ISSN 0026-2617, Microbiology, 2007, Vol. 76, No. 6, pp. 639–653. © Pleiades Publishing, Ltd., 2007.
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REVIEW

Bioremediation of Soils and Sediments Contaminated by Polychlorinated Biphenyls

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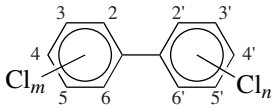
Received January 22, 2007

Abstract—This review discusses the prospects of using the potential of microorganisms for bioremediation of PCB-contaminated natural environments (soil, sediments, and sewage sludge) under anaerobic and aerobic conditions. A detailed analysis of the research conditions of original works has shown that the efficiency of bioremediation of PCB-contaminated matrices strongly depends on the character and degree of contamination. In the case of aerobic bioremediation, the best results were obtained with moderately contaminated soils and sediments (20 to 700 PCB/kg), in which the level of contamination decreased by 40–75%. These results could be achieved by repeated inoculation of a consortium of specific microorganisms (isolated or engineered) with concurrent addition of biphenyl as an inducer and of biosurfactants; their effect increased in a slurry bioreactor. PCB concentration decreased mainly due to the degradation of congeners with one to three chlorine atoms. The content of higher-chlorinated PCB can be noticeably decreased only under sequential anaerobic/aerobic treatment; the best effect was achieved with anaerobic granules. However, only in individual cases, mainly in laboratory experiments with freshly spiked PCB at moderate concentrations, was it possible to reduce their content to a level permissible for technogenic soils. The review begins with the description of the main metabolic pathways and patterns of biodegradation of these pollutants in natural and artificial environments.

DOI: 10.1134/S002626170706001X

INTRODUCTION

Polychlorinated biphenyls (PCB) are oily liquids comprising a mixture of compounds with the total of 1 to 10 chlorine atoms in two C–C bound aromatic rings. Theoretically, 209 congeners are possible comprising the entire set of homologues and isomers, with an ordinal number given to each of them [1]. Hereinafter, homologous PCB groups with the same number of chlorine atoms will be designated as monoCB, diCB, …, and nonaCB.



$m + n = 10$

Since 1929, PCBs have been produced in different countries under the trade marks of Sovol, Sovtol, Hexol, Aroclor, Clophen, Delor, Kanechlor, Phenclor, and Pyralene [1–3]. Commercial mixtures usually contain 20 to 60 PCB congeners, mainly with 3–6 and sometimes with 2, 7, and 8 Cl atoms. Due to their thermal and chemical stability, flame resistance, and dielectric properties, PCBs have been widely used as dielectric liquids, heat-exchange fluids, hydraulic liquids, plastifiers, adhesive substances, etc. In the 1970–1980s, after their persistence in the environment, toxicity, and ability to accumulate in human and animal adipose tissues had been demonstrated, PCB production in many countries was banned and their application was drastically restricted. Now these compounds are considered among the most hazardous pollutants in the world [1, 4].

Before the mid 1980s, worldwide PCB production was about 1.5 million tons; presently about 750 thousand tons of PCBs are still used, mainly in closed systems, and approximately the same amount is present in the biosphere [5, 6]. The biggest part of PCBs in the environment is localized in soil and in water sediments close to the places of their former production and application. In contaminated places, concentrations of these chemicals in soil and sediments may reach $10–10^4$ mg/kg [2, 7]. This is several orders of magnitude higher than the permissible level, which varies from 0.01 to 50 mg/kg depending on the country and land use (in Russia, 0.06 mg/kg) [8]. In Canada, all soils containing over 50 mg PCB/kg must be kept in hazardous waste landfills or decontaminated. According to the calculations of the United States Environmental Protection Agency (US EPA), elimination of 525 million tons of PCB-containing waste will require about 400 billion dollars [4, http:// www. epa. gov/superfund/sites/query/basic.htm]. Approximately 0.3–0.5 million tons of PCBs have been produced in Russia, mainly in Dzerzhinsk (Nizhny Novgorod oblast) and Novomoskovsk (Tula oblast). Soils heavily contaminated by PCBs are located also in Chapaevsk

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(Samara oblast) and in the vicinity of the plant manufacturing capacitors and transformers in Serpukhov (Moscow oblast) as well as in some sites of application and storage of electrochemical equipment [2].

The most-widely used methods for utilization of PCB-contaminated waste (high-temperature incineration and burial) require heavy material and energy expenses and result in the loss of soil fertility and formation of dioxins, highly dangerous toxicants [4]. Bioremediation is one of the alternative methods for purification of PCB-contaminated soils and sewage sludge. There are several recent reviews discussing the advantages and limitations of bioremediation of PCB-contaminated soils [1, 5, 9, 10] and sediments [5, 11, 12]. However, most works consider mainly the genetic and biochemical diversity of degrading microorganisms and the methods of isolation and construction of PCB-degrading strains, and state some techniques for acceleration of PCB biodegradation, mostly not taking into account the conditions of their application.

This work is devoted to discussion of the prospects of using the potential of microorganisms for bioremediation of PCB-contaminated natural matrices. The efficiency of different methods for bioremediation of PCB-contaminated objects is compared with consideration of the character of contamination. The review begins with description of the main metabolic pathways and patterns of biodegradation of these pollutants in natural and artificial environments.

PCB BIODEGRADATION IN CULTURE AND IN NATURAL ENVIRONMENTS

Microorganisms are known to play a key role in biodegradation of PCBs and other stable pollutants in liquid and solid natural environments. Microorganisms capable of PCB transformation were shown to be present in many natural and anthropogenic environments even in the absence of pollutants. However, specific microflora has already formed in historically contaminated places under the action of selective factor (PCBs and other chlorinated organic compounds), and the process of adaptation goes on [5].

PCBs can be transformed in aerobic (surface soil, surface sediments and sewage sludge, aerobic bioreactors) and anaerobic media (flooded soils, sediments of rivers and ponds, anaerobic bioreactors). Microbial processes in the surface biofilm, which is formed at the interface between anaerobic and aerobic conditions, plays a special role in their degradation. Figure 1 presents the generalized scheme of major pathways of PCB biotransformation in natural and artificial environments.

Most of the PCB congeners are degraded mainly under conditions of co-metabolism and cannot be growth substrates for soil microorganisms. However, PCBs, particularly higher-chlorinated congeners, are highly oxidized compounds and can serve as electron acceptors for energy storage in anaerobic environments under electron acceptor deficit; in the course of this process, they undergo reductive dechlorination [11, 12].

Reductive Dechlorination of PCBs

In the sediments of natural reservoirs, this process is accomplished by **anaerobic bacteria** capable of anaerobic respiration with sulfates, carbonates, nitrates, and other oxidized compounds as electron acceptors. In the presence of chloroorganic pollutants including PCBs, some anaerobic bacteria are able to switch to the process of dehalorespiration. Such reorganization needs some time; hence, dechlorination of freshly spiked PCBs begins after a lag period of one to several months. PCB dechlorination in sediments is performed by obligate anaerobes: methanogens, sulfate reducers, etc. As a rule, PCB dechlorinators form consortia with other microflora, which provides them with carbon sources and maintains anaerobic conditions and the optimal level of hydrogen [11, 13]. Under the effect of dehalogenase, chlorine atoms in PCBs are one by one replaced by hydrogen atoms (usually from H_2) and are cleaved as chloride ions. Microbial communities formed in natural sediments have varied sets of dechlorinating enzymes, which differ in their selectivity to individual congeners and in the composition of products. The rate of dechlorination in sediments usually decreases from higher- to low-chlorinated congeners.

The number of PCB dechlorinators in natural environments is usually low (about 10^2 cells/g), which explains the low rates of dechlorination. At cultivation in selective media, PCB dechlorinators are accumulated slowly. Only recently, mixed bacterial cultures and individual strains similar to desulfovibrios *Degalococcoides* spp. have been isolated, which are able to dechlorinate PCB in the presence of easily accessible organic carbon (or CO_2) and H_2 [5, 14]. In liquid medium, the mixed culture dechlorinated all the 64 congeners of Arochlor 1260 (50–500 mg/l); after four months of incubation, 76% congeners with 6–9 chlorine atoms were transformed into congeners with 3–5 chlorine atoms [14]. *Degalococcoides* spp. had a rare ability to cleave chlorine from the *ortho* position [5].

Apparently, reductive dechlorination of PCBs is also possible in water-logged soils. Apart from anaerobic bacteria, micromycetes can also participate in this process. The ability of **white rot fungi** *Phanerochaete chrysosporium* to dechlorinate 2,2',4,4',5,5'-hexaCB (25 mg/l) even under aerobic conditions in the presence of carbon sources and nitrate as a nitrogen source (non-white rot conditions) has recently been proved. Nitrate reductase has been shown to participate in this process; its gene was first found in this micromycete by PCR. Commercial nitrate reductase also revealed the ability to dechlorinate PCBs [6].

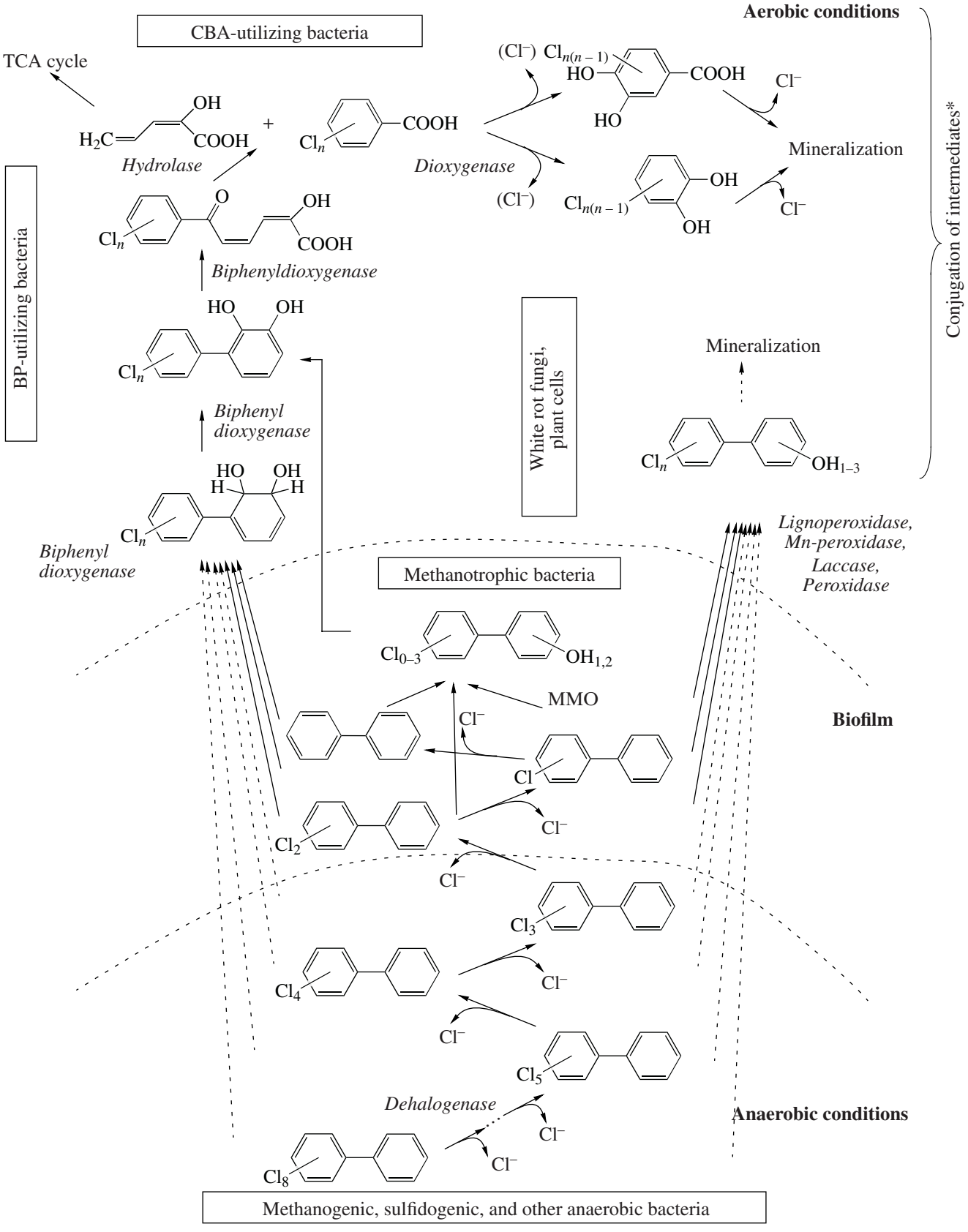

Fig. 1. Schematic representation of the main pathways of PCB biotransformation in solid natural and artificial media under aerobic and anaerobic conditions, as well as in aerobic biofilms under microaerophilic conditions. Based on [7, 11, 15, 16, 22, 26].*Conjugation of metabolites with humic acids and cell components.

Aerobic Degradation of PCBs

Aerobic degradation of PCBs is of particular interest for researchers, because their complete mineralization is possible only under aerobic conditions. In natural aerobic environments, PCBs are degraded mainly by aerobic bacteria or nonspecific exoenzymes secreted by white rot fungi and other organisms, including plants.

Bacterial degradation by the BP pathway. Bacteria utilizing biphenyl (BP) as the sole carbon and energy source play a key role in PCB degradation. BP-utilizing bacteria metabolize PCBs via the pathway of primary catabolism of biphenyl (BP pathway) into chlorobenzoic acids (CBA), which are usually mineralized by other bacteria. CBA-mineralizing bacteria are widespread in soils contaminated with various chloroaromatic compounds and can degrade chlorobenzoates via different pathways [10].

Microbial PCB degradation proceeds stepwise, involving four groups of enzymes encoded in the BP genes, which are organized into a single operon. The rate of transformation of chlorinated derivatives is usually much lower than that of BP proper (at least 50 times). It strongly depends on their structure, and enzyme specificity to different PCB congeners varies in different strains. Dozens of PCB-degrading strains are already known, and the newly isolated strains, as a rule, possess still broader substrate specificity to PCBs and still greater ability to degrade highly chlorinated congeners. Among the isolated PCB degraders are gram-negative bacteria of the genera *Pseudomonas*, *Alcaligenes*, *Achromobacter*, *Burkholderia*, *Comamonas*, *Ralstonia*, *Sphingomonas*, *Acinetobacter* and gram-positive *Rhodococcus*, *Bacillus*, etc. [15].

The rate and depth of microbial PCB degradation correlates negatively with the number of chlorine atoms in a molecule. All of the isolated strains are able to degrade mono-, di-, and triCB, some of them degrade tetraCB, but only few (*Burkholderia xenovorans* LB400, *Pseudomonas pseudoalcaligenes* KF707, *Rhodococcus globerulus* P6, etc.) slowly degrade congeners with 5 or more chlorine atoms (up to nonoCB). The most active strains have a high activity and broad substrate specificity to PCB congeners, but the oxidized products formed during this process often cannot undergo further enzymatic transformation. Nonplanar congeners (with two and more chlorine atoms in the *ortho* position), especially with *ortho*-chlorines located in both rings, are the most stable for the majority of bacteria. Many PCB destructors can grow on monoCB, and some of them also on individual representatives of di- and even triCB, utilizing in most cases only the nonchlorinated phenyl ring. Some strains are able to utilize monoCB as a carbon source, which allows them to completely mineralize monoCB [1, 3, 9, 10, 15, 16].

The genes responsible for the synthesis of enzymes of the BP pathway are usually expressed during growth in the presence of BP or its utilized analogues. Therefore, degradation of the preparations proceeds much faster and deeper with bacteria grown on BP as compared to the cells grown on glucose, glycerol, etc.; the degradation of nearly all PCB congeners by growing cells (in the presence of 0.01–0.1% BP) is higher and more effective than in the absence of growth. For example, the week-long degradation of ^{14}C-Arochlor 1254 (10 mg/l) by two strains grown on BP, *Acinetobacter* sp. P6 and *Arthrobacter* sp. B1B, was 8–17% in case of resting cells and 23–32% under growth conditions (in the presence of BP); many congeners with 4–6 Cl atoms were degraded only by the growing cells [17].

There are two main reasons for the poor activity of BP degraders against average- and high-chlorinated PCB congeners in culture media. First is the formation of highly toxic PCB metabolites (chlorinated dihydroxybiphenyls, chlorocatechols, etc.), which impede complete degradation of these xenobiotics. The possibility of cross inhibition of the enzymes involved in the degradation of some congeners by the products of primary metabolism of other PCB or CBA congeners has been proved [10]. Second, extremely high hydrophobicity determines the accumulation of these pollutants in the cytoplasmic and intracellular membranes of cells. At low PCB concentrations, it reduces their accessibility to the enzymes; at high concentrations, it causes the liquefaction and degradation of the membranes and, finally, cell death [9].

At present, active research is being carried out on isolation and construction of PCB degrading strains by the methods of genetic engineering [9, 10, 15, 16]. The most practical progress has been made in the creation of strains with broader substrate specificity and strains with multiple sets of genes encoding the enzymes of degradation of not only PCB but also of CBA. Russian researchers have isolated a highly active strain *Micobacterium* sp. B51, which can utilize 2-CB, 2,2'-diCB, and 2-CBA, as well as oxidize 2,4'-di-, 4,4'-di-, 2,4,2'-tri-, and 2,4,4'-triCB. A mixed culture of this strain with *Arthrobacter* sp. H5 utilizing 4-CBA grew on a medium with 2,4'-diCB (up to 1000 mg/l) as a sole carbon and energy source [18]. A similar consortium of PCB and CBA destructors immobilized on polyurethane foam or glass beads efficiently eliminated lower-chlorinated PCBs from water in a flow biofilm reactor. The decrease of the total concentration of Arochlor 1221 or the artificial mixture of diCB and monoCBA from 1–2 mg/l at the input to <0.001 mg/l at the outlet was accompanied by the stoichiometric release of chloride ions [19]. In most of the other studies of microbial degradation of commercial mixtures in culture media in a wide range of PCB concentrations (0.26 to 500 mg/l), the rate of oxidative degradation sharply decreased after several hours or days. The degree of direct aerobic degradation of Arochlor 1221, 1242, 1248, 1254, 1260, and their analogs (the prevailing congeners with 1 to 2, 3 to 4, 4 to 5, 5 to 6, and 6 to 7 chlorine atoms, respectively) varied within 60–100, 50–95, 40–60, 30–50, and 0–30%, respectively [11, 17, 20, 21].

Degradation by white rot fungi. White rot fungi play an important role in the degradation of PCBs and their metabolites in soil [7]. It is assumed that degradation of these and other stable xenobiotics involves extracellular white rot enzymes (ligno-peroxidase, Mn-dependent peroxidase, laccase), which cleave natural polymer compounds of complex structure, in particular, lignin. Due to their low specificity and free-radical mechanism of action, white rot enzymes can oxidize or reduce many stable xenobiotics; the products of this process are polymerized and/or bound to soil humus [22].

The ability to degrade low PCB concentrations (0.3–2.5 mg/l) was demonstrated for several strains of the genera *Phanerochaete*, *Bjerkandera*, *Pleurotus*, *Trametes*, *Rhizobium* etc. In liquid media under white rot conditions (nitrogen limitation), these strains degraded many PCBs (up to hexaCB), but mono- and diCB were transformed at the highest rate. In culture media, mineralization of labeled ^{14}C-mono-, di-, or triCB (0.3 mg/l) by micromycetes varied from 0.4 to 1% in 1–2 months, while most of the radioactivity (about 40%) was bound to the fungal mycelium. Trace amounts of mono- and dihydroxy derivatives of PCBs, their methylated analogs, and chlorine derivatives of benzyl alcohol, benzaldehyde, and benzoic acid were identified among the metabolites [7, 23].

The possibility of PCB transformation by these fungi in soil (up to 30–45 mg/kg) has also been proved. For example, in the experiments with sterile soil (C_{org} 0.8%, pH 5.2) contaminated by Delor 103 (10 mg PCB/kg), 60% of PCB was quickly bound by the soil matrix due to abiotic processes, and another 2–20% (depending on the congener) of di-, tri-, and tetraCB was degraded and/or additionally bound by the fungal mycelium after two months of incubation in the presence of inoculated white rot fungi [24]. However, micromycetes are sensitive to higher concentrations of these xenobiotics in soil; they are therefore nearly absent at >500 mg PCB/kg soil [11]. Probably, PCBs are degraded in the same way by the only yeast strain *Hansenula californica* AT known among PCB degraders [25].

Degradation by enzymes of plant origin. Plant peroxidases, both endogenous and exogenous, also have a substantial ability to transform PCBs [7]. In plants, they perform antioxidant and certain other functions, including transformation and mineralization of xenobiotics. Plant peroxidases can degrade many PCB congeners (particularly mono-, di-, and triCB) and CBA. Many mono- and diCB (1–2 mg/l) were degraded in a medium with purified peroxidases of PCB from black nightshade (morel) and tobacco in the presence of 0.02% H_2O_2; their metabolites included (chloro)benzoic acids, mono-, di-, and trihydroxy derivatives of PCB, and less and more chlorinated PCB congeners [7]. In the cell culture of root fibrils of black nightshade (*Solanum nigrum* L.), 72% Delor 103 (mainly mono- and diCB) was almost completely transformed after 7 days; the intermediate products (mono- and dihydroxy derivatives of mono- and diCB) quickly formed conjugates with cell metabolites [7].

PCB Degradation by Anaerobic–Aerobic Consortia

PCB degradation by anaerobic–aerobic consortia plays a particular role, because it offers the prospect of complete biodegradation of highly chlorinated PCBs. Natarajan et al. [26] developed the technique of obtaining anaerobic granules from activated sludge of municipal and other treatment facilities. The unadapted anaerobic bacterial consortium of these granules exhibited high dechlorinating activity against many chloroorganic pollutants. This consortium dechlorinated many PCB congeners with 1 to 8 chlorine atoms but, unlike the mixed culture isolated from sediments, it was more efficient against low-chlorinated congeners, particularly monoCB, and the process was accompanied by a decrease of the molar concentration of biphenyls. It is assumed that PCB degradation involves, besides anaerobes, also aerobic bacteria that form a biofilm on the surface of anaerobic granules.

As a rule, the biofilms on the surface of anaerobic matrices protect anaerobes, consuming trace contaminants of oxygen present in the medium. Methanotrophic bacteria utilizing methane as the major growth substrate play an important role among them. Methane oxidation occurs under the influence of a low-specific **methane monooxygenase (MMO)**, which is able to hydroxylate both non-chlorinated and chlorinated compounds, including low-chlorinated PCBs. The indication of these processes is accumulation of low amounts of 2- and 4-hydroxybiphenyl and their monochlorinated derivatives under anaerobic/aerobic degradation of BP/PCBs in the presence of sewage sludge from treatment facilities [26].

In culture media, the mixed anaerobic consortium isolated from uncontaminated groundwater, as well as the pure culture of a methanotrophic strain II of type CSC1 in the presence of methane, hydroxylated 2- and 4-monoCB, hydroxyl-monoCB, 2,4- and 2,4'-diCB (20 mg/l) with predominant oxidation of the *ortho*-chlorinated congeners. The resulting hydroxylated products are believed to be further transformed by aerobic BP-utilizing heterotrophs present in the biofilm [27]. Similar processes probably take place in some of the sediments, which also show dechlorination of low-chlorinated PCBs and BP degradation [26, 28, 29].

PCB Biodegradation in Natural Environments

In spite of the abundance of PCB-degrading microorganisms in the environment, PCBs are characterized by extremely high persistence. This fact is caused by the low activity of native PCB-degrading microorganisms, and the great diversity and high toxicity of PCB congeners and/or their metabolites. However, the most

important reason for their high stability in natural matrices is poor accessibility of PCBs for microorganisms, resulting from their very low water solubility and extreme hydrophobicity, together with the slow mass transfer of PCB molecules in natural matrices.

Since PCB congeners in soil and sediments concurrently participate in several processes, the contribution of which depends significantly on their structure, it may be difficult to determine the degree of biodegradation, volatilization, or binding at decreasing PCB concentrations. The difficulties of studying PCB behavior in natural objects are aggravated by the great diversity of congeners and the difficulty of their chemical analysis. At present, very few works describe long-term observations of PCBs spiked into soil or sediments. Most conclusions of their persistence have been made on the basis of long-year field observations of naturally contaminated sites or short-term experiments with freshly contaminated samples.

At the current stage, the following concept of the change of PCB bioavailability and ability to volatilize in natural environments has been developed. After penetration into soil or sediment, PCBs are quickly adsorbed by the solid phase, primarily by the natural organic substance (humus) and hydrophobic particles of "black carbon" (soot-like carbonaceous particles of pyrogenic origin, incompletely decomposed plant residues, and weathered oil hydrocarbons). This adsorption results in incomplete detection of a substantial part of spiked preparations during the chemical analysis of samples almost immediately after introduction. Usually, quick physical binding of PCBs varies within the 5–30% range depending on the composition of congeners and may reach 10–50% at low concentrations (<1 mg/kg) [24, 30, 31]. In the course of "aging" (months), the degree of binding might increase somewhat due to the penetration of PCB molecules into finer pores of humus or soot-like particles. However, there is also the possibility of a reverse process of releasing the bound PCB residues, e.g., in the case of biodegradation of the binding structures. The quantitative and qualitative composition of these fractions varies significantly depending on the initial level of contamination, congener composition, pollutant concentration, and time. Usually two, and sometimes three, fractions of pollutants are distinguished: readily available, potentially available, and bound to the soil matrix; the latter is practically unavailable to plants, microbes, and other biota. The first two fractions are sometimes termed exchangeable and unexchangeable, or labile and unlabile. Their total amount is determined by analysis of PCB content in natural matrices by officially approved methods based on extraction by a mixture of solvents, most often acetone–hexane/methylene chloride. The fraction of bound PCBs can be extracted by a boiling solvent of aromatic nature, e.g. toluene [32]. The content of labile fraction in an individual sample can be determined by mild extraction with the mixture of water and alcohols or by passive extraction with water in the presence of floating grainy resin (TENAX, XAD, etc.). This fraction is in a state of near-equilibrium with the pore solution and, hence, is readily biodegraded under optimal conditions. However, at very low PCB concentrations, when the content of pollutant molecules in pore water falls below the threshold level, the synthesis of inducible enzymes and, consequently, microbial degradation stops. On the other hand, in heavily contaminated soils, either PCBs themselves or their transformation products may exceed the critical level of toxicity, which also impedes the processes of self-purification of environmental objects. The processes of biotransformation of these chemicals therefore usually begin after long lag periods, and biodegradation almost stops after reaching a certain threshold level. The optimal concentrations, at which comparatively rapid PCB biodegradation occurs in soil and sediments, both under anaerobic [11, 13, 33, 34] and aerobic (see Fig. 2) conditions, are within 10–60 to 500–1000 mg/kg; at lower and higher concentrations, the process decelerates substantially.

Many-year field observations of **river and marine sediments**, particularly deepwater ones, which are moderately contaminated by PCBs, have shown a slow decrease in the content of higher-chlorinated and accumulation of lower-chlorinated PCB congeners, mainly *ortho*-substituted. In laboratory conditions, this process begins after a long lag period (several months). Depending on the initial type of contamination and the medium conditions, the congeners with 4 to 7 chlorine atoms are dechlorinated and those with 1 to 3 and sometimes 4 and 5 chlorine atoms accumulate. In the course of this process, the molar PCB concentration remains practically unchanged [11]. However, in historically contaminated sediments with low PCB content, the share of the nonlabile fraction is usually very high, which strongly complicates their bioremediation. In sediments from the Gulf of Venice with a low PCB content (1.6 mg/kg), dechlorination started only after 5 months and was insignificant. On the contrary, about 90% of coplanar congeners with 4 to 6 Cl atoms specially introduced into these sediments (100 mg/kg in total) was transformed during the same period into congeners with 1 to 4 Cl atoms. The introduced congeners did not accelerate dechlorination of the previously present PCBs [13]. The degree of dechlorination of Arochlor 1242 introduced into pure sediments from the Hudson River (Michigan, United States) at 700 mg PCB/kg was high as well. After four months, 87% of the major tetra- and penta-PCB was converted into mono- and diCB, although it was impossible to achieve microbial dechlorination of all PCBs [11].

In soil and other aerobic environments, on the contrary, low-chlorinated congeners (mono-, di-, and triCB) are the most effectively degraded by microorganisms and are partially volatilized, while higher-chlorinated congeners are more adsorbed and become less mobile. During the first 1–3 months after PCB introduction into open systems, about a half of less

chlorinated PCBs (with 2–5 chlorine atoms) can volatilize from the topmost soil layer (1–5 cm), but later on the soil consolidates, and so the actual contribution of volatilization to a decrease in PCB concentration is insignificant. This finding has been demonstrated, for example, in vegetation experiments in a wide range of PCB concentrations: 90–4200 mg/kg [35].

Under optimal conditions, biodegradation of PCB mixtures in aerobic environments starts after a short lag period (1 to 2 months) and initially proceeds comparatively quickly. The T_{50} of different homologous groups under optimal conditions is 1 to 10 months. High-chlorinated PCBs are degraded together with low-chlorinated ones. However, as the degree of chlorination increases, the rate and degree of degradation noticeably decrease, although the persistence of isomers within homologous groups may also significantly differ. After PCB concentration decreases 2–5 times, degradation as a rule abruptly slows down. According to the expert estimates, T_{50} of the aged PCB residues present in natural soils at low concentrations (<1–5 mg/kg) is 3–10 years or more depending on the homologous group [36]. In a field experiment with three types of soils (C_{org} 1.1; 2.1; and 21%) under typical conditions of Great Britain, T_{50} of disappearance of the five spiked PCB congeners with 3 to 7 chlorine atoms (0.3 mg/kg in total) varied within 4 to 29 months; for the aged pollutants (a year after introduction), this parameter was 6 to 280 months (0.5–23 years) depending on the homologous group [37]. In most of the experiments with ^{14}C-labeled lower-chlorinated congeners spiked into soil at low concentrations (1–2.5 mg/kg), the indigenous soil microflora oxidized PCB molecules only partially under normal conditions. The resulting reactive products (mainly mono- and dihydroxy derivatives) were quickly bound with soil humus, and the mineralization was <1% [24, 30].

The persistence of high PCB concentrations in soil is also very long. In our microfield experiments with historically contaminated soil taken near the Kondensator plant in Serpukhov (1900 and 4100 mg PCB/kg, major congeners with 3 to 5 chlorine atoms), the content of chemicals decreased by only 26–45% in 3.5 years, mainly due to the degradation of triCB and, to some extent, tetraCB [32]. In the work of Fava et al. [38], the content of Arochlor 1260 aged in soil (980 and 9500 mg PCB/kg) decreased by only 2 and 6%, respectively, after 5 months of incubation in solid and slurry bioreactors.

Thus, in order to accelerate PCB biodegradation in historically contaminated soil, it is necessary to create the optimal conditions for activation of microbial PCB destructors and to solve the problem of the optimal ratio of accessibility of pollutants and concurrent decrease of their toxic effect on microflora and plants.

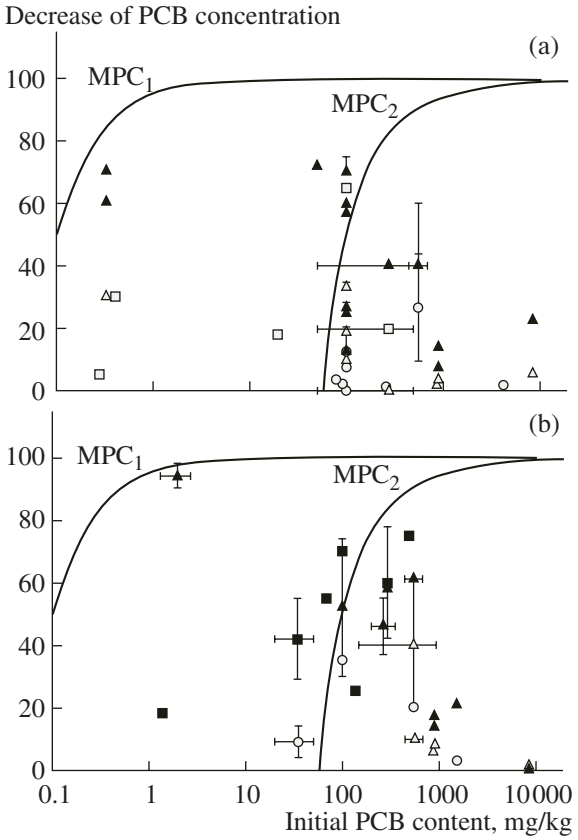

Fig. 2. Decrease of the total PCB content observed by different authors in natural and anthropogenic matrices in the course of experiments on bioremediation in solid media (a) and in slurry bioreactors (b) under aerobic conditions (in the control, under stimulation of indigenous specific microflora, △; at inoculation of microorganisms with different additives, ▲; under anaerobic–aerobic conditions in bioreactors, ■; at phytoremediation, □). The curves represent the relation between the initial level of contamination and the necessary decrease of PCB content for reaching the minimal and maximal permissible levels (MPC_1 = 0.05 and MPC_2 = 50 mg/kg, respectively). Initial data are taken from original sources [20, 24, 26, 28, 29, 32, 34, 35, 37, 38, 43–45, 47, 51, 57, 59–61, 64].

BIOREMEDIATION OF SOILS, SEDIMENTS, AND SEWAGE SLUDGE

The methods to enhance the efficiency of PCB biodegradation in solid natural matrices have been studied mainly under laboratory conditions in solid and slurry bioreactors and in microfield (pilot) experiments. The dozens of works published so far consider the possibilities of anaerobic, aerobic, and anaerobic–aerobic bioremediation of PCB-contaminated natural environments. These works focused primarily on the methods of biostimulation of specific indigenous microflora that can co-metabolize PCBs by means of introduction of specific inducers and optimization of the media conditions. In some studies, increase of the quantity and diversity of PCB destructors or reinforcement of their degradative capacities were achieved by bioaugmenta-

tion, i. e., by inoculation of the native microbial destructors obtained by the method of enrichment cultures or genetically engineered strains (separately or in mixture). Particular attention was paid to the increase of PCB accessibility to microorganisms and decrease of their toxic effect on microorganisms and other biota.

Anaerobic Bioremediation of Sediments

The technologies of in situ anaerobic bioremediation were developed mainly to reduce contamination of the sediments of rivers, lagoons, and other reservoirs in situ. Although anaerobic dechlorination does not result in complete degradation of xenobiotics, it still contributes to the detoxification of the environment due to formation of less toxic and less cumulative products [12].

Activation of indigenous PCB dechlorinators. Laboratory research into anaerobic bioremediation of PCB-contaminated sediments was carried out in anaerobic boxes or under thick water layers. ***The optimal temperature, pH, salinity, and presence of biogenic elements*** varied within a wide range. In the Northern hemisphere, dechlorination in freshwater and marine environments starts at 12°C, reaches its highest rate at 25°C, and abruptly slows down at 37°C [11, 12]. The optimal acidity of the medium is close to neutral (pH 6–8), and the process decelerates abruptly at pH ≤ 5 and pH ≥ 9; some macro- and microelements play a certain role in the process [11, 35].

In laboratory studies, intensive PCB dechlorination in sediments started after a lag period under ***strictly anaerobic (methanogenic) conditions***, i.e. at the redox potential of < –400 eV; the rate and completeness of the process depended on the rate of the redox potential decrease [11, 39]. For example, in submerged sediment, dechlorination of the introduced Arochlor 1248 started after one month, while in the water-logged sediments, only after three months, when the numbers of methanogenic bacteria increased by three orders of magnitude. During the next 12 months, the average chlorine content in the PCB molecules decreased from 3.9 to 3.2 in the former case and only to 3.6 in the latter case [40].

PCBs and their brominated analogs are ***inducers*** of dechlorination, provided that they are present in optimal concentrations. The relation between dechlorination rate and the concentration, composition, and duration of contamination was discussed in the previous section of this review. Dehalogenation of PCBs was also accelerated when ***nonspecific inducers*** (electron acceptors and/or donors) were added. However, the composition and quantity of these additives should be adjusted individually depending on other environmental conditions. Since no strict rules presently exist for the selection of these parameters, the optimal concentrations of inducers are usually selected empirically. For example, sulfate heavily inhibited PCB dechlorination in sediments, but $FeSO_4$ reduced the toxicity of the formed sulfides due to their precipitation as FeS. As a result, after sulfate was consumed completely, the process intensified and dechlorination of *meta*- and *para*-chlorine was more extensive [11, 12].

The electron/hydrogen donors may be easily utilizable carbon sources, e.g. glucose, fatty acids (lactate, pyruvate, acetate, formate, etc.), alcohols (methanol, ethanol), as well as zero-valence iron and hydrogen. The introduction of these additives, however, only shortened the lag period or accelerated the initial process of dechlorination and had no effect on the final result. The introduction of additional carbon sources seems to be justified only in the sediments deficient in organic matter [11, 39]. Introduction of Fe^0 at low concentrations (0.1 g/kg) and continuous feeding of H_2 (0.001 atm) into the microcosms with sediments resulted in the shortening of the lag period of degradation of di-*ortho*-substituted PCBs by 100 days [41].

Bioaugmentation of PCB-contaminated sediments was suggested to improve the dechlorinating capacity of indigenous microflora by the introduction of microorganisms with the complementary dechlorination pathways which were originally lacking in the sediments. This also led to a drastic increase of the quantity of dechlorinators [12]. However, in most cases the contribution of inoculated bacteria was minor due to the medium toxicity or to other aspects of the medium composition. The most effective results were obtained with the above described anaerobic granules, which were spiked into heavily contaminated sediments (10% of the volume) together with carbon sources (glucose, organic acids, alcohols, or ground wood). After eight months of incubation of historically PCB-contaminated sediments (920 mg/kg) in anaerobic bioreactors under a thick water layer at 12 or 22°C, the biggest part of congeners with 3–7 Cl atoms (50 to 90%, depending on the homologous group) was converted into mono- and di-CB, whereas dechlorination in the control was insignificant [26]. In the presence of anaerobic granules, 70% of Arochlor 1254 (250 mg PCB/kg) introduced into pure sediments from Lake Michigan (United States) was dechlorinated after six months. At the same time, the concentrations of congeners with 2, 4, 5, 6, and 7 Cl atoms decreased by 100, 14, 75, 81, and 78%, respectively, and only the triCB concentration increased. Complete dechlorination (up to BP formation) of some low-chlorinated congeners under these conditions has been demonstrated [26]. Although the application of anaerobic granules acclimated to PCBs shortens the lag period, economically it is more profitable to use a mixture of PCB-nonacclimated commercial granules obtained from bioreactors of different treatment facilities [28].

At present, the efficiency of bioaugmentation of sediments has been demonstrated only under bioreactor conditions, while experimental introduction of the biomass of PCB-dechlorinating microorganisms into the Hausatonic River (United States) under field conditions

produced no desirable results [11]. High volumes of anaerobic granules required for bioaugmentation make unlikely their application in situ.

Aerobic Bioremediation of Soils and Other Natural Environments

In the recent 10–15 years, high emphasis has been placed on search for the methods of intensification of this process in soil and other natural environments. Some aspects of aerobic bioremediation of PCB-contaminated objects are considered in reviews [1, 9, 10], but a number of conclusions have been based on detailed analysis of the original sources mentioned in this section.

Activation of indigenous destructor microorganisms. For aerobic bioremediation of natural objects contaminated by many organic chemicals, the ***optimal hydrothermal conditions, acid–salt and oxygen regimes, as well as composition of the main macro- and microelements***, are rather close; these conditions can be achieved by techniques similar to those described in detail in [42]. For maximal oxygenation during land farming, addition of a loosening material to soil followed by regular plowing, the mixture in bioreactors is bubbled with air and intensively agitated or repeatedly supplemented with hydrogen peroxide [38, 43]. The known destructors of PCB (and of many other pollutants) are usually mesophiles; their optimal growth conditions are in the range of 22–35°C and 60–70% of the total field water capacity [42]. However, some psychrotolerant bacteria are known to carry out PCB degradation in Arctic soils at lower temperatures: 3 to 20°C [37]. The conditions required for optimal functioning of most degrading microorganisms include near-to-neutral reaction of the medium (pH 6.0–7.5), an optimal level of salts, which does not create high osmotic pressure in the pore water, and an adequate supply of macro- and microelements. Additional introduction of mineral sources of nitrogen, phosphorus, and potassium noticeably accelerated PCB biodegradation in some soils and sediments [38].

As in the case of utilization of commercial mixtures, ***inducers*** of aerobic degradation by PCB degraders in soil and other matrices, were most often BP and more degradable brominated PCB analogues or their degradation products, chlorobenzoates [1, 5, 9, 10]. The introduction of BP into soil, particularly repeated "feeding" with moderate doses of BP, significantly accelerated not only PCB transformation but also mineralization of the biphenyl skeleton. For example, incubation of soil supplemented with ^{14}C-Arochlor (100 mg/kg) resulted in formation of 37% $^{14}CO_2$ in the presence of 0.33% BP, as compared with 2% in the absence of BP. The formation of bound ^{14}C was also higher in the presence of BP than in control (37 and 26%, respectively), which indicates the key role of BP degraders in mineralization and formation of covalently bound PCB residues; at the same time, they need an inducer for activation [31].

Nontoxic plant inducers, e.g. terpene, when added to soil, also supported the growth of indigenous PCB degraders and enhanced the survival of the inoculated strains. This fact was evidenced by the 10–100-fold increase of the amount of the PCB-degrading strain *Pseudomonas pseudoalcaligenes* KF107 bearing the inserted fluorescent gene in the presence of terpene, as compared with the control soil samples without inducers [44]. However, a noticeable effect on bioremediation degree could be achieved only at repeated introduction of terpene, which may be due to its low bioavailability in soil [45]. At the same time, composting of the soil with plant resides that contain large amounts of terpenes (orange skin, pine needles, etc.) significantly increased the quantity of BP degraders and accelerated PCB degradation [5, 10].

PCB bioavailability in solid media can be enhanced in several ways. To increase the contact of microorganisms with pollutants, bioremediation was carried out under maximal dispersion and agitation of soil, which was best achieved ***under conditions of a slurry bioreactor***. In the studies of Fava et al. [38], bioremediation of historically or intentionally PCB-contaminated and aged soils of Italy in a slurry reactor was 1.5 to 3 times more rapid than in a solid phase reactor.

The most popular trend is ***application of surfactants*** for changing PCB bioavailability; this issue is discussed in the review [46]. The effect of both chemically synthesized nonionic surfactants (Twin, Triton X-100, etc.) and biosurfactants of plant origin (lipopeptides, maltose ethers, and saponins) has been studied. Though the cost of chemical surfactants is much lower, their toxic effect on cells may decrease the rate of PCB biodegradation. A good effect was obtained upon application of a comparatively inexpensive commercial preparation RAMEB (technical mixture of irregularly methylated β-cyclodextrines), which is produced biologically. It is nontoxic for microflora and is slowly metabolized in soil [38]. Incubation of moderately contaminated soil (890 mg PCB/kg, mainly with 4–6 Cl atoms) in solid and slurry reactors with background introduction of 0.4% BP and 0.5 to 1% RAMEB for six months resulted in the degradation of 14 to 17% PCB as compared with 2 to 6% in the control. In the presence of cyclodextrines, the quantity of PCB degraders noticeably increased and the phyto- and biotoxicity of soil decreased. At the same time, the introduction of RAMEB into heavily contaminated soil (9500 mg PCB/kg) had a certain positive effect only in solid phase reactor, whereas degradation in slurry reactor, on the contrary, slowed down [38], probably due to the increase of the PCB toxic effect on the microflora.

The solubility and bioavailability of PCBs in soil and sediments can increase under the influence of ***biosurfactants*** (rhamnolipids etc.), which are usually accumulated in nature in the course of metabolism of

hydrocarbon-utilizing microorganisms. The deficiency of N and P in soil resulted in more intensive accumulation and long preservation of rhamnolipids (at least, up to 70 days); biosurfactants, however, were quickly degraded after depletion of readily available organic from the medium [47]. Incubation of sandy soil contaminated by PCBs and mineral lubricating oils (1200 and 11400 mg/kg, respectively) with minor additions of ammonium phosphate in a slurry bioreactor resulted in rapid accumulation of rhamnolipids: up to 1 g/l (i.e. 20 times higher than the critical micellar concentration). As a result, after six days of incubation the PCB content in the medium decreased from 1200 to 14 mg/kg due to PCB emulsification in the slurry, although the actual degradation of PCBs was insignificant [48]. However, the presence of aged oil and petrochemicals in soil may retard bioremediation, because the capacity of oil residues in soil to bind PCBs is very high, even higher than that of pyrogenic particles (soot, activated carbon, etc.) [49].

Bioaugmentation of soil and other objects in the course of their bioremediation was studied in a number of works, yielding both encouraging and negative results. The low efficiency of specific microorganisms amendment was explained by the presence of a barrier formed by the microflora of the indigenous community, by the absence or low content of inducers, and by the limited availability of nutrient elements and/or PCBs, as well as by the inhibiting effect of toxic compounds including toxic PCB metabolites. In most cases, the quantity of PCB degraders introduced without additional BP inducers decreased by several orders of magnitude after 2 to 4 weeks. Therefore, specific microflora was inoculated into soil, as a rule, the presence of BP or other inducers of expression of the BP genes, and sometimes together with surfactants or surfactant-forming microbial strains.

The following laboratory experiments can be mentioned as positive examples:

Inoculation of native strains (the mixture of the PCB-degrading strain *Pseudomonas testosteroni* B-256 and the surfactant-producing, hydrocarbon-degrading strain *Alcaligenes faecalis* B-556, 10^8 cells/kg of each) on the background of regular introduction of minor BP doses (0.01%) resulted in a 25% decrease of the concentration of the spiked Arochlor 1242 (100 mg/kg) after three weeks, as compared to a 16% decrease without inoculation. However, the degradation of the preparation slowed down one month after the disappearance of the major part of di- and triCB [50].

Inoculation of the mixture of *gfp*-transformed psychrotolerant *gfp* strains (*Pseudomonas* sp. Cam-1-*gfp*1 and Sag-50G-*gfp*1 bearing the gene of green fluorescent protein) into soil microcosms with subsequent incubation at 4 and 22°C resulted in the degradation of 59–75% of 2,3-diCB (C_0 = 50mg/kg), while the cell number in soil remained at a level of 10^6–10^8 cells/g for 4–5 months [51].

In a liquid medium without BP but in the presence of yeast extract (0.01%), strain *Janibacter* sp. MS3 degraded all Arochlor 1242 congeners (10 mg/l) by 70–100% after 7 days. When it was introduced into soil contaminated with the same preparation (1 mg/kg), only diCB, partially triCB (0 to 60%, depending on the congener), and a very small portion of tetraCB were degraded [52].

Considerable positive effect was achieved by repeated introduction into soil of BP-degrading strains *Arthrobacter* sp. B1B and *Ralstonia eutrophus* H850 (10^6 cells/g each) together with terpene, salicylic acid, and surfactant sorbitol trioleate (0.01–0.05% in total). It provided a 55–59% decrease of the concentration of freshly spiked Arochlor 1242 (100 mg/kg), while the indigenous microflora with the same additions degraded 30–36% of the PCB [45].

Certain progress was made at ***introduction of microbial consortia*** of PCB-cometabolizing and CBA-mineralizing bacteria [5, 9, 10, 38]. The use of constructed organisms and/or CBA degraders enhanced not only the degradation but also the mineralization of PCBs in model systems. However, in most cases, the effect of the introduction of these strains into soil was not very high, which is explained by the poor survival of these microorganisms in the environment and low availability of the pollutant. Besides, the use of genetically modified organisms is strictly limited in many developed countries. For example, in the United States only one strain, *Pseudomonas fluorescens* HK 44 bearing the plasmid of naphthalene degradation and the bioluminescence gene *lux*, has been approved for use in bioremediation [16].

A positive effect was also achieved by the introduction into soil of ***a native consortium of nonspecific microorganisms*** (bacteria, fungi, etc.) formed in the course of utilization of municipal waste and containing BP and CBA degraders. One of these commercial preparations, Enzyveba, was used for bioremediation and reduction of toxicity of soils historically contaminated by Arochlor 1248 and 1260 (920 mg/kg). Its application increased the quantity of heterotrophic and specific bacteria in soil by several orders of magnitude and resulted in removal of nearly all diCB and 75% of triCB in one month. However, the total effect for all congeners was no more than 3 and 8% in solid phase and slurry bioreactors, respectively [53].

At present, very few studies have been carried out with historically contaminated soil under field conditions or in laboratory conditions close to the field ones [10]. Among these, the study of the possibility of stimulation of aerobic PCB degradation in historically contaminated sediments of the Hudson River, with prevailing mono- and diCB (the products of many-year dechlorination of Arochlor 1242), is of particular interest. The sediments at the river bottom (3 m from the shore, 1 m deep) were enclosed in steel cylinders 1.8 m in diameter (total height, 5 m) the interior of the cylin-

ders was supplemented with nitrogen, phosphorus, and potassium (NPK) salts, H_2O_2, and biphenyl. Aerobic conditions, introduction of BP (0.05%) and salts stimulated the accumulation of indigenous microbial degraders. As a result, after 2.5 months the PCB concentration decreased by 37–55% (from 20–50 to 12–19 mg/kg) as compared with 4–14% in untreated control variants. The rate of PCB degradation by indigenous microorganisms was maximal after 2–4 weeks, when temporary accumulation of monoCB and minor amounts of diCB in the water was registered. At the same time, additional augmentation of some of the sediments by the PCB-degrading strain H850 proved ineffective. The quantity of introduced bacteria decreased from 10^8 to 10^5 cells/ml during the first 10 days, and their contribution to PCB degradation was insignificant [43].

Sequential Anaerobic–Aerobic Bioremediation of Natural Environments

Since only highly chlorinated PCB congeners are most effectively dechlorinated under anaerobic conditions, while their transformation products (mainly *ortho*- and *para*-substituted mono-, di-, and some of triCBs) can be effectively degraded by BP degraders [54], it was suggested that sequential processing of soil or sediments first in anaerobic and then in aerobic conditions would solve the problem of bioremediation of high-chlorinated PCBs. This concept was first developed by J. M. Tiedje (Michigan State University, United States). His laboratory carried out field tests in the state of Mississippi (United States) in order to stimulate the development of indigenous anaerobes in soil. However, six-month flooding of soil in situ with additions of readily available organic compounds did not result in dechlorination of the PCB present there. Low pollutant concentrations in soil solution and/or a longer period required for microflora acclimation were among the main obstacles to microbial dechlorination [5]. Somewhat greater progress was made under greenhouse conditions by Zeeb et al. [35], who studied phytoremediation of the soil weakly contaminated with PCBs (20 mg/kg) under the thrice-repeated cycle of marsh plants cultivation (sedge, cane, etc.) for 3.5 months in water-logged soil with additions of easily available carbon and nitrogen sources followed by a one-month aerobic period. After a year and a half of such treatment, the concentration of aged Arochlor 1260 decreased by 17–18%. A higher degree of degradation of PCB homologues in the row of pentaCB < hexaCB < heptaCB and the positive effect of additions of readily available carbon (straw, starch) and nitrogen provided indirect evidence of the role of PCB dechlorinators in this process [55].

Dechlorination of Arochlor 1260 in arctic soils of Canada was stimulated by inoculation of anaerobic river sediments adapted to chloroorganic pollutants. In laboratory experiments, PCB dechlorination in inoculated flooded soil at 30°C started when methanogenic conditions were established (in 2 months). In the next two months of incubation at 21°C, the average chlorine content decreased from 6.7 to 5.1 in the samples with freshly spiked Arochlor 1260 (100–106 mg PCB/kg) and from 6.5 to 4.6 in the samples with aged PCBs [56]. In other studies, the sediments specially contaminated by Arochlor 1242 (70 mg/kg) were maintained for one year under anaerobic conditions in the presence of an acclimated anaerobic consortium from river sediments. A bacterial consortium comprising two recombinant strains, LB400(ohb) degrading *ortho*-chlorinated PCB congeners and *Rhodococcus* sp. RHA1(*fcb*) growing on 4-CBA was then introduced (10^4–10^6 cells/g each). Subsequent incubation in aerobic conditions for one month resulted in 54–57% PCN degradation [57].

In Gary (United States), the US EPA used the anaerobic–aerobic method for bioremediation of the sediments stored on a special testing ground. They were regularly ploughed after the introduction first of anaerobic sludge from municipal treatment facilities and then of aerobic PCB degraders. After 4–9 months, the content of PCBs decreased by 75% at 500 mg/kg and by 25% at 140 mg/kg [58].

The possibility of anaerobic–aerobic bioremediation of the PCB-contaminated sewage sludge excavated from municipal treatment facilities after ten-year storage on special landfill was studied in municipal treatment facilities. The initial of priority PCB congeners with 4–7 chlorine atoms in this sludge was 1.7 mg/kg. Incubation of the 3.2% suspension of this sludge in laboratory anaerobic bioreactors for 40 days at 35°C resulted in dechlorination of about 40% of priority PCBs. Their subsequent incubation in an aerobic bioreactor at 22°C under daily feeding of readily available organic compounds (0.1% of solution weight) resulted in a decrease of the total PCB concentration in the sludge to 1.4 mg/kg (with complete degradation of tetraCB). Nevertheless, current limit value in France (0.8 mg PCB/kg), which permits the application of sludge as an organic fertilizer for arable lands, was not achieved [59]. Apparently, complete purification of weakly contaminated sewage sludge requires more intensive treatment at the second stage. This was indicated by the results of Japanese researchers obtained by incubation of sewage sludge in bioreactors under regular introduction of strains *Commamonas testosterone* TK102 or *Rhodococcus opacus* TSP203 and BP; such treatment resulted decrease of PCB concentration (mainly of tri and tetraCB) from 1.4–2.7 to 0.05–0.14 mg/kg (by 90–98%) in 2 months [60].

Rhizoremediation

The possibility of using plants for purification of PCB-contaminated soils is considered in review [7] and in some recent publications. Although some plants can accumulate considerable amounts of PCBs (e.g., the roots and stems of zucchini, up to 6700 and 470 mg/kg), no noticeable decrease of Arochlor 1260

concentration in moderately and heavily contaminated soils (90–4200 mg/kg) due to phytoextraction was observed [35]. Much better results were obtained in greenhouse experiments in soil with aged Arochlor 1260 (20 mg PCB/kg). In a year and a half, after three cycles (3.5-month growing of wetland plants in waterlogged soil supplemented with C and N with a subsequent one-month aerobic period), PCB concentration decreased by 17–18%. The involvement of anaerobic dechlorinators in PCB transformation was confirmed by the increasing degree of disappearance in the order of pentaCB < hexaCB < heptaCB; addition of readily available carbon (straw, starch) and nitrogen [55].

Rhizoremediation is one of the most promising lines of development of techniques for biological soil purification from PCBs and other stable pollutants. Its basis is as follows: the major part of soil microflora is concentrated in the plant rhizosphere, and the relative quantity of PCB and particularly CBA degraders among rhizosphere microorganisms is much higher than in the remaining volume of planted and especially unplanted soil. PCR has demonstrated that genome DNA of rhizobial strains possess a genetic potential similar to that of strain *C. testosteroni* B-356 in regard to its ability for BP degradation [7]. The enhanced activity of PCB degraders in the plant rhizosphere is associated with the presence of inducers of PCB-degrading enzymes in root exudates. The most active BP inducers are phenylpropanoids (flavonoids, etc.), which are excreted by both living and dead plant roots. Alfalfa, black nightshade, and particularly tobacco grown on a soil with a long-time PCB contamination have a positive effect on the rate of PCB degradation; in the latter case, 34% more PCBs was degraded than in the unsowed control [61]. The potential of microbial PCB degradation in soil increased in the presence of the root system of not only various grasses but also trees [7].

The rhizosphere was supposed to be an ideal place for penetration of PCB-degrading strains, and the most successful of these must be strains that can easily colonize plants. The strain able to colonize the alfalfa rhizosphere was used as a basis for constructing the PCB-degrading strain *Pseudomonas fluorescens* F113L:1180. Its inoculation into soil contaminated by Delor 103 gave better results as compared to non-inoculated soil or to the soil inoculated with strain LB300 incapable of rhizosphere colonization [62]. Moreover, the plasmid of PCB degradation pE43 was transferred into a strain of the root nodule bacterium *Sinorhizobium meliloti*. Its inoculation together with the seeds of symbiotic alfalfa plants contributed to accelerated degradation of 2,3',4-triCB in soil. Under conditions of a growth chamber in 1.5 months after contamination and alfalfa sowing, the concentration of 2,3',4-triCB decreased from 0.33 to 0.24 mg/kg in the case of alfalfa treatment with the wild type strain of *S. meliloti* and to 0.11 mg/kg in the case of treatment with a GMO (genetically modified) strain (i.e., for 30 and 60%, respectively). The concentration of introduced 2,3',4-triCB decreased still more effectively (to 0.09 mg/kg) in the soil seeded with alfalfa seeds pretreated with a mixture of unidentified indigenous PCB degraders, which contained root nodule bacteria [63]. However, the possibility of rhizoremediation of heavier contaminated soils has not been proved as yet.

CONCLUSIONS

Figure 2 presents the generalized results obtained by different authors during the study of bioremediation of soils, sediments, and bottom deposits contaminated with PCBs. The figure shows that the degree of purification of soil, sediments, or sewage sludge strongly depends on the initial level of contamination and on purification conditions. The best results were obtained at aerobic bioremediation of moderately contaminated soil (50 to 700 mg PCB/kg), when PCB concentration decreased by 40–75%. The PCB content significantly decreased only at repeated inoculation of a bacterial consortium of CBA and PCB degraders (isolated or constructed) together with surfactants and biphenyl as an inducer. The efficiency of purification in slurry bioreactors was generally 10–20% higher than in solid phase reactors. As a rule, the concentration decreased mainly due to the degradation of congeners with 1–3 and, to a lesser extent, 4 chlorine atoms. In case of higher-chlorinated PCB preparations, noticeable purification could be achieved only by anaerobic–aerobic treatment of samples for several months. However, the permissible level for technogenic soils could be achieved only in rare cases, mainly with freshly spiked PCBs in moderate concentrations; in only one case of purification of weakly contaminated sewage sludge (1–3 mg PCB/kg), the concentration of PCBs was reduced to a level permitting its application as a biofertilizer. Thus, the method of bioremediation of PCB-contaminated environmental objects still requires significant improvement. There are certain prospects of increasing the effect of bioremediation by the application of anaerobic granules for transformation of high-chlorinated PCBs, by isolation or construction of aerobic strains of PCB and CBA degraders; the search for appropriate nontoxic inducers of the BP genes and surfactants improving the availability of PCBs in historically contaminated objects is also promising. Certain hopes are pinned on rhizoremediation. At the same time, the use of bioremediation for purification of heavily PCB-contaminated soils and sediments is unlikely as of yet. Probably, it will be possible to solve this problem in future by way of introduction of sorbents (activated carbon, etc.) that significantly reduce the toxicity of these soils [32].

ACKNOWLEDGMENTS

The authors are grateful to Prof. J.J. Ortega-Calvo, P.J. Shea, and J.P. Schwitzguebel for their assistance,

and to the Ministry of Industry and Science of Moscow oblast and Russian Foundation for Basic Research (grant 04-05-97203) for financial support.

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