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Effects of Temperature, Substrate Concentration and pH on the Polycyclic Aromatic Hydrocarbon Pyrene Biodegradation by *Arthrobacter* sp. NJ5 Strain

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Abstract. **Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment and may persist for extended periods of time. PAHs are one of the most important classes of persistent organic contaminants. High molecular weight (HMW) PAHs (compounds containing four or more fused benzene rings) are generally recalcitrant to microbial attack. Until recently, only a few genera of bacteria have been isolated with the ability to utilize four-ring PAHs as sole carbon and energy sources. Because of the toxic, mutagenic, and carcinogenic characteristics of some, PAHs have been studied extensively by many scientists around the word. This paper presents research results, where 10 microbial strains belonging to genus *Arthrobacter* sp. and obtained from culture collection of JSC „Biocentras" were tested for the best biodegradation of HMW PAH pyrene. Pyrene degradation experiments were conducted in liquid mineral medium. Pyrene concentration was 0.2 mg/mL at the beginning of degradation experiments. After 72 h incubation with ten *Arthrobacter* sp. strains, gas chromatography analysis revealed that highest pyrene degradation (19%) was reached by *Arthrobacter* sp. NJ5 strain. The effect of medium pH, pyrene concentration and temperature on the intensity of the degradation by the most active strain *Arthrobacter* NJ5 was investigated.**

Keywords – Arthrobacter **sp., biodegradation, pyrene, polycyclic aromatic hydrocarbons, (PAHs).**

I INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) constitute a large and diverse class of organic compounds consisting of three or more fused aromatic rings in various structural configurations. The inertness, their low water solubility and strong lipophilic properties of these compounds lead to very high accumulation levels in the environment [1, 2]. They have been detected in air, soil, sediments, surface water, ground water and road runoff [3, 4, 5, 6]. Soil is the most important reservoir and reemission source of PAHs. Soil pollution with polycyclic aromatic hydrocarbons can have a bad influence on human health. Polycyclic aromatic hydrocarbons are resistant to degradation and can bio-accumulate through the food chain, so PAHs also may pose threat to human health over a long period [7]. These compounds enter the environment in many ways. PAHs and their derivatives are widespread products of incomplete combustion of organic materials arising, in part, from natural combustion such as forest, chemical fires and volcanic eruptions, but for the most part by human activities [8, 9, 10, 11, 12]. In recent decades the major sources of PAH pollution are industrial production, transportation, refuse burning, gasification and plastic waste incineration [13, 14, 15, 16].

Many PAHs are highly toxic, mutagenic, carcinogenic and teratogenic in nature; exposure to PAHs represents public health risks and raises environmental concerns [17, 18, 19, 20, 21, 22, 23, 24, 25, 26]. Numerous studies have indicated that one-, two- and three-ring compounds are acutely toxic, while higher molecular weight PAHs are considered to be geotoxic [4].

Because many PAHs are so toxic there is a big interest in understanding the physicochemical processes and microbial degradation reactions that affect the mobility and fate of these compounds in groundwater and soil sediment systems.

When dissolved in water or adsorbed on particulate matter, PAHs can undergo photodecomposition upon exposure to ultraviolet light from solar radiation. In the atmosphere PAHs can react with pollutants such as ozone, nitrogen oxides and sulfur dioxide, generating diones, nitro- and dinitro-PAHs and sulfonic acids, respectively. PAHs may also be degraded by some microorganisms [1, 27] (Fig.1).

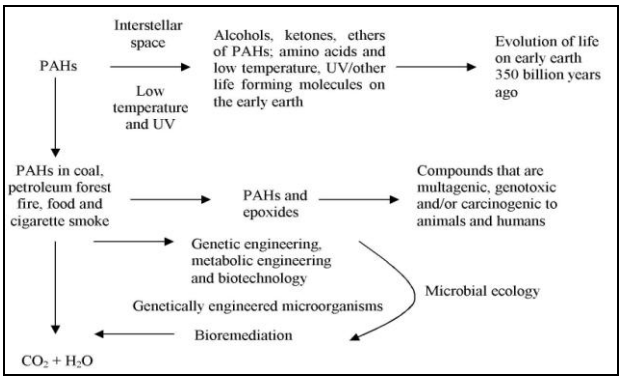

Fig. 1 Fate, toxicity and remediation of polycyclic aromatic hydrocarbons (PAHs) in environment [23]

Photodegradation is an important degradation pathway in aquatic systems for high molecular weight PAHs. Photooxidation can chemically transform PAHs and the resulting products may be more carcinogenic and toxic than the parent compounds.

Volatilization plays an important role in the removal of low molecular weight PAHs from aquatic systems.

Biodegradation using microorganisms is usually the preferred and major route of PAH removal from contaminated environment because of some inherent advantages such as its cost effectiveness, the safest and comparatively better cleanup [17].

PAHs are subject to biodegradation by various microorganisms such as bacteria, fungi, and certain algae that live in soils, in sediment substrate, or are suspended in the water column [4, 28].

Site contamination with complex mixtures of organic compounds such as creosote, petroleum, or their combinations results in the selection of a mixed population of microorganisms with improved abilities to tolerate and extract energy from the contaminants [20, 28]. Among bacteria *Xanthobacter, Serratia, Acitenobacter, Bacillus* [24], *Alcaligenes, Arthrobacter, Burkbolderia, Cychoclasticus, Pseudomonas* [29, 30], *Ralstonia, Nocardia, Rhodococcus, Sphinggomonas* [31], *Terrabacter, Mycobacterium* [32] and among fungi *Penicillium, Phanerochaete, Bjerkandera* [12] and *Trametes* are the frequently identified microorganisms involved in PAH bioremediation [28, 33, 34].

II MATERIALS AND METHODS

Polycyclic aromatic hydrocarbon

Pyrene($C_{16}H_{10}$) was obtained from Germany Merck-Schuchardt Co. with a purity >96%. Pyrene has 4 benzene rings; molecular weight is 202.26; the octanol–water partition coefficient ($logK_{ow}$) is 4.88 [35].

Microorganisms

The following strains of hydrocarbon degrading bacteria belonging to genus *Arthrobacter* and obtained from culture collection of JSC „Biocentras“ were used: sp N3, NJ1, NJ5, NJ9, NJ6, Pr82, Mž811, Kl1, M1 and M2.

Media

Nutrient agar (Oxoid, Basingstone, UK) was used for plating microbial strains, and nutrient broth (Oxoid, Basingstone, UK) was used for the subculture and preculture of the strains. To investigate the ability of the strains to degrade pyrene, a mineral medium was used. The mineral medium had the following composition (g/L): 0.01 $(NH_4)_2HPO_4$; 0.2 NH_4Cl; 0.25 K_2HPO_4; 0.25 KH_2PO_4; 0.02 $MnSO_4$; 0.01 $(NH_4)_2Fe(SO_4)_2*6H_2O$; 0.01 $CaCl_2$ and 0.05 $(CH_3COO)_2Zn$.

Biodegradation of pyrene

The ability of the strains to degrade pyrene was investigated under sterile conditions in 250 mL flasks with 50 mL of mineral medium. Pyrene was used at a concentration of 0.2 mg/mL. Pyrene was entered into the nutrient medium as a 10 mg/mL pyrene/hexane solution. Hexane has been evaporated while intensive mixing and heating in the temperature of 75 °C temperature. Pyrene suspension in nutrient medium prepared with such method was used for experiments. Experimental flasks with 10% (v/v) of inoculum added and blank flasks were incubated in a rotary shaker at 30 °C and 200 rpm. The effect of medium pH, pyrene concentration and temperature on the intensity of the degradation by the most active strain was investigated under the same cultivation conditions.

Pyrene degradation dependency on medium pH was investigated at pH values of: 4; 5; 6; 7; 8 and 9.

Pyrene degradation dependency on temperature was evaluated at 5; 20; 25; 30; 35; 40 ^oC; and dependency on concentration was investigated with 0.1; 0.2; 0.3; 0.4; 0.6; 0.8 mg/mL of pyrene.

After 72 h incubation, pyrene was extracted with 20 mL of hexane from experimental and blank flasks.

GC analysis of pyrene

Samples were quantified with a GC system (GC-2010 Plus, Shimadzu, Japan) equipped with a flame-ionization detector and MXT-1 capillary column (Siltek treated stainless steel). Operation conditions were as follows: nitrogen was used as the carrier gas; the injector temperature and detector temperature were 330 and 350 ^oC, respectively; the column oven temperature was kept at 40 ^oC for 1 min and then raised to 320 ^oC at a rate of 10 $^oC\ min^{-1}$.

Pyrene degradation intensity was calculated using the formula (1).

$$\text{Degradation intensity}(\%) = \frac{c_0 - c_x}{c_0} \times 100 \quad (1)$$

c_0-pyrene concentration in blank flasks; c_x-pyrene concentration in experimental flasks.

III RESULTS AND DISCUSSION

Pyrene has been used as a model compound to study biodegradation of high molecular weight polycyclic aromatic hydrocarbons because it is one of the sixteen toxic, mutagenic and carcinogenic PAHs, that have been considered as priority pollutants by US Environmental Protection Agency [28, 36] (Fig. 2).

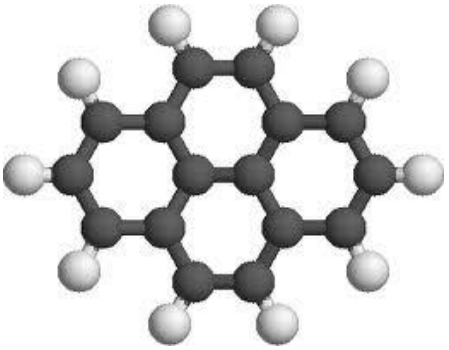

Fig.2 Structure of high molecular weight (HMW) polycyclic aromatic hydrocarbon pyrene ($C_{16}H_{10}$) [37]

After 72 h incubation with ten *Arthrobacter* strains in aerobic conditions in a rotary shaker, gas chromatography analysis revealed that the highest pyrene degradation was accomplished by *Arthrobacter* sp.NJ5 strain (Fig. 3), which was chosen for the later experiments.

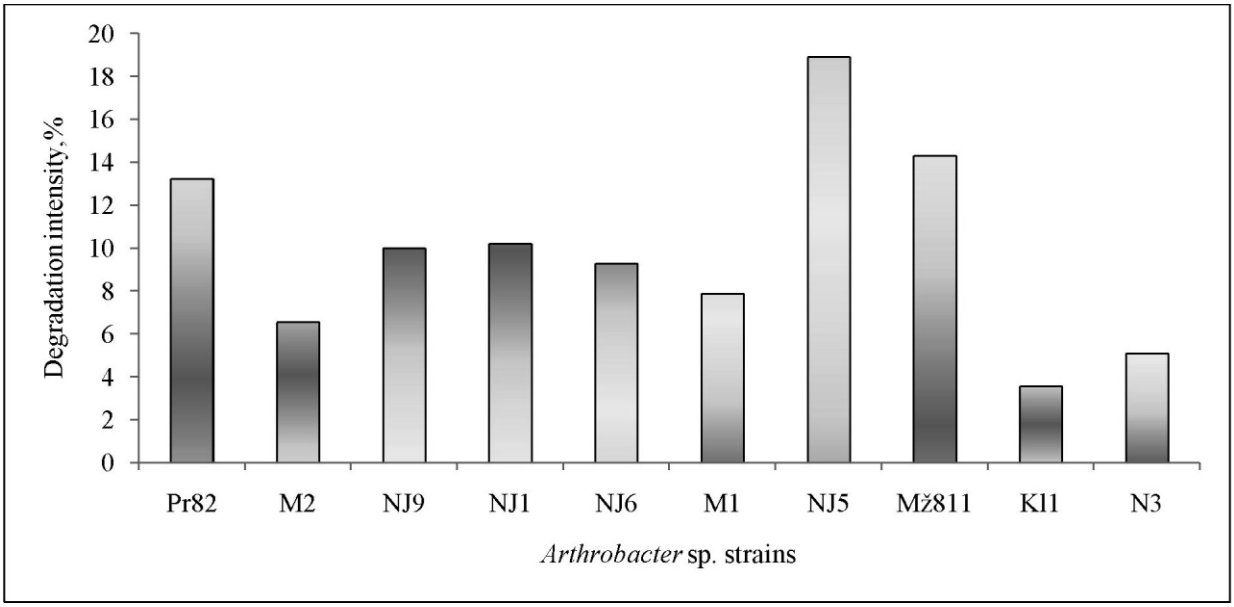

Fig.3 The effect of *Arthrobacter* sp. strains on pyrene biodegradation

Arthrobacter sp. NJ5 strain was isolated from clay contaminated with crude oil near Nefteyugansk in Russia.

The rate of degradation of hydrocarbons is mainly influenced by environmental limiting factors (salinity, nutrients, pH, temperature, oxygen) and therefore may not be due to the enzymatic capacities of the endogenous hydrocarbon degrading bacterial strain [38, 39].

The effect of initial medium pH, temperature and substrate concentration on pyrene degradation with *Arthrobacter* sp. NJ5 strain was investigated in this paper (Fig. 4; 5; 6). During the selection of an optimal medium pH, the maximum substrate degradation was achieved at pH 7 (Fig. 4).

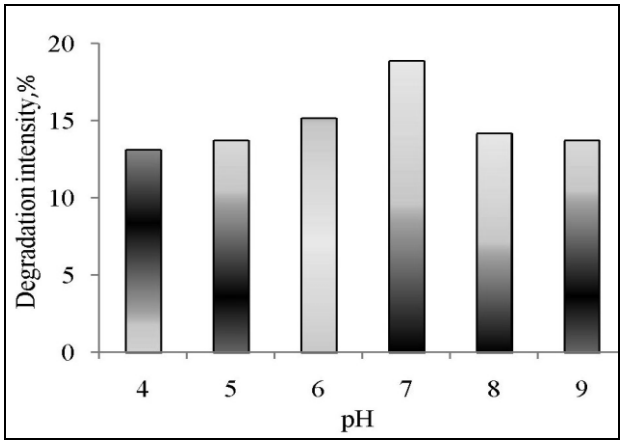

Fig. 4 Dependency of pyrene degradation by *Arthrobacter* sp. NJ5 strain on medium pH

After determining the dependency of degradation on medium pH, temperature regime was selected for the most efficient pollutant degradation. It was found that the rise in temperature from 5 to 35 ^{o}C promotes degradation of substrate and it is the highest at 35 ^{o}C, however, degradation level drops at a temperature range of 35-40 ^{o}C (Fig. 5).

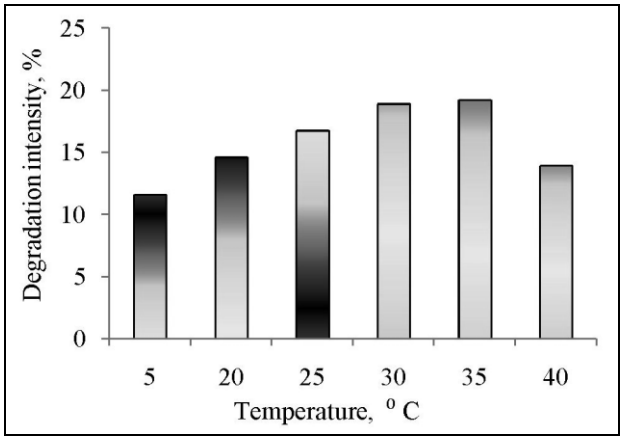

Fig. 5 Dependency of pyrene degradation by *Arthrobacter* sp. NJ5 strain on temperature

The effectiveness microbiological hydrocarbon degradation process is influenced by the substrate concentration as well, which can be inhibitory or even toxic to oil-oxidizing microorganisms [29, 40]. Therefore, the *Arthrobacter* sp. NJ5 strain oxidizing properties were tested against the polycyclic aromatic hydrocarbon substrate. Results revealed that, when substrate concentration in medium was increased from

0.1 to 0.4 mg/mL, substrate degradation level has risen, but higher concentrations (up to 0.8 mg/mL) slowed the intensity of degradation down. The best substrate degradation was observed when the initial pyrene concentration was 0.4 mg/mL (Fig. 6).

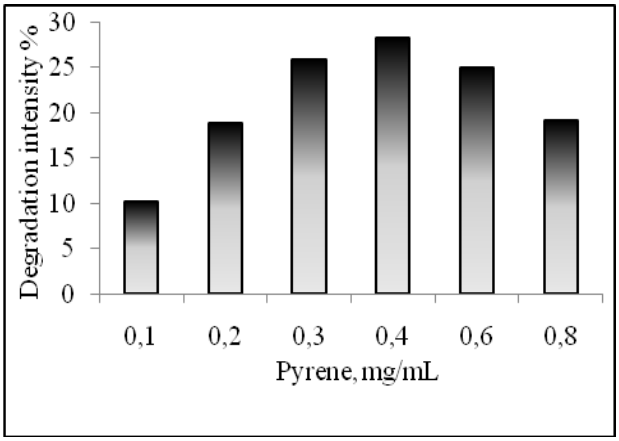

Fig. 6 Dependency of pyrene degradation by *Arthrobacter* sp. NJ5 strain on pyrene concentration

Results from previous experiment indicate that degradation of lower molecular weight polycyclic aromatic hydrocarbons is much easier. Experiments with antracene, where its concentration in the medium is 0.1 mg/mL, achieved 51.8 % degradation intensity of *Arthrobacter* sp. NJ5 within five hours [41]. Using the same concentration of pyrene, degradation intensity after 72 hours is only 10 %.

Gas chromatography analysis was carried out to investigate quantitative and qualitative changes of pyrene during biodegradation by the selected *Arthrobacter* sp. NJ5 strain (Fig. 7; Table 1). In biodegradation experiment initial concentration of pyrene and inoculate in mineral medium was 0.4 mg/mL and 10 %, respectively. Inoculated flasks were incubated at 35 ^{o}C and 200 rpm for 72 h. Control test was performed at the same conditions, except for the inoculate.

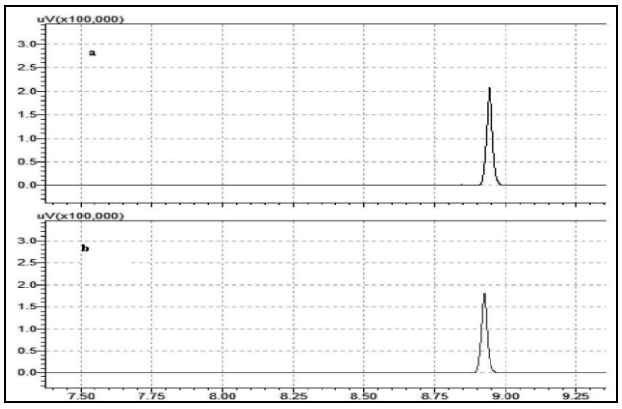

Fig. 7 Gas chromatograms of pyrene (a-control test; b-after biodegradation by *Arthrobacter* sp. NJ5 strain)

Gas chromatography data shows no qualitative structural changes of pyrene after biodegradation with *Arthrobacter* sp. NJ5 strain. According to lower pyrene peak plot it is considered that quantity of pyrene has changed during biodegradation.

TABLE 1.

QUANTITATIVE ANALYSIS OF PYRENE

No	Peak retention time	Peak area	Peak height	Concen-tration
a	8.907	321111.6	208099.0	0.24384
b	8.914	224021.1	104803.5	0.17609

IV Conclusion

Among all 10 *Arthrobacter* strains used for experiments, *Arthrobacter* sp. NJ5 showed the best results in polycyclic aromatic hydrocarbon pyrene degradation.

The highest intensity of pyrene degradation by *Arthrobacter* sp. NJ5 strain occurred at the intial medium pH value of 7.0.

Temperature range of 30-35 ^{o}C was found to be the most optimal for pyrene biodegradation by *Arthrobacter* sp. NJ5 strain.

The highest substrate degradation level with *Arthrobacter* sp. NJ5 was reached, when the initial pyrene concentration was 0.4 mg/mL, medium pH=7 and temperature 30 ^{o}C.

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