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ANTHRACENE AND PHENANTHRENE DEGRADATION BY BACTERIA IMMOBILIZED ON ACTIVATED CARBON AND ZEOLITES

Degradation of anthracene and phenanthrene in the concentration of $5 \cdot 10^{-6}$ mol/dm^3 by two single strains of *Bacillus alvei* and *Arthobacter* sp. and a defined mixed culture of seven microbe strains immobilized by sorption on activated carbon, clinoptilolite and zeolite 5A was tested. All three sorbents colonized by *B. alvei* immobilized on all sorbents tested removed 66–85% of anthracene and 61–69% of phenanthrene, while *Arthrobacter* sp. and mixed bacterial culture removed the compounds specified above in 66–94%, 54–94% and 92–100%, and 90–100%, respectively. The highest efficiency was achieved on activated carbon bed, and the lowest – on zeolite 5A. Metabolites of anthracene and phenanthrene were identified.

1. INTRODUCTION

High degree of contamination of water used for municipal purposes with, among others, polycyclic aromatic hydrocarbons makes it necessary to use complex technological systems for water purification. Adsorption process and spontaneous microbiological activity associated with it have become an integral part of these systems. The organic substances dissolved and undissolved on the sorbents are used by the bacteria immobilized on them as a source of energy. The pollutants, which appear in a very little amount, are concentrated on the sorbents, and until then can be used as a source of nutrients by the immobilized bacteria. Such processes may have two advantages:

higher absorption capacity of the sorbents,

unblocked surface of the sorbents [1], [2].

Control of these processes depends on our knowledge about biotransformation, biocumulation and degradation of PAHs in water [3], [4] and the ability of bacteria to

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immobilize, since the degree of microorganisms dispersion significantly affects the efficiency of pollutant degradation [5].

The aim of the investigation was to design a model system of PAH biodegradation with possibly the highest level of efficiency using single bacterial strains and a mixed bacterial culture composed of metabolically active strains as well as to identify the products of biodegradation of anthracene and phenanthrene as secondary pollutants.

2. MATERIALS AND METHODS

From a collection of isolated and selected bacterial strains, two most active strains were chosen, i.e. *Arthrobacter* sp. (4_1) and *B. alvei* (16A), and a specially prepared mixed culture composed of *B. thiaminolyticus* (14), *B. circulans* (17), *B. laevoliticus* (18), *Arthrobacter* sp. ($4'_1$) and *B. alvei* (16A).

The systems for testing the sorption and biodegradation of hydrocarbons consisted of glass columns, ∅ 1 cm, filled with 20 cm^3 of mineral sorbents (zeolite 5A and clinoptilolite) and activated carbon. Two tricyclic PAHs, linear anthracene and angular phenanthrene, were used in the concentration of 5×10^{-6} mole/dm^3 in a mineral medium composed of: K_2HPO_4 (1.0 g/dm^3), NH_4NO_3 (0.1 g/dm^3), KH_2PO_4 (0.5 g/dm^3), $MgSO_4$ (0.5 g/dm^3), $(NH_4)_2SO_4$ (0.5 g/dm^3), NaCl (0.5 g/dm^3), $CaCl_2{\cdot}2H_2O$ (0.02 g/dm^3), $FeSO_4{\cdot}7H_2O$ (0.02 g/dm^3) and microelements [6].

The anthracene and phenanthrene concentrations were determined directly by means of the UV spectrophometer Shimadzu at the wavelength of 254 nm. In order to identify the products of PAH degradation, the cultures were extracted three times with ethyl acetate. The samples were analysed by means of GC/MS method with Shimadzu QP 2000 in SPB 5 column.

Effluents from sorption columns were analysed in the same way after 5 days of operation.

In the first phase of the investigation, the strains of bacteria were gradually adapted to anthracene and phenanthrene in a batch culture. Immobilization of single strains and the mixed culture on sorbent beds was performed for 24 hours with a constant recirculation. The colonisation of sorbents by bacteria culture was confirmed by scanning micrographs.

3. RESULTS

Metabolites produced by the microorganisms were determined by batch tests during the time of passaging which was done in order to achieve metabolic activation. The following products of anthracene and phenanthrene degradation were identified: anthracene, 9,10-dihydro-9(10)-anthracenone, 9,10-anthracenedion,

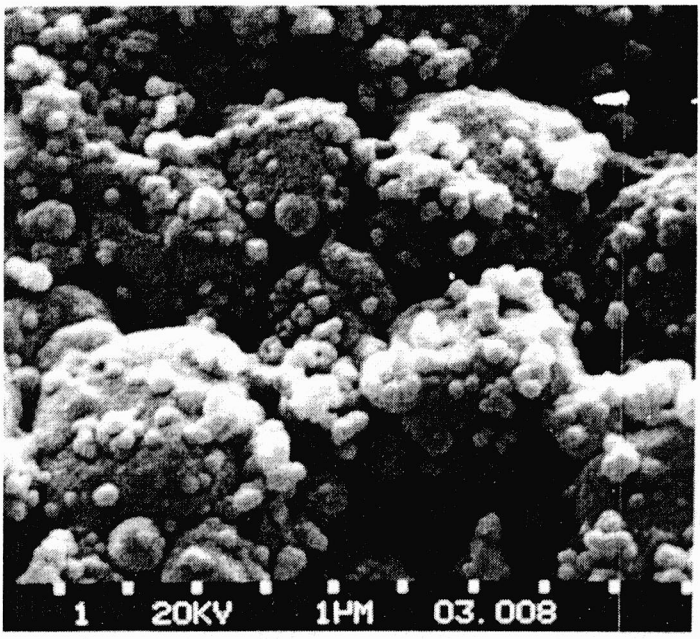
phthalic anhydride, esters of 1,2-benzene-dicarboxy-acid, methyl derivatives of naphthalene and esters of phthalic acid. Metabolites of the first phase of phenantrene biodegradation were not identified. Only in two samples of the effluents from the beds, the traces of 1,9-anthracene dione were determined. Chromatograms consisted of a series of peaks of low intensity which represented fatty acids, aldehydes and alcohols of chain length varying from C5 to C16. These compounds were the natural products of cell metabolism and did not arise from PAH degradation. On each sorbent two single strains and a mixed culture of bacteria were immobilized individually (figure 1). The efficiencies of the anthracene and phenanthrene removal were determined during the first 5 hours of three consecutive days of the bed work. The results are given in table 1. Three sorbents colonized by *B. alvei* removed 66–85% of anthracene. The best effects were observed for activated carbon, and the worst for zeolite 5A. The efficiency of anthracene removal increased with time of the bed operation. Under analogous conditions, 61–69% of phenanthrene were removed independently of the time of the bed operation.

Table

Efficiency of anthracene and phenanthrene removal in the sorption–biodegradation system

Sorbent	Time (days)	Anthracene			Phenanthrene		Mixed culture
		Bacillus alvei	*Arthrobacter* sp.	Mixed culture	*Bacillus alvei*	*Arthrobacter* sp.	
	1	76.2	93.8	100	60.7	90.3	100
Activated carbon	2	74.3	91.1	100	68.3	94.1	100
	3	84.9	93.5	99.8	68.2	93.0	100
	1	67.6	84.0	100	46.8	66.2	100
Clinoptilolite	2	66.1	66.8	95.2	61.3	59.0	99.9
	3	83.3	68.8	91.6	62.1	67.3	93.6
	1	69.5	72.4	100	61.1	58.5	1000
Zeolite 5A	2	70.2	53.6	95.1	62.6	41.2	98.4
	3	84.0	71.2	92.5	68.4	62.4	90.3

Anthracene removal by *Arthrobacter* sp. ($4'_1$) was clearly dependent on the type of sorbent on which the strain was immobilized. The removal efficiency was the highest, approaching 91–94%, in the column filled with activated carbon. The efficiency of a column filled with clinoptylolite was lower – approximately 73%, and that with zeolite 5A – 66%. Results of phenanthrene removal in the column with activated carbon were similar to those for anthracene, while both zeolite beds had considerably poorer efficiencies (approx. 64% and 54%, respectively).



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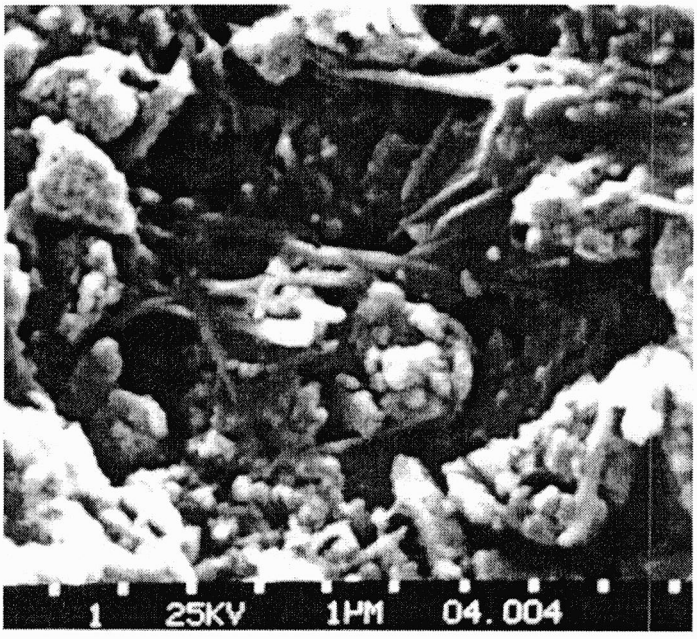

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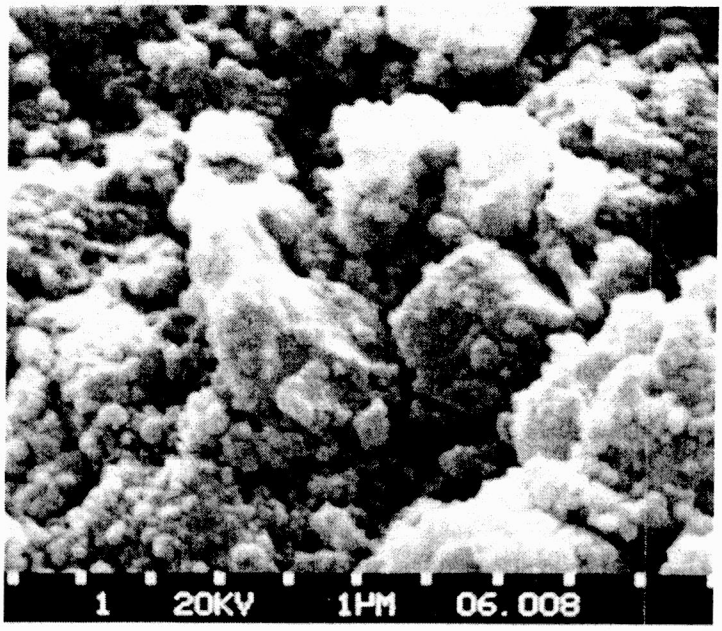

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Fig. 1.Scanning micrographs of bacterial culture immobilized on:
1 – zeolite 5A, 2 – clinoptilolite, 3 – activated carbon

The best performance of the sorption–biodegradation system was achieved with the mixed bacterial culture, where besides the above-mentioned strains the microorganisms specially selected for metabolic transformations were present. The efficiencies of anthracene and phenanthrene removal by the systems with activated carbon reached 100%. Zeolite beds removed completely the PAHs tested only in the first day of the system operation. Their efficiency was reduced with time to 90–92% in the third day.

4. DISCUSSION

The investigation confirmed that bacteria, unlike other water organisms, can utilize PAHs as a sole source of carbon and energy and degrade them by dioxygenation and dehydrogenation. Alternative degradation pathways exist both for anthracene and phenanthrene which is supported by identification of a number of diverse intermediates [7], [8].

Batch tests proved that phenanthrene is more readily degraded by bacteria. This is probably the reason why it is not possible to isolate its biodegradation intermediates. It is likely that in the experiments presented anthracene metabolites were more stable or were not easily available substrates particularly in the mixed bacterial culture [9].

Biodegradation of PAHs by the microorganisms tested was associated with the formation of identified intermediate products which could bind to cell elements in coupling reactions or could be removed from water by sorption. Therefore, biodegradation process was enhanced by immobilization of bacteria on the sorbents chosen. The efficiency of PAH removal was dependent both on biological models used and on sorption capacity of the supports.

The efficiency of anthracene removal by both bacterial strains immobilized on zeolites was lower approximately by 10% compared to the efficiency of phenanthrene removal. This was in contradiction with the results of batch tests which proved that phenanthrene was a more easily available substrate [7]. However, the time of enzymatic adaptation of *B. alvei* was prolonged. *B. alvei* and *Arthrobacter* sp. immobilized on activated carbon removed anthracene and phenanthrene with a similar efficiency of 92%. The highest efficiency, over 96%, was found for the mixed culture of bacteria on all supports tested. Activated carbon beds colonized by the mixed culture had a constant 100% efficiency, while the efficiency of zeolites decreased with the time of operation. This suggests that the capacity of the aluminosilicates for sorption is lower compared to that of activated carbon. The importance of adsorption processes [1] is also greater.

Results of the investigation lead to the conclusion that the most effective technology of removal of toxic and carcinogenic micropollutants from water may be the combination of sorption and biological degradation. Specially selected and adapted mixed bacterial cultures are capable of biodegrading. This method is beneficial because on one hand it extends the time of column operation due to the phenomenon of sorbent bioregeneration, and on the other hand it increases the process efficiency by the removal of the metabolites produced which results in complete elimination of persistant micropollutants from environment.

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ROZKŁAD ANTRACENU I FENANTRENU PRZEZ BAKTERIE IMMOBILIZOWANE NA WĘGLU AKTYWNYM I ZEOLITACH

Zbadano rozkład antracenu i fenantrenu w stężeniach 10^{-5} mol/dm^3 przez dwa pojedyncze szczepy *Bacillus alvei*, *Arthrobacter* sp. oraz zmieszaną z siedmiu szczepów kulturę bakterii. Bakterie były immobilizowane w procesie sorpcji na węglu aktywnym, klinoptylolicie i zeolicie 5A. Wszystkie trzy sorbenty z obrostem *Bacillus alvei* usuwały 66–85% antracenu i 61–69% fenantrenu. Natomiast *Arthrobacter* sp. usuwał antracen w 66–94%, fenantren w 54–94%, a mieszana kultura bakterii odpowiednio 92–100% i 90–100%. Najwyższą efektywność uzyskano dla złóż węgla aktywnego, a najniższą dla zeolitu 5A. Zidentyfikowano metabolity antracenu i fenantrenu.