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REVIEW

Aerobic Bacterial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs) and Its Kinetic Aspects

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Abstract—Aerobic bacterial degradation of PAHs is reviewed. Particular attention is paid to its kinetic aspects (rate and specificity). The general concepts of PAH biodegradation in nature and the role of aerobic bacteria in this process are described. The problem of PAH bioavailability and the mechanism of PAH penetration through bacterial cell wall are discussed. The key role of the reaction of PAH hydroxylation in controlling the rate and specificity of PAH biodegradation process is substantiated. The effects of competitive inhibition, intermediate inhibition, cross induction, and cometabolism are considered. The importance of microbial communities for PAH biodegradation in natural ecosystems is shown. The review contains the list of 138 references.

Keywords: PAH, biodegradation, bioavailability, dioxygenases, kinetics, specificity, inhibition, microbial communities

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Polycyclic aromatic hydrocarbons (PAHs) are substances with molecules consisting of condensed aromatic rings (Fig. 1; [1]). PAHs are formed during pyrolysis of organic compounds in various natural and technogenic processes and are present in coal tar and oil at high concentrations. PAHs and their conversion products are toxic for living organisms [2], and some of these compounds have mutagenic and carcinogenic properties [3]. While environmental pollution with these compounds is often diffused [4], places of local contamination with oil, coal tar, and the products of their refinement present the greatest problem [5–7].

Microbial activity is one of the key factors of PAH degradation in the environment [8–10]. Therefore, bioremediation technologies such as natural attenuation [11], land farming [12], composting [13], bioreactor processing [14, 15], and phytoremediation [16] are developed together with the physicochemical methods for purification of polluted soils, bottom sediments and ground waters.

PAH biodegradation is carried out by bacteria, fungi, and plants. Most eukaryotes are capable only of transforming PAH molecules in the reactions with cytochrome P450, and only ligninolytic basidiomycetes mineralize PAHs in the reactions catalyzed by laccases and peroxidases [17]. Bacteria, in contrast to eukaryotes, can utilize PAHs as a growth substrate and grow in PAH-containing medium in the absence of other carbon and energy sources. PAH biodegradation under aerobic conditions is much more rapid and complete than under anaerobic conditions [18–20]. The mechanisms of aerobic and anaerobic PAH biodegradation are different in principle [21]. In this review we will limit ourselves to consideration of aerobic PAH degradation by bacteria, focusing our attention on its kinetic aspects, i.e., will discuss the factors that determine the rate and specificity of PAH biodegradation.

PAH TRANSFER TO THE BACTERIAL CELL

In the environment, PAHs exist as vapors in the air and solutions in water; they can also be sorbed by solid bodies and water-immiscible liquids [22–24]. As a rule, not all the substrate in the ecosystem is available to microorganisms. The issue of the physical localization of the bioavailable PAH fraction in polluted soil is still under discussion. All PAH-degrading bacteria are able to take up the substrate from the aqueous phase; however, most of the soil pore volume is inaccessible for bacterial cells for reasons of geometry [25]. Some bacteria take up PAHs only from the aqueous phase [26–28]. In other cases, direct PAH intake from interphase surfaces [29–32] or from surfactant micelles [33, 34] was reported.

In addition to the bioavailable fraction of the substrate, there is a reversibly bound (bioaccessible) fraction, which is not available directly but exchanges the substance with the bioavailable fraction, and an irreversibly bound fraction, from which no substance transfer into the bioavailable fraction is possible [35].

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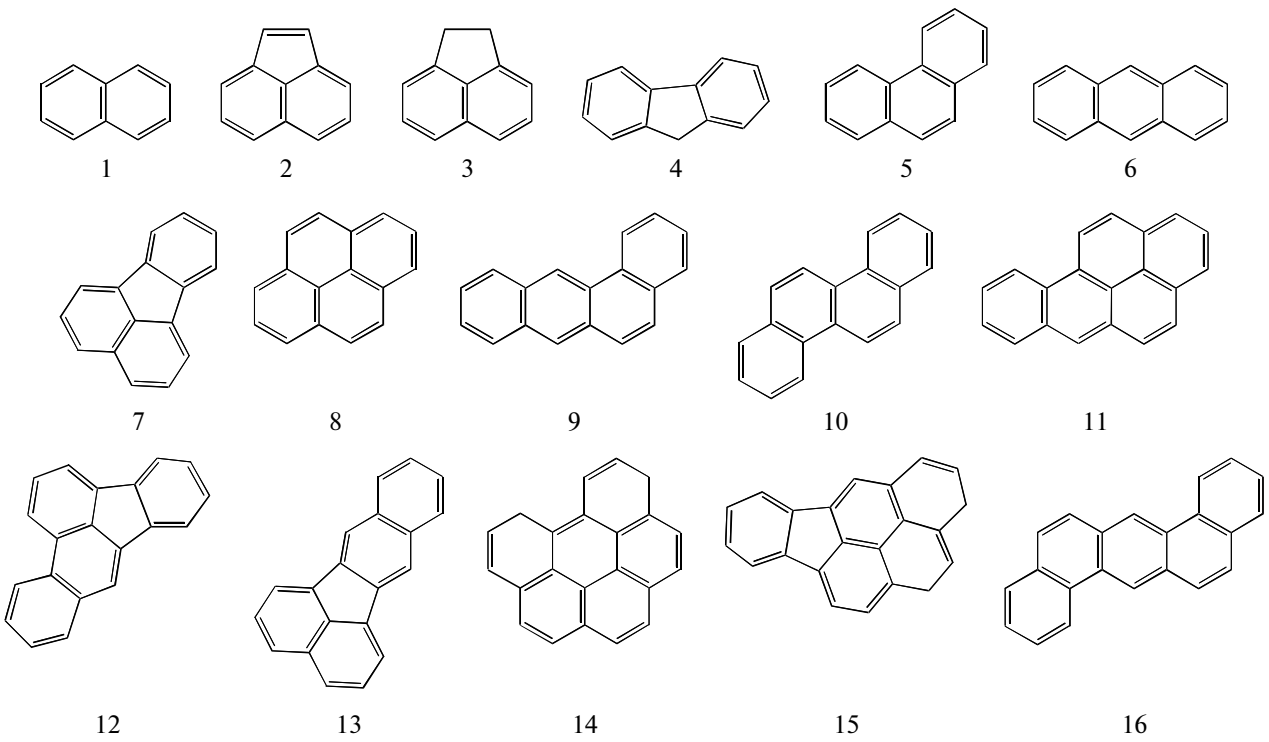

Fig. 1. PAHs included in the list of priority environmental pollutants by the United States Environmental Protection Agency (US EPA) [1]: naphthalene (1), acenaphthylene (2), acenaphthene (3), fluorene (4), phenanthrene (5), anthracene (6), fluoranthene (7), pyrene (8), benz[*a*]anthracene (9), chrysene (10), benzo[*a*]pyrene (11), benzo[*b*]fluoranthene (12), benzo[*k*]fluoranthene (13), benzo[*g,h,i*]perylene (14), indeno[1,2,3-*c,d*]pyrene (15), and dibenz[*a,h*]anthracene (16).

PAH biodegradation may be considered as a successive two-stage reaction:

$$C_B \leftrightarrow C_A \rightarrow Products, \quad (1)$$

where C_A is the bioavailable fraction and C_B is the reversibly bound fraction. The first stage is PAH transfer to the site of conversion and the second one is PAH conversion. If the steady-state biodegradation rate is completely controlled by the stage of conversion and the stage of mass transfer is in the equilibrium, then such process mode is termed kinetic; if the steady-state rate of biodegradation is completely controlled by the stage of mass transfer, then such process mode is termed diffusion. In the natural ecosystems such as soil or bottom sediments, biodegradation usually occurs in the diffusion mode [36].

The rate of contaminant biodegradation in the diffusion mode does not depend on biomass concentration; therefore, linear growth is observed at a constant rate of PAH transfer to the bioavailable fraction [28, 37]. Fed-batch culture serves as an experimental model of substrate conversion in the diffusion mode [38, 39]. Under the real conditions of polluted soil, the rate of microbial growth slows down with time, either due to exhaustion of the reversibly bound substrate or as a result of an increase in the cumulative amount of the substrate consumed for biomass maintenance. In the latter case, microbial population enters the stationary growth phase in spite of large stocks of the substrate in the reversibly bound fraction: there is so much biomass that the entire substrate entering the bioavailable fraction is consumed for biomass maintenance [38, 40].

One should distinguish between the kinetic and thermodynamic aspects of bioavailability. The kinetic aspect is characterized by the size of the bioavailable and reversibly bound fractions and by the rate of mass transfer between them; these parameters determine the rate of biodegradation in the diffusion mode and the total amount of PAH that can be degraded. The thermodynamic aspect is characterized by the ratio of the bioavailable and reversibly bound fractions after the establishment of equilibrium between them [41]. At a sufficiently high coefficient of substrate partition between the reversibly bound and bioavailable fractions, its equilibrium bioavailable concentration may become less than the saturating concentration for microorganisms, and the rate of biodegradation in the kinetic mode will be lower than the anticipated value. On the other hand, reversible sorption of the toxic substrate reduces its inhibitory effect [42].

The irreversibly bound PAH fraction in soil increases with the time since the moment of contamination. This process is called soil aging; during soil aging, both the possibility of biodegradation and the

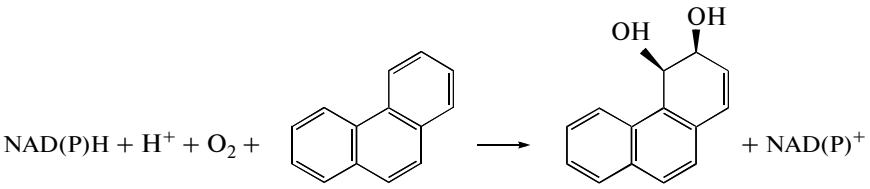

Fig. 2. The reaction catalyzed by hydroxylating dioxygenase (phenanthrene oxidation to (3*S*,4*R*)-dihydroxy-3,4-dihydrophenanthrene is shown).

toxicity of the contaminant decrease, because the irreversibly bound substance is nontoxic [23].

PAH bioavailability may be enhanced in different ways. The possibilities for enhancing the substrate bioavailability follow from the Fick's equation of diffusion [40], which can be simplified as follows:

$$J = DA(S_0 - S)/x, \quad (2)$$

where J is the substrate flux from the source (place of occurrence) to the sink (place of degradation), mol/s; S_0 is the substrate concentration in the source, mol/m^3; S is the substrate concentration in the sink, mol/m^3; x is diffusion path length, m; A is the cross-sectional area of diffusion, m^2; and D is the diffusion coefficient, m^2/s.

Diffusion path (x) may be decreased via biofilm formation at the interphase surfaces [43], which is facilitated by changes in the cell wall chemical composition in response to PAH appearance in the medium [44]. Some bacteria also use chemotaxis to decrease the substrate diffusion path [45].

Many PAH-degrading bacteria synthesize surface-active substances, i.e., biosurfactants [46, 47]. Biosurfactants increase the cross-sectional area of diffusion (A) due to oil phase dispersion and increase the diffusion coefficient (D) due to lowered interphase surface tension and better moistening, as well as to direct interaction with adsorbed PAH molecules [48].

Low PAH concentration in the sink (S) is attained due to the high value of specific affinity (q_{max}/K_S) of a given microorganism to the relevant PAH [40].

PAH degradation occurs inside bacterial cells under the influence of intracellular enzymes. PAH molecules, small and lipophilic, can freely penetrate through bacterial cell walls and biomembranes. PAHs are probably transferred into bacterial cells by way of simple diffusion. This point of view is confirmed by the following facts:

(1) No specialized PAH transporting systems have been found in the PAH-degrading strains which have been well-studied at the molecular level; and

(2) Recombinant *E. coli* strains carrying the genes of hydroxylating dioxygenases (the enzymes catalyzing PAH oxidation) can oxidize PAHs in spite of the fact that *E. coli* obviously has no proprietary PAH carriers.

Although assumptions about the existence of systems for the active PAH transport to bacterial cells may be found in the literature [49, 50], they are not sufficiently substantiated, because the existence of such systems has not been confirmed by molecular methods. On the other hand, it has been shown that PAHs may be an object of active transport from the cells into the environment [51, 52]; the probable function of this transport system is to remove free fatty acids from the cells under stress [53].

HYDROXYLATION OF THE AROMATIC RING

Aerobic bacterial PAH degradation begins with hydroxylation of one of the aromatic rings of the substrate (Fig. 2). This reaction is catalyzed by aromatic ring hydroxylating dioxygenases (ARHDs). ARHDs are two- or three-component systems, including a flavoprotein reductase that performs NAD(P)H oxidation and electron transfer to an oxygenase; this electron transfer is usually mediated by ferredoxin, a small FeS-containing protein. The oxygenase component of ARHD is a homo- (α_3) or hetero- ($\alpha_3\beta_3$) oligomer. Each α-subunit of ARHD consists of the so-called Rieske domain transferring the electron to the active site of the neighboring α-subunit and the catalytic domain forming the ARHD active site [54]. Conservative sequences of the α-subunit are used in the design of universal primers for amplification of the Rieske domain region [55] and primers specific to certain phylogenetic groups of ARHD genes [56, 57]; the review of this subject can be found in the work [58].

According to their amino acid sequences, bacterial ARHDs comprise a single protein family. In addition to PAH-oxidizing ARHDs, this family includes ARHDs catalyzing the oxidation of benzene [59], alkylbenzenes [60, 61], chlorobenzenes [62, 63], nitroaromatic compounds [64], aromatic amines [65], biphenyl and chlorobiphenyls [66, 67], aromatic acids [68, 69], and heterocyclic compounds [70]. ARHD classifications were constructed on the basis of the similarity between amino acid sequences of the α-subunit [71–73] or of the structure of all components of the dioxygenase system [74]. Phylogenetically, ARHDs are divided into several subgroups. The best studied of them are toluene/biphenyl dioxygenases, PAH-oxidizing dioxygenases of gram-negative bacteria, PAH-oxidizing dioxygenases of gram-positive

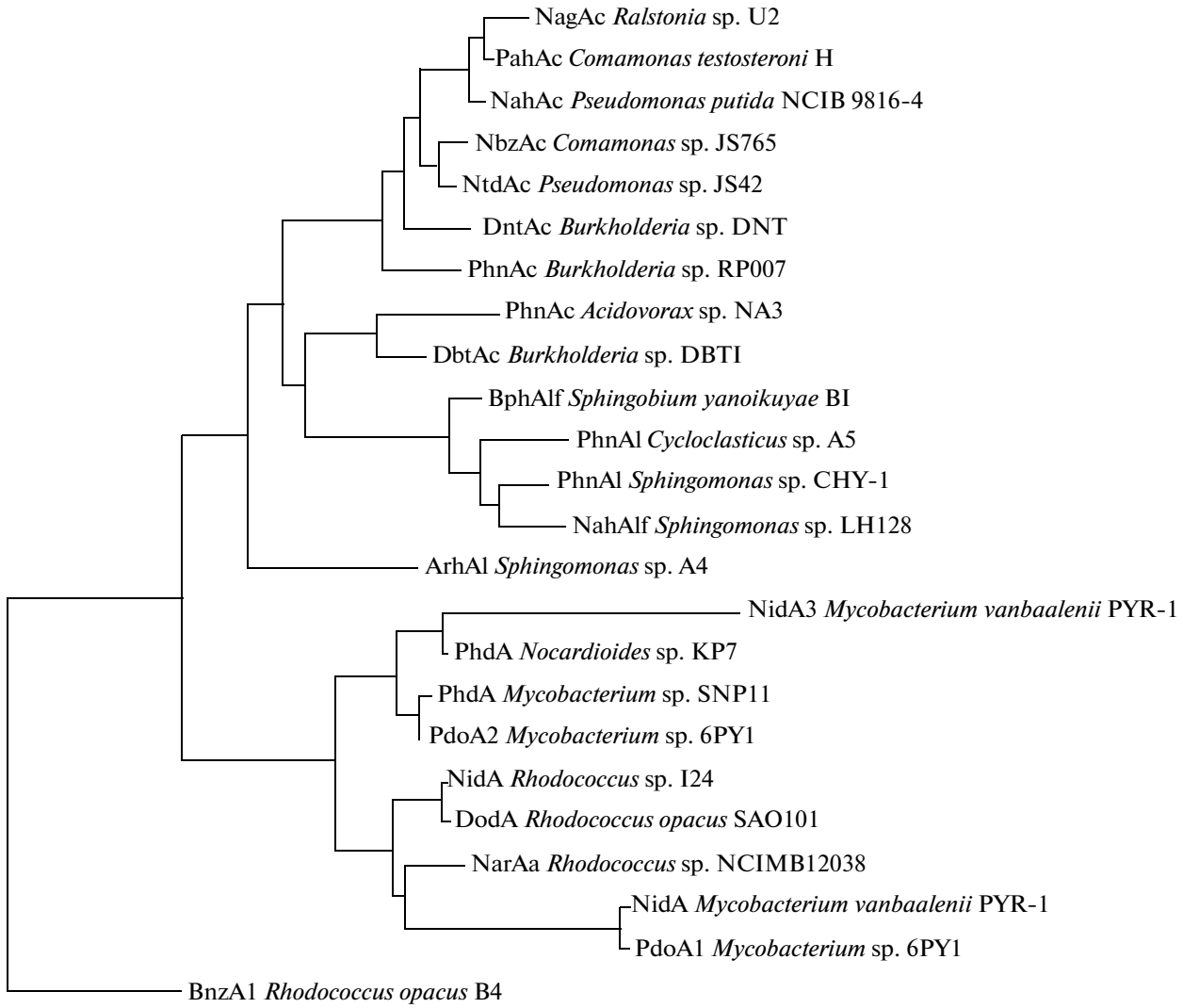

Fig. 3. The phylogenetic tree of α-subunits of PAH-oxidizing ARHD. The amino acid sequences were revealed using the PSI-BLAST and BLAST software (http://www.ncbi.nlm.nih.gov/blast). The sequences were aligned using T-Coffee (http://tcoffee.crg.cat/apps/tcoffee) [75]. The tree was constructed by the neighbor-joining method using TREECON [76]; the root was the amino acid sequence of the α-subunit of benzene dioxygenase BnzA1 from the strain *Rhodococcus opacus* B4 (YP_002777102). The sequences for tree construction were selected by the following necessary characteristics: (1) ARHD is functionally characterized and (2) ARHD is involved in the metabolic pathway of PAH degradation or falls into the same cluster with such ARHD.

bacteria, and benzoate dioxygenases. The names of the ARHD phylogenetic groups are rather conventional and correspond to one of the prevailing enzymatic activities in the phylogenetic cluster. A considerable part of the ARHD sequences determined as a result of genome sequencing have not been functionally annotated, because they did not exhibit sufficient similarity with any enzyme with known activity [73]. Classification of PAH-oxidizing ARHDs by the amino acid sequence of their α-subunits generally corresponds to the 16S rRNA classification of bacteria. The ARHDs of proteobacteria and actinobacteria form two separate clusters (Fig. 3). Although a correspondence to the subdivision into the α, β and γ-proteobacteria may be deduced within the cluster of proteobacterial ARHDs, it is not absolute, probably due to the horizontal gene transfer within this subgroup.

Some enzymes of the metabolic pathways for the degradation of nitro compounds [77, 78] and heterocyclic compounds [79] are closely related to PAH-oxidizing ARHDs. They often possess an activity against PAHs as well; for example, nitrobenzene dioxygenase from the strain *Pseudomonas* sp. JS42 [78] and dinitrotoluene dioxygenase from the strain *Burkholderia* sp. DNT [77] related to naphthalene dioxygenases of the Nah subfamily are able to oxidize naphthalene. On the other hand, many PAH dioxygenases oxidize also nitro compounds [77], heterocyclic compounds [80–82], and biphenyl [80, 83, 84].

Three-dimensional structures of PAH-oxidizing ARHDs from *Pseudomonas putida* NCIB 9816-4 [85], *Rhodococcu*s sp. NCIMB12038 [86], *Sphingobium yanoikuyae* B1 [87], and *Sphingomonas* sp. CHY-1 [88] have been determined by now; the latter two enzymes can oxidize PAHs with more than three aromatic rings. In spite of the low identity between the primary structures, their tertiary structures are very similar. The differences in substrate specificity of these enzymes may result from the minor differences in the geometry of their active sites [54], particularly in the volume of the substrate-binding pocket [87–89].

ARHDs show broad specificity against PAHs. For example, naphthalene dioxygenase from the strain *Pseudomonas* sp. NCIB 9816 catalyzes conversion of more than 50 various aromatic substrates [90] but not of high-molecular-weight PAHs; the enzyme from the strain *Sphingomonas* sp. CHY-1 oxidizes various PAHs containing 2 to 5 aromatic rings (in some cases, oxidation of the products with the formation of bis-dihydrodiols was observed [84]); the dioxygenase of the strain *Sphingomonas* sp. A4 capable of growth on acenaphthene and acenaphthylene, apart from these two substrates catalyzes the oxidation of naphthalene, phenanthrene, anthracene, and fluoranthene, but not pyrene [91]; ARHD from the strain *Sphingomonas* sp. LH128 oxidizes various PAHs and heterocyclic compounds at different rates [82]; the strain *Mycobacterium* sp. 6PY1 has two ARHDs responsible one for phenanthrene biodegradation and the other for pyrene biodegradation, which are, however, not quite specific [92]; the two ARHDs of the strain *Mycobacterium* sp. PYR-1, well-characterized at the functional level, show the highest activity to pyrene and fluoranthene, respectively; both of them also oxidize phenanthrene and other PAHs [89]. Thus, the specificity of PAH conversion by bacterial ARHDs is characterized by the following properties: (1) the same ARHD catalyzes the oxidation of several PAHs; (2) activity of an ARHD is different against different PAHs; and (3) different ARHDs vary in substrate specificity.

The other side of substrate specificity of ARHDs is the selectivity of product formation; it may be high when one product is predominantly generated from many potential isomers. For example, phenanthrene oxidation by the dioxygenase from the strain *Pseudomonas* sp. NCIB 9816-4 results in formation of (3*S*,4*R*)-dihydroxy-3,4-dihydrophenanthrene with 90% selectivity [93] (Fig. 2); this isomer is a metabolite of the best known pathway of bacterial phenanthrene degradation. Selectivity of formation of a PAH hydroxylation product may be low. For example, phenanthrene oxidation by the enzyme NidA3B3 from *Mycobacterium* sp. PYR-1 results in the formation of 3,4-, 9,10- and 1,2-*cis*-dihydrodiols at a ratio of about 2 : 1 : 1 [94]. PAH hydroxylation products are promising intermediates for organic synthesis [95].

The reaction of aromatic ring hydroxylation occupies a central place in PAH metabolism. It is irreversible for thermodynamic reasons, being the first irreversible stage of PAH metabolism, with consideration of the absence of diffusion limitations for PAH transfer into the cell. The enzymes catalyzing such reactions usually control the substance flux through the metabolic system to a considerable degree. In particular, as follows from the theory of metabolic control analysis, the enzyme catalyzing the first irreversible reaction completely controls the flux if it does not undergo negative feedback regulation by the metabolites of the system [96–98]. There are no data on ARHD regulation in the literature; the allosteric sites of metabolite binding in ARHD molecules have not been described. NAD(P)H and $NAD(P)^+$, being the respective substrate and a product of the reaction, cannot regulate the enzyme activity by negative feedback, because the functioning of the degradation pathway as a whole results in NAD(P)H concentration increase and $NAD(P)^+$ concentration decrease, i.e., the feedback is positive. Thus, there are grounds to believe that ARHDs control the rate of PAH biodegradation to a considerable degree, or even completely determine it. Unfortunately, no experimental assessment of the control coefficient of PAH biodegradation rate by ARHD has been performed.

The data on the kinetic properties of ARHDs are very scarce; it is caused by the difficulty of obtaining ARHD enzyme preparations and performing the kinetic studies. For example, in the work [84] the Michaelis constant (K_M) and the specificity constant (k_{kat}/K_M) could be determined only for the two most soluble out of eleven substrates. ARHD activities against different substrates were usually assessed by comparing not the specificity constants but the rates of individual PAH conversion by a recombinant strain or (rarely) by a purified enzyme [82, 84, 94]. Such analysis is inadequate in the context of enzyme kinetics and allows only tentative conclusions concerning ARHD specificity.

Kinetic analysis of PAH conversion by microbial cultures was given more attention compared to PAH conversion by purified enzymes. The rate of PAH conversion in the kinetic mode is proportional to biomass concentration, with exponential growth observed in the absence of complicating factors. The rate of PAH conversion at rather low, "unsaturating", PAH concentrations depends on PAH concentration. This dependence is generally well described by the Monod equation [99], although substrate inhibition probably occurs sometimes [99]. The substrate constant (K_S) is usually much lower than the water solubility of PAH under consideration, suggesting the high affinity of bacteria to the substrate. Specific affinity (q_{max}/K_S) of the same culture to various substrates may differ several times [99, 100] or even by two orders of magnitude [101], which is evidence of the high selectivity of bacterial PAH degradation.

The study of the biodegradation of PAH mixtures is of great interest, because, first, PAHs are present in the environment as mixtures and, second, such experiments provide for a better understanding of the mechanism of PAH biodegradation. PAH mixture conversion by a microbial culture usually follows the multisubstrate model, according to which all components of the mixture compete for a single active site in accordance with the principles of the Michaelis kinetics [102–107]. As follows from this fact, the rate of PAH conversion by bacteria is determined by the reaction in the active site of the enzyme (supposedly ARHD) and does not depend on the stage of substrate diffusion into the cell; if the process rate was controlled by the stage of PAH diffusion through the cell wall, no competition for the metabolic system between different PAHs would be observed.

Sometimes, two active sites need to be considered to explain the kinetics of PAH mixture conversion by bacterial cultures [100]. Based on the kinetic data, we suggested the existence of two ARHDs with different substrate specificity in the strain *Sphingomonas* sp. VKM B-2434: one enzyme converting acenaphthene and acenaphthylene and the other converting phenanthrene and fluoranthene [100]. This hypothesis was subsequently confirmed by molecular methods (Baboshin and Golovleva; unpublished data).

METABOLISM OF THE REACTION PRODUCTS OF AROMATIC RING HYDROXYLATION

Dihydrodiols formed during PAH hydroxylation undergo further metabolism. Dihydrodiols are oxidized in the dehydrogenase reaction with the formation of PAH dihydroxy derivatives. PAH dihydroxy derivatives are subjected to the action of ring-cleaving dioxygenases; incorporation of two oxygen atoms into the PAH dihydroxy derivative molecule is accompanied by a cleavage of the aromatic ring. An aromatic ring may be cleaved in the intradiol manner, i.e., between the carbon atoms carrying hydroxy groups (also called *ortho*-cleavage), or in the extradiol manner (*meta*-cleavage), when another C–C bond is cleaved. Intradiol ring cleavage results in the formation of dicarboxylic aromatic acids, while extradiol cleavage leads to the formation of monocarboxylic aromatic acids, most often aromatic substituents of 2-keto-3-butenoic acid. This product is cleaved in the aldolase reaction into pyruvate and a PAH derivative with the number of rings less by one. If the microorganism possesses the respective enzyme activities, all rings comprising the PAH molecule are sequentially cleaved with formation of the compounds of central metabolism. The degradation pathways of various PAHs coincide at certain stages, varying only in the initial reactions. The metabolic pathways of bacterial PAH degradation are described in detail in the reviews [108–110].

PAH biodegradation leads to the formation of three fractions of products: biomass, intermediates, and final metabolic products (CO_2 and H_2O). Stoichiometry of PAH biodegradation is characterized by the distribution of electrons [111, 112] or carbon atoms [113, 114] between the products. When bacteria utilize PAHs as growth substrates, the biomass yield is usually low [113, 114]. Accumulation of the intermediates may vary from nearly zero to a high percentage of the converted substrate [112–114]. Accumulation of the intermediates is caused by incomplete substrate oxidation due to the presence of dead-end branches in the metabolic scheme. In particular, the broad substrate specificity of ARHDs and the far-from-absolute selectivity of formation of the PAH hydroxylation products favor the accumulation of non-metabolized intermediates, especially if the medium contains a PAH mixture. Incomplete PAH oxidation by bacteria is accompanied by accumulation of ketones and quinones [115–117] or *o*-hydroxycarboxylic acids [118, 119]. Accumulation of the intermediates is of interest, because these products inhibit PAH biodegradation [112, 115, 116, 120, 121] and may be stable and environmentally hazardous [6, 117].

The presence of other PAHs in the medium may have not only an inhibitory, but also an activating effect on the biodegradation of a given PAH [122]. Such activating effects may result from cross induction [123], cometabolism [124], and increase in the biomass concentration [103]. Cometabolism is particularly important for biodegradation of some high-molecular PAHs, which are not utilized by bacteria as a sole carbon and energy source, e.g., for biodegradation of benzo[*a*]pyrene [125]. Benzo[*a*]pyrene is mineralized by microbial cultures when pyrene [126], oil, or oil fractions [127] are used as a co-substrate.

In view of the great diversity of PAHs in the environment and still greater diversity of their bioconversion products, efficient bioremediation requires the presence of a great diversity of enzyme activities in the ecosystem, i.e., the activity of microbial communities. Indeed, experience shows that microbial communities degrade PAHs, and especially PAH mixtures, more efficiently than pure bacterial cultures [126, 128]. The effect of a microbial community is not a mere sum of the effects of its individual members because of the interactions between the members of this community [129]. In particular, the rate of PAH mixture degradation by a mixed culture may be much higher than the sum of the rates of degradation of this PAH mixture by individual strains comprising this mixed culture [122]. Such cooperative effect is based on the compensation for the competitive inhibition between PAH mixture components, as well as the removal from the medium of toxic products inhibiting PAH biodegradation [128]. For example, the strain *Arthrobacter* sp. F101 utilizes fluorene as a growth substrate, but the side product is 9-fluorenone which inhibits its growth; the mixed culture of *Arthrobacter* sp. F101 and the strain

Pseudomonas mendocina MC2 growing on 9-fluorenone but not consuming fluorene is not susceptible to such inhibition [120].

If the contaminant is degraded by a microbial community, the metabolic pathway is distributed between several species of microorganisms which may be interconnected by complex trophic relationships. Therefore, the rate of contaminant degradation is not necessarily determined by the microorganism performing the first stage of biodegradation; this rate may strongly depend on the microorganism utilizing the toxic intermediate. For describing the substance flux regulation in the ecosystem, application of the theory of metabolic control analysis originally developed for enzyme systems was proposed [130]. This approach has never been used for PAH biodegradation analysis but seems to be promising for revealing the "bottlenecks" of the metabolic pathway in the ecosystem. Such analysis obviously must be preceded by description of the structure of the microbial community involved in the degradation of the contaminant under consideration in the studied ecosystem (e.g., polluted soil). The microbial degraders of the contaminant are revealed by the cultivation-independent techniques [131]. One of the best methods for this purpose is stable-isotope probing (SIP), when a substrate (PAH) enriched with the heavy carbon isotope ^{13}C is introduced into the ecosystem. Assimilation of the labeled PAH is accompanied by the synthesis of biomolecules with enhanced content of the heavy isotope; qualitative analysis of such biomolecules (e.g., heavy DNA sequencing) makes it possible to determine the systematic affiliation of microorganisms–destructors, while quantitative analysis (e.g., quantitative PCR) makes it possible to determine the abundance of each bacterial species involved in the degradation of the given PAH. SIP was used for PAH biodegradation analysis in a number of studies [132–137]. The strains, which are PAH destructors according to SIP, may sometimes be isolated from the ecosystem under study as a pure culture [132, 138], but many of the found DNA sequences belong to "uncultivated" bacteria, and some of them cannot be ascribed to any of the known bacterial genera.

In the study of PAH-degrading microbial community, the most important questions seem to be as follows: (1) what microorganisms are involved in PAH degradation; (2) to what extent does each microbial species control the rate of PAH degradation; and (3) what properties do have the microorganisms controlling the rate of PAH degradation to a greater extent than other microorganisms? The existing methods of investigation of microbial communities can give only approximate answers to these questions; therefore, further progress in this subject will depend on the development of new experimental techniques.

CONCLUSION

In terms of kinetics, aerobic bacterial PAH biodegradation is a process consisting of three sequential stages: (1) PAH transfer into the bacterial cell; (2) PAH hydroxylation; and (3) utilization of hydroxylation reaction products. The rate of PAH biodegradation may be determined by the first stage (diffusion mode) or by the two later stages (kinetic mode). The diffusion mode is conditioned by the limitations of PAH transfer to the bacterial cell surface. The rate of PAH biodegradation in the kinetic mode is to a large degree controlled by the dioxygenase that performs PAH hydroxylation. ARHD specificity determines the spectrum of PAHs undergoing conversion and the spectrum of products formed. PAH biodegradation is influenced by the effects such as competitive inhibition, inhibition by intermediates, cross induction, and cometabolism. PAH biodegradation in natural ecosystems is performed by microbial communities, and the metabolic system of PAH degradation is distributed between different members of the microbial community.

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