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EXPERIMENTAL ARTICLES

Phenanthrene Degradation by Bacteria of the Genera *Pseudomonas* and *Burkholderia* in Model Soil Systems

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Abstract—Degradation of phenanthrene by strains *Pseudomonas putida* BS3701 (pBS1141, pBS1142), *Pseudomonas putida* BS3745 (pBS216), and *Burkholderia* sp. BS3702 (pBS1143) were studied in model soil systems. The differences in accumulation and uptake rate of phenanthrene intermediates between the strains under study have been shown. Accumulation of 1-hydroxy-2-naphthoic acid in soil in the course of phenanthrene degradation by strain BS3702 (pBS1143) in a model system has been revealed. The efficiency of phenanthrene biodegradation was assessed using the mathematical model proposed previously for assessment of naphthalene degradation efficiency. The efficiency of degradation of both phenanthrene and the intermediate products of its degradation in phenanthrene-contaminated soil is expected to increase with the joint use of strains *P. putida* BS3701 (pBS1141, pBS1142) and *Burkholderia* sp. BS3702 (pBS1143).

Key words: biodegradation, phenanthrene, mathematical model, *Pseudomonas, Burkholderia.*

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Polycyclic aromatic hydrocarbons (PAHs), such as naphthalene, phenanthrene, fluorene, and pyrene, enter the environment as a result of natural processes and human activity (oil, peat, and coal processing; garbage incineration; wastes coking, petrochemical, and gas processing industries).

A negligible portion of PAHs undergoes abiotic degradation in the air as a result of interaction with free radicals; some PAHs are adsorbed on dust particles and precipitated on the soil surface. Due to their low solubility and, consequently, low bioavailability for microorganisms, high-molecular PAHs tend to accumulate in soil. Water solubility is 34 mg/l for naphthalene and 1.3 mg/l for phenanthrene. The solubility of PAHs decreases with an increasing number of benzene rings [1].

Although certain reduction of PAH concentration in soil is possible due to abiotic processes, microbial populations play the main role in the degradation of these compounds [2]. Soil samples, including those of soils not contaminated by PAHs, have been shown to contain significant numbers of naphthalene-degrading bacteria: 10^4 cells/g of dry soil. The bacteria degrading phenanthrene and anthracene are present in the amount of 10^3–10^5 cells/g of dry soil, but only in PAH-contaminated soil samples [3].

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In gram-negative microorganisms, the genes of PAH biodegradation are often localized on conjugative plasmids. Recently, the involvement of plasmid genes of naphthalene biodegradation in the degradation of phenanthrene, anthracene, and fluorene has been reported more and more often [4].

To this point, two different pathways of phenanthrene degradation have been described (Fig. 1). First, phenanthrene is transformed to 1-hydroxy-2-naphthoic acid as a result of sequential reactions. Further biochemical pathways of degradation of this compound may be different: 1-hydroxy-2-naphthoic acid is metabolized either via salicylate and catechol or via formation of *o*-phthalate and protocatechuate. Catechol and protocatechuate are then cleaved by the *ortho*- or *meta*-pathway to the TCA cycle intermediates [5]. There is an alternative pathway (Fig. 1) of oxidation of salicylic acid through gentisic acid [6, 7].

One of the methods of cleaning territories from PAH contamination is the introduction of bacteria capable of rapid PAH degradation into soil. Before the introduction of microorganisms into the environment, it is necessary to forecast their survival and behavior and to assess the efficiency of biodegradation of specific compounds.

The goal of the present work was to study the microbial degradation of phenanthrene and to assess the effi-

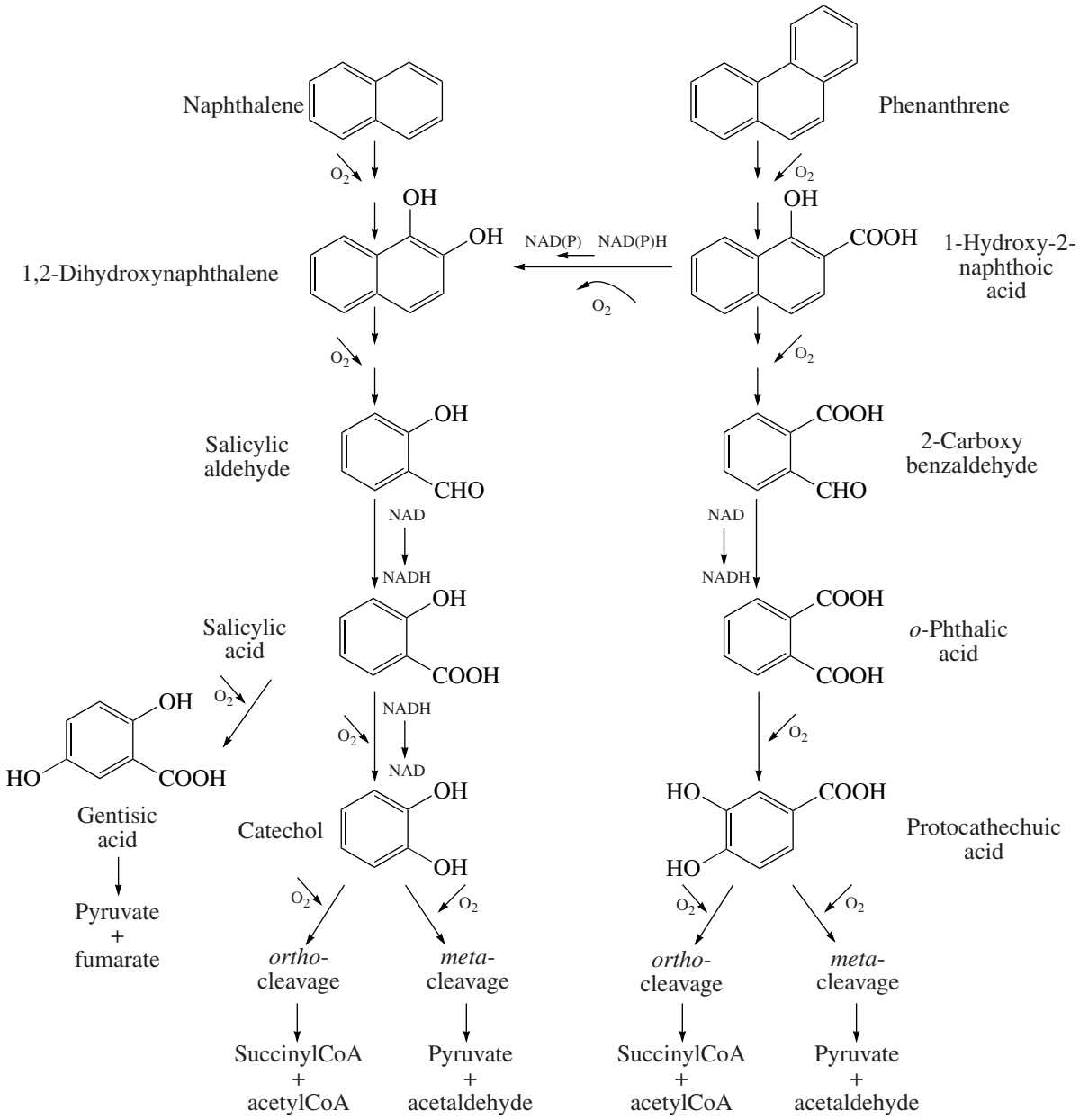

Fig. 1. Pathways of microbial degradation of naphthalene and phenanthrene.

ciency of this process carried out by various strains in model soil systems, using mathematical simulation.

MATERIALS AND METHODS

Bacterial strains and cultivation conditions. Bacterial strains *Pseudomonas putida* BS3701 (pBS1141, pBS1142) and *Burkholderia* sp. BS3702 (pBS1143) isolated from soil samples contaminated by petroleum products and coking industry wastes from the territory of the Moscow oblast were used in the work. Strain *P. putida* BS590 (pBS216) was obtained from the collection of Laboratory of Plasmid Biology, Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino. Strain *Pseudomonas* sp. WD25 was kindly provided by Dr. W. Duetz (Institute of Health and Environmental Protection, Bilthoven, the Netherlands). Strain BS3745 (pBS216) has been obtained in the course of this work by conjugative transfer of plasmid pBS216 from strain BS590 to strain WD25.

Strains were grown at 24°C for 24 h on rich media and for 3 to 5 days on minimal or selective media.

Evans medium was used as the minimal medium [8]. Glucose, sodium succinate, and sodium salicylate in concentrations of 0.1–0.2% were used as carbon

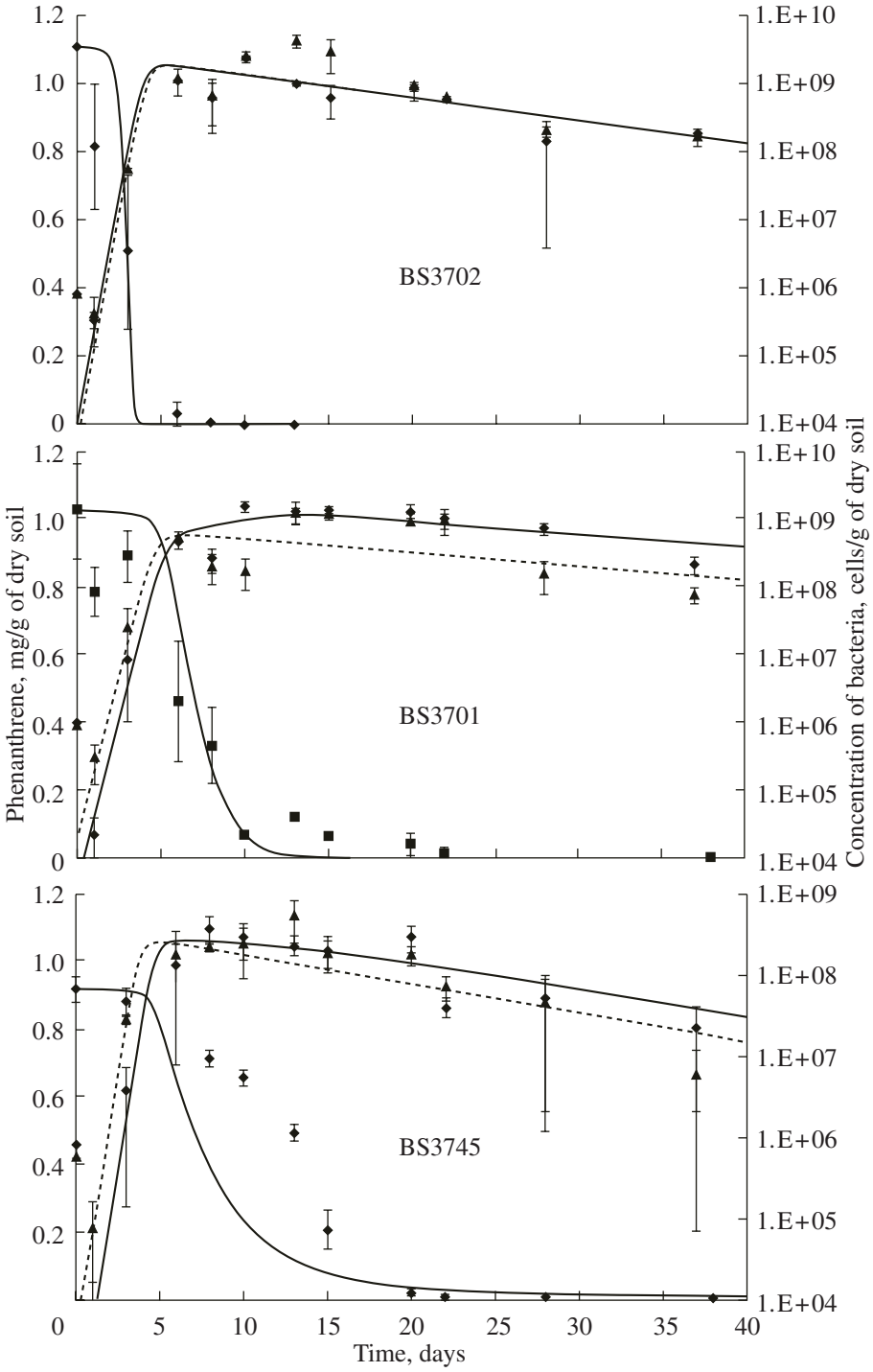

Fig. 2. The dynamics of quantity of bacteria (BS3702 (pBS1143), BS3701 (pBS1141, pBS1142), BS3745 (pBS216)) in model soil systems with phenanthrene (diamonds), without phenanthrene (triangles), and phenanthrene concentration (squares): experimental data (diamonds, triangles, squares) and solution of the model (continuous and broken lines).

sources. When the microorganisms were grown in a liquid medium, phenanthrene was added as powder in the concentration of 0.5 g/l. King B medium (KB [9]) was used for the monitoring of strains to assess their competitiveness under growth on phenanthrene.

Specific growth rate in batch culture with phenanthrene was determined by growing microorganisms in flasks with Evans medium. Phenanthrene was added as a powder (500 mg/l). The cultures grown on the Evans medium with phenanthrene were used as inocula. The

initial concentration of bacteria in the flasks was 1–2 × 10^6 cells/ml. The optical density of the culture liquid was determined in a FEK-56M-U42 photocolorimeter at a wavelength of 540 nm and cuvette thickness of 0.5 cm. The samples were taken every three hours. Concentrations of microorganisms in the growth medium were determined by standard serial dilutions followed by inoculation on plates with agarized LB medium [10].

The maximal specific growth rate was calculated by minimization of the sum of squares of the deviations of an exponential function from the data on the kinetics of microbial growth in the exponential growth phase according to [11]. Minimization was carried out by the method of polyhedron and the quasi-Newton method realized in MINUIT software package [12].

Conjugative transfer of bacterial plasmids was carried out by a modified method of Dunn and Gunsalus [13].

Preparation of model soil systems. Model soil systems were prepared from gray forest soil collected in the vicinity of Pushchino, Moscow oblast, containing: ash, 91.00%; C, 2.89%; H, 1.05%; N, 0.25%; P, 0.06%; Ca, 0.48%; Mg, 0.14%; Fe, 1.20%; K, 2.47%; and SiO_2, 72.5%. The pH of the aqueous extract was 7.05. Before use, the soil was sieved through a 2-mm sieve; then a weighed portion was sterilized (1 atm for 30 min) three times with 24-h intervals. The soil with phenanthrene was prepared using the phenanthrene solution in a low-boiling solvent (pentane). Thus prepared soil (40 g) with phenanthrene (at a final concentration of around 1 mg/g of dry soil) was placed into petri dishes, 120 mm in diameter and 20 mm in height (the soil layer was about 8 mm thick). Model soil systems were placed into a desiccator and incubated at 18–20°C for 7 weeks. The plates were weighed daily to control moisture evaporation; if necessary, sterile water was added to compensate for its loss.

Introduction of inoculum into soil. Bacteria were grown in liquid Evans medium with phenanthrene as a sole carbon and energy source to the end of the exponential growth phase. Then, using the turbidity standard, the culture was diluted with phosphate buffer to the concentration of 2 × 10^8 cells/ml. Thus prepared bacterial suspension was mixed with sterile tap water; this mixture was then introduced into soil. The introduced amount of water was calculated from the humidity required (40%). The amount of the introduced bacterial suspension was calculated so that the final concentration was 1 × 10^6 cells per 1 g of dry soil. Then the soil in the petri dishes was thoroughly homogenized with a spatula.

Sampling. The total quantity of microorganisms and concentration of phenanthrene were determined by taking averaged samples (0.5 g and 1.0 g, respectively) from three to four different soil sites. The 0.5-g samples were suspended in 4.5 ml of phosphate buffer and stirred in a Paramix 2 mixer (Germany) for 1 min at room temperature; appropriate standard dilutions of the samples were plated on LB agar. The dishes were incubated at 24°C for 24 h. The quantity of colony-forming units was calculated per 1 g of dry soil.

Extraction of phenanthrene and 1-hydroxy-2-naphthoic acid from soil samples. Soil (1 g) was treated with 20 ml of methanol. Methanol extract samples (1 ml) were taken to determine phenanthrene concentration. Then, the residual soil methanol extract was acidified to pH 1.0–2.0 to make samples for analysis of 1-hydroxy-2-naphthoic acid content.

Determination of the content of phenanthrene and 1-hydroxy-2-naphthoic acid in soil extracts. Methanol extract (1 ml) was centrifuged in a Beckman microcentrifuge (United States) for 3 min to remove mechanical admixtures. Phenanthrene concentration was determined in an LKB-2150 HPLC chromatograph (Sweden). The extract of soil samples (25–200 μl) was applied to a column and analyzed under the following conditions: the column was reversed phase C-18 (Marcherey-Nagel Nucleosil, 3 μm, 4.6 × 125 mm); precolumn was a Serva HPLC Column; the carrier was Octadecyl Si-60, 20–40 μm, 4.6 × × 75 mm; eluent was 83% methanol and 17% deionized distilled water; detection wavelength was 254 nm; flow rate was 0.8 ml/min; and column temperature was 50°C.

The concentration of 1-hydroxy-2-naphthoic acid in methanol extracts under study was determined under the same conditions as that of phenanthrene, except for the eluent: 60% methanol in 0.001 M formaldehyde solution and 17% deionized distilled water.

The absorption peaks of phenanthrene and 1-hydroxy-2-naphthoic acid were registered by the interface of Nelson Analytical 900 series and Olivetty M-24. The results were processed using the Nelson Analytical applied software package. The concentrations of analyzed compounds were calculated by peak area as compared with the peak area of a control sample.

Detection of intermediate products of phenanthrene degradation in the culture liquid. The qualitative analysis of metabolites accumulated in the culture liquid of the microorganisms grown on phenanthrene was performed by high-pressure liquid chromatography (HPLC). The culture was grown in a test tube with 10 ml of liquid Evans medium supplemented with phenanthrene.

The analysis was carried out in an LKB-2150 chromatograph; reversed phase column C-18 (Serva HPLC Column, Octadecyl Si-100, 30 μm, 4.6 × 250 mm); precolumn (Serva HPLC Column, Octadecyl Si-60, 20–40 μm, 4.6 × 60 mm); flow rate 0.7 ml/min; elution solutions A (45% methanol, 25 mM orthophosphoric acid) and B (100% methanol, 25 mM orthophosphoric acid); and a gradient of elution 0 to 15% of solution B. The wavelength of detection was 230 nm. Absorption peaks were registered and processed as described in the previous section.

Mathematical simulation of phenanthrene biodegradation. The efficiency of phenanthrene degradation by different strains was assessed using a mathematical model which described bacterial growth and consumption of substrates in soil [14].

RESULTS AND DISCUSSION

Phenanthrene Degradation by Introduced Strains in Model Soil Systems

Investigation of the process of pollutants degradation in model soil systems is among the stages of the study of degrader strains before their introduction into the environment for bioremediation of contaminated sites is.

Model soil systems used in this work had a humidity level of 40%, which is optimal for naphthalene-degrading pseudomonades [15].

In the beginning of experiments on phenanthrene degradation in model soil systems, the soil was checked for the presence of phenanthrene-degrading bacteria. The experiments showed the absence of endogenous soil microflora capable of phenanthrene degradation in the gray forest soil used in the experiment.

The study of phenanthrene degradation (1 m/g dry soil) in the model system with sterile soil showed that phenanthrene concentration was actually unchanged during 37 days. In our experiments, strains BS3701 (pBS1141, pBS1142), BS3702 (pBS1143), and BS3745 (pBS216) degraded phenanthrene in sterile soil at different rates. In the model soil system with phenanthrene, this compound was degraded 4 days after the introduction of the naphthalene- and phenanthrene-degrading strain BS3702 (pBS1143) and 21 days after the introduction of strain BS3701 (pBS1141, pBS1142) or BS3745 (pBS216) (Fig. 2).

The study of microbial growth kinetics in model soil systems with and without phenanthrene showed that the maximal concentration of bacteria in the soil with phenanthrene was lower than (for strain BS3702 (pBS1143)) or comparable to (for strains BS3701 (pBS1141, pBS1142) or BS3745 (pBS216)) the maximal concentration of the these microorganisms in model soil systems in the absence of phenanthrene.

Manilal and Alexander showed that phenanthrene concentration (0.5 mg/g) in sterile mineral medium and in sterile soil remained unchanged during 30 days [16]. Möller and Ingvorsen, who studied the possibility of acceleration of phenanthrene degradation in soil by introduction of a pure culture of *Alcaligenes* sp. isolated from oil-contaminated soils, found that phenanthrene concentration (initial amount 1 mg/g of dry soil) in sterile control soil decreased by 12% in 42 days of incubation [17]. Inoculation of the strain into a model system with nonsterile soil resulted in the degradation of 1 mg of phenanthrene in 11 days. It is interesting to note that *Alcaligenes* sp. was unable to degrade phenanthrene in sterile soil. This strain is probably capable of phenanthrene metabolism only in the presence of indigenous soil microorganisms.

Estimation of the Efficiency of Phenanthrene Degradation by Different Strains Introduced into the Model Soil System Using Mathematical Simulation

One of the problems of bioremediation is the low rate of microbial degradation of contaminants which are weakly soluble in water. Due to restrictions of mass transfer, the rate of microbial degradation of such compounds in an open environment is often much lower than under laboratory conditions, because microorganisms cannot degrade organic compounds which are not in a dissolved state. At present, various mathematical models exist for description of naphthalene and phenanthrene degradation processes. Volkering et al. [18] showed that the mass transfer of weakly soluble substrates could limit microbial degradation of these compounds, resulting in a nonexponential growth of microorganisms. Later on, these researchers [19] proposed a model for a system without a constant rate of mass transfer (the system with low naphthalene concentration), describing the growth of microorganisms under the given conditions. The proposed model, which combines the kinetics of substrate dissolution and the kinetics of microbial growth, may be used to describe bacterial growth in a system where the concentration of available substrates is limited by mass transfer.

In this work, we have assessed the efficiency of phenanthrene degradation by different strains using the mathematical model used previously for the estimation of the efficiency of naphthalene biodegradation [14]. The model presumes a two-stage mechanism of PAH utilization in soil: at the first stage, bacteria degrade PAHs with accumulation of some intermediate products; at the second stage, bacteria utilize these products for growth. Further, soil organic matter is considered as a potential carbon and energy source. Based on these assumptions, the bacterial growth is described by the following differential equations:

$$dx/dt = M_P xP + M_S xS - m_x x,$$

$$dN/dt = -Q_N xN - a_N N,$$

$$dS/dt = -Q_S xS,$$

$$dP/dt = Q_N xN - Q_P xP,$$

where x is the concentration of bacteria, N is the concentration of phenanthrene, S is the concentration of soil organic matter, and P is the concentration of intermediate products of phenanthrene degradation.

The efficiency of phenanthrene degradation by different degrader strains was assessed on the basis of the parameters presented in Table 1.

The solutions of the model for different strains are given in Fig. 2. Quantitative assessment of the parameters for different strains is presented in Fig. 3.

Table 1. The kinetic parameters of cell growth and utilization of PAHs, their intermediates, and available organic soil substances (AOSS)

Parameter	Physical meaning
M_S	Characterizes the rate of microbial growth on AOSS
Q_S	Characterizes the rate of AOSS utilization
$Y_S = M_S/Q_S$	Economic coefficient of AOSS utilization
M_P	Characterizes the rate of microbial growth on PAH intermediates
Q_P	Characterizes the rate of utilization of PAH intermediates
$Y_P = M_P/Q_P$	Economic coefficient of utilization of PAH intermediates
Q_N	Characterizes the rate of PAH utilization
m_x	Specific rate of microbial death
a_N	Specific rate of abiotic PAH degradation
X_0	Effective initial concentration of bacteria

Note: AOSS, available organic soil substances.

Table 2. Accumulation of metabolites of phenanthrene degradation by different strains grown in a liquid mineral medium with phenanthrene

Strain	Metabolite, mg/l		
	1-hydroxy-2-naphthoate	2-hydroxy-1-napthoate	dihydroxy-2-naphthoate
BS3701 (pBS1141, pBS1142)	37	6	<0.1
BS3702 (pBS1143)	42	–	–
BS590 (pBS216)	1.5	1	–

The following parameters are most informative for the estimation of efficiency of degrader strains: the rate of utilization of PAHs (Q_N) and their intermediates (Q_P); specific rate of death of microorganisms (m_x); initial effective concentration of bacteria (X_0), which characterizes the rate of adaptation of microorganisms to new environmental conditions; and the economic coefficient (Y_P). When selecting microorganisms for soil bioremediation, preference should be given to the strains which degrade PAHs and their intermediates at a high rate, quickly adapt to new environmental conditions, and are quickly eliminated after xenobiotic degradation, i.e. have the highest values of the parameters Q_N, Q_P, m_x, X_0 and the lowest values of Y_P.

The comparison of kinetic parameters of microbial growth and substrate consumption (Fig. 3) has shown that BS3702 (pBS1143) is the most effective degrader strain of phenanthrene. However, the analysis of the parameters characterizing bacterial growth on phenanthrene (Q_N), organic soil substances (M_S and Y_S), and intermediates of phenanthrene degradation (M_P and Y_P) suggested that strain BS3702 (pBS1143) transformed only phenanthrene, while no growth occurred at the expense of intermediates of phenanthrene degradation. Soil organic matter was the nutrient source for growth. However, phenanthrene concentration in the soil inoculated with strain BS3702 (pBS1143) decreased drastically and after 3 days phenanthrene practically disappeared from the system. We therefore suggested that the intermediates of phenanthrene degradation were accumulated in soil.

The Study of the Intermediate Products of Phenanthrene Metabolism In Analyzed Strains

The accumulation of metabolites in the culture liquid of the analyzed strains was studied at cultivation under conditions of batch cultivation on phenanthrene (Table 2). The HPLC study of metabolites accumulating in the culture liquid at cultivation on phenanthrene at different periods of growth of strains BS3701 (pBS1141, pBS1142) and BS3702 (pBS1143) revealed differences in the accumulation of metabolites. Strain BS3702 (pBS1143) growing on the medium with phenanthrene accumulated and utilized only 1-hydroxy-2-naphthoic acid, while in the culture liquid of BS3701 (pBS1141, pBS1142), 1-hydroxy-2-naphthoic acid, 2-hydroxy-1-naphthoic acid, and β-naphthol were actively accumulated during the initial growth phase. Strain BS3702 (pBS1143), in contrast to BS3701 (pBS1141, pBS1142), was unable to grow on the minimal medium with exogenous 1-hydroxy-2-naphthoic acid as a sole carbon and energy source (Table 3). These differences in accumulation and uptake of the intermediates of phenanthrene degradation by strains BS3701 and BS3702 (pBS1143) are probably associated with the differences in the genetic systems of phenanthrene catabolism in these strains. It is known that genetic systems of PAH degradation revealed in the microorganisms of the genus *Burkholderia* are different from the "classical" *nah* systems of degradation, which have been described for pseudomonads [20].

As a result of conjugative transfer of plasmids pBS1141 and pBS1142 into recipient strains, transformation, and hybridization analysis, plasmid pBS1141 (100 kbp) was shown to control the *meta*-pathway of catechol oxidation and to carry the "silent" genes of salicylate hydroxylase. Plasmid pBS1142 (50 kbp) does not carry the genes of naphthalene and phenanthrene biodegradation [21]. The attempts to transfer plasmid pBS1143 (120 kbp) by conjugation and transformation into the recipient strains available in the laboratory collection failed as well as the attempts to eliminate this plasmid.

At cultivation of strain BS3701 (pBS1141, pBS1142) on 2-hydroxy-1-naphthoate as a sole carbon

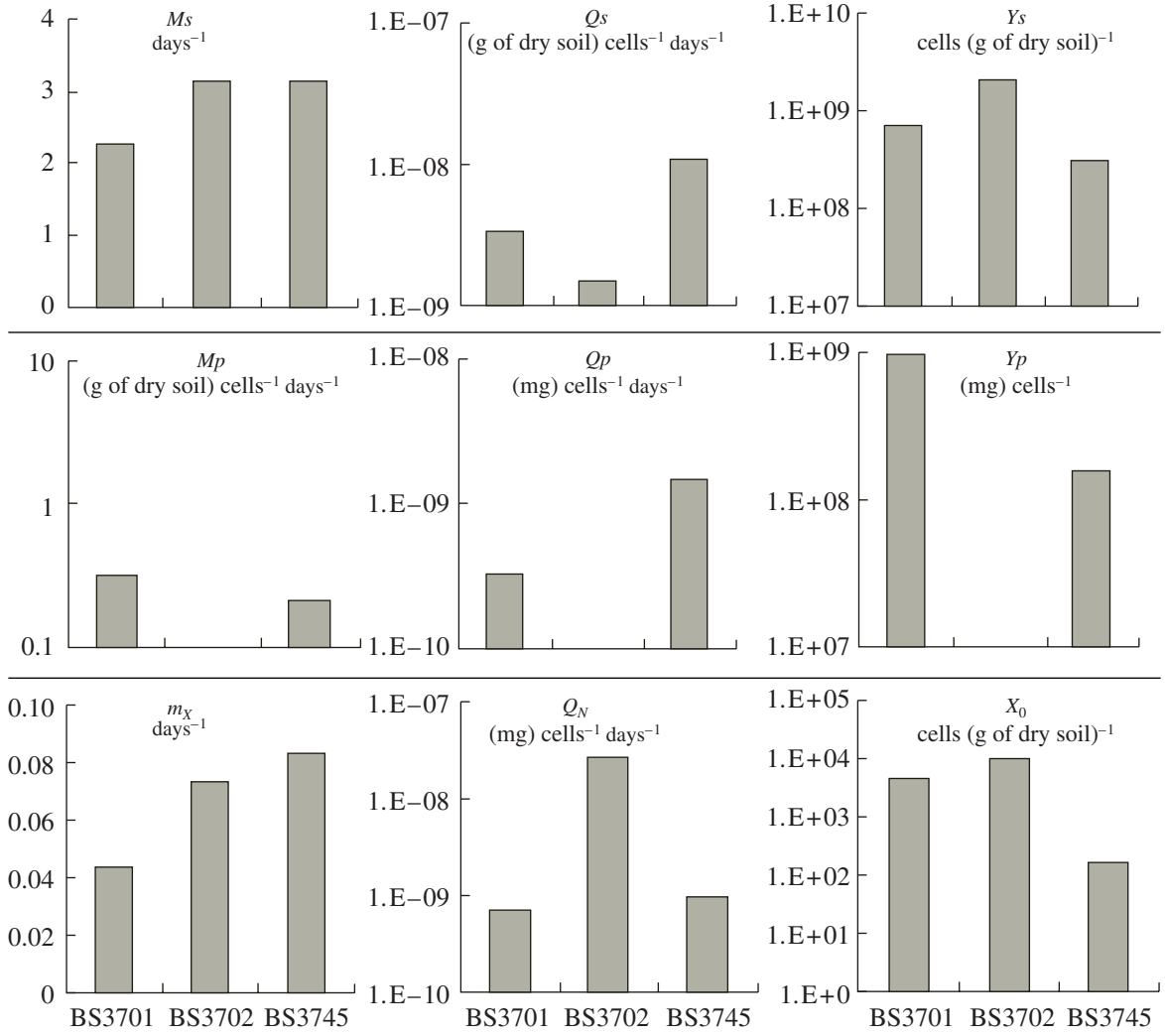

Fig. 3. The kinetic parameters of strains BS3701 (pBS1141, pBS1142), BS3702 (pBS1143), and BS3745 growth and utilization of phenanthrene, its intermediates, and available organic soil substances.

and energy source, culture growth did not occur but β-naphthol was accumulated as the major product. Further experiments showed that β-naphthol was formed in the course of spontaneous oxidation of 2-hydroxy-1-naphthoate in aqueous solutions.

The assessment of specific growth rate on the medium with 1-hydroxy-2-naphthoic acid as a sole growth factor showed that strains BS3701 (pBS1141, pBS1142) and BS590 (pBS216) (plasmid donor for soil experiments) could grow on this compound (specific growth rate μ = 0.27–0.28 h^{-1}), whereas BS3702 (pBS1143) did not grow on the medium with exogenous 1-hydroxy-2-naphthoic acid.

Therefore, we supposed the possibility of accumulation of 1-hydroxy-2-naphthoic acid in soil in the course of phenanthrene degradation by strain BS3702 (pBS1143). The change of conditions of extraction of soil samples made it possible to reveal 1-hydroxy-2-naphthoic acid. Accumulation of this compound was observed in soil inoculated with strain BS3702 (pBS1143) from day 7 on: its concentration was 0.1 mg/g of dry soil on day 14.

The Study of Competitiveness of Strains BS3701 (pBS1141, pBS1142) and BS3702 (pBS1143) in Liquid Mineral Medium with Phenanthrene

The behavior of strains BS3701 (pBS1141, pBS1142) and BS3702 (pBS1143) in mixed culture was studied under batch cultivation in liquid mineral medium with phenanthrene as a sole carbon and energy source. The monitoring of the strains was performed using agarized King B medium, because strain BS3701 (pBS1141, pBS1142) was fluorescent under UV illumination, in contrast to BS3702 (pBS1143), which forms no fluorescent pigments.

The observed fluctuations of the numbers of analyzed strains (Fig. 4) at cultivation in the liquid mineral

Table 3. Characteristics of growth of degrader strains on phenanthrene and 1-hydroxy-2-naphthoate

Strain	Substrate	μ_{max}, h^{-1}	Metabolites
BS3701 (pBS1141, pBS1142)	Phenanthrene	0.05	1-hydroxy-2-naphthoate, 2-hydroxy-1-napthoate, dihydroxy-2-naphthoate
	1-hydroxy-2-naphthoate	0.27	Salicylate
	Phenanthrene	0.12	1-hydroxy-2-naphthoate
BS3702 (pBS1143)	1-hydroxy-2-naphthoate	–	No growth
	Phenanthrene	0.10	1-hydroxy-2-naphthoate, 2-hydroxy-1-napthoate,
BS590 (pBS216)	1-hydroxy-2-naphthoate	0.28	Salicylate

Note: μ_{max}, maximal specific growth rate.

medium with phenanthrene are probably associated with their metabolic characteristics (utilization of phenanthrene and its intermediate products at different rates), because strain BS3701 (pBS1141, pBS1142) degrades phenanthrene with the formation of salicylate, which is further oxidized to catechol and then to the TCA cycle intermediates, whereas strain BS3702 (pBS1143) oxidizes salicylate to gentisate, which is further cleaved to the TCA cycle intermediates.

Phenanthrene Degradation in Model Soil System by Transconjugant Strain BS3745 (pBS216)

Discussion of the possibility of using genetically modified microorganisms (GMM) for bioremediation is still under way. On the one hand, GMM proved to be efficient; on the other hand, there are restrictions on the release of in vitro constructed organisms into the environment [22]. In nature, genetic information is transferred due to the processes of conjugation, transformation, transfection, or transduction. Therefore, an ecologically safe alternative of GMM is production of microorganisms with desired properties using the above natural mechanisms of genetic exchange with the application of native strains and gene clusters.

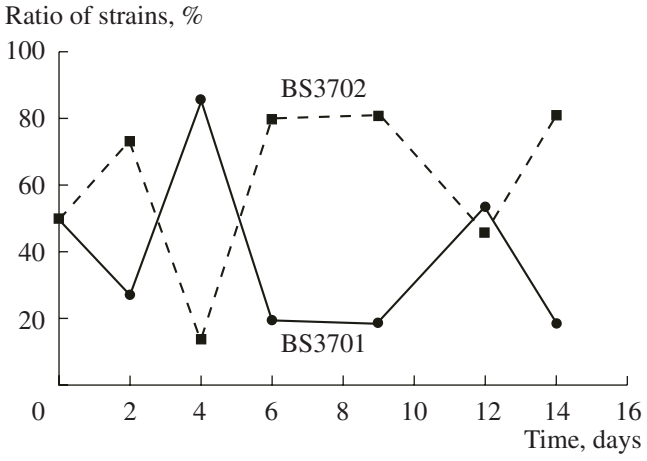

Fig. 4. The dynamics of the quantity of strains BS3701 (pBS1141, pBS1142) and BS3702 (pBS1143) under batch cultivation of mixed culture in liquid mineral medium with phenanthrene as a sole carbon and energy source.

Transconjugant BS3745 (pBS216) was used in our work. Strain BS3745 (pBS216) was obtained by conjugation transfer of plasmid pBS216 controlling the $Nah^+Sal^+Phn^+$ phenotype into the plasmid-free strain WD25, a derivative of strain *P. putida* mt-2 [23]. We have shown (Fig. 2) that the rate of phenanthrene degradation by strain BS3745 (pBS216) in model soil systems is five times lower as compared with strain BS3702 (pBS1143). However, the specific growth rate of strain BS3745 (pBS216) grown in liquid medium with phenanthrene was comparable to that for BS3702 (pBS1143). The mathematical model describes the experimental data well; however, in the case of BS3745 (pBS216), the observed phenanthrene consumption was different from the expected one (Fig. 2). To elucidate the causes of the above deviation, the stability of plasmid in strain BS3725 (pBS216) was studied. Cultivation of the strain on rich medium showed that only 84% of BS3745 (pBS216) cells remained capable of naphthalene and salicylate utilization after 80 generations. The loss of these features in transconjugants resulted from the loss of plasmids. The low rate of phenanthrene degradation in the model system by strain BS3745 (pBS216) was probably caused by the loss of the plasmid controlling phenanthrene degradation.

Thus, in the course of this work we have proposed a method of estimation and selection of the most active phenanthrene-degrading strains using mathematical simulation. This research has shown significant differences in phenanthrene degradation as well as in the accumulation and rate of consumption of intermediates in the strains under study. Since the strain *Burkholderia* sp. BS3702 (pBS1143) can quickly degrade phenanthrene to 1-hydroxy-2-naphthoic acid and *P. putida* BS3701 (pBS142, pBS1142) can utilize this intermediate at a high rate, joint use of these strains in phenanthrene-contaminated soil may increase the efficiency of degradation of this pollutant.

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