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The Study of Biodegradation of Diesel Fuels by Different Strains of *Pseudomonas*

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Abstract. The analysis of diesel fuel's hydrocarbon composition before and after biodegradation is carried out by the methods of photocolorimetry. It was determinated, that the hydrocarbon composition of the diesel fuels is changed on influence of microorganisms. It has been shown that changes in composition hydrocarbons of diesel fuels and individual hydrocarbons in soil observe during growth of microorganisms on this soil.

Introduction

The extent of hydrocarbon biodegradation in contaminated soils is critically dependent upon four factors, namely the creation of optimal environmental conditions to stimulate biodegradative activity, the predominant petroleum hydrocarbon types in the contaminated matrix and the bioavailavility of the contaminants to microorganisms. The petroleum hydrocarbon degradation is also affected by the molecular composition of the hydrocarbons, characteristic which is directly related with the bioavailability of these compounds, and as a consequence, the biodegradation rate may be altered [1].

Biodegradation is the metabolic ability of microorganisms to transform or mineralize organic contaminants into less harmful, non-hazardous substances, which are then integrated into natural biogeochemical cycles. The intensity of biodegradation is influenced by several factors, such as nutrients, oxygen, pH value, composition, concentration and bioavailability of the contaminants, chemical and physical characteristics and the pollution history of the contaminated environment. Bioremediation, a non-destructive, cost- and treatment-effective and sometimes logistically favorable cleanup technology, attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of limiting conditions [2-4].

Material and methods

In the present work are given results of laboratory experiments on investigation of biodegradation's hydrocarbons of diesel fuels and individual hydrocarbons processes in soil before and after the bring into aerobic oiloxidative microorganisms by method of spectrophotometry. Summer brands of diesel fuels of joint stock company "PetroKazakhstan Oil Products", Pavlodar petrochemical factory (PPCF) and Russian refinery "LukOil" were chosen as hydrocarbon raw materials. The choice of the given fuels is conditioned by that they are characterized by different chemical composition and properties. Bacterial cultures *Pseudomonas mendocina H-3* and *Pseudomonas alcaligenes H-15* were isolated from oil-polluted soil [5-7]. Cultivation was carried out on liquid medium *E-8* with adding diesel fuel as a source of carbon and energy in concentration of 5 and 10 % per 100 ml of medium suspension.

Synthetic broth medium E8 used for growth of microorganisms, composition of this medium (Distilled water g/l): KH_2PO_4 - 0,7; $(NH_4)_2HPO_4$ - 1,5; NaCl - 0,5; $MgSO_4$ – 0,8; bactoagar – 20. Every component sterilize in autoclave no less 30 minute under pressure of 0,1 atmosphere. Un-inoculated mineral medium supplemented with the same oil but without bacteria was used as the control.

Dynamics of growth of bacterial cultures was studied in with volume 500 ml under aerobic conditions in a liquid mineral medium (E8). Oil was added as the sole source of carbon and energy at concentrations of 40 and 100 g l^{-1} oil addition in medium after sterilization. Cultivations were carried out in three times on a rotary shaker at 220 rpm at room temperature 20º C. Increase in biomass was controlled by the change in optical density (OD) at 540 nm using photocolorimeter KVK-2 MP. It is possible to calculate the bacterial biomass from these data, i.e. does a calibration curve exist comparing OD and actual biomass. OD measurements were done after 3 h of incubation and afterwards, every 24 h over a period of 7 days.

Results and discussion

Figures 1-3 show the results of the spectrophotometric measurements performed over a time period of 7 days. Both *Pseudomonas* strains were found to grow when diesel fuel was added as the sole source of organic carbon and energy. Optical density (OD_{540}) of the bacterial cultures and hence microbial biomass increased many times over in comparison with the optical density of control flasks with diesel fuels but without bacteria (compare Fig. 1-3). Furthermore, this finding indicates that the growth conditions (temperature, shaking and oxygen supply, mineral content of the medium) were generally suitable for the microbes so that a growth-supporting microenvironment could be established.

The diesel fuels were decomposed and chemically modified during bacterial treatment due to their oxidation and subsequent utilization as growth substrate. As the result, the optical density increased indicating a substantial microbial growth. *P. alcaligenes H-15* showed the best results for all diesel fuels tested and reached the maximal biomass on day 4 of cultivation. *P. mendocina H-3* was less efficient but also degraded the diesel fuels to some extent with a biomass maximum already on the second cultivation day.

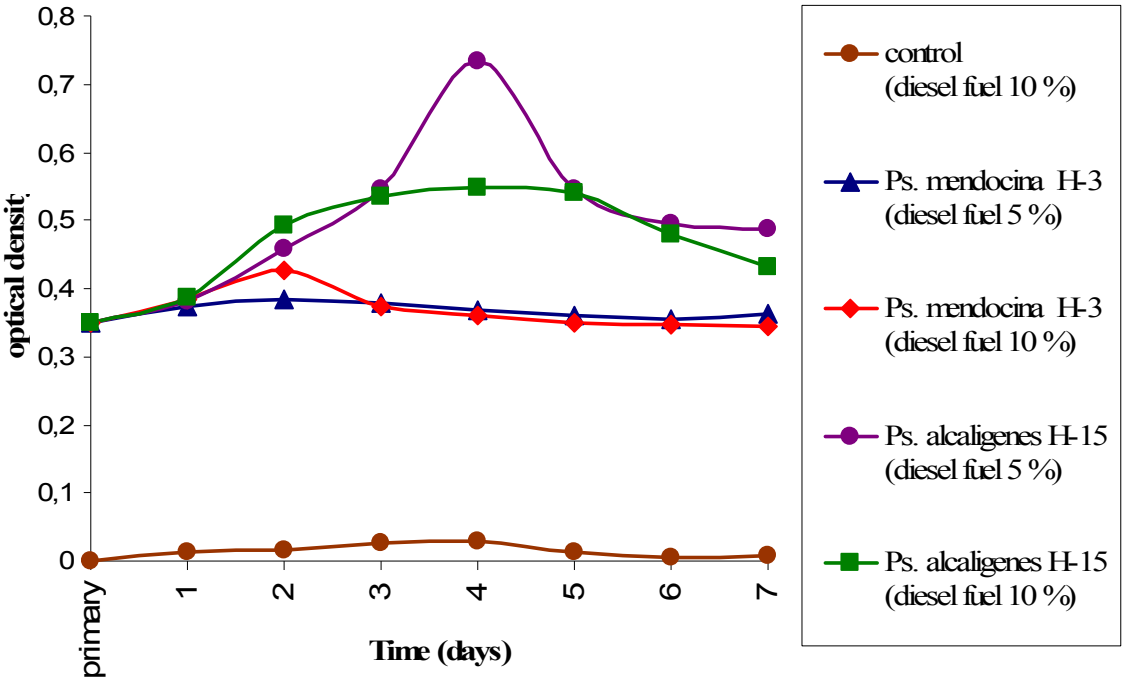

Fig.1. Dynamics of bacterial growth on diesel fuel of the Joint Stock company *"PetroKazakhstan Oil Products"*

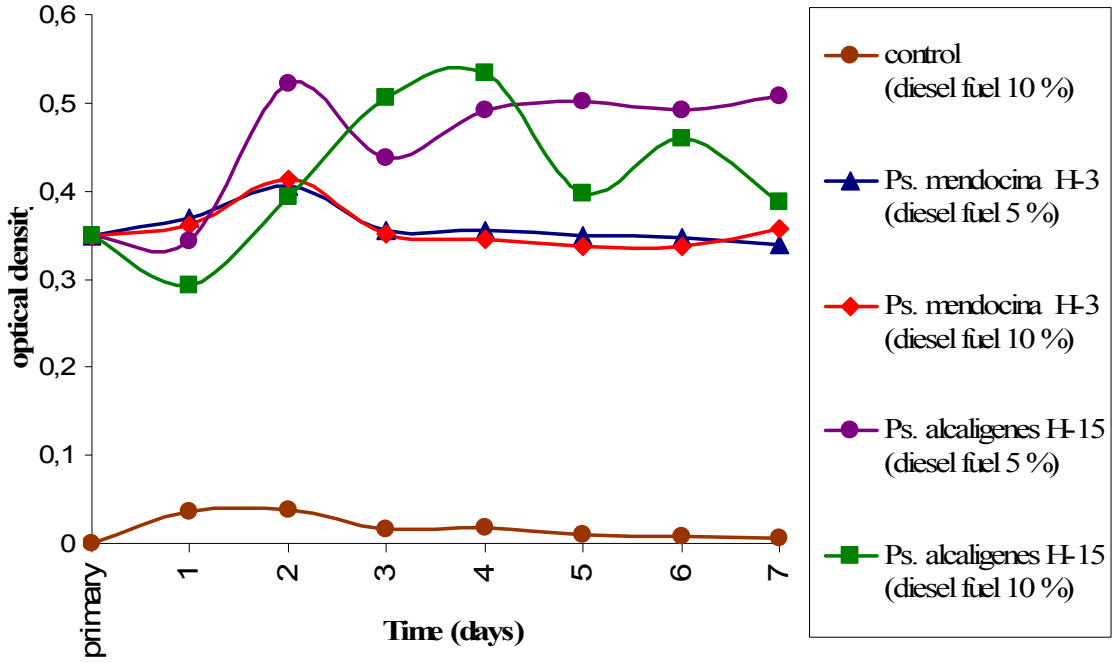

Fig.2. Dynamics of bacterial growth on diesel fuel of the Pavlodar Petrochemical factory *"PPCF"*

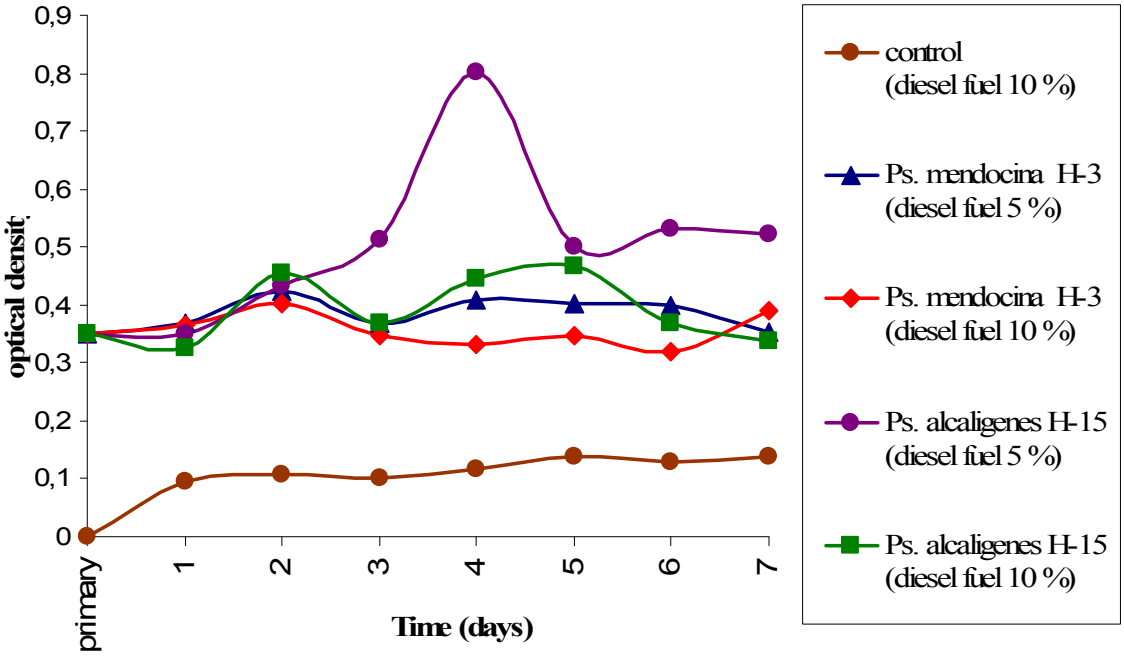

Fig.3. Dynamics of bacterial growth on diesel fuel of Russian refinery *"LukOil"*

The biomass of *P. alcaligenes H-15* increased about 1.5-fold (according to OD_{540} on day 4) in comparison to the initial optical density (OD_{540} = 0.326) in the medium supplemented with diesel fuel of the Joint Stock company (*PetroKazakhstan Oil Products*) both at a con-centration of 5 and 10%. *P. mendocina* H-3 showed the best growth on day 2 of cultivation and the maximal biomass corresponded to an OD_{540} of 0.406 (at a diesel fuel concentration of 5%) and 0.413 (10% diesel fuel). Compared to the initial index of biomass (OD_{540}: 0.354), i.e. a moderate increase of 0.05 units that was also observed for other diesel fuel samples.

The growth of *Pseudomonas* spp. on paraffinaceous diesel fuels (products of Joint Stock company *"PetroKazakhstan Oil Products"* and Russian refinery *"LukOil"*) did not occur permanently and it seems that the bacterial cells oxidized the hydrocarbons rather cyclically in the so-called catabolic phase (compare the oscillating OD_{540}/biomass curves in Fig. 2-4). In the

anabolic phase of metabolism, pure and mixed cultures of microorganisms mainly utilize pre-oxidized products of hydrocarbons with a simpler structure and lower molecular mass (e.g. *n*-alkanols or organic acids). So far as microorganisms cleave a certain number of carbon atoms (one or two) from aliphatic and/or cyclic compounds at each phase of the metabolic cycle, the irreversible destruction of the crude-oil constituents is obvious. Thus at a concentration of 5% fuel, both strains grew well on the diesel hydrocarbons from the Russian refinery (*LukOil*), and at a concentration of 10%, they did so either on diesel fuel from the Pavlodar petrochemical factory (*PPCF*).

Summary

To summarize, the data obtained by GC-MS analysis strongly indicate that the content of *n*-alkanes decreases in diesel fuels as the result of bacterial growth while the relative amount of *iso*-alkanes, cycloalkanes and aromatic hydrocarbons tends to increase (even though individual molecule species of the latter fractions may also be degraded by the bacteria). Moreover, the results show that the bacterial strains tested are capable of utilizing crude-oil hydrocarbons from diesel fuels as growth substrate, i.e. they use them as sole source of carbon and energy (productive biodegradation: mineralization of hydrocarbons and conversion into biomass). Last but not least, our findings support the idea of using bacteria for cleaning the environment by the biological removal of hazardous crude-oil hydrocarbons from contaminated soils. To this end, additional degradation studies and further isolation of bacterial strains from contaminated soil will be necessary. The results presented here demonstrate that bacteria of the genus *Pseudomonas* are capable of degrading and productively utilizing hydrocarbons from a persistent Kazakh crude-oil which makes them a promising biotechnological target for the development of bioremediation and cleaning technologies.

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