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Advances and perspective in bioremediation of polychlorinated biphenyl-contaminated soils

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Abstract In recent years, microbial degradation and bioremediation approaches of polychlorinated biphenyls (PCBs) have been studied extensively considering their toxicity, carcinogenicity and persistency potential in the environment. In this direction, different catabolic enzymes have been identified and reported for biodegradation of different PCB congeners along with optimization of biological processes. A genome analysis of PCB-degrading bacteria has led in an improved understanding of their metabolic potential and adaptation to stressful conditions. However, many stones in this area are left unturned. For example, the role and diversity of uncultivable microbes in PCB degradation are still not fully understood. Improved knowledge and understanding on this front will open up new avenues for improved bioremediation technologies which will bring economic, environmental and societal benefits. This article highlights on recent advances in bioremediation of PCBs in soil. It is demonstrated that bioremediation is the most effective and innovative technology which includes biostimulation, bioaugmentation, phytoremediation and rhizoremediation and acts as a model solution for pollution abatement. More recently, transgenic plants and genetically modified microorganisms have proved to be revolutionary in the bioremediation of PCBs. Additionally, other important aspects such as pretreatment using chemical/physical agents for enhanced biodegradation are also addressed. Efforts have been made to identify challenges, research gaps and necessary approaches which in future, can be harnessed for successful use of bioremediation under field conditions. Emphases have been given on the quality/efficiency of bioremediation technology and its related cost which determines its ultimate acceptability.

Keywords Polychlorinated biphenyls (PCBs) · Dechlorination · Bioremediation · Pretreatment · Plant-microbe interaction · Genetically modified organisms (GMOs) · Phytoremediation

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Introduction

Increased industrialization in developed/developing countries has resulted in many types of pollutants contaminating the environment over recent decades. Anthropogenic activities have introduced numerous xenobiotic hydrophobic organic compounds into the natural environment. Many of these contaminants have multiple toxic and mutagenic effects on human health as well as environment. Polychlorinated biphenyls are among one such group of notorious contaminant. Polychlorinated biphenyls (PCBs) are members of chlorinated organic chemicals which may theoretically contain 209 different congeners. These congeners are formed with different number and position of chlorine atoms on the biphenyl ring (Bedard 2003; Pieper 2005; Tu et al. 2011; Passatore et al. 2014). However, only 60–90 congeners have actually been reported in commercial chemical PCB formulations (Wiegel and Wu 2000; Field and Sierra-Alvarez 2008). Highly chlorinated congeners are more stable and tend to have lower solubility in aqueous solution and also have higher octanol water

partition coefficients (K_{OW}) than low molecular weight PCBs (Hawker and Connell 1988). The high K_{OW} is partly responsible for their persistence and enables them to strongly absorb in the soil (Wiegel and Wu 2000; Passatore et al. 2014). The semi-volatility is the reason of their spread throughout the environment according to the "grasshopper effect" which bioaccumulate, volatilize in warmer conditions and deposits in colder climates (Gomes et al. 2013). They are persistently showing their presence in the list of top 10 most toxic priority pollutants of the US Agency for Toxic Substances and Disease Registry (ATSDR) (Agency for Toxic Substances and Disease Registry 2011; Meggo and Schnoor 2013). Considering the environmental and ecological impacts of PCBs, an international chemical treaty, i.e. Stockholm Convention has listed them among the priority persistent organic pollutants (POPs) which also documents that the PCBs must be eliminated from the environment by the year 2025 (Egorova et al. 2013). Commercially, PCBs were sold under several trade names at various parts of the globe, e.g. Aroclor (Monsanto, USA), Santotherm (Mitsubishi, Japan), Clophen (Bayer, Germany) and Phenoclor and Pyralene (Prodolec, France) on the basis of percent of chlorine (by weight).

It is reported that between 1920 and early 1980s, approximately 1.5 million tons of PCBs was manufactured throughout the globe and as a result of which a significant amount of PCBs has been released in the environment (Pieper 2005; Sharma et al. 2014). Due to their extensive use in the past and lack of appropriate disposal technologies, PCBs and their congeners have attained ubiquitous distribution from the Arctic to the Antarctic. Although, the extent of global PCB contamination is still to be studied; Holoubek (2000) made an attempt to examine sites contaminated with PCBs throughout the world and provided detailed information with respect to its production, import/export, fate, contaminated sites and management approaches. However, inventorization of sites contaminated with PCBs has to be carried out by each signatory country to Stockholm Convention which is to be documented under their respective national implementation plans (NIP) POPs.

According to a report of the USEPA (2011), 350 sites are contaminated with PCB in the USA, while 148 sites in Canada are contaminated with PCBs as per Federal Contaminated Sites Inventory (TBCS 2011). Meijer et al. (2003) estimated global soil total PCB burden of 21,000 tons in an inventory on atmospheric deposition in background surface soil. The threshold concentration for contaminated soil varies between 10 and 50 mg kg^{-1} in some countries, while in some countries, it may be as low as 0.5 mg kg^{-1} (CCME 1999; EPA 2009; UKEPA 2004; USEPA 2012).

PCBs enter the environment during their production; due to accidental spills and leaks; and during transportation, use, disposal etc. PCBs own a "dioxin-like toxicity" which makes it a probable carcinogen due to this reason (Baars et al. 2004). Some PCB congeners posses dioxin-like activity with associated toxicity. PCBs have recently been categorized as carcinogen class I by the International Agency for Research on Cancer (IARC) (Lauby-Secretan et al. 2013). Initially, toxicity testing was restricted to animals only which with time was extrapolated to humans also. Three decades ago, Safe (1984) has reported development of hepatic tumours in rats due to exposure to PCBs. Reduced reproduction and mass mortality of sea birds are reported as a result of bioaccumulation of PCBs (O'Riordan 1995; Borja et al. 2005). Several studies have reported a variety of exposure-related effects of PCBs in humans, including endocrine disruption, non-specific reproductive effects, dermal abnormalities including chloroacne and abnormal neurobehavioral effects in children (ASTDR 2000; Ross 2004). Serious health effects among approximately 14,000 people have been reported in Yusho, Japan, after ingestion of PCB-contaminated rice oil and the effects of which can still be observed (Gomes et al. 2013). PCBs also indirectly affect humans by entering the food chain through transfer by phytoplanktons to invertebrates, fish and mammals. Owing to these hazards to both humans and environment as a result of PCB bioconcentration, it is necessary to deal with this toxic contaminant in an eco-friendly and cost-effective manner.

Remediation measures for PCBs

Due to their long persistence and deleterious environmental and health impacts, it is important to secure and decontaminate the polluted sites. This is highlighted in many national and international studies. To address this challenging problem, UNIDO has prepared a toolkit on investigation and management of sites contaminated with POPs (UNIDO Contaminated site toolkit 2010). Several methods have been reviewed and suggested for effective remediation/destruction of PCBs which include landfilling, landfarming, incineration, thermal desorption, chemical dehalogenation, plasma arc, catalytic hydrogenation, ultrasonic technology and advanced oxidation processes (Li et al. 2007; Gomes et al. 2013). The selection of appropriate technology for remediation depends upon the socio economical and climatic conditions of a particular place and availability of particular technology plus concentration, volume and matrix of the PCB contamination along with any co-contaminant. The half life of PCBs varies with respect to different environmental conditions. Further, Weber (2007) has suggested a list of criteria for evaluation of PCBs/POPs destruction technologies which include (a) applicability (target contaminants); (b) overall cost reliability and maintenance safety; (c) residuals produced (byproducts: PCDD/PCDF, other POPs, other toxic compounds); (d) minimum achievable concentration; (e) public acceptability; (f) development status; (g) environmental impacts; (h) performance dependency on site characteristics; (i) clean-up time required; (j) decontaminated soil quality and (k) site data needed.

Several technologies listed earlier have their own advantages and disadvantages with respect to time required, efficiency and effectiveness, amount of waste/unintentional byproduct generated etc. Therefore, development of cost-effective and environmentally sound technology becomes an urgent need of the time. In this context, bioremediation has incredible potential to satisfy the requirement and holds faith for protection of environmental and its management (Juwarkar et al. 2014). Bioremediation is an increasingly popular alternative to conventional methods for treating pollutants with the possibility to degrade contaminants, since it uses natural microbial activity mediated by different consortia of microbial strains. Many studies on bioremediation have been reported, and the scientific literature has revealed the progressive emergence of various advances in bioremediation techniques (Vidali 2001; Juwarkar et al. 2010; Aken et al. 2010; Nanekar and Juwarkar 2015).

The bioremediation process is divided into two techniques as ex situ and in situ. The in situ techniques are preferred compared to ex situ techniques as they cut down the cost of excavation and also restrict the contaminant transfer by carrying out the remediation process at the site of contamination. In situ techniques comprise of both bioremediation (including microbial and fungal) and phytoremediation techniques which when clubbed can emerge as an effective weapon for eradication or breakdown of organic contaminants like PCBs. Remediation using fungal strains in some cases proved as a highly effective remediation approach (Kubatova et al. 2001; Juwarkar et al. 2014).

The superhydrophobicity of PCBs makes its biodegradation a very difficult task. The rate and efficiency of biodegradation of PCBs can be increased using a chemical reduction/oxidation process which modifies molecular structures that are resistant to biodegradation (Dercová et al. 1999; Baciocchi et al. 2005; Prządo et al. 2007). Chemical reduction/oxidation is a process in which a hazardous contaminant is chemically converted into a non-toxic or less hazardous compound resulting in more stable, less mobile and inert products (Li 2006). Chemical oxidation is an effective and innovative technology for degradation of a range of contaminants in which chemical oxidants are delivered to contaminated media either to destroy the contaminants or to convert them into easily biodegradable compounds (Goi et al. 2006). This process combined with biodegradation forms an emerging technique having a chemo-biological approach towards breakdown of PCBs and is briefed in this article.

Bioremediation: strategies and outline

Bioremediation is defined as the breakdown of contaminant by biological mechanisms that include organisms in order to clean a contaminated site. Use of microorganisms capable of utilizing organic contaminant as a carbon source is the essence of bioremediation. Some microorganisms survive in the contaminated site by degrading the contaminant using an enzyme or cofactor during the oxidation or reduction of carbon containing complex organic compounds. Indeed, many congeners can be degraded by multiple pathways (Bedard 2003; LaRoe et al. 2014). Additionally, the rate of degradation strongly depends on the nature of microbial population and their metabolic specificity which actually depends on the number and position of chlorine atoms along with the presence of electron donors (Wiegel and Wu 2000). This intrinsic property of catabolism possessed by microorganisms favours their use in the bioremediation process. Ideally (Chávez et al. 2006), microbes can be used for bioremediation of PCB-contaminated sites if it posses properties such as (a) PCB tolerance, (b) surfactant production which increases bioavailability of PCBs, (c) chemotactic towards PCBs, (d) possession and expression of various dehalogenating enzymes responsible for dechlorination of PCBs, (e) degradation of PCBs without or with minimal generation of toxic intermediates and (f) able to survive till the completion of a clean-up process.

Further, it has been comprehensively explained by Wiegel and Wu (2000) that various environmental factors including temperature and pH affect the growth and variety of metabolic processes of different microorganisms and hence affect their ability of dechlorination of PCBs. Accordingly, a better understanding of whether and to what extent environmental factors are affecting is important and this knowledge will help in predicting potential for PCB degradation in soil and will support in developing PCB bioremediation plans. Isolation of these kinds of organisms and their augmentation at contaminated site ensures that the contaminant is degraded completely or transformed into a non-toxic compound.

Bioremediation technology can be effective in both aerobic and anaerobic environments which has been one of the reasons for its wide acceptance. Bioremediation may be either aerobic or anaerobic (Wiegel and Wu 2000; Juwarkar et al. 2014). Owing the problem associated with either of this method to treat highly complex compounds, sometimes, sequential anaerobic-aerobic bioremediation processes are also adopted to remediate contaminated sites (Master et al. 2002).

Anaerobic bioremediation

Anaerobic bioremediation is facilitated by anaerobic organisms which break chemical compounds in the soil to release energy required for their metabolic processes. Anaerobic bacteria respire by means of electron acceptors like sulphates and nitrates in uncontaminated soils. However, in case of PCB-contaminated soils, they switch to dehalorespiration (May et al. 2008; Payne et al. 2011). Dehalorespiration is a process in which bacteria attack chlorine substituents in para and meta

position, replacing them with hydrogen. The numbers of these bacteria are small or negligible in soil which explains the reduced natural anaerobic degradation of PCBs. The process of dehalorespiration transforms higher chlorinated congeners to less chlorinated congeners thus decreasing their toxicity and further making them available for aerobic degradation (Lehtinen 2010).

Biostimulation of anaerobic organisms

Biostimulation simply refers to a stimulation of the indigenous flora by providing optimum survival conditions. In case of soil, anaerobic organisms, creating anaerobic conditions can be stimulation. Several researchers in the 1990s have performed dechlorination of Aroclor congeners under anaerobic conditions and have found promising results and concluded that anaerobic bioremediation can act as the only way to breakdown highly chlorinated PCBs (Quensen et al. 1990; Tiedje et al. 1993; Alexander 1999). For activation of these organisms, anaerobic conditions can be created by flooding of soil with water.

Microbial dechlorination of Aroclor 1260 was stimulated with the use of other halogenated aromatic compounds (DeWeerd and Bedard 1999). Although, dechlorination of PCBs can be stimulated by using some non-specific inducers such as fatty acids, alcohols, glucose, hydrogen and zero valent metals; but the final products formed are the same (Rysavy et al. 2005). Moreover, a significant increase in microbial activity was found after the addition of defined minimal medium comprising of suitable nutrients and trace elements. The addition of $FeSO_4$ to the Aroclor 1242-contaminated soils showed promising results in stimulation of dechlorination process for PCBs as it stimulated the growth of sulphate-reducing microorganisms that were responsible for PCB dechlorination (Borja et al. 2005; Anyasi and Atagana 2013). It was also reported that the direct addition of Fe^0 to contaminated sediments might significantly reduce the lag period before dechlorination (Rysavy et al. 2005). The concentration of sodium bicarbonate was also found to affect the dechlorination of 2,3,4,5-CB in sediments (Yan et al. 2006).

Bioaugmentation of anaerobic organisms

The term bioaugmentation stands for the addition/supplementation of microbial strains capable of degrading the pollutants at respective contaminated site. The success of the process generally depends on the efficient and reliable microbes which are to be augmented with knowledge of their survival and metabolic activities. The availability of robust information on augmented population can provide insight for an effective management of the contaminant (Chi et al. 2013).

Laboratory as well as in situ reductive dechlorination of PCBs using anaerobic microorganisms has been demonstrated extensively. Bioaugmentation of granular anaerobic methanogenic microbial consortium along with a suitable carbon source was successfully demonstrated (Nollet et al. 2005). In this line, anaerobic dechlorination of PCBs has also been reported for various contaminated sites (Pakdeesusuk et al. 2005). Macedo et al. (2007) observed a transformation of highly chlorinated PCB congeners as an effect of adaptation of microbial communities in contaminated soil. A group of microorganisms within the dechlorinating *Chloroflexi* that appear to be common in PCB-contaminated sites may catalyse reductive dechlorination activity (Watts et al. 2005).

Moreover, several studies on dechlorination of Aroclor (1260 and 1254)-contaminated soils demonstrated positive results, by priming the indigenous microorganisms in sediments with PCBs (Rysavy et al. 2005). Yan et al. (2006) found that chemistry and origin of contaminated soil considerably affected the activity of bioaugmented PCB-degrading cultures. Certain macro- and microelements are crucial in the process of dechlorination of PCBs in soil (Zeeb et al. 2006). Recently, it was showed that paddy field soils have the potential for anaerobic microbial degradation of a wide range of PCB congeners (Baba et al. 2007; Chen et al. 2014). However, it was also found from the biological data estimation (e.g., biomarkers of contamination, structure of the microbial community) that the results were comparatively similar in both biostimulated soils as well as in soils without addition of *Dhc* (*Dehalococcoide*) consortium indicating specialized PCB dechlorinators were not adopted to the harsh conditions (high PCB concentrations) existing in the contaminated soil (Matturro et al. 2016).

Aerobic bioremediation

Aerobic bioremediation makes use of microorganisms that use atmospheric oxygen to perform breakdown of contaminants. The main approach towards encouraging aerobic degradation of PCBs has been via addition of oxygen, co-substrates, inducers, surfactants and sometimes bioaugmentation of PCB-degrading bacteria (Abraham et al. 2002; Ohtsubo et al. 2004; Field and Sierra-Alvarez 2008). Biphenyl, which is vital the primary substrate that supports PCB co-metabolism, has been successfully employed to stimulate degradation of PCB-contaminated soil aerobically (Field and Sierra-Alvarez 2008). During aerobic degradation, oxidative destruction of PCBs takes place involving several genes and their associated enzymes (Pieper 2005; Field and Sierra-Alvarez 2008; Hashmi et al. 2016). These genes are mainly *Bph* gene clusters viz. *BphA*, *BphB*, *BphC*, *BphD*, *BphE*, *BphF*, *BphG* which give rise to enzymes like BphB (dehydrogenase), BphC (ring cleavage dioxygenase), BphD (hydrolase), BphE (hydratase),

BphF (aldolase) and BphG (acetaldehyde dehydrogenase). These enzymes are major enzymes required in PCB degradation pathway as documented by Ohtsubo and co-workers (2004). Figure 1 represents one of the most accepted and uncomplicated schematic representations of pathways of aerobic degradation of PCBs as described by Bedard (2003) and Pieper (2005). According to the process stated by Lehtinen (2010), bacteria first transform PCBs to chlorobenzoic acid (CBA) using biphenyl as a carbon and energy source. Furthermore, CBA-degrading bacteria transform CBA to less toxic end products and aerobic degradation is known to breakdown lower chlorinated congeners.

Biostimulation of aerobic organisms

For biostimulation of PCB degradation, pH in neutral range, optimum salt concentrations, adequate availability of macro- and microelements along with addition of mineral sources of nitrogen, phosphorus and potassium distinctly accelerate PCB biodegradation in some soils and sediments (Fava et al. 2003). It majorly covers several remedial technologies which enhance biodegradation of the contaminant by supplementing soils with growth substrates/co-substrates. The most common biostimulation agents include bulking agents, nutrient supplementation, halogenated priming compounds (halopriming) and surfactants (Passatore et al. 2014; Nanekar et al. 2015). The rate of PCB dechlorination can be increased by priming with a halogenated compound. During this process, a halogenated aromatic substrate is used to stimulate PCB-degrading indigenous organisms (Bedard et al. 1998; Passatore et al. 2014). This process is based on the assumption that high concentration of dehalogenation substrate will enhance the growth of dehalogenating microorganisms selectively as they use the compound as an electron acceptor. This enhanced population of dehalogenators will further dechlorinate PCBs in the contaminated site. In a study by Haggblom and co-workers (2003), a 74% decrease in sediment associated PCBs was observed in a year by addition of 26-BB (bromobiphenyl) as a priming agent which activated PCB-dechlorinating indigenous bacteria.

Biphenyls, chlorobiphenyls and some more easily degradable brominated analogues of PCBs along with CBAs (chlorobenzoic acids/chlorobenzoate) have been postulated to be inducers of aerobic biodegradation of PCBs in soils (Pieper 2005). Ferrer et al. (2003) have documented the use of maltotriose fatty acid monoesters drastically increases the bioavailability which in turn accelerates the biodegradation of higher PCBs. Addition of nutrients such as ammonia-nitrogen and phosphate, biphenyl and oxygen enhances PCB

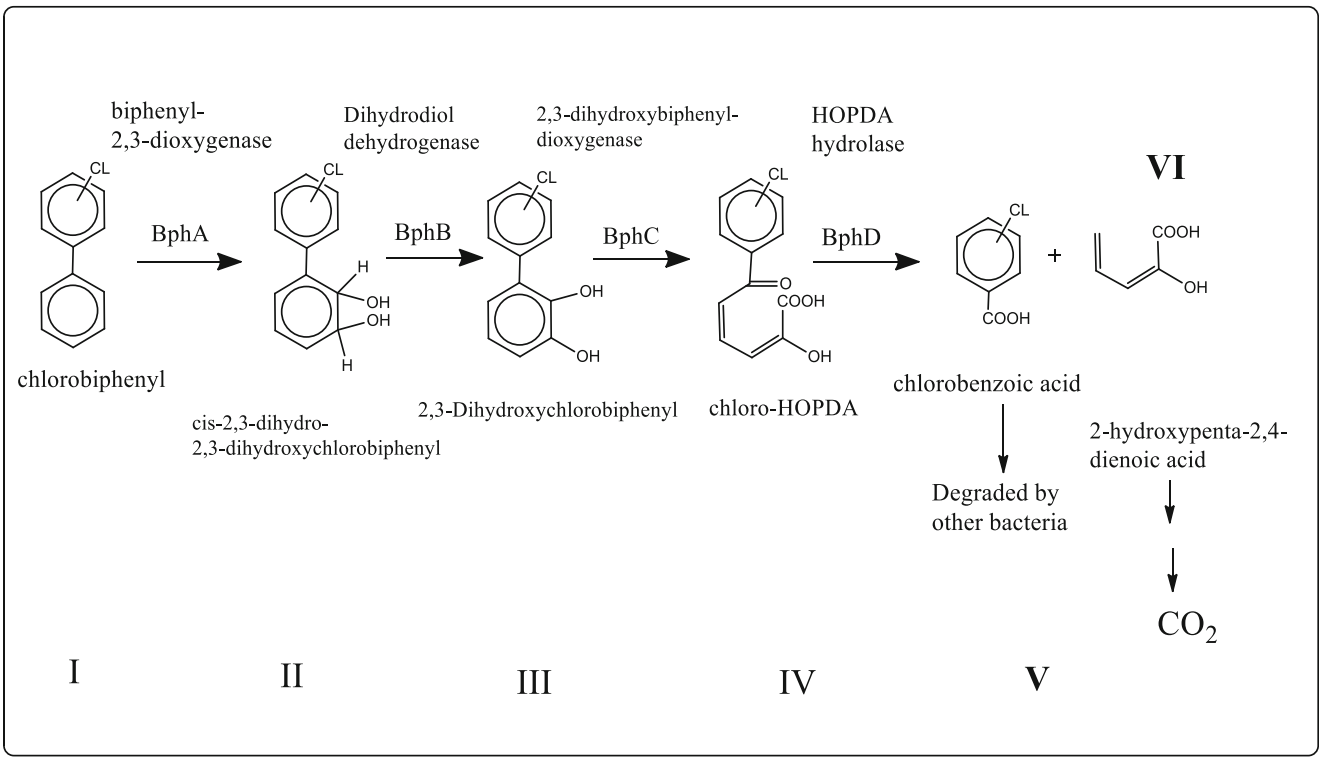

Fig. 1 Pathway of aerobic PCB degradation by biphenyl-oxidizing bacteria (Novakova et al. 2002; Bedard 2003; Pieper 2005). (*I*) biphenyl, (*II*) 2,3-dihydroxy-4-phenylhexa-4,6-diene, (*III*) 2,3-dihydroxybiphenyl, (*IV*) 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid, (*V*) chlorobenzoic acid, (*VI*) 2-hydroxypenta-2,4-dienoic acid. (*bphA*) biphenyl 2,3-dioxygenase, (*bphB*) dihydrodiol dehydrogenase, (*bphC*) 2,3dihydroxybiphenyl 1,2-dioxygenase, (*bphD*) 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase

biodegradation (Wiegel and Wu 2000). Several studies conducted in soil microcosms spiked with known mixture of PCBs have revealed that they can be significantly biodegraded, particularly when they are amended with biphenyl and oxygen and inoculated with PCB-degrading bacteria. Reports show that studies conducted on PCB-contaminated soils have confirmed this finding (Field and Sierra-Alvarez 2008). Bacteria of the species *Rhodococci*, such as strain RHA1, are recognized to be effective PCB degraders which have good survival in soil (Leigh et al. 2006). A study demonstrates isolation of three aerobic bacterial strains from Nigerian polluted soils, which could grow on all mono-CBs and on a wide range of di-CBs (68 to 100% removal) (Adebusoye et al. 2007). When dealing with hydrophobic contaminants like PCBs, certain surfactants can be augmented to enhance the process of degradation. Several bacterial and fungal species produce metabolic products which mimic surfactants when they are grown on hydrophobic compounds. These are called biosurfactants which are cell wall associated and secreted externally. The excreted ones can be used for emulsification and hence enhanced remediation of PCBs (Zhang et al. 2012). Biosurfactants increase the surface area of hydrophobic compounds and increase their bioavailability owing to their amphiphilic structure. Moreover, these compounds are biodegradable and non-hazardous (Pacwa-Płociniczak et al. 2011). In some studies, it was reported that biosurfactants can increase the bioavailability of non-aqueous and soil-bound phases of PCBs through desorption and solubilization (Robinson et al. 1996; Fiebig et al. 1997; Cho et al. 2004; Viisimaa et al. 2013). It could be presumed that biosurfactants can not only enhance the bioavailability of PCBs but also their availability to chemical oxidants as both processes are predetermined by similar functionalities (Viisimaa et al. 2013). Biosurfactants also reduced the lag time before dechlorination. Amendment of biosurfactant can be accomplished through in situ enrichment of biosurfactant-producing microorganisms or direct application of biosurfactants (Cho et al. 2004; Field and Sierra-Alvarez 2008).

Bioaugmentation of aerobic organisms

Aerobic organisms actively degrade mono- and dichlorbiphenyls (Egorova et al. 2013). However, many aerobic bacterial strains like *Pseudomonas*, *Burkholderia* and *Rhodococcus* display degradative activity to lower and highly chlorinated biphenyls (Pieper 2005; Egorova et al. 2011; De et al. 2006; Hatamian-Zarmi et al. 2009; Petric et al. 2011). Fava and Bertin (1999) have reported that exogeneous PCB- and CBA-degrading bacteria can be used in slurry phase in presence of biphenyl and oxygen for effective bioremediation of PCB-contaminated soil. The following bacteria showed encouraging results: a mixture of gfp-transformed strains in soil microcosm (*Pseudomonas* sp. Cam-1-gfp1 and Sag-50G-gfp1); a mixture of strain *Pseudomonas testosteroni* B-356 along with a surfactant-producing, hydrocarbon-degrading strain in soil microcosm (Ahn et al. 2001); a strain of *Janibacter* sp. in liquid medium and soil (Sierra et al. 2003); *Pseudomonas fluorescens* HK 44 bearing a naphthalene-degradation plasmid and the bioluminescence gene lux in field condition (Ang et al. 2005); biphenyl-degrading strains of *Arthrobacter* sp. B1B and H850 in the presence with carvone, salicylic acid and surfactant sorbitol trioleate in soil (Singer et al. 2000). Among the most methodically studied are *Burkholderia xenovorans* LB400 and *Rhodococcus jostii* RHA1 (Pieper 2005; Furukawa and Fujihara 2008). In studies carried out by various researchers, PCB degrader strains *Rhodococcus ruber* P25 and *Microbacterium* sp. B51 degraded a broad range of PCB congeners. Strains P25 and B51 were found to degrade chlorinated biphenyls efficiently from mono to hexachlorobiphenyls which includes planar congeners too. It was recognized that these strains are able to utilize a variety of chlorobiphenyls as medium for growth without requiring supplementary carbon source and accumulated non-/less-toxic byproducts in the environment (Rybkina et al. 2003; Egorova et al. 2011; Plotnikova et al. 2012). In the recent past, *R. ruber* P25 and *Microbacterium* sp. B51 demonstrated a high degradation capability to all type of congeners present in Sovol (Egorova et al. 2013).

Effectiveness of sequential anaerobic and aerobic treatment for PCB degradation

Anaerobic microbes use reductive dechlorination to reduce the number of chlorine atoms whereas aerobic oxidation is brought about by addition of oxygen to biphenyl ring. The replacement of a chlorine substituent by a hydrogen and the departure of chlorine as chloride ion is termed as reductive dechlorination. Furthermore, decrease in chlorine number results in decreased anaerobic reductive dechlorination rates while the same results in increased aerobic oxidation rates (Anid et al. 1993). Owing to high redox potentials of highly chlorinated congeners, they are less susceptible to aerobic degradation. Low chlorinated congeners are reduced to greater extent and hence more vulnerable oxidation. These processes (dechlorination and oxidation) are dependent on substitution position of chlorine and not only on its number. Therefore, sequential anaerobic-aerobic biodegradation has been proposed as an efficient strategy for treatment of PCB-contaminated soils and sludges which have been tested successfully in sediment microcosms (Klasson et al. 1994; Rodrigues et al. 2006). A study conducted by Master et al. (2002) revealed that the higher chlorinated congeners were converted into lesser chlorinated congeners predominantly tetrachlorobiphenyls which were subsequently degraded by *Burkholderia* LB400 in a sequential anaerobic-aerobic treatment of Aroclor 1260. A decrease in average chlorine content by 20–30% has also been observed in Canadian arctic soils

contaminated with Aroclor 1260 upon inoculation of anaerobic river sediments (Kuipers et al. 2003). Later on, several two-step anaerobic-aerobic bioremediation experiments were carried out for Aroclor 1242-contaminated sediment (Rodrigues et al. 2006). A biological tilled soil reactor which functioned under sequential anaerobic-aerobic conditions has achieved 75% reduction of total PCB concentration in sludge from Ralston street lagoon which was heavily contaminated by Aroclor 1248 (Tharakan et al. 2006). More recently, in a two-stage organic composting, 25% reduction of PCBs was observed during a 98-day experiment (70 days anaerobic and 28 days aerobic) suggesting benefits of sequential approach for remediation. The study also suggested that further research on two-stage composting to get better results for tackling of higher chlorinated biphenyls (Long et al. 2015).

Scope of metagenomics approach

Although degradation of PCB by isolated microbes is well characterized as discussed earlier, knowledge on the role of uncultivable microbes in PCB bioremediation is very limited. Considering >99% environmental microbes are uncultivable (Singh 2010), the diversity of majority of the degrading but uncultivable microbes and their physiological capabilities are still to be discovered. Recent advancements in technologies provide unprecedented opportunities to generate in-depth knowledge and harness them for better, effective, economic bioremediation technologies (Ray et al. 2012). For example, metagenomics not only provides an enormous opportunity to characterize the PCB-degrading uncultivable bacteria but can be also exploited to provide novel gene enzyme systems which can increase the efficiency of transgene-based bioremediation technologies. However, environmental microbes are extremely diverse and metagenomics approach to find new a gene/enzyme system for bioremediation may be too costly and time-consuming to be practically and economically feasible in some cases. However, because several microbes can utilize PCB as a carbon source, stable-isotope probing (SIP) combined with metagenomes can overcome this problem to some extent (Singh 2009). In the approach, soil is first incubated with 13C PCBs and cellular components including genetic materials (DNA, RNA) of microbes which will utilize PCB as a source of energy get enriched 13C. Heavy DNA (13C DNA) of PCB-degrading microbes can be separated from other environmental microbes by ultracentrifugation before metagenomic analysis. Such an approach has already produced a number of genes for bioremediations (Sul et al. 2009).

Integrated chemo-biological approach

One of the most effective approaches for enhanced degradation of PCBs is the application of a pretreatment step prior to the biological degradation (Aronstein et al. 1995; Dercová et al. 1999). For this pretreatment, advanced oxidation process is among the most commonly used methods which are based on generation of hydroxyl radicals to initiate oxidation of organic compounds (Aronstein et al. 1995; Dercová et al. 1999; Manzano et al. 2003; Prządo et al. 2007). These hydroxyl radicals can be generated using hydrogen peroxide in the presence of catalysts, i.e. ferrous ion and is commonly referred as the Fenton's type reaction. The Fenton's type reaction is effective owing to its non specificity towards the aromatic rings during the chemical oxidation process. Generally, partial chemical oxidation increases the water solubility of organics that in turns increases bioavailability and facilitates biological action for enhanced degradation (Dercová et al. 1999). By combination of these two processes, one can expect more efficient degradation of PCBs at a low cost and lesser time when compared with the classical bioremediation technologies. Dercová et al. (1999) have suggested that partial chemical oxidation is responsible for increased water solubility of the organic compounds which leads to increase in biosusceptibility and thus facilitates microbial action which in turns enhances the biodegradation rate. Scanty work has been carried out on chemical pretreatment of PCBs using the Fenton's type reaction (Viisimaa et al. 2013); however, researchers have documented the process for pretreatment of different organic compounds such as PAHs, phenols, PCPs and chlorinated organic pesticides for their subsequent biodegradation. Yang (1994) reported that the pretreatment of 4,4′-dichlorobiphenyl (DCB) and 2,2′,4,4′,6,6′-hexachlorobiphenyl (HCB) using Fenton's reagent in which the biodegradation rate constant for pretreated sample was 5 times faster than the untreated sample. Two possible means of enhanced biodegradation of PCBs post Fentos's pretreatment were suggested by Aronstein et al. (1995), i.e. (a) utilization of partially oxidized compounds in the system and (b) direct microbial attack on transformed compound. Subsequently, various researchers have reported the effectiveness of Fenton pretreatment for degradation of PCBs (Dercová et al. 1999; Manzano et al. 2003; Prządo et al. 2007). Studies have also been carried out using UV radiations, ozone as well as photo-Fenton process for pretreatment of PCBs (Quiroga et al. 2009; Javorská et al. 2009; Dasary et al. 2010; Liu et al. 2011). Liu et al. (2012) demonstrated the combined effect of pretreatment of PCB-contaminated soils using biosurfactant washing and UV irradiation on subsequent increase in biodegradation rate. More recently, it was also reported that joint application of microorganisms, biosurfactant and oxidizing chemicals in moderate quantity to PCB-contaminated soil led to increase in soil respiration along with dehydrogenase activity as compared to that obtained by microbial consortium alone, demonstrating stimulation of microflora integrating these processes (Viisimaa et al. 2013).

Another approach for pretreatment has been the use of activated carbon (AC) which is used as an adsorbent for many volatile/hydrophobic/organic contaminants because of its high specific surface area and microporous structure (Payne et al. 2011; Kjellerup et al. 2013). It has an inherent property to attract PCB degraders to form biofilm and also to keep PCB adsorbed to the surface. Hence, use of dechlorinating bacterial biofilm-coated activated carbon at contaminated site ensures close proximity of PCB and its degraders. Use of biofilm-adsorbed AC also provides high density of PCB degraders on its surface increasing the direct interaction between PCB and bacteria required for electron transfer and subsequent PCB degradation. The adsorption also protects the dechlorinating bacteria from being washed off and scavenging from indigenous organisms which helps in their long-term presence at the contaminated site (Edwards and Kjellerup 2013).

Further, nanoscale zero valent metals have great potential for in situ PCB remediation. Zero valent iron (ZVI) oxidizes to the environmentally friendly Fe(III) and can be applied through direct subsurface injection (Gardner et al. 2004). Researchers are now focusing on iron-reducing cultures that may dechlorinate PCBs co-metabolically. Wiegel and Wu (2000) have studied PCB dechlorination to occur under iron(III)-reducing conditions. Use of nanoscale ZVI reduces the oxidation reduction potential of contaminated sediments and stimulates anaerobic organisms. Such conditions are favourable for sulphate reducers and methanogens. Thus, indigenous/augmented cultures were stimulated by the use of zero valent metals to enhance the reductive dechlorination (Mikszewski 2004). A very recent research conducted by Le et al. (2015) revealed that bimetallic nanoparticles Pd/nFe used for pretreatment of Aroclor 1242 resulted in dechlorination of tri-, tetra-, penta-, and hexachlorinated biphenyls upto 99, 92, 84 and 28%, respectively. The resulted biphenyls were later subjected to rapid biodegradation by *B. xenovorans* LB400 in which benzoic acid was formed as an intermediate.

White rot fungi as an attractive candidate for bioremediation (mycoremediation)

Breakdown of PCB's is majorly restricted by their hydrophobic nature making them less bioavailable for microbial breakdown. White rot fungi are a group of basidiomycetes considered the most efficient organisms in mineralizing lignin in nature. Lignin degradation (ligninolysis) is brought about by a group of extracellular lignin-modifying enzymes (LME) which comprise of lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. The non specificity of these enzymes provides white rot fungi with the unique ability to degrade a wide range of environmental pollutants such as dioxins, PCBs, petroleum hydrocarbons, munition wastes (such as trinitrotoluene), industrial dyes, herbicides and pesticides. Alike ligninolysis, degradation of a number of pollutants by these organisms is activated by limitation for nutrients such as N and C and is also temporally correlated to lignin mineralization. Furthermore, they utilize other available sources of energy in the environment, such as sugars and polysaccharides and not the pollutants and in turn needlessly breakdown various pollutant chemicals, which are usually present in minute amounts (Marco-Urrea and Reddy 2012).

These organisms have become a positive option for remediation due to the following features/characteristics (Baldrian 2008; Pinedo-Rilla et al. 2009):

- The availability of organisms for bioremediation studies due to their wide distribution in the nature
- The flexibility to degrade a range of chlorinated organic pollutants either individually or in consortium
- Inherent biodegradation enzymes help in acclimatization in the polluted environment
- Extracellular enzymes peroxidases and laccases break down the pollutants via oxidation and avoids internalization of substrates
- Their growth via hyphal extension helps in attaining better contact to few contaminants which accumulate in small pores in soil

Phanerochaete chrysosporium decreased PCB concentration of Aroclors 1242, 1254 and 1260 (Yadav et al. 1995; Borazjani et al. 2005; Gomes et al. 2013). Additionally, congeners of lower chlorine numbers were shown to be degraded more extensively (Borazjani et al. 2005; Gomes et al. 2013). Beaudette et al. (1998) evaluated biodegradation of six selected PCB congeners using 12 white rot fungi. However, only a few PCB degradation studies were performed in soil systems. Lower concentration of surfactants increased fungal mineralization of PCB congeners (Beaudette et al. 2000). The ability of fungi to degrade low PCB concentrations has been demonstrated for several strains (Kamei et al. 2006).

Phytoremediation of PCBs

Microbial remediation faces some difficulties due to the presence of a wide range of congeners and their low bioavailability and also due to their positional selectivity in attacking the chlorine substituent. In addition, there is complexity in the interaction of contaminated sites with microbes and individual congeners (Borja et al. 2005). Metabolites of PCB degradation can also affect the viability of the organisms involved in degradation due to their high toxicity effects (e.g. dihydrodiols and dihydroxybiphenyls) (Cámara et al. 2004). Though microbial degradation is effective till some extent, the cost constraints increase when we aim the maximum degradation of

PCBs (owing to the cost of augmented biosurfactants, bacterial consortiums and other co-substrates). Therefore, it needs to be clubbed with some other remediation techniques like phytoremediation for better and harmless degradation outcomes. Use of plants will help to overcome these issues and help in maximum degradation of PCBs. The details are discussed further in this article.

Phytoremediation is an emerging technology that uses living green plants and associated bacteria or fungi for in situ treatment of contaminated soil, sludges, sediments and groundwater through removal, degradation or containment of the contaminant (Aken et al. 2010). Although, phytoremediation is a natural process; investigation of its efficiency and progress in its application as a modern and innovative treatment technology at waste sites are not very old (Newman and Reynolds 2004; Liu and Schnoor 2008; Aken et al. 2010; Abhilash et al. 2012). Very recently, Arslan et al. (2015) have provided a critical view of factors that affect absorption and translocation of POPs in plants along with the limitations that plants have to deal with during the POPs remediation. Phytoremediation of PCBs may take place by one of several ways: pollutants can be taken up inside the plant tissues (phytoextraction/phytoaccumulation); enzymatic transformations of PCBs can occur within the plant (phytotransformation) or volatilize into the atmosphere through the leaves (phytovolatilization). The secondary metabolites released by plants also enhance microbial activity, improving the degradation of PCBs in the root zone (rhizoremediation); it can be adsorbed to the roots (rhizofiltration) or contained to the soil material (phytostabilization) (Aken et al. 2010). Out of the above processes, phytoextraction and rhizoremediation are found to be most effective ways of PCB degradation and are discussed further in this article. Figure 2 represents different processes and phases for phytoremediation of PCBs from contaminated soil.

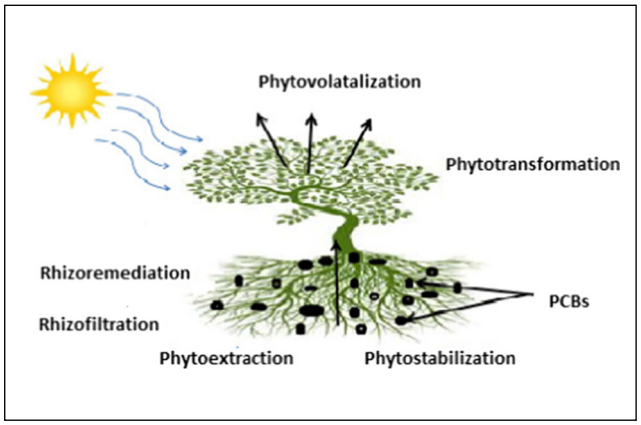

Fig. 2 Phytoremediation of polychlorinated biphenyls (PCBs) may involve several processes viz.; pollutants from contaminated soil, sediment and groundwater can be taken up by the plant tissues (*phytoextraction*) or adsorbed to the roots (*rhizofiltration*); pollutants inside plant tissues can be transformed by plant enzymes (*phytotransformation*) or can be volatilized into the atmosphere (*phytovolatilization*); pollutants in soil can be degraded by microbes in the root zone (*rhizoremediation*)

Phytoextraction of PCBs

Phytoextraction involves two major processes, i.e. phytoaccumulation and translocation. Metabolism of the contaminant starts from absorption of PCBs to roots followed by active translocation to the shoots. Once accumulation of the contaminant is complete, transformation of the contaminant to less toxic metabolites takes place which are then phytoevaporated/evapotranspirated through the plant leaves (Prasad 2011). PCBs diffuse into the free spaces in the endodermis of the root and then must bypass the Casparian strip, where they can be translocated up into the shoots via the vascular tissues (Zeeb et al. 2006). Several studies highlighted the potential of phytoextraction for PCBs from contaminated soils (Teng et al. 2010; Ficko et al. 2011; Wang et al. 2011).

Many researchers have studied the uptake and translocation of PCBs in different plant species such as corn (*Zea mays* L.), cabbages (*Brassica oleracea* var. *capotata* L.), pumpkin (*Cucurbita pepo* ss *pepo* cv. Howden), carrots (*Daucus carota* L.), squash (*C. pepo* ssp. *ovifera*), zucchini (*C. pepo* ssp. *pepo*), beets (*Beta vulgaris*), turnips (*Barssica rapa* L.) and beans (*Phaseolus vulgaris*) (Iwata and Gunther 1976; Fries and Marrow 1981; Webber et al. 1994; White et al. 2006; Low et al. 2010). For the same reason, they are taken up by plant tissues in negligible amounts. However, there are some plant species belonging to Cucurbitaceae family which are known to accumulate PCBs in the roots as well as shoots. It is reported that pumpkins are efficient in taking up and translocating PCBs from soil (Aslund et al. 2008). Also, it has been documented that *C. pepo* ssp. *pepo* plants maximize phytoextraction of PCBs when the plant shoot has reached its maximum biomass (Low et al. 2010). Also, Hulstler and his co-workers (1994) have studied the uptake of PCBs in zucchini and found that zucchini fruits could accumulate two orders of magnitude of more polychlorinated dibenzo-*p*-dioxins and dibenzofurans than other fruits and vegetables in the same contaminated site. Findings of some researchers (Mattina et al. 2007; Aslund et al. 2007, 2008; White et al. 2006) support the hypothesis that plant species *C. pepo* spp. *pepo* are efficient in phytoextraction of PCBs containing POPs by uptake and translocation to the shoots via roots. Currently, the research focuses on the uptake mechanisms of *C. pepo* for POP-containing PCBs. The higher uptake of PCBs in this plant can be an induced phytoextraction process wherein the compounds secreted by plant roots into the soil facilitate the uptake of PCBs (Dakora and Phillips 2002). A list of selected plants species studied for phytoextraction of PCBs along with the major findings is given in Table 1.

Table 1 Plant species studied for phytoextraction of PCB's

Plant(s)	Contaminant(s)	Accumulation in plant	Major findings	Reference
Cucurbita pepo ssp. *pepo* cv. Howden	Aroclor 1248	Roots and shoots	PCBs accumulated in roots and shoots during the growth period with stable concentration	Low et al. 2010
Cucurbita pepo ssp. *pepo* cv. Howden	Aroclor 1254–1260	Roots and shoots	Greater accumulation of PCBs in shoots as compared to roots owing to higher uptake and translocation	Aslund et al. 2008
Medicago sativa L.	PCB-contaminated soil	Roots and shoots	Inoculation with rhizhobium and fungal species increased the accumulation of PCBs in shoots along with higher yield of alfalfa	Teng et al. 2010
Cucurbita pepo ssp. *pepo* *Bidens cernua* *Chenopodium album* *Daucus carota* *Plantago major* *Rumex crispus*	PCB-contaminated soil	Roots and shoots	Equal distribution of PCBs in roots and shoots was observed. Root exudates were found to affect uptake and transport of contaminants within specific plant species	Ficko et al. 2011
Zea mays	Mix of PCB congeners (15, 28, 47)	Roots and shoots	Higher translocation factor of PCBs resulted in higher PCB concentration in shoots as compared to roots	Wang et al. 2011
Nicotiana tabacum *Solanum nigrum*	Mix of PCB congeners (total PCB concentration 110 mg kg^{-1})	Roots, leaves and berries	Both plant species differed in the amount of accumulation and distribution of PCBs within the plant species. Uptake of PCBs was observed in roots, leaves and berries	Kurzawova et al. 2012
Amaranthus retroflexus *Ambrosia artemisifolia* *Brassica nigra* *Cirsium vulgare* *Daucus carota* *Echinochloa crusgalli* *Lythrum salicaria* *Polygonum persicaria* *Setaria viris* *Solidago canadensis* *Soncus asper* *Symphyotrichum ericoides* *Symphyotrichum novae-angliae* *Vicia cracca* *Chrysanthemum leucanthemum*	Mixture of Aroclors 1254/1260 Aroclor 1248	Roots and shoots	All the studied plant species showed significant accumulation of PCBs in their root and shoot tissues. However, it was also observed that changes in environmental conditions such as precipitation and temperature possibly affected plant growth, and therefore potentially the plant tissue concentrations	Ficko et al. 2010
Zea mays L.	e-waste-contaminated soil	Roots and shoots	In this comparative study on effect of chelant and plant-growth-promoting bacteria (PGPB) on PCB uptake, P GPB resulted in higher accumulation of PCBs in shoot owing to higher plant growth and biomass	Luo et al. 2015

Rhizoremediation of PCBs

Rhizoremediation is a promising phytoremediation strategy that banks on the ability of plant roots to facilitate growth and activity of pollutant-degrading bacteria present in its rhizosphere. Researchers have reported significant reduction of PCBs in planted soils as compared to that of unplanted controls (Chaudhry et al. 2005; Gerhardt et al. 2009). PCBs got sorbed to soil particles strongly owing to their hydrophobic nature. Bittsánszky and his co-workers (2011) have compiled a review on different species of plants belonging to Cucurbitaceae family having the potential to accumulate PCBs in plant roots and shoots. Tall fescues can accumulate considerable amount of PCB in the roots and are hence good candidates for phytoaccumulation (Pinsker 2011) Although PCBs are accumulated within the plant, reports on their transformation to non-toxic components are not available. Hence, the fear of introduction of accumulated PCBs in the soil matrix upon death of plant persists. In view of this problem, breakdown of the contaminant is an apt solution for its remediation. This is brought about by rhizospheric bacteria, and the process is called rhizodegradation. Leigh et al. (2006) documented that the indigenous PCB-degrading microorganisms are associated with the plants growing in the contaminated soils. Research also suggest the significance of the plant and rhizospheric microbial interactions (Mackova et al. 2006) concerning their ability to degrade PCBs. Arslan et al. (2015) have comprehensively reviewed and compiled information on plant-rhizobacteria partnership for remediation of POPs including PCBs.

Fate of PCBs in the rhizosphere Degradation of organic pollutants can still be a problem even with sufficient microbial biomass and availability of the contaminant. This can be due to uninduced degradation pathway genes or insufficient energy for performing the degradation process. The part of gene inducers and surfactants in making the compound available for both rhizospheric bacteria and plants has been discussed earlier. Energy supply to the degrading cells is also of equal importance while studying the degradation of organic contaminants. It is quite possible that the energy produced by the cells after degradation of the contaminant is not enough for the survival of the organisms. Plants are capable of ameliorating this deficit of energy (McCutcheon and Schnoor 2004). Aerobic degradation of higher chlorinated compounds in rhizospheric soil is either very slow or negligible. Plants secrete certain compounds in root exudates that are similar to biphenyl and act as co metabolites for stimulation of PCB-degrading microorganisms in the rhizosphere (Singer et al. 2000; McCutcheon and Schnoor 2004). Plant roots also provide certain compounds that drive metabolism of PCBs as secondary substrates (McCutcheon and Schnoor 2004).

It has been reported that few compounds present in root exudates released by plant may stimulate microbial degradation of PCBs (Mackova et al. 2006). *Brassica nigra* directly contributed to the enhanced removal of PCBs in Aroclor 1242-contaminated soil (Singer et al. 2003). *Carex aquatalis* and *Spartina pectinata* are predicted to be among the most efficient and effective plants for phytoremediation of PCBs (Smith et al. 2007). Efforts were undertaken to expand the degradation capacity of rhizosphere-competent bacteria. Strain F113 has been found to be an excellent colonizer in several plant rhizospheres which helped in co-metabolism of PCBs better than strain LB400 (Villacieros et al. 2005). Zeeb et al. (2006) recently studied the phytoremediation of a soil with slight contamination of Aroclor 1260. However, no considerable PCB removal was found in highly contaminated soil, but the plants performed well in lower contamination. Table 2 presents the PCB-metabolizing bacteria isolated from various rhizospheres of plant species. Further studies are needed to characterize both cultivable and uncultivable microbes which can mineralize PCB in the rhizosphere. Emerging technologies such as metagenomics, transcriptomics and proteomics not only provide huge opportunity to do so but also allow an understanding about their physiological capabilities. Such information is key for successful exploitation of microbial capability for industrial processes including bioremediation. For example, these technologies can be employed to know whether intrinsic microflora have degrading capability of PCB (by examining degrading genes/proteins) and what nutritional amendment is needed for biostimulation.

However, phytoremediation has a limitation that this technology is quite slower and is climate dependent. To overcome this limitation, genetically modified organisms and transgenic plant species for speeding up the remediation process can be implemented. The rhizopheric degradation can be enhanced by inducing degradation genes in organisms as well as plants to reduce the time required. Also, transgenic plants which are able to survive in given climatic conditions can be designed. The use of both in the remediation process is discussed further.

Genetically modified organisms and transgenic plants for phytoremediation of PCBs

In the face of the complex detoxification pathway present in plants, their slow generation time compared with that of microorganisms means that plants have had less time to evolve efficient methods for detoxifying these synthetic compounds (Aken et al. 2010). Although bacteria isolated from contaminated soil can rapidly detoxify PCBs in laboratory cultures, the fact that these PCBs persist in the environment suggests that bacteria do not possess enough biomass or metabolic activity to decontaminate these areas significantly (Liste and Alexander 2000; Zhuang et al. 2007). The rhizosphere

Table 2 PCB-metabolizing rhizospheric bacteria isolated from rhizospheres of respective plant species

Bacteria	Plant(s)	Contaminant(s)	Probable effect	Reference
Arthrobacter sp.	Austrian pine (*Pinus nigra* L.)	PCB-contaminated soil	Biphenyl degradation	Leigh et al. 2007
Rhodococcus sp.				
Staphylococcus sp.				
Pseudomonas sp.				
Burkholderia cepacia	Barley (*Hordeum vulgare*)	2,4-Dichlorobiphenyl	Biotransformation of PCBs	Petroutsos et al. 2008
Pseudomonas plecoglossicida	Nightshade (*Solanum nigrum*)	PCB mixture Delor 103	Phyto/rhizoremediation of PCBs	Mackova et al. 2009
Ochrobactrum anthropi	Horseradish (*Armoracia rusticana*)			
Achromobacter xylosoxidans				
Pseudomonas sp.	Thistle (*Silybum marianum*)			
P. stutzeri	Alfalfa (*Medicago sativa*)			
P. fluorescens	Tobacco (*Nicotiana tabacum*)			
P. putida				
P. fluorescens				
P. pseudoalcaligenes				
Hydrogenophaga palleronii (AF019073)	Horseradish (*Armoracia rusticana*)	PCBs	Metabolism and catabolism of biphenyl in the rhizosphere	Uhlik et al. 2009
Achromobacter xylosoxidans subsp. *xylosoxidans* (AJ491839)				
Methylobacillus flagellatus (CP000284)				
Rhizobium meliloti	Alfalfa (*Medicago sativa* L.)	PCBs	Enhanced removal of PCBs	Xu et al. 2010

provides a natural environment for in situ bioremediation of the contaminant in soil. Inserting genes encoding biphenyl pathway into the host bacteria which already exists in the rhizosphere ensures rapid degradation. Also, exudates from plants can act as co-substrates that biostimulate degradation activity of microbes (Rein 2006). Plant roots in turn help to reduce leaching of contaminants, aerates the soil and release exudates that foster selective microorganisms (Amos and Younger 2003). This kind of rhizoremediation technology which conjugates use of GMOs and plants is a promising bioremediation technology.

The microbial and mammalian catabolic genes possess the metabolic enzymes for complete mineralization of organic molecules. These genes can therefore be used to harmonize the metabolic abilities of the plants. Transgenic plants have been developed for the phytoremediation of PCBs. Recently, the use of transgenic plants for PCBs phytoremediation has been evaluated by several researchers (Cherian and Oliveira 2005; Eapen et al. 2007; Doty 2008; Aken 2008; Aken et al. 2010). In an innovative study, Francova et al. (2003), developed transgenic tobacco plants (*Nicotiana tabacum*) by inserting a gene responsible for 2,3-dihydroxybiphenyl ring cleavage, *bph*C, from the PCB degrader *Comamonas testosteroni*. Similarly, *bph* genes from *B. xenovorans* LB 400 were cloned into tobacco plants, one of the most efficient PCB-degrading bacteria. *bph*AE, *bph*F and *bph*G which are essential components of the *bph* operon needed for dioxygenation of the biphenyl ring were independently cloned and expressed in transgenic plants. It was observed that purified enzymes extracted from plants were capable of oxidizing 4-chlorobiphenyl into 2,3-dihydro-2,3-dihydroxy-4′-chlorobiphenyl. Sylvestre et al. (2009) reported that transgenic plants can also generate three components of the biphenyl dioxygenase and the 2,3-dihydroxybiphenyl dioxygenase which catalyse vital steps in bacterial PCB degradation. Taking this into account, this type of microbe-assisted phytoremediation wherein transgenic plants initiate PCB metabolism and exude nutrients for rhizospheric degradation can be implemented. Table 3 presents a list of transgenic plants and bacteria engineered for phytoremediation of PCBs. Recently, Novakova et al. (2009) successfully cloned 2,3-dihydroxybiphenyl-1,2-dioxygenase, *bph*C gene obtained from *P. testosteroni* B-356 into one of the highly suitable tobacco (*N. tabacum*) plant during which the growing transgenic plants in presence of 2,3-dihydroxybiphenyl show higher resistance to the toxic compounds in comparison to wild type plants. The growing transgenic plants in presence of 2,3-dihydroxybiphenyl show higher resistance to the toxic compounds in comparison to wild type plants. In an effort to improve rhizoremediation performances, several researchers have cloned key catabolic genes of known PCB degraders into specific rhizosphere bacteria. Villacieros et al. (2005) isolated *bph* operon from *B. xenovorans* strain LB400 and introduced

Table 3 List of genetically engineered plants for phytoremediation of PCB's using gene from bacterial source

Gene	Source	Target	Contaminant(s)	Effect	Reference
Biphenyl dioxygenase, *bph*, located on transposon, TnPCB	*Pseudomonas* sp. strain LB400	Sugar beet seeds (cv. Rex)	PCBs	Rhizodegradation of PCBs by *Pseudomonas*	Brazil et al. 1995
Biphenyl dioxygenase, *bph*C	*Comamonas testosteroni* B-356	Tobacco (*Nicotiana tabacum*)	PCBs	Phytodegradation of PCBs	Francova et al. 2003
Oxygenolytic *ortho*-dechlorination (*ohb*) gene	*Pseudomonas aeruginosa* strain 142	*Sinorhizobium meliloti* colonizing Alfalfa (*Medicago sativa*)	2′,3,4-Trichloro-biphenyl	Enhanced *ortho*-dechlorination of PCBs	Toure et al. 2003
Biphenyl dioxygenase, *bph* Green fluorescent protein, *gfp*	*Burkholderia xenovorans* LB400	Alfalfa roots (*Medicago sativa,* var. Resis, DLF Trifolium)	3-Chloro-biphenyl	Rhizosphere degradation of PCBs	Boldt et al. 2004
PCB-degrading genes, *ortho*-halobenzoate 1,2-dioxygenase (*ohb*) genes	*Pseudomonas aeruginosa* strain 142	*Sinorhizobium meliloti* colonizing Alfalfa (*Medicago sativa*)	2′,3,4-Trichloro-biphenyl	Enhanced bioremediation of PCB	Chen et al. 2005
Biphenyl dioxygenases, *bph*A, *bph*E, *bph*F, *bph*G	*Burkholderia xenovorans* LB400	*Nicotiana tabacum* *Nicotiana benthamiana*	4-Chloro-biphenyl	Oxygenation of 4-chloro-biphenyl	Mohammadi et al. 2007
Biphenyl dioxygenase, *bph*	*Burkholderia xenovorans* LB400	*Pseudomonas fluorescens* F113 colonizing willow (*Salix* sp.) rhizosphere	Individual PCBs congeners	Degradation of PCB congeners	Rein et al. 2007
Biphenyl dioxygenase, *bph*	*Burkholderia xenovorans* LB400	*Pseudomonas fluorescens* colonizing rhizosphere of willow (*Salix* sp.)	PCB-contaminated soil	Rhizoremediation of PCBs through genetically modified organisms	de Carcer et al. 2007
2,3-Dihydroxybiphenyl-1,2-dioxygenase, *bph*C	*Pseudomonas testosteroni* B-356	Tobacco (*Nicotiana tabacum*)	2,3-Dihydroxybiphenyl	Increase the efficiency of the biodegradation of PCBs	Novakova et al. 2009
2,3-Dihydroxybiphenyl-1,2-Dioxygenase, *bphC*	*Pandoraea pnomenusa*	Tobacco (*Nicotiana tabacum*)	PCBs (Delor 103)	Increased efficiency degradation of total PCBs	Viktorová et al. 2014
2,3-Dihydroxybiphenyl-1,2-Dioxygenase, *bphC*	*Agrobacterium tumefaciens*	Alfalfa (*Medicago sativa*)	PCBs and 2,4-dichlorophenol (2,4-DCP)	Expression of bphC enhanced tolerance of plants and remediation of PCBs and 2,4-DCP	Wang et al. 2015

into strain F113 under the influence of a strong promoter, nodbox 4, from *Sinorhizobium meliloti*. The modified strain, F113::1180, expressed a high level of biphenyl dioxygenase which was capable of metabolizing biphenyl and various PCB congeners at a much higher rate than strain F113pcb (Rein et al. 2007). Mesocosm experiments with PCB-contaminated soil demonstrated a good survival capability of F113 strains in willow plant rhizosphere, signifying that alliance of transgenic rhizosphere bacteria with plants represents a promising approach for the management of PCB-contaminated soils.

Basidiomycetes like white rot fungus produces unique extracellular oxidative enzymes like lignin peroxidase (Lip), manganese-dependent peroxidase (Mnp) and laccase (Lac) which are found to be important in degradation of PCBs. In the study conducted by Sonoki and co-workers (Sonoki et al. 2007), genes responsible for production of Lip, Mnp and Lac enzymes produced by *Phaenerochete chrysoporium* have been introduced into the DNA of *Arabidopsis thaliana* to make a transgenic species that efficiently degrades PCBs. More research needs to be done on plant-microbe interactions both GMO and inbuilt rhizhospheric bacteria and plant. The in-depth knowledge of the fate of the contaminant inside the plant is of utmost importance to avoid undesired effects in field application. Exploiting the knowledge on molecular communication between plants and microbes will be helpful in achieving better results in the elimination of contaminants and will be a fascinating area of research.

These studies might disclose the microbe-plant interactions and can be used to study the induction of catabolic pathways in polluted soils undergoing rhizoremediation. These emerging techniques will also allow to monitor or selection of catabolic genes to improve remediation strategies (Kiely et al. 2006). The development of metagenomic analysis will perhaps reveal new degradative genes that will be worth introducing into strains with other interesting qualities like superior root colonization capability. To find the perfect combination of plant and augmented organism for degradation, the signals that plants and microbes exchange when they identify each other will have to be understood along with dissection of molecular basis of the specific interactions between certain plant genotypes and specific bacteria (Segura et al. 2009).

However, the impact of transgenic plants on the environment is still being debated and we strongly recommend the use of native plant species of the contaminated site as primary option for phytoremediation owing to their adaptability to the conditions.

Estimation of global cost for remediation

Most of the above-mentioned technologies are going through their developmental stages. Additional information regarding field data and pilot scale experiments is required to evaluate the effectiveness and efficiency of these technologies. As discussed earlier, owing to the complexity of PCBs, a single technology does not seem to be conveniently applicable to both ex situ and in situ remediation of PCB-contaminated soil. Every case is different and various factors need to be considered while calculating the cost constraint (Gomes et al. 2013). Investigations on application of bio- and phytoremediation are growing rapidly all over the globe because of its advantages over conventional physico-chemical treatments. The ever-growing demand of developing countries has pushed the international community to harness biological remediation technologies, wherever applicable and as well as to assess the estimated cost for remediation of contaminated sites. There is a vastly growing market for environmentally sound management of hazardous waste and remediation of contaminated site which was estimated around US$ 1 trillion (Masons Water Yearbook 2000–2001).

The cost including the risk associated with a particular technology is of high priority. The financial aspects include costs of capital and operation, installation and management, energy consumption, chemicals, manpower, monitoring, pretreatment and post-treatment. On the other hand, risk assessment should include loading flexibility, emergency management and transient control (Rahuman et al. 2000; Li et al. 2007). The literature shows that the worldwide market for the contaminated site remediation is possibly in the range between US$30 and 35 billion (Singh et al. 2009). On the other hand, it is calculated that approximately US$1.5 billion is the global requirement of bioremediation technologies per annum (Passatore et al. 2014). The market for bioremediation of contaminated sites is ready to explore in various countries such as Western European countries, Canada, Australia, Japan and the USA. Whereas, developing countries such as Asian, Latin American and Eastern European countries correspond to the budding market for contaminated site bioremediation. However, it is very complicated to assess the cost of this promising market for remediation because of lack of an established comprehensive catalogues for contaminated sites in several countries (Singh et al. 2009). Nonetheless, Li et al. (2007) has comprehensively assembled a few recognized bioremediation technologies under the assignment of United Nations Industrial Developmental Organization (UNIDO) viz. DARAMEND®, Xenorem™, estimating approximately US$55 to $360/m^3 for remediation of sites contaminated with chlorinated POPs. USEPA has also calculated the costs for phytoremediation of halogenated POPs (specifically chlorinated) including PCBs which estimates in the range of $150 and $630/m^3. These estimates may vary according to different essential factors including site characteristics, availability of expertise and national law and legislation. Table 4 represents comparison of various technical aspects/requirements between bio/phytoremediation and other physico-chemical technologies (Rahuman et al. 2000; Li et al. 2007; Gomes et al.

Table 4 Comparison between different factors affecting bio/phytoremediation and other physico-chemical technologies

Factors (dependency/ requirement)	Bio/phytoremediation	Other physico-chemical technologies, e.g. incineration, pyrolysis and solvent extraction
Soil temperature	High	Average to high
Soil moisture	Average to high	Average to high
Particle size	High	Average to high
Permeability/clay content	High	Low
Space requirement	Depends whether in situ or ex situ	Average to high
Pretreatment	Low to average	Average
Power	Low	High
Water	Average	Low to average
Monitoring	Low	High
Skilled labour	Average	High
Transportation	Low	High
Excavation	Low	High
Post-treatment	Low	Low to average
Impact on environment	Low	Average (sometimes high)
Hazardous byproducts	Low	Average to high
Field testing	Limited to average	Average to high
Developmental stage	Initial to practical	Practical
Societal acceptance	Average to high	Low to average
Efficiency	Average to high	High
Cost	Low to average	High

2013). Inspite of all these factors, the worldwide market for bioremediation of contaminated sites is experiencing a qualitative transformation and evidently, it will attain market maturity and stability (Passatore et al. 2014).

Conclusions and future perspectives

Owing to the physico-chemical properties of PCBs such as persistence, low bioavailability and toxicity, sustainable remediation measures are warranted from time to time. Rapid progress has been made in developing effective, economical and socially viable bioremediation processes. It is expected that in the future, bioremediation using microbes, plants (including transgenic plants) and plant-microbe interactions will be used widely to significantly reduce PCBs from the environment. As pretreatment for enhanced biodegradation of PCBs, an integrated approach for chemo-biological treatment seems to be an effective tool for remediation. However, to exploit these possibilities on large scale, several scientific, regulatory and social aspects are needed to be addressed as follows:

1. Up scaling laboratory finding to field scale needs further understanding of microbial and plant's physiological requirements. In this case, examples of petroleum industries that have successfully implemented bioremediation technology on field scale can be followed for remediation of PCB-contaminated sites.
2. Emerging consensus is that phytoremediation in combination with microbial degradation can proved to be an effective technology to remediate PCB contamination. On this front, further understanding of rhizospheric microbial interactions is fundamental for effective remediation. Novel knowledge and investigations on mode of interactions and communication between different biotic factors in rhizospheric zone will provide further tools for effective remediation technology.
3. Combining phytoremediation with other economic and environmental benefits is needed. For example, use of biofuel plants for bioremediation will provide both remediation of the site and biomass for energy generation. This in turn will make technology economically attractive and environmental-friendly. Therefore, there is a need to study such plants with additional benefits.
4. Characterization of uncultivable PCB-degrading microbes and evaluating their physiological capabilities and nutritional requirements using emerging technologies of omics will provide a strong platform for exploitation of intrinsic microflora for bioremediation.
5. Discovery of novel and more efficient genes/enzymes using metagenomics and their expression in plants and microbes will benefit bioremediation efficacy. Here,

improvement in bioinformatics' support for metagenomic works is needed which is considered to be the main bottleneck for this technology.

6. The adoption of an integrated approach for enhanced degradation of PCBs needs to be investigated with a minimal requirement of chemical dechlorinators which may lead to complete mineralization of PCBs. Here, it is important to carry out further advanced investigations on optimum utilization of readily available strong oxidizing agents along with employment of several physico-chemical agents such as H_2O_2, nanoscale zero valent metals, activated carbon, ozone and UV radiations.
7. Social and regulatory acceptance of transgenic technology needs to be settled as soon as possible. Until then, non-transgenic technologies such as designer plants can be exploited for multi-purpose bioremediation.
8. Use of GMOs has difficulties in field applications even after their known and reported benefits. There are legal restrictions on release of recombinant organisms in the field in many countries which should be studied (along with its scientific concerns) before release of GMOs into the environment (Bloom and de Serres 1995). An important obstacle for field application of transgenic plants for bioremediation is linked with the true or apparent risk of horizontal gene transfer to wild or cultivated plants. Therefore, need for more risk-benefit analysis and risk mitigation plan becomes significant to guarantee that transgenic biotechnologies would result in wide recognition and application of bioremediation (Aken et al. 2010).

Additionally, it is necessary to achieve modifications in the existing legislation, overcome regulatory obstructions and educate the public to improve their views on GM plants and microbes. Current knowledge implies that bioremediation is an effective technology but requires time and needs to be tailored to achieve desired results for decontamination of PCB-contaminated sites.

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