrichment of cleaning soil with screened active microbial strains and addition of mineral nutrients resulted in a significantly stronger hydrocarbon biodegradation. For example, a mixed culture of microorganisms degraded 42.9% of the heavy fraction of oil in 28 days in sandy soil when a phosphate and nitrate source was provided and only 11.9% without these components [6]. Sodium, potassium and ammonium phosphates are usually used in the media for the growth of fungi. Various rates of mineral fertilizers in soil bioremediation processes are used.

It is important to select phosphorous fertilizers suitable for microbial degrading avtivity and reduce the costs when big amounts of fertilizers are needed for soil cleaning *in situ* or *ex situ*. The aim of the investigation was to select fungal strains able to degrade oil hydrocarbon and to find the optimal concentrations of phosphorous fertilizers for accelerating the fungal growth and heavy oil (mazut) degradation.

METHODS

Phosphorous fertilizers such a superphosphate (containing about 23% of P), bone-meal (about 13% of P) and complex Kemira Horti-2 6–12–24 NPK fertilizers (28.6% of P) used instead of the standard reagent K_2HPO_4 and various their concentrations suitable for the growth of fungi and degradation of oil products were investigated. Modified Czapek medium containing 1.5 g of NH_4NO_3 instead of $NaNO_3$ was used as a control medium. It was calculated that 1 g of KH_2PO_4 was equivalent to 1.6 g of bone-meal, 1g superphosphate and 0.8 g of Kemira Horti-2 fertilizers. In the experiment 0.5, 1, 1.5 and 2 times larger fertilizer concentrations as compared to the control were used. The pH was adjusted to 4.8–5.0.

The ability of fungi to grow on the media with various kinds and concentrations of phosphorous fertilizers and to degrade hydrocarbons was estimated according to the growth intensity in solid or liquid media containing 1% of heavy fuel oil (mazut), diesel fuel or naphthalene as a sole carbon source. The residual oil products in the media were assayed by IR-spectrophotometry.

RESULTS AND DISCUSSION

The fungal strains isolated from oil-polluted soils had been shown in previous laboratory tests to be able to grow on the test media containing mazut or diesel fuel as a sole carbon source. The best growth was shown by *Trichoderma harzianum* VNB-16 and *Fusarium oxysporum MG-4-1* strains. The selected fungal strains were used to investigate phosphorous fertilisers suitable for their growth. The results showed that the linear growth rate of colonies of fungi was more extensive on the media with test fertilizers than on control media with KH_2PO_4. Nevertheless, different fertilizers and their concentrations influenced the mycelium density and its pigmentation and thus could possibly be of great importance for the biodegrading activity of fungi.

The growth of fungi on the media containing various concentrations of fertilizers and 1% of diesel fuel are shown in Table 1. The influence of various fertilizers on the growth of fungal strains studied was rather different. The growth of *Arthroderma* sp. MG-1-1 and *Fusarium oxysporum* MG-4–1 strains was the best on the media containing 0,8 g/l Kemira Horti-2, *Penicillium funiculosum* VAU-4 – 1.2 or 1.6 g/l, *P. verruculosum* MN-1 – 0.8 or 1.6 g/l of these fertilizers. All concentrations of complex NPK fertilizers were suitable for the growth of *Trichoderma harzianum* VNB-16. Superphosphate was available for the growth of studyed strains mainly at a concentration of 0.5 g/l. Some changes in the cultural and morphological properties of fungal strains were noticed. The colonies of *Fusarium oxysporum* MG-4-1 and *Penicillium funiculosum* VAU-4 had a typical pigmentation, were rather compact, aerial mycelium formed intensively on the media with Kemira Horti-2 (especially at a concentration of 1.6 g/l) and with supherphosphate (0.5 and 1 g/l). *Trichoderma harzianum* VNB-16 under the action of diesel fuel formed leather-like colonies without well-expressed aerial mycelium, but when NPK (0.4 or 1.2 g/l) or superphosphate (1 g/l) was added, the mycelium became similar to the control. *Penicillium verruculosum* MN-1 colonies did not differ from control.

The main attention was paid to the degradation of heavy oil – mazut. Only two strains – *Penicillium verruculosum* MN-1 and *Trichoderma harzianum* VNB-16 – grew intensively on the media containing

Table 1. **The linear growth rate of fungal colonies on the media containing different phosphorus sources and diesel fuel after 7 days of incubation**

Phosphorus source	Concentration of P fertilizer (g/l)	Diameter of colonies, mm				
		Fusarium oxysporum Schltdl. MG-4-1	*Penicillium funiculosum* Thom VAU-4	*Penicillium verruculosum* Peyronel MN-1	*Trichoderma harzianum* Rifai VNB-16	*Arthroderma* sp. MG-1-1
Control (KH_2PO_4)	1	52.5 ± 0.4	18.0 ± 1.2	27.5 ± 1.2	90	9.0 ± 0.2
Superphosphate	0,5	54.5 ± 1.5	28.5 ± 1.8	33.5 ± 1.8	90	13.0 ± 0.4
–“–	1	52.5 ± 3.4	–	32.5 ± 1.2	90	–
Kemira Horti-2	0,4	39.5 ± 0.2	15.0 ± 1.2	32.0 ± 1.2	90	17.8 ± 0.2
–“–	0,8	60.0 ± 0.0	19.2 ± 1.8	38.2 ± 3.2	90	18.5 ± 0.8
–“–	1,2	–	22.0 ± 1.8	28.0 ± 0.8	90	13.5 ± 0.2
–“–	1,6	49.8 ± 0.2	21.5 ± 1.0	37.5 ± 1.8	90	12.5 ± 1.5

Table 2. **The linear growth rate of fungal colonies on the media containing different phosphorus sources and mazut after 7 days of incubation**

Phosphorus source	Concentration of P fertilizer (g/l)	Diameter of colonies, mm	
		Penicillium verruculosum Peyronel MN-1	*Trichoderma harzianum* Rifai VNB-16
Control (KH_2PO_4)	1	13.8 ± 0.2	90
Superphosphate	1	12.5 ± 0.5	90
–“–	2	14.5 ± 1.2	90
Kemira-Horti 2	0.8	18.0 ± 0.0	90
–“–	1.6	16.0 ± 1.0	90
Bone-meal	1.8	17.5 ± 0.1	57.0 ± 2.7
–“–	3.6	15.5 ± 0.1	48.0 ± 2.5

different P sources and mazut (Table 2). Kemira Horti-2 fertilizers were suitable for the growth of *Penicillium verruculosum* MN-1. *Trichoderma harzianum* VNB-16 used NPK fertilizers (0.4 and 0.8 g/l) and superphosphate (1 g/l) for its growth. Growing on media with these fertilizers, the strain formed thick, pigmented colonies with a dense aerial mycelium. The bone-meal wasn't so available for the fungal growth because of its low solubility.

In liquid media containing a mixture of mazut and diesel fuel in the ratio of 1:1 and 0.8 g/l of Kemira Horti-2 the dry biomass of fungus *Trichoderma harzianum* VNB-16 came to 150% comparing with control media containing KH_2PO_4. 34.5% of mazut degraded in polluted soil fertilized with Kemira Horti-2 0.8 g/kg and inoculated with the fungus *Trichoderma harzianum* VNB-16 in laboratory tests after 40 days of incubation. The results indicate that Kemira Horti-2 fertilizers at a concentration of 0.8 g/l most effectively stimulated the growth of the fungal strains in various media containing oil products. That means a stimulation of fungal biodegradation activities. From this point of view KH_2PO_4 was less effective.

The results of oil product degradation tests in liquid mineral media by *Trichoderma harzianum* strain VNB-16 showed that 10% of mazut and 44% of naphthalene were degraded during 7 days of cultivation. At that time mazut decomposed to small drops, and after 20 days only a light brown liquid was observed. The deeper and faster utilization of mazut was achieved by a combined cultivation of bacteria and fungus strains. The *Trichoderma harzianum* strain VNB-16 was able to degrade a wide spectrum of oil products, so it seems to possess high abilities to grow in oil-polluted sites and to enhance hydrocarbon degradation.

The results of the investigation showed that the influence of the study fertilizers on fungal biodegradation activity was positive. Cheaper phosphorous fertilizers produced in or imported to our country successfully changed pure phosphorous salts in the nutrition media. Kemira Horti-2 NPK fertilizers may be recommended for acceleration of fungal growth and activation of biodestruction of oily pollutants in soil cleaning processes when active fungal strains are inoculated into polluted soil. The selected *Trichoderma harzianum* strain VNB-16 is of essential importance for biodegradation of mazut and may be used for bioremediation of hydrocarbon-polluted areas. Its abilities to survive in highly polluted soils, to utilize such a hard-to-degrade fraction as mazut, to decrease the toxicity and improve the structure of soil are of great practical importance. The selected *Trichoderma harzianum* strain VNB-16 was offered to be patented.

References

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FOSFORO TRĄŠŲ ĮTAKA MIKROMICETŲ AUGIMUI IR NAFTOS PRODUKTŲ DEGRADACIJAI

S a n t r a u k a

Teršalų poveikis aplinkai labai priklauso nuo mikroorganizmų sugebėjimo juos utilizuoti ir nukenksminti. Biodegradaciniams procesams užterštoje aplinkoje pagreitinti naudojamos azoto ir fosforo trąšos. Valant užterštą gruntą *in situ,* svarbu pakeisti cheminį reagentą KH_2PO_4 pigesnėmis ir plačiai naudojamomis fosforo trąšomis. Tyrimų rezultatai parodė, kad tinkamiausios grybų augimui ir naftos produktų degradacijai buvo kompleksinės Kemira Horti-2 trąšos. Atrinkti aktyvūs – angliavandenilius oksiduojantys mikromicetų štamai, augantys terpėse su mazutu ir dyzelinu. Aktyviausiai mazutą skaidė *Trichoderma harzianum* VNB-16 štamas, kuris pateiktas patentavimui. Šį mikromiceto štamą galima panaudoti įvairių naftingų atliekų utilizavimui pagreitinti ir užterštam dirvožemiui rekultivuoti *ex situ* ir *in situ*.