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EVALUATION THE ABILITY OF THE FUNGUS *ASPERGILLUS* TO REMOVE OIL FROM CONTAMINATED SOILS

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Abstract

The paper aims to present the capacity to consumption of petroleum products by the fungus Aspergillus. The Aspergillus sp. was isolated in an area that is often polluted with crude oil as a result of oil pipes breaking from Olt County. The best activity towards gasoline and diesel was presented by the Aspergillus sp. *strain noted I2. However, it is interesting that the Aspergillus strain noted I3 is more active against crude oil than other strains used. During the period of 40-days of the experiment the isolated strains were able to degrade petroleum by-products in percentages ranging over a wide range between 32.46% and 78.42%. The degree of degradation depends on the composition of petroleum and their chemical structure.*

Key words*: oil pollution, bioremediation, Olt County, Aspergillus* sp.

INTRODUCTION

Population growth and therefore increasing energy and fuel needs in the recent decades has led to an increase in unwanted areas polluted with oil products both oceans and seas as well as of soil. The soil pollution problem is more complicated in oil extraction areas because, while oil residues pollution takes place and wastewater pollution, salt, capable of causing a strong salinization of soils polluted with oil. Under the conditions of such pollution is virtually unproductive soil and is removed completely from the economic circuit.

Most studies on the remediation of soils polluted by oil have used for bioremediation processes different microorganisms such as bacteria (*Bacillus, Dietzia, Ochrobactrum, Alcanivorax, Peudomonas, Sphingomonas sp., Stenotrophomonas, Gordonia, Micrococcus, Marinobacter, Microbulbifer, Sphingomonas, Cellulomonas,),* fungi (*Fusarium, Aspergillus, Penicillium, Amorphoteca, Paecilomyces, Talaromyces, Graphium, Neosartorya)* or yeasts *(Candida, Yarrowia sau Pichia)* (Jain et al. 2011; Margesin and Schinner, 1997; Mueller et al. 1996; Matei et al. 2004; Matei et al. 2007).

In most studies, selected microorganisms for bioremediation came from the oil polluted areas (Mariano et al. 2007; Santhini et al. 2009; Wang et al. 2011, Chibuike and Obiora, 2014).

In bioremediation it is necessary that petroleum products to be accepted as carbon source by microorganisms and to have at their disposal an electron acceptor which may be oxygen or nitrates. In addition, microorganisms require also nutrients for growth and the lack of specific inhibitors that can block the development of the maximum capacity of microbial selected (Collins 2007).

Thus in different experiments has been shown that by increasing soil nitrogen and phosphorus there has been an degradation of oil up to 45.5% over a period of about 50 days (Mariano et al., 2007).

In this context, the paper presents an analysis of the efficiency of *Aspergillus* sp microorganisms isolated from soils polluted with oil in 2012 in the Icoana village, Olt County, Romania.

MATERIALS AND METHODS

In order to characterize the efficiency of fungus *Aspergillus* on oil products, were used three *Aspergillus* sp. Isolates obtained in September 2012 from soils of Icoana village, which were polluted in May 2012 by damaging of an oil pipeline. About 0.005g of soil sample was scattered on the bottom of a sterile Petri dish and molten cooled agar medium (PDA) was added, which was then rotated gently to disperse the soil particles in the medium. The Petri dishes were then incubated at around 28^0C in dark for three days.

Fungal morphology were studied macroscopically by observing colony features and microscopically by staining with lacto phenol cotton blue and observe under compound microscope for the conidia, conidiophores and arrangement of spores in order to identify the fungi. The fungi were identified as *Aspergillus* sp. The *Aspergillus* strains were noted I1, I2 and I3.

Unpolluted soil samples were dried at 105^0 for 2 hours to kill the own microorganisms and portions of 5 kg were placed in special containers. Soil samples were polluted with gasoline, diesel and crude oil in proportion of 50 g oil product to 1 kg of soil. In order to have the necessary moisture of soil to develop microorganisms in each sample was added 1 L of nutrient solution (25 g $(NH_4)_3PO_4$, 25 g K_2HPO_4, 2g $MgSO_4$ and 100g NaCl in 1 L water). The soil samples so prepared were inoculated with selected microorganisms. Each soil sample was inoculated with about 10 mg mycelium, collected from the mycelium developed on the plates. Evaluation of the oil concentration was carried out by the gravimetric method. For this evaluation was used as the extraction solvent a mixture of hexane and petroleum ether in the ratio of 1: 1. Each soil sample was extracted three times to obtain reproducible results and has been calculated the average for values differed no more than 5% each. Evaluation of the consumption / destruction of fuel (diesel, gasoline and crude oil) of soils were carried out at intervals of 5-10 days.

RESULTS AND DISCUSSIONS

The results of the average values obtained are shown in Table 1. Oil content was very high at pipe breakage (of 61.16 times higher), but also has high values between 11.57 to 43.44 times higher than the maximum limit allowed (MAL is 500 ppm for land used for agriculture).

Table 1. The average oil content from soil samples

Place of soil sampling	Oil content, g/kg	Report towards the maximum limit allowed
0	122.32	61.16
200 m	83.46	41.73
400 m	68.53	34.27
600 m	86.87	43.44
800 m	34.45	17.23
1000 m	23.14	11.57

The results obtained regarding evaluation of the consumption of fuel by *Aspergillus* sp. are presented in Table 2.

The data table shows that the nature of the fuel led to a different consumption specific to each *Aspergillus* isolate for different oil products. Thus it appears clearly that AI2 strain is best fitted to oil products consumption.

Table 2. The evolution of oil products content in soil at different periods of time (g/kg)

Oil product	Microorganism	Day, oil content g/kg					
		5	10	15	20	30	40
Diesel	*Aspergillus* I1	49.25	46.85	39.40	33.40	29.78	28.15
	Aspergillus I2	47.13	42.07	34.08	27.87	22.49	19.80
	Aspergillus I3	48.01	44.11	38.31	34.71	27.77	21.24
Gasoline	*Aspergillus* I1	44.96	38.77	33.16	26.19	22.13	19.63
	Aspergillus I2	43.93	39.44	32.15	24.37	17.70	10.79
	Aspergillus I3	45.48	38.79	32.72	26.95	22.84	13.07
Crude oil	*Aspergillus* I1	48.63	45.64	45.97	38.24	36.01	33.77
	Aspergillus I2	46.33	40.41	34.30	33.33	31.50	25.75
	Aspergillus I3	48.16	42.21	36.71	37.59	31.24	25.06

To better visualize the microorganisms behaviour towards different fuel, these were plotted in Figures 1, 2 and 3.

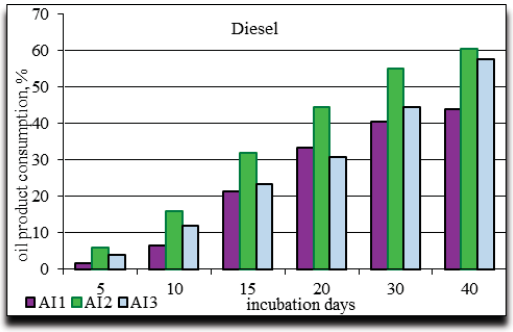

Figure 1 Degradation evolution of diesel by *Aspergillus* sp strains.

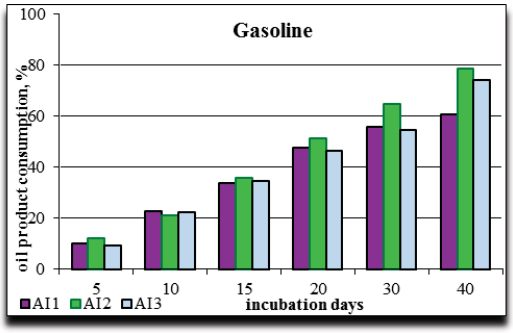

Figure 2. Degradation evolution of gasoline by *Aspergillus* sp. strains

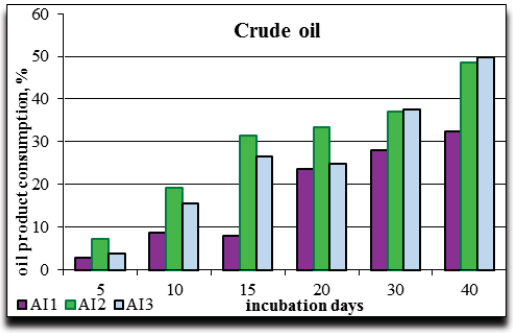

Figure 3. Degradation evolution of crude oil by Aspergillus sp. strains

All *Aspergillus* sp. isolate have the ability to faster reduce gasoline followed by diesel and then crude oil.

It is also clear the slow progress of petroleum products degradation. Thus, it is noted that none of the microorganisms used was not able to decompose at least one fuel completely even after 40 days of incubation at room temperature. However, different studies have shown that microorganism's consortium own polluted soil in a shorter time. For example a study shown that the microorganisms own of soil degrade petroleum by-products between 16-31% in unfertilized soils and between 27-53% in fertilized soils after 20 days at a temperature of about 10^0C. So, microorganism's consortium is more effective for decontamination process than a particular species isolated separately (Margesin and Schinner, 1997).

It should be noted however, that *Aspergillus* sp I2 strain manages to decompose gasoline and diesel up to 80% respectively 60% but after 40 days. Meanwhile, *Aspergillus* sp. I3 strain becomes more active after 30 days of incubation being the most active to decomposition of crude oil.

In addition some studies show that the most effective microorganisms in the degradation of hydrocarbons are bacteria such as *Flavobacterium* sp., *Brevibacterium* sp. or Micrococcus sp. (Santhini et al. 2009; Wang et al. 2011)

CONCLUSIONS

Pollution degree of areas where accidents occur by breaking petroleum products pipelines is over 10 times higher than the limit for less sensitive use areas and more than 200 times higher than those accepted for normal soils (500 ppm).

Among *Aspergillus* sp. isolate obtained from the polluted area and used in laboratory experiments, I2 has proven to be the most active for gasoline and diesel, while I3 is the best for crude oil.

As a general conclusion it can be said that the use of contaminated soil microorganisms their own in bioremediation processes and enriching them with nutrients to help these microorganisms in their work is an affordable way of regenerating polluted areas.

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