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Review

Polychlorinated biphenyls and their biodegradation

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Abstract

Polychlorinated biphenyls (PCBs) are stable organic molecules that were widely used during 1930s and 1940s. Because of their widespread use, PCBs have entered the environment through both legal and illegal use and disposal and are persistent in the environment contaminating various environmental matrices worldwide. The environmental persistence of PCBs results primarily from the inability of natural aquatic and soil biota to metabolize the compound at a considerable rate. Several studies have been conducted on PCBs biodegradation to determine how the degradation rate can be improved. This paper is a review of literature and studies on the biodegradation of PCBs. Studies show that there are two biologically mediated PCBs degradation processes: anaerobic and aerobic. The anaerobic process removes chlorine atoms of highly chlorinated PCBs, which are then mineralized under aerobic condition. The degradation route is dependent on the complexity of the PCB congener coupled with the type of microorganism employed and the interaction among the microorganisms.

Keywords: Biodegradation; Anaerobic transformation; Dechlorination; Metabolic pathway; Oxidative degradation; Polychlorinated biphenyls

Contents

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1. Introduction

Polychlorinated biphenyls (PCBs) are organic chemicals with characteristics similar to that of DDT [1]. They were produced commercially by catalytic chlorination of biphenyls producing a complex mixture of multiple isomers with different degrees of chlorination yielding up to 209 products called congeners [2]. PCB congeners with the same number of chlorine atoms are known as homologs, and the homologs with different chlorine positions are called isomers.

Polychlorinated biphenyls (PCBs) were produced from 1930s, with particularly large volume to 1970s [3], producing a complex mixture containing 60–90 congeners [4]. They were used in a wide range of industrial applications because of their excellent physical and chemical properties. PCBs had been marketed in the US under the trademark Aroclor. Aroclor is identified by a four-digit number; the first two digits represent the number of carbon atoms and the last two digits represent the percent chlorine in the mixture. Other international trade names include Kaneclor (Japan), Fenclor (Italy), Pyralene (France), and Clophen (West Germany) [4]. Between 1930 and 1979, over 600 million kilograms of PCBs were used in North America alone [5], 15% of which entered the environment through legal and illegal use and disposal [6] and accidental releases [7]. Since they are highly resistant to degradation, they remain in soils and bodies of water for many years, and because they are liphophilic in nature, they bioaccumulate in cells and are passed up the food chain.

Concerned over the impact of PCBs on the environment and their persistence; their manufacture, use and importation was banned in Sweden (1970) and Japan (1972). In the US, the manufacturing, processing and distribution of PCBs were banned under the 1976 Toxic Substances Control Act. Electrical applications of PCBs have been phased out, and stringent requirements for handling, storage and disposal have been specified. More recently, the Chemical Treaty on Persistent Organic Pollutants (POPs) lists PCBs as priority chemicals for eventual elimination by 2025 [8].

The environmental persistence of PCBs results primarily from the inability of natural soil and aquatic biota to degrade the compound at a significant rate. Studies on the biodegradation of PCBs show that the compound can either be transformed to less toxic substances or mineralized. There are two biologically mediated processes for the degradation of PCBs: anaerobic reductive dechlorination and aerobic oxidative degradation [6]. This paper reviews related literature and studies on PCBs and their biodegradation.

1.1. Properties of PCBs

Polychlorinated biphenyls (PCBs) are made up of a biphenyl nucleus with 1–10 chlorine atoms having a chemical formula of $C_{12}H_{10-n}Cl_n$. The basic structure of PCBs is given by Wiegel and Wu [9] and is shown in Fig. 1.

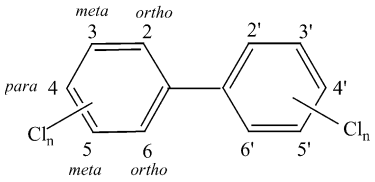

Fig. 1. Structural formula of PCB (Wiegel and Wu [9]).

Their manufacture produces a mixture of compounds with molecular weight ranging from 188 to 439.7 depending on the number of chlorine atoms attached to the biphenyl ring. Toxic congeners carry between 5 and 10 chlorine atoms, mostly in the *para*- and *meta*-positions [10], however, the congener substituted at the 3,4-*ortho* positions are considered the most toxic [11].

The properties of each PCB congener are dependent on the degree of chlorination. The industrially produced PCBs have properties ranging from highly mobile liquids that are colourless and oily to more viscous and increasingly darker liquids, to yellow and then black resins. Lower chlorinated PCBs (the mono-, di-, tri-, and tetra-chlorinated PCBs) tend to be colourless oily liquids. Pentachlorobiphenyls are heavy, honey-like oils. The most highly chlorinated PCBs are greases and waxy substances. Flash points can be as low as 140 °C to 200 °C; however, most have no flash points at all as measured by standard test. The vapour is invisible and has a characteristic strong odour [12].

PCBs are poorly soluble in water but extremely soluble in oils and fats. Their solubility in water decreases with increase in the degree of chlorination. It ranges from 6 ppm for monochlorobiphenyl to 0.007 ppm for octachlorobiphenyl. Decachlorobiphenyl, although it has higher chlorine content, has solubility twice that of octachlorobiphenyl. The solubility also varies among congeners of the same number of chlorine atoms [13].

The properties of PCBs that made them valuable for industrial applications include thermal stability, chemical inertness, non-flammability, high electrical resistivity or high dielectric constant and low acute toxicity [14].

1.2. Uses of PCBs

For several decades PCBs were used extensively in a wide range of industrial applications such as: oil in transformers, dielectrics in capacitors, hydraulic fluids in hydraulic tools and equipment and heat exchange liquids. PCBs also found widespread use as lubricants for turbines and pumps, in the formulation of cutting oils for metal treatment, and, to a lesser extent, in applications such as plasticizers, surface coatings, adhesives, pesticides, carbonless copy paper, inks, dyes, and waxes [15].

1.3. Sources of PCBs

There are no known natural sources of PCBs. They persist in the environment and are found in air, water, soil, and food.

PCBs entered the air, water, and soil during their manufacture, use, and disposal; from accidental spills and leaks during their transport; and from leaks or fires in products containing PCBs. PCBs can travel long distances in the air and can be deposited in areas far away from where they were released [16]. Municipal waste combustion, hazardous waste incineration, and medical waste incineration account for a significant portion of PCB emissions to air. Additional sources of PCB emissions include treatment, storage, and disposal facilities and landfills; hazardous waste sites; steel and iron reclamation facilities (e.g., auto scrap burning); accidental releases (PCB spills and leaks, and transformer fires); and environmental sinks of past PCB contamination [17].

In water, PCB concentrations are generally higher near human activity and near shorelines. The major source of PCBs in surface waters is from environmental cycling (i.e., from sediment, air and land). Sediments at the bottom of a water body can act as a reservoir from which PCBs can be released in small amounts to water. PCBs in fish can be hundreds of thousands of times higher than in water because they accumulate in the fish.

Polychlorinated biphenyls (PCBs) attach strongly to soil and may remain there for several years. Environmental cycling is suspected of being the current major source of PCBs in soil outside of disposal and spill sites [16]. Another source of PCB exposure is the workplace. Workplace exposure can occur during repair and maintenance of PCB transformers, accidents, fires, spills, or disposal of PCB-containing material by breathing contaminated air and touching materials containing PCBs. Old appliances and electrical equipment are also believed to be the primary source of household contamination, since they may contain PCBs. PCB levels in indoor air are often much higher than in outdoor air.

1.4. Health and environmental effects of PCBs

Polychlorinated biphenyls (PCBs) have low-to-moderate toxicity. Treated samples of animals show an LD_{50} ranging from 0.5 g/kg to 11.3 g/kg of body weight [18]. Most of the effects are the result of repetitive or chronic exposure.

Polychlorinated biphenyls (PCBs) are absorbed by humans and animals through the skin, the lungs, and the gastrointestinal tract [19]. Once inside the body, they are transported through the blood stream to liver, to various muscles and adipose tissue where they accumulate. Research shows that PCBs cause a variety of adverse health effects depending on the route of exposure, age, sex, and area of the body where PCBs are concentrated. Studies on animals show conclusive evidence that PCBs are carcinogenic. Animals that ate food containing large amount of PCBs for short periods of time had mild liver damage and some died. PCBs have also been implicated as a cause of mass mortalities in seabirds [20]. Environmental concerns over PCBs first surfaced in the late 1960s, some 30 years after PCBs were introduced. A Swedish scientist found eggshell thinning among seabirds due to bioaccumulation of PCBs, leading to reduced reproductive capacity. PCBs have anti-estrogen properties that can inhibit calcium deposition during eggshell development, leading to insufficiently strong shells and premature loss. Anti-estrogen effects of PCBs may lead to adverse effects on male reproductive capabilities of birds and animal species.

In addition to animal studies, a number of epidemiological studies of workers exposed to PCBs have been performed. Results of human studies show that PCBs are probable carcinogens [17]. PCB workers were found to have increases in rare liver cancers and malignant melanoma. The presence of cancer in the same target organ (liver) was found in both humans and animals exposed to PCBs. Research also shows that exposure to PCBs in high concentration can have various acute effects that include a skin disease known as chloracne (skin lesions), liver damage including clinical hepatitis; other non-cancer short-term effects like body weight loss, impaired immune function; and clinically diagnosable damage to the central nervous system, causing headaches, dizziness, depression, nervousness, and fatigue. Other adverse health effects of PCBs are liver, stomach and thyroid gland injuries, behavioral alterations, and impaired reproduction [16].

Polychlorinated biphenyls (PCBs) can affect the productivity of phytoplankton and the composition of phytoplankton communities. Phytoplankton is the primary food source of all sea organisms and a major source of oxygen in the atmosphere. The transfer of PCBs up the food chain from phytoplankton to invertebrates, fish, and mammals can result in human exposure through consumption of PCB-containing food source [16].

2. Biological transformation of PCBs

A recent publication has reviewed the physical and chemical treatment options for PCB degradation [21]. This section reviews the biological degradation of PCBs.

Organisms may modify organic pollutants such as PCBs in such a way that the negative effects are minimised. Microorganisms participate in the biodegradation by producing enzymes, which modify the organic pollutant into simpler compounds. Biodegradation is of two forms, mineralization and co-metabolism [22,23]. In mineralization, competent organisms use the organic pollutant as a source of carbon and energy resulting in the reduction of the pollutant to its constituent elements. Co-metabolism, on the other hand, requires a second substance as a source of carbon and energy for the microorganisms but the target pollutant is transformed at the same time. If the products of co-metabolism are amenable to further degradation they can be mineralized, otherwise incomplete degradation occurs. This may result in the formation and accumulation of metabolites that are more toxic than the parent molecule requiring a

consortium of microorganisms, which can utilize the new substance as source of nutrients.

The effectiveness of biodegradation depends on many environmental factors. Rates vary depending on the conditions present in the environment. These factors include the structure of the compound, the presence of exotic substituents and their position in the molecule, solubility of the compound and concentration of the pollutant. For aromatic halogenated compounds, a high degree of halogenation requires high energy by the microorganisms to break the stable carbon–halogen bonds [22]. Chlorine as the substituent alters the resonant properties of the aromatic substance as well as the electron density of specific sites. This may result in the deactivation of the primary oxidation of the compound by microorganisms [24]. Additionally, the positions occupied by substituted chlorines have stereochemical effects on the affinity between enzymes and their substrate molecule [24,25].

The water solubility of the compound has a vital role in its degradation. Compounds with high aqueous solubility are easily accessed by microorganisms than those with low solubility. For PCBs, highly chlorinated congeners are very insoluble in water. This could account for the resistance of highly chlorinated PCB congeners to biodegradation.

Pollutant concentration is also a major factor affecting biodegradation. In general, a low pollutant concentration may be insufficient for the induction of degradative enzymes or to sustain growth of competent organisms. On the other hand, a very high concentration may render the compound toxic to the organisms [25]. At a low concentration range, degradation increases linearly with increase in concentration until such time that the rate essentially becomes constant regardless of further increase in pollutant concentration [22].

Other environmental factors affecting degradation are temperature, pH, presence of toxic or inhibitory substance and competing substrates, availability of suitable electron acceptors, and interactions among microorganisms. All these factors interplay and make the rates of biodegradation unpredictable.

The use of microorganisms, both anaerobic and aerobic, is the only known process to degrade PCBs in soil systems or aquatic environments. Anaerobic bacteria possess characteristics that are well adapted to pollutants with high carbon concentration because of the diffusional limitation of oxygen in high concentration systems [22]. The environment of anaerobes is conducive to reductive transformations where chlorine is displaced by hydrogen [23]. The dechlorinated compound is suitable for the oxidative attack of the aerobic bacteria. Aerobic bacteria grow faster than anaerobes and can sustain high degradation rates resulting in mineralization of the compound. Theoretically, the biological degradation of PCBs should result to give CO_2, chlorine, and water. This process involves the removal of chlorine from the biphenyl ring followed by cleavage and oxidation of the resulting compound [26].

2.1. Anaerobic transformation of PCBs

Anaerobic transformation of chlorinated organic compounds involves reductive dehalogenation where the halogenated organic compound serves as the electron acceptor [27], the halogen substituent is replaced with hydrogen [28].

$$R{-}Cl + 2e^- + H^+ \rightarrow R{-}H + Cl^-$$

Electron acceptors are generally the factors limiting metabolism in anaerobic environments. Thus, any microorganism that could use PCBs as terminal electron acceptors would be at a selective advantage [29].

Anaerobic dechlorination can attack a large array of chlorinated aliphatic and aromatic hydrocarbons. Several anaerobic dechlorinating bacteria have been isolated [30] These include *Desulfomonile tiedjei* [31], *Desulfitobacterium*, *Dehalobacter restrictus*, *Dehalospirillum multivorans*, *Desulforomonas chloroethenica*, *Dehalococcoides ethenogenes* and the facultative anaerobes *Enterobacter* strain MS-1 and *Enterobacter agglomerans*. Some of these microorganisms reductively dechlorinate the chlorinated compound in a co-metabolism reaction; others utilize the chlorinated compounds as electron acceptors in their energy metabolism. The characteristics common to dehalogenators are: (a) aryl reductive dehalogenation is catalyzed by inducible enzymes, (b) these enzymes exhibit distinct substrate specificity, (c) aryl dehalogenators function in syntrophic communities and may be dependent on such communities and, (d) aryl dehalogenators derive metabolic energy from reductive dehalogenation. Microorganisms with distinct dehalogenating enzymes each exhibit a unique pattern of congener activity.

Under anaerobic condition, reductive dechlorination of PCBs occurs in soils and sediments. Different microorganisms with distinct dehalogenating enzymes are responsible for different dechlorination activities and dehalogenation routes. The rate, extent, and route of dechlorination is dependent on the composition of the active microbial community, which in turn are influenced by environmental factors such as availability of carbon sources, hydrogen or other electron donors, the presence or absence of electron acceptors other than PCBs, temperature, and pH [9].

The first evidence of an anaerobically mediated dechlorination of PCB was based on the observed modification of the substance in anaerobic sediments of the Hudson River (HR) and Silver Lakes (SL), Massachusetts [29]. Comparison between the distribution patterns of PCB in the anaerobic sediments and those of commercial mixtures discharged into the river showed that the sediments have an increase in the proportion of low-chlorinated congeners (mono- and di-) and a decrease in the highly chlorinated congeners (tri-, tetra-, and higher chlorobiphenyls). These observations were consistent with reductive dechlorination through *meta*- and *para*-chlorine removal. These findings were later confirmed under laboratory

conditions [28]. Evidence was obtained that the sediment microbial population could reductively dechlorinate most of the congeners of Aroclor 1242 at the *meta*- and *para*-positions and proportions of mono- and di-chlorobiphenyls increased from 9% to 88%.

Laboratory experiments in the dechlorination of commercial PCB mixtures using microorganisms eluted from anaerobic sediments showed that the rate and extent of dechlorination tended to decrease with increase in the degree of chlorination [32]. Also, dechlorination was associated with syntrophic communities attacking PCB at different sites with specificities for PCB dechlorination.

Quensen et al. [28] reported the dechlorination of Aroclors 1242, 1248, 1254, and 1260 by microorganisms from the HR sediments, and of Aroclors 1242 and 1260 using microorganisms from the SL sediments. The rate of dechlorination by the SL microorganisms was similar for Aroclors 1242 and 1248 showing extensive dechlorination from the *meta*-plus *para*-positions within 8 weeks of incubation leaving predominantly *ortho*- substituted mono- and di-chlorobiphenyls. Aroclor 1254 was dechlorinated at a somewhat lesser rate with 63% of the chlorine in the *meta*-plus *para*-positions in 25 weeks. For Aroclor 1260, only 15% of the *meta*- and *para*-chlorines were removed even after 50 weeks. Major products from the dechlorination of Aroclors 1242, 1248, and 1254 were 2-chlorbiphenyl, 2,2-chlorobiphenyl and 2,6-chlorobiphenyl while dechlorination of Aroclor 1260 followed a different pattern where 2′,5′-2′5′-chlorobiphenyl and 2′,3′,5′-2′,5′-chlorobiphenyl were major products.

The dechlorination of Aroclor 1242 by microorganisms from the SL sediments was less extensive compared to the HR microorganism as only 46% of the *meta*-plus *para*-chlorines were removed even after 16 weeks. In contrast, dechlorination of Aroclor 1260 was more rapid than with the HR inoculum. Quensen et al. [33] attributed these different dechlorination activities to the previous exposure of the microorganism to the particular Aroclor present at the site. Sonzogni [34] reported a dechlorination pattern in the Sheboygan River believed to be contaminated with Aroclor 1248 and 1254. It was observed that congener profiles shifted from higher chlorinated to the lower chlorinated congeners. The *meta*- and *para*-chlorinated congeners were depleted more than the *ortho*-congeners.

The use of organic substrate as electron donors has also been shown to increase the rate of dechlorination of Aroclor 1242 using HR microorganisms [35]. The separate addition of acetate, acetone, methanol, and glucose showed similar patterns of dechlorination for each substrate, however, the extent and rate of dechlorination were different. The rate of dechlorination was, in decreasing order, greatest for methanol, glucose, acetone, and least for acetate. As before, dechlorination occurred primarily on the *meta*- and *para*-positions of the highly chlorinated congeners, resulting in the accumulation of less-chlorinated, primarily *ortho*-substituted products. The use of pyruvate and acetate as electron donors was also tested using HR microorganisms [35]. Aroclors 1242, 1248, 1254, and 1260 were dechlorinated primarily at the *meta*-positions of the biphenyl molecule. The extent of dechlorination was greatest for Aroclor 1254. When acetate was used there was a delay in dechlorination.

The presence of potential electron acceptors also affects the removal of chlorine atoms [27]. Electron acceptors compete with halogenated compounds for reducing potential and the compounds impose different selective pressures on growing communities. It has been found that sulfate and bromoethane sulfonate inhibit dechlorination, but is enhanced with the use of carbon dioxide and nitrate as electron acceptors.

The addition of $FeSO_4$ to PCB-contaminated sediments was also found to achieve nearly complete *meta*- plus *para*-dechlorination of Aroclor 1242 [36]. The $FeSO_4$ stimulated the growth of sulfate reducing organisms responsible for PCB dechlorination, while Fe^{2+} reduced the sulfide bioavailability and toxicity by forming the insoluble precipitate FeS.

Williams [37] reported the dechlorination sequence in six trichlorobiphenyls with all chlorines in one ring. Each of the trichlorobiphenyls was incubated in slurries of sediments from HR, SL and Woods Pond. In each of the slurries, the chlorine between the two other chlorines was removed first. Dechlorination of every trichlorobiphenyl occurred in the HR sediments with all *meta*- and *para*-chlorines removed but no *ortho*-dechlorination was observed. In contrast, only one chlorine, *meta*- or *para*- , was removed in the SL and Woods Pond sediment slurries. *Ortho*-dechlorination was also observed in these slurries.

The first experimental demonstration of a biologically mediated *ortho*-dechlorination of PCB was in the use of microorganisms eluted from the sediments of the Woods Pond, Massachusetts, which was contaminated with hydrocarbon oil and Aroclor [38]. The PCB congener 2,3,5,6-tetrachlorobiphenyl was dechlorinated to 2,5-CB (21%), 2,6-CB (63%) and 2,3,6-CB (16%) in 37 weeks.

Studies were also conducted on the dechlorination of PCBs that have persisted in Housatonic River Sediments [38–40]. Slurries of Aroclor 1260-contaminated sediment from Woods Pond that were primed with 2,5,3′,4′ tetrachlorobiphenyls resulted in a selective *para*-dechlorination that decreased the penta- through heptachlorobiphenyls containing 2,3,4-, 2,3,5- , or 2,3,4,5-chlorophenyl groups by up to 83% in 12 weeks. The products were tetra- and pentachlorobiphenyls containing 2,3-, 2,5-, and 2,3,5-chlorobiphenyl groups [39].

A sustained *meta*-dechlorination of Aroclor 1260 was attained by priming the indigenous microorganisms in sediments [41]. To stimulate dechlorination, 2,3,4,5,6-pentachlorobiphenyl, 2,3,4,6-tetrachlorobiphenyl; and 2,3,6-trichlorobiphenyl were used. The dechlorination targeted most of the hexa-, hepta-, and octa-chlorobiphenyls

and converted them to tetra- and penta-chlorobiphenyls containing mostly *ortho*- and *para*-chlorines.

The enrichment of microorganisms from sediments with 2,3,4,5,6-pentachlorobiphenyls resulted in a sequential *meta*- and *para*-dechlorination of Aroclor 1260 [40]. The hexa- through nonachlorobiphenyls in the sediments was reduced from 66.3 mol% to only 16.7 mol% through *meta*-chlorine removal from serial transfers of actively dechlorinating slurries. The enrichment also fostered *para*-dechlorination that caused further conversion of the meta-dechlorination products to tri- and tetra-chlorobiphenyls.

Studies on the primary and enriching effect of several PCB congeners on the dechlorination of Aroclor 1260 are believed to be mediated by two distinct populations of PCB dechlorinators with different specificities. In addition, the primary congeners were good substrates for the respective dehalogenases and support growth of the dechlorinators by acting as electron donors.

Two different enrichment cultures (BK 24 and BK 27) previously enriched from marine sediments with a history of PCB contamination was able to dechlorinate four octachlorobiphenyls (2,3,4,5,6,2′,3′,4′-octachlorobiphenyl; 2,3,4,5,2′,3′,4′6′-octachlorobiphenyl; 2,3,4,5,2′,3′,5′,6′-octachlorobiphenyl and 2,3,4,5,6,2′,4′,5′-octachlorobiphenyl) and three nonachlorobiphenyls (2,3,4,5,6,2′,3′,4′,5′-nonachlorobipheny; 2,3,4,5,6,2′,3′4′,6′-nonachlorobiphenyl; and 2,3,4,5,6,2′,3′,4′,5′-nonachlorobiphenyl). The single congeners each with a concentration of 50 ppm were added separately to the microbial enrichment cultures. For a period of 16 weeks, all seven congeners were reductively dechlorinated. The predominant dechlorination pattern showed *meta*-dechlorination of doubly flanked *m*-chlorines followed by *meta*-dechlorination of singly flanked *m*-chlorines. Some *ortho*- and *para*-dechlorination were also observed. The extent of the dechlorination was only 1.4% removal for congener 2,3,4,5,6,2′,3′,4′,5′-nonachlorobiphenyl [42].

Studies on the effect of temperature on dechlorination show that temperature has a significant effect on the growth of the microorganisms and the catalytic activity of the enzymes [9]. The investigation of the dechlorination of added 2,3,4,6-chlorobiphenyl and residual Aroclor 1260 in Woods Pond revealed that Aroclor 1260 was marginally dechlorinated at 8–34 °C and at 50–60 °C with an optimal temperature of 18–30 °C. Between 8–34 °C and 50–60 °C, it was observed that flanked *meta*-dechlorination occurred, whereas unflanked *para*-dechlorination was observed only between 18 and 34 °C. Dechlorination of doubly unflanked *para*-chlorines occurred only in the temperature range of 18–30 °C. For 2,3,4,6-chlorobiphenyl optimal temperature for overall chlorine removal was 20–27 °C. Unflanked *ortho*-dechlorination was observed at 8–30 °C. Wiegel and Wu [9] proposed a temperature-dependent route of microbial reductive dechlorination of spiked 2,3,4,6-chlorobiphenyl in Woods Pond as shown in Fig. 2.

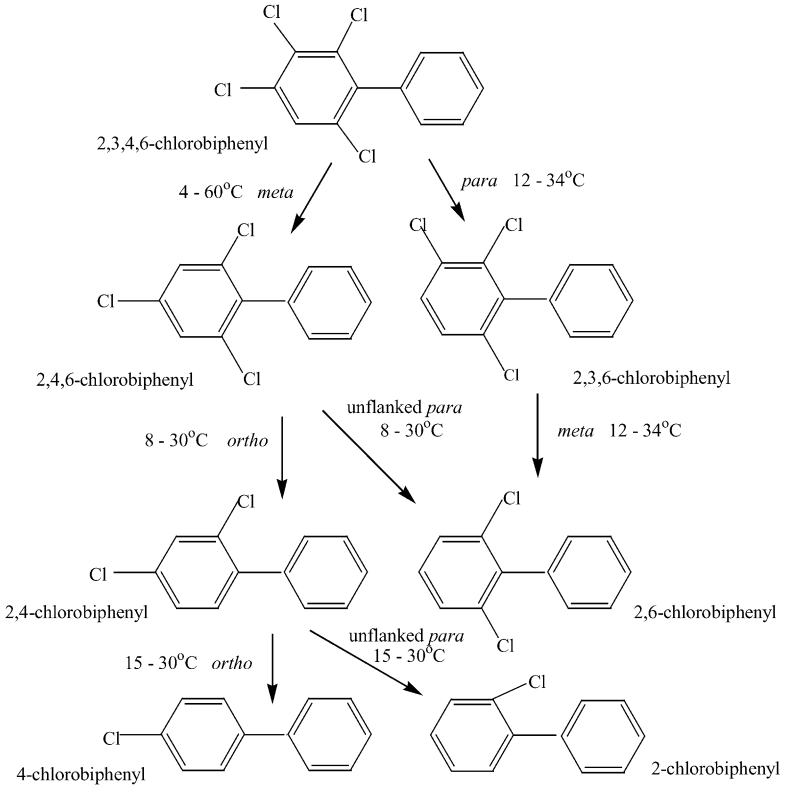

Fig. 2. Temperature-dependent routes of microbial reductive dechlorination (Wiegel and Wu [9]).

The effect of pH on the dechlorination of PCBs in sediments is complex because of the interaction of the different dehalogenating and non-dehalogenating microbial population. Further, the bioavailability of PCBs is affected by the equilibrium between PCBs that are dissolved and those that are adsorbed to organic matter [18]. The dechlorination of residual PCBs in Woods Pond and of 2,3,4,6-chlorobiphenyl added as primer was studied at pH values between 5.0 and 8.0 at temperatures where dechlorination was observed [9]. Some dechlorination was observed at all temperatures and pH except for 34 °C at pH 5.0. Overall optimal pH for chlorine removal was at 7.0–7.5. The flanked *meta*-dechlorination occurred at pH 5.0–8.0, unflanked *para*-dechlorination at pH 6.0–8.0, and *ortho*-dechlorination at 6.0–6.5. At pH 7.0 and 15 °C, *ortho*-dechlorination dominated, whereas at 18 and 25 °C, unflanked *para*-dechlorination outpaced the other dehalogenation reactions. The optimal pH for overall chlorine removal was at 6.0–7.5.

All laboratory investigations of microbial PCB dechlorination have been carried out using sediment slurries, both as a source of microorganism and growth matrix [29]. These investigations show that microorganisms with different characteristic specificities for PCB dechlorination existed in PCB-contaminated sites. The microbial population present in the sediments have distinct dehalogenating enzyme, each exhibiting a unique pattern of congener selectivity resulting in various patterns of PCB dechlorination [43]. However, a similarity between degradation patterns exists. The *para*- and *meta*-substituted congeners are more commonly degraded than *ortho*-substituted congeners as shown in Fig. 3.

Only a few *ortho*-substituted congeners have been reported to biodegrade [44]. Moreover, anaerobic degradation has most commonly been observed under methanogenic conditions. This may lead one to conclude that anaerobic reductive dechlorination occurs under methanogenic conditions, if not inhibited by sulfate-reducing conditions. Sulfates have a higher affinity for electrons than the chloroaromatics [43].

Anaerobic PCB dechlorination reduces the potential risk and potential exposure to PCBs. Moore [45] reported that carcinogenic potential of PCBs correlates with total chlorine levels. PCBs with two *para*, two or more *meta*, and no *ortho* substituents exhibit dioxin-like activities [46]. Coplanar PCB congeners like 3,4,5,3′,4′-, 3,4,3′,4′-, and 3,4,5,3′,4′,5′-chlorobiphenyl exhibit strong binding to the dioxin receptor. The preferential loss of *meta*- and *para*-chlorines catalyzed by anaerobic dechlorination results in dramatic reductions in the levels of coplanar, dioxin-like congeners in the PCB mixtures [47]

The decrease in risk is manifested in two ways. First, lightly chlorinated congeners produced by dechlorination can be readily degraded by indigenous bacteria. Second, dechlorination significantly reduces bioconcentration potential of the PCB mixture through conversion to congeners that do not significantly bioaccumulate in the food chain [45].

2.2. Aerobic biodegradation of PCBs

The lightly chlorinated PCB congeners resulting from the dechlorination of highly chlorinated congeners are substrates for aerobic bacteria [6,42]. Aerobic oxidative destruction involves two clusters of genes. The first one is responsible for the transformation of PCB congeners to chlorobenzoic acid, and the second cluster is responsible for the degradation of the chlorobenzoic acid. A common growth substrate for PCB-degrading bacteria is biphenyl or monochlorobiphenyl. When biphenyl is utilized by bacteria yellow *meta*- ring cleavage product is produced. This has been observed in most bacteria studied especially by the *Pseudomonas* sp. [26], which was also observed in *Micrococcus* sp. [48]. The metabolic pathway used by this family of bacteria is illustrated in Fig. 4.

By way of 1,2-dioxygenative ring cleavage, benzoate results as a common by-product of biphenyl degradation. Although different bacterial species seem to produce benzoate through PCB metabolism, further breakdown of benzoate seems to differ among the different microbes. Nevertheless, the by-products produced are less toxic compounds to people and the environment [48]. Since PCBs are more persistent with increasing chlorination of the congener, aerobic biodegradation involving biphenyl ring cleavage, is restricted to the lightly chlorinated congeners.

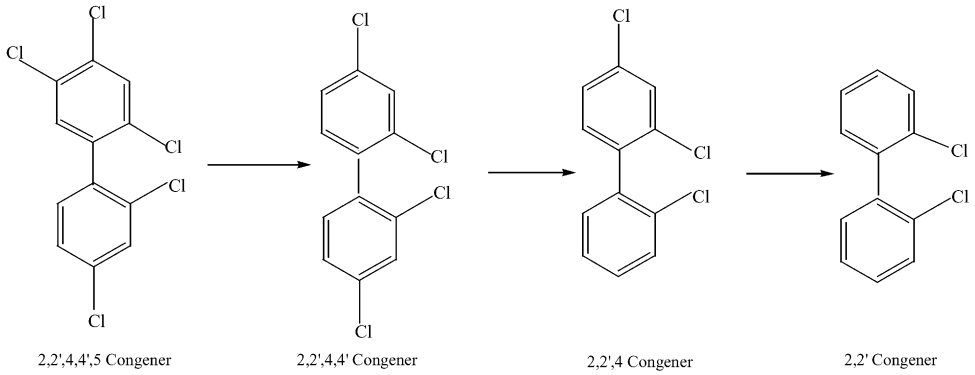

Fig. 3. Potential pathway for anaerobic dechlorination of a highly chlorinated congener (Fish and Principe [44]).

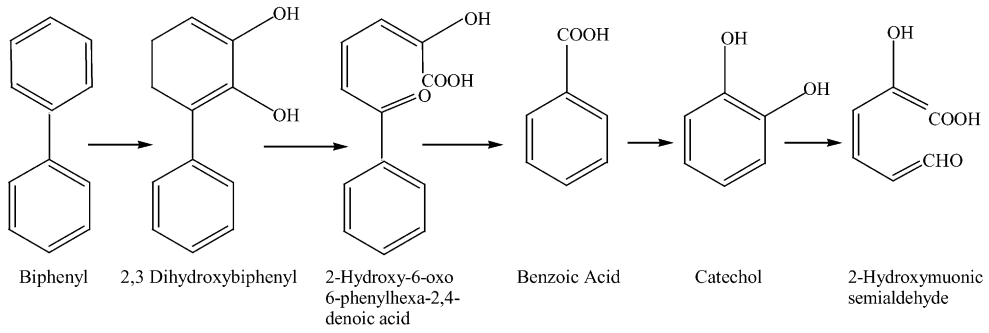

Fig. 4. Possible pathway for the aerobic oxidative degradation of biphenyl (Bevinakatti and Ninnebar [48]).

While biphenyl and monochlorobiphenyl can serve as growth substrates, the degradation of PCB congeners with more than one chlorine atom proceeds by a co-metabolic process [49] in which biphenyl is used as carbon and energy source while oxidizing PCBs. Biphenyl also serve as an inductor of degrading enzymes. Ahmed and Focht [50] first reported that two species of *Achromobacter* are capable of growing on biphenyl and 4-chlorobiphenyl. The degradation of PCBs by *Nocardia* sp. and *Pseudomonas* sp. increased upon addition of biphenyl [51]. Clark et al. [52] described the enhanced co-metabolism of Aroclor 1242 in the presence of acetate using mixed cultures of *Alcalegenes odorans*, *A. denitrificans*, and an unidentified bacterium. Focht and Brunner [53] observed the increased mineralization of Aroclor 1242 by *Acinetobacter* strain P6 by the addition of biphenyl. Furukawa et al. [54] reported that *Acinetobacter* strain P6 and *Arthrobacter* strain B1B grows well on biphenyl and 4-chlorobiphenyl. These microorganisms also co-metabolized Aroclor 1254 in the presence of biphenyl. *Alcalegenes* H850 utilized biphenyl and three monochlorobiphenyls as growth substrates and oxidized all detectable di-, tri-, and tetra-chlorobiphenyls in Aroclors 1242 and 1248, and partially degrading Aroclor 1254. The observed oxidation of PCBs in the presence of a second substrate was attributed to the increased biomass that even a slight oxidation by each microorganism would lead to a more complete degradation of PCBs for the system as a whole [6].

In a recent study, a new bacterium, *Janibacter*, MS3-02, was isolated from soil [55]. It is interesting to note that the degradation of Aroclor 1242 was significantly higher in the liquid medium without biphenyl (70–100% after 7 days). When biphenyl was added in the medium, degradation was only 84%. For the studies on soil medium, it was observed that the soil native population was not able to degrade the PCBs present in Aroclor 1242. On the other hand, inoculation of the soil with MS3-02 produced a decrease in some of the chromatographic peaks. Comparison of the result obtained in the liquid medium with that obtained in soil shows that the degradation was less efficient in soil because of the lower bioavailability of PCBs and the interactions with the indigenous soil microorganisms.

Several studies on the microbial degradation of commercial PCB mixtures show that certain patterns of chlorine substitution seriously hinder PCB degradation. For lightly chlorinated PCB congeners, a sequential enzymatic steps involved in the degradation had been developed [50,56–59]. The metabolic pathway is shown in Fig. 5.

Molecular oxygen is introduced at the 2,3 position of the non-chlorinated or lesser chlorinated ring of PCB to form cis-dihydrodiol compounds (2,3-dihydroxy-4-phenylhexa-4,6-diene) by the action of oxygenase. The dihydrodiols are then dehydrogenated to yield 2,3-dihydroxy-biphenyl by a dihydrodiol dehydrogenase. The 2,3-dihydroxy biphenyl is cleaved at the 1,2 position by a 2,3-dihydroxy biphenyl dioxygenase to produce the *meta*-cleavage compound, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate. The *meta*-cleavage compound is hydrolyzed to the corresponding chlorobenzoic acid by a hydrolase. Congeners with chlorine

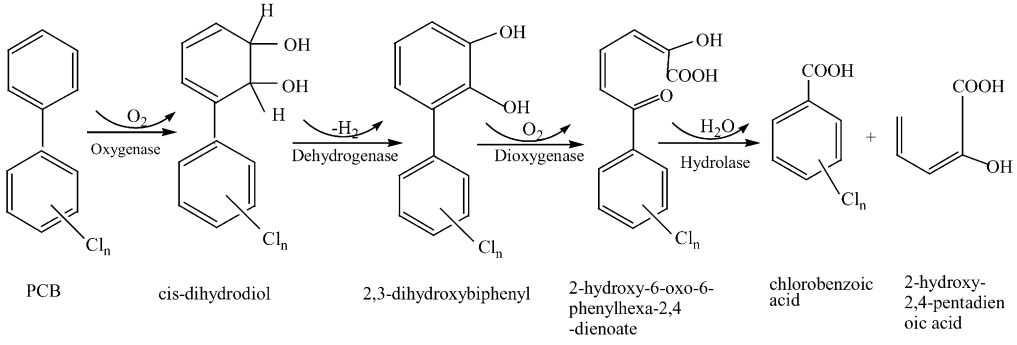

Fig. 5. Major steps in the conversion of PCBs into chlorobenzoates (Sylvestre and Sandossi [25]).

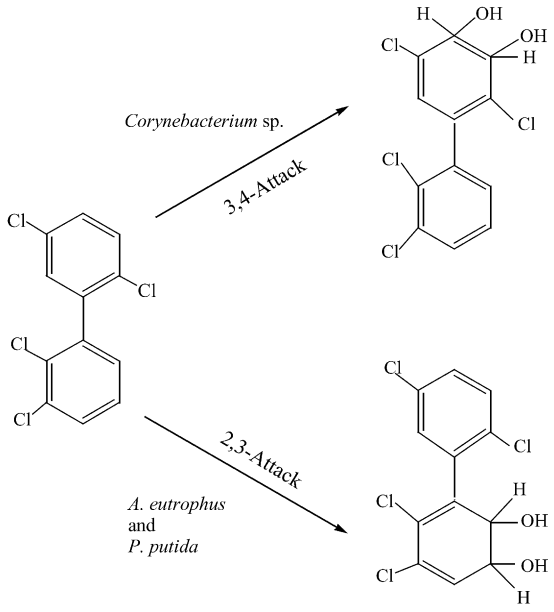

Fig. 6. Microorganism-specific nature of PCB degradation (Unterman et al. [65]).

at the 2,5,4′-, 2,3,2′,5′-; 2,4,5,2′,3′- positions are accessible to both 2,3- and 3,4-dioxygenase attack but would be increasingly difficult to degrade due to the degree of chlorination. The degradation of 4,4′-dichlorobiphenyl proceeds through a series of hydroxylation, the two rings are cleaved and cleaved products eventually enter the natural pathway [60]. However, congeners blocked at the 2,3- and 5,6-positions can be degraded by *A. eutrophus* H850. On the other hand, *P. putida* LB400 has the ability to degrade 2,4,5,4′,5′-hexachlorobiphenyl, a congener with no adjacent unchlorinated carbon. Generally, highly chlorinated PCB congeners showed resistance to biodegradation0 [54,61–64].

The complete degradation of PCBs requires various microbial strains with specific congener preferences. In addition, the position and number of chlorines on the molecule can influence the rate of the first oxygenase attack. Unterman et al. [65] proposed a mechanism for the oxidation of PCBs by *A. eutrophus*, *P. putida*, and a *Corynebacterium* sp. *Alcalegenes eutrophus* and *P. putida* strains degrade tetrachlorobiphenyl via 2,3- attack while *Corynebacterium* degrades the compound via 3,4-attack. This is illustrated in Fig. 6.

In a study conducted by Komancova et al. [49] using cells of *Pseudomonas* sp. 2 immobilized on SIRAN carrier, the degradation of individual congeners (2,4,4′-trichlorobiphenyl, 2,2′,5-trichlorobiphenyl, 2,2′,5,5′-tetrachlorobiphenyl, 2,2′,4,5′-tetrachlorobiphenyl and 2,2′,5,6′-tetrachlorobiphenyl) with biphenyl as growth substrate show a common metabolic pathway starting by oxidation at the 2,3-position of the less chlorinated ring. The degradation for 2,4,4′-trichlorobiphenyl, a 2,3-dioxygenase attack of the less chlorinated ring was the primary reaction used by *Pseudomonas* sp. 2 resulting in the formation of the yellow metabolite 3-chloro-2-hydroxy-6-oxo-6-(2,4-dichlorobhenyl)hexa-2,4-dienoic acid; and a final product, 2,4-dichlorobenzoic acid as shown in Fig. 7.

A similar pathway was observed for 2,2′,5-trichlorobiphenyl (Fig. 8) but this congener was also degraded via 3,4-dioxygenase attack (Fig. 9) with the formation of 2′,5′-dichloroacetophenone and trichlorodihydroxybiphenyl in addition to the 2,5-dichlorobenzoic acid.

The congener 2,2′,5,5′-tetrachlorobiphenyl was degraded via 2,3-dioxygenase attack (Fig. 10) with the formation of 2,5-dichlorobenzoic acid and trichlorobiphenyl. The identified metabolites indicate that *Pseudomonas* sp. 2 was capable of dehalogenating PCBs.

The ability of the bacterial strain to dehalogenate PCBs was confirmed by the degradation of 2,2′,5,6′-tetrachlorobiphenyl (Fig. 11). Degradation for this compound was via 2,3-dioxygenase attack and products formed corresponded to (based on molecular weights) 4-(2,5-dichlorophenyl)-oxobutanoic acid. Two other compounds, 2-chloro-3-(2,5-dichlorophenyl)-2-acrylic acid and monochloroacetophenone, were also detected. These products are consistent with 3,4-dioxygenase attack (Fig. 12).

In the case of 2,2′,4,5′-tetrachlorobiphenyl degradation was via 2,3-dioxygenase attack forming on the

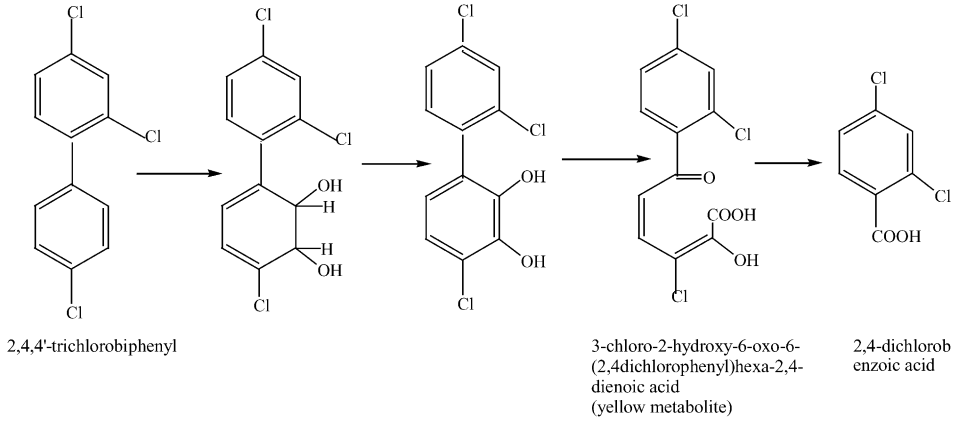

Fig. 7. Proposed metabolic pathway of 2,4,4′-trichlorobiphenyl (Komancová et al. [49]).

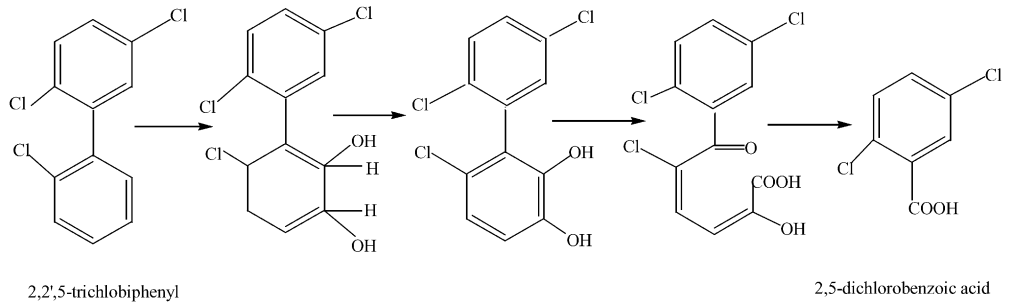

Fig. 8. Proposed metabolic pathway of 2,2′,5-trichlorobiphenyl via 2,3 attack (Komancová et al. [49]).

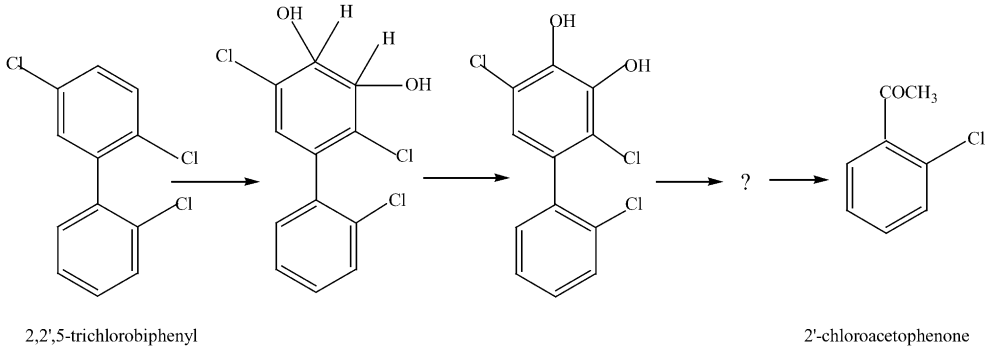

Fig. 9. Proposed metabolic pathway of 2,2′,5-trichlorobiphenyl via 3,4-dioxygenase attack (Komancová et al. [49]).

2,4-dichlorophenyl and was preferred to the 3,4-oxygenase attack (Fig. 13). The identified degradation products were 2,5-dichlorobenzoic acid and 4-(2,5-dichlorophenyl)-4-oxobutanoic acid.

The bulkiness of the chlorine atoms prevents access to the enzyme's active site. Furthermore, the chlorine atoms may prevent oxygenation if they occupy the carbon positions that are most susceptible to the oxygenase attack [61]. The *ortho*-positions are also the most resistant to microbial attack [54,62].

Some aerobic bacteria have the capacity to degrade highly chlorinated PCB congeners using a different initial oxygenase reaction involving a 3,4-hydroxylation instead of 2,3-hydroxylation. This is the case for *P. putida* LB400 [66] and *P. testosterone* B356 [11]. In *P. testosterone*, the oxygenase attack was always on the *ortho*- and *meta*-carbon while for *P. putida*, the oxygenase attack on PCB congeners with chlorine atoms on both rings was usually accompanied by a *para*- or *ortho*-dehalogenation of the molecule.

Furukawa [67] summarized the relationship between chlorine substitution and the microbial breakdown of PCBs as follows:

1. The degradation rate of PCBs decreases as chlorine substitution increases.
2. PCBs containing two chlorines in the *ortho*-position of a single ring (i.e., 2,6-) and each ring (i.e., 2,2′) show a striking resistance to degradation.
3. PCBs containing all chlorines on a single ring are generally degraded faster than those containing the same number on both rings.

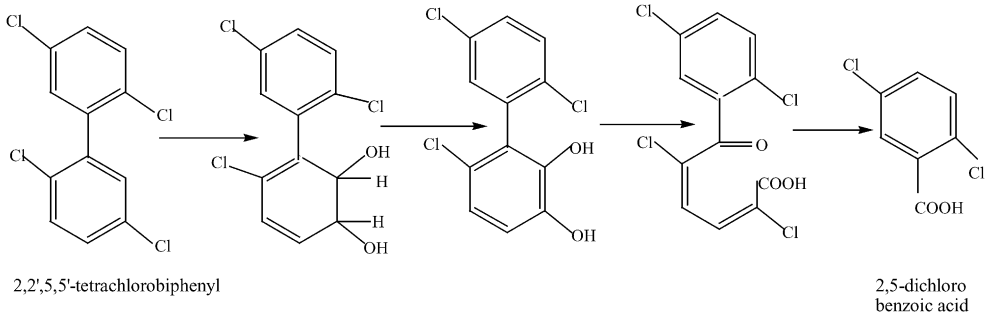

Fig. 10. Proposed pathway of 2,2′,5,5′-tetrachlorobiphenyl (Komancová et al. [49]).

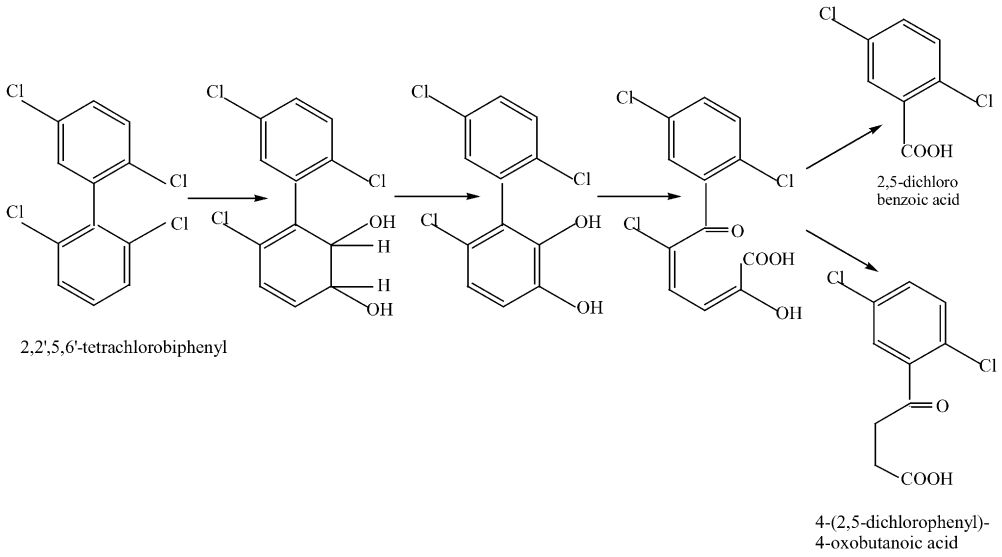

Fig. 11. Proposed metabolic pathway of 2,2′,5,6′-tetrachlorobiphenyl via 2,3-dioxygenase attack (Komancová et al. [49]).

4. PCBs having two chlorines at the 2,3 position of one ring such as 2,3,2′,3′-, 2,3,2′,5′-, 2,4,5,2′,3′-chlorobiphenyls are susceptible to microbial attack compared with other tetra- and penta-chlorobiphenyls, although this series of PCBs is metabolized through an alternative pathway.
5. Initial dioxygenation followed by ring cleavage of the biphenyl molecule occurs with a non-chlorinated or less chlorinated ring.

The usual products from the co-metabolic degradation of PCBs are chlorinated benzoic acids [67,68] but other metabolites have been observed. *Acinetobacter* sp. rapidly degraded Kaneclor 200 (dichlorobiphenyl) after 4 h of incubation showing predominant accumulation of monochlorobenzoic acid. Kaneclors 300 (trichlorobiphenyl) and 400 (tetrachlorobiphenyl) produced various intermediate metabolites such as mono- and di-chlorobenzoic acid, dihydroxybiphenyl compounds with two and three chlorines and the ring *meta*-cleavage compounds with two and three chlorines. In addition to these products large amounts of unknown compounds with two chlorines in the molecule were also produced. On the other hand, Kaneclor 500 was resistant to degradation and hardly metabolized. The degradation of 4-chlorobiphenyl by an *Achromobacter* sp. and a *Bacillus brevis* strain generated the same metabolites with 4-chlorobenzoic acid as the major metabolite [69]. *Alcalegenes eutrophus* H850 can degrade dichlorobiphenyl yielding the corresponding chlorobenzoic acid and a novel metabolite 2′,3′-dichloroacetophenone while 2′,4′,5′-2′,4′,5′-hexachlorobiphenyl with no adjacent unsubstituted carbons was oxidized to 2′,4′,5′-trichloroacetophenone. In the metabolism of PCBs with four chlorine substituents by *Alcaligenes* sp. JB1, monochlorobenzoates and dichlorobenzoates were detected as metabolites [63]. Resting cell assays with chlorobenzoates showed that JB1 could metabolize the monochlorobenzoates and dichlorobenzoates containing only *meta*- and *para*-chlorine substituents but not dichlorobenzoates possessing an *ortho*-chlorine substituent. The chlorobenzoates formed tend to accumulate in the reaction mixture together with other intermediate metabo-

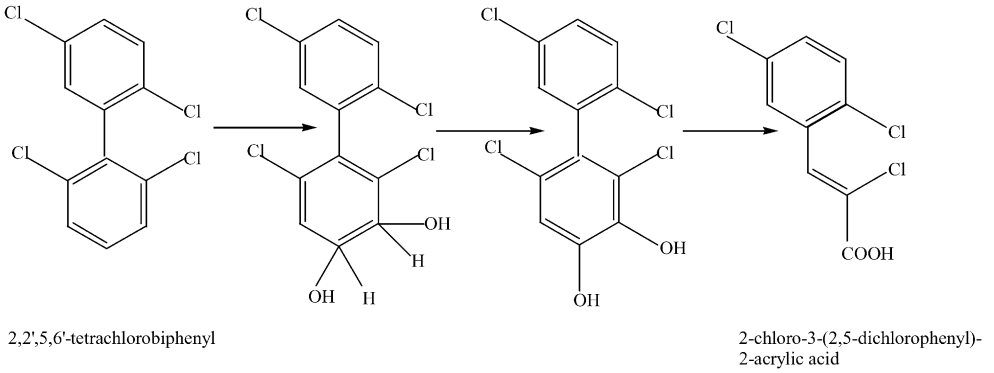

Fig. 12. Proposed pathway of 2,2′,5,6′-tetrachlorobiphenyl via 3,4 attack (Komancová et al. [49]).

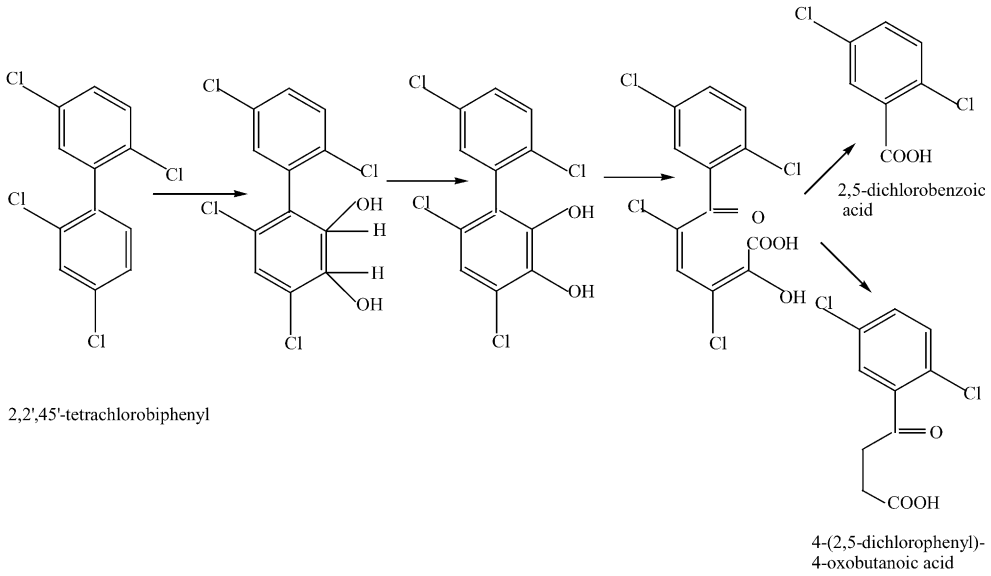

Fig. 13. Proposed pathway of 2,2′,4,5′-tetrachlorobiphenyl via 2,3 attack (Komancová et al. [49]).

lites such as dihydroxy and trihydroxy compounds and other unidentified products [51]. Cultures capable of co-metabolizing PCBs are usually unable to grow on these substrates. This suggests that no single organism is responsible for the degradation of multiple chlorinated biphenyls. Previous work have shown that a more efficient degradation of monochlorinated biphenyls by bacterial consortia occurs in the presence of a bacterial strain capable of degrading chlorobenzoates produced by the PCB-degrading members, either directly [65] or by co-metabolism [70]. This may suggests that chlorobenzoates are in general involved in the regulation of the PCB aerobic degradation process [71]. The incorporation of chlorobenzoate degraders such as *Pseudomonas aeroginosa* and *Pseudomonas putida* [72] can greatly effect complete mineralization of PCBs. A mixed microbial consortium is also needed for microbial synergism and co-metabolism Synergism is also important in enhancing the overall rate of degradation of PCBs in mixed cultures. This increased rate is a result of combined metabolic attack at different sites on the organic compound, increasing overall degradation rate.

2.3. Sequential anaerobic–aerobic transformation of PCBs

The biodegradation of PCBs by aerobic bacteria had been well studied. However, it was observed that only lightly chlorinated PCB congeners, those with four or less chlorine atoms, was degraded. Highly chlorinated PCB congeners, those with five or more chlorine atoms, remain biorefractory to aerobic bacteria, although there had been few reports on the aerobic degradation of penta- and hexa-chlorobiphenyls.

Studies have also been conducted on the transformation of PCBs using anaerobic bacteria eluted from PCB-contaminated sediments. Under anaerobic condition, highly chlorinated PCB congeners have been found to be reductively dechlorinated through a preferential *meta*- and *para*-chlorine removal producing less chlorinated congeners that are amenable to aerobic biodegradation. This biotransformation pattern appears to be common among halogenated aromatic compounds.

The results of the studies conducted using solely aerobic or anaerobic microorganism suggests that mineralization of chlorinated organic compounds can be attained through sequential exposure to specialized anaerobic and aerobic microbial cultures. This was demonstrated by the degradation of hexachlorobenzene, tetrachloroethylene and chloroform [73]. The chlorinated compounds were added to the influent of a constant flow anaerobic reactor containing mixed methanogenic cultures. The compounds were dechlorinated to the level of tri- and di-chlorinated products in the anaerobic reactor. These products were subsequently transformed into non-volatile intermediates and carbon dioxide in the aerobic reactor.

A sequential anaerobic–aerobic treatment of PCBs has been successfully tested in microcosms of HR sediments [74]. Batch cultures were incubated anaerobically for 20 weeks in sealed serum bottles with methanol. The tri-, tetra-, penta-, and hexa-chlorobiphenyls were reduced and an increase in mono- and di-chlorobiphenyls was observed. After 20 weeks of incubation the anaerobic cultures were purged with oxygen and inoculated with an aerobic bacterium isolated from HR. After 96-h incubation most of the mono- and about 25% of the di-chlorobiphenyl were degraded.

Rogers and Julia [75] reported a sequential anaerobic–aerobic treatment of PCBs in soil microcosms. Results of the batch soil–slurry microcosm showed dechlorination

of several hexachlorobiphenyl to penta- and tetrachlorobiphenyl by indigenous microorganisms. The aerobic microcosm experiment demonstrated the presence of microorganisms capable of degrading the tri- and tetrachlorobiphenyl.

A sequential anaerobic dechlorination, and subsequent aerobic degradation of PCB in soil spiked with Aroclor 1242 was reported by Anid et al. [76] and Shannon et al. [77]. Likewise, Master et al. [78] reported a sequential anaerobic–aerobic laboratory scale treatment of soil contaminated with weathered Aroclor 1260. Using an initial concentration of 59 ppm, the major components of Aroclor 1260 were either completely or partially transformed to less chlorinated PCB congeners within 4 months of anaerobic treatment. The major products of reductive dechlorination were 2,4,2′,4′-tetrachlorobeiphenyl and 2,4,2′,6′-tetrachlorobiphenyl. There was no decrease in the total PCB. These products were degraded in the subsequent aerobic treatment using *Bukholderia* sp. strain LB400. The concentration of PCBs was reduced to 20 ppm after 28 days of aerobic treatment.

3. Concluding remarks

Both anaerobic and aerobic metabolism modes transform PCBs. Different microorganisms show preferential attack on PCBs resulting in different patterns of degradation. The degree of chlorination of the congener is a major factor, which influences degradation potential of the compound. In addition, environmental factors such as temperature, pH, and the presence of other substrates affect the composition and growth of the microorganisms. These factors have to be optimized to obtain high degradation efficiency.

4. Challenges in the degradation of PCBs

Much effort has been directed towards the selection of technology options for the destruction of PCBs. Although the baseline remediation technology for PCBs is incineration and despite its high efficacy, incineration is expensive and may produce undesirable by-products such as polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs) due to incomplete combustion.

Several alternative PCB destruction technologies have been proposed during the last two decades or are already commercially applied. However, to date, there is no state of the art technology for a destruction method alternative to incineration.

There are several factors that have prevented the commercialization of several non-incineration technologies. To cite:

- No single non-incineration technology is deemed applicable to all PCB-contaminated media.
- Significant data gaps still exist on possible emissions and end-product contaminants for most alternative technologies.
- Many technology options have been found to work only on site-specific circumstances thus, site-specific information and treatability studies are necessary.
- Cost is often a key factor that has prevented the commercialization of many technologies.

The factors cited above may pose as challenges to researchers and governments in coming up with a technology alternative to incineration. Therefore, an extensive review of the extent of the PCB problem of an individual country will be critical before an appropriate technology maybe selected. The greatest challenge is in the bioremediation of PCB-contaminated sites. The complexity of the microbial processes responsible for degradation and the complexity of the compounds themselves make it difficult to degrade the compounds. There are 60–90 congeners present in commercial PCBs that were discharged to the environment and PCB degraders have positional selectivity in attacking the chlorine substituent. In addition there is complexity in the interaction of contaminated sites with microbes and individual congeners. All these factors have to be considered when remediating contaminated sites.

Acknowledgment

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