Journal of Food Health and Bioenvironmental Science (September - December 2019), 12(3): 58-65

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Bioremediation of Persistent Organic Pollutants in Environment: Alternatives and Limitations

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Article info

Article history:
Received: 16 October 2019
Revised: 9 December 2019
Accepted: 12 December 2019

Keywords:
Bioremediation, Biodegradation, Persistent Organic Pollutants

Abstract

The contamination of persistent organic pollutants (POPs) in environment has been increasing in recent years due to anthropogenic activities. The toxicity of POPs even in the concentration at nanogram levels make them a concern for harmful effects on human health and environment. Human and animal exposure to POPs can lead to various effects ranging from skin and eye irritation, liver and kidney toxicity, nervous system damage, to cancer and death. So far, proper techniques for remediation of POPs are still unresolved. The chemical treatment methods are considered not environmental-friendly and cost-effective; therefore, remediation has favorably turned to biological techniques. Natural microorganisms have been shown by many studies to be able to degrade hazardous pollutants; however, the direct use of only environmentally-isolated microorganisms does not always result in effective remediation of POPs due to their toxic and recalcitrant nature. Therefore, effective strategies should be considered before applying the techniques for bioremediation of POPs. By examining the most recent research and studies for biodegradation of POPs, this review aims to describe the alternatives of bioremediation of POPs, which are the implementation of specific or adapted strains, the application of plant-microorganism interconnected relationships, and the utilization of enzymes. Moreover, the factors that can limit the complete bioremediation of POPs are also discussed. The gaps for improvement provided by previous studies should be able to pave the way for further studies to develop new techniques for bioremediation of POPs in contaminated sites.

Introduction

In addition to the increasing industrial and agricultural activities, with the advancement of electronics, pharmaceuticals, and medicines, more organic chemicals have been newly synthesized for human uses. Some of these chemicals showed the evidence of high persistence, potential bio-accumulation and adverse effect on human health or the environment, and also the potential for long-range transport; These compounds or persistent organic pollutants (POPs) have been a contamination problem for decades. In 2001, the

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Stockholm Convention on POPs listed twelve compounds as the initial POPs; as of 2017, the list is now expanded to cover sixteen more compounds (Table 1). These POPs can be categorized into three groups, e.g., pesticides, industrial compounds and unintentional by-products depending on the source information or use purposes. When released into the environment, POPs are considered as the emerging pollutants since their contamination is new to our generation and quite unknown for the methods of remediation. The toxicity of POPs, ranging from skin irritation to nervous damage or cancer, makes them harmful not only for human but also environment even in only small concentrations. Studies showed that these POPs can stay in environment for a very long time and hard to be degraded by light and oxygen alone (Arslan et al., 2017).

Various technologies have been proposed for the treatment of POPs. Some of the effective treatment methods such as advanced oxidation processes seem to rely on the ex-situ treatment where the portion of contaminated soil or water is removed from the field. These techniques require high capital cost and need an expert for maintenance. Inexpensive remediation techniques where they can be applied in-situ are preferable.

Bioremediation is a remediation technique that can be operated with a low-cost investment. A very successful bioremediation is commonly achieved in laboratory conditions; however, application of bioremediation needs to overcome some challenges on diverse environmental conditions that can affect not only the microorganisms but also the conditions for degradation of POPs.

This review is divided into 3 sections. First, the contamination and effects of POPs are given out to emphasize on the severe effects of them on environment and why they requires treatment. Next, the options for applying bioremediation in the contaminated sites are presented. Since there are many uncertainties in environment that can hinder the successful bioremediation, the factors limiting the bioremediation of POPs will be described in the last section.

Contamination and effects of POPs

Besides agricultural and industrial uses, POPs can be found from the residues of everyday products including cosmetics, disinfectant, antibiotics, anti-inflammatories, wood preservatives, paint additives, antidepressants, plasticizers and phthalates (Tripathi et al., 2015). There have been many reports on the contamination of POPs in soil and water ecosystems. It was found that wind speed, seasonal change, temperature variation, and land-use activities play an important role for environmental contamination and effects of POPs (Alharbi et al., 2018). The concentrations of conventional POPs such as PCBs, DDT, and some PAHs were found in soil ranging from 2 ng up to 7 µg (Zhu et al., 2014). Halogenated POPs such as trichloroethylene (TCE) have been a concern for over a decade due to the prevalence in soil and water. Since the POPs are hydrophobic in nature, they tend to sorb with the organic partitions, which are organic matter in soil particles and organic constituents in water and sediment (Ren et al., 2018b). The problem of POPs in Asia is projected to worsen according to the environmental standards that have been set to the higher levels than the guidelines set by the US EPA and EU. For example, the amount of organochlorine compounds as hexachlorocyclohexane (HCHs) is recommended below 950 ng/L by the US EPA's National Recommended Water Quality Criteria for aquatic life and is at 500 ng/L for the EU Drinking Water Regulations while the China environmental quality standards for surface water limits at 2,000 ng/L (Han & Currell, 2017). In Thailand, according to the Pollution Control Department, the national surface water quality standards for the organochlorine compounds are set at 5×107 ng/L for total organochlorine pesticides and at 20,000 ng/L for Heptachlor and Heptachlorepoxide (source: www.pcd.go.th).

POPs have been reported to generate oxidative stress, which creates an inflammatory response in the cellular level (Petriello et al., 2014). Organisms such as fish and insects exposed to POPs can result in birth defects and abnormalities (Chakraborty & Das, 2016). Also, it was reported that POPs can accumulate not only in animals but also in plants (Zhu et al., 2014). In case of humans, because POPs are highly soluble in lipid, the accumulation in human tissue is likely to happen. This leads to inflammatory diseases and the increased risk of chronic diseases (Petriello et al., 2014). Furthermore, many of POPs are known to be carcinogenic and mutagenic (Zhu et al., 2014). The stability of POPs in environment is high, and it will be longer for the higher chlorinated compounds (Arslan et al., 2017).

Alternatives for bioremediation of POPs

The chemical treatment techniques like advanced oxidation processes (AOPs) have been popularly used against the recalcitrant organic pollutants in soil and water. Since the structure of POPs is hard to break down, the most effective techniques rely on the generation of hydroxyl radicals (•OH), which is a very powerful oxidant, to break or destroy the specific chemical bonds in the target organic pollutants. It has been proved that various AOPs such as ozonation, Fenton oxidation, or photocatalysis were able to remove both conventional POPs such as organochlorine insecticides, solvents, and polychlorinated biphenyls and emerging POPs such as antibiotics, hormones, and drugs from water and wastewater (Ikehata et al., 2008). However, the generated hydroxyl radicals attack compounds non-specifically, which means not only the targeted POPs but also the surrounding organic matters; The excavation of contaminated soil or water, and also the extraction of POPs are required. Accordingly, an operation and maintenance cost can be the drawback when using the chemical or physicochemical treatments. Also, not many studies have been conducted on the mineralization and the bioavailability of the metabolites and degradation products after using AOPs (Ikehata et al., 2008). Furthermore, remediation techniques using chemicals will be considered 'not green' for environment. Even though some studies have successfully used environmentally friendly biopolymer for adsorption of POPs (Pariatamby & Kee, 2016), further management of the biopolymer waste containing POPs is required.

Microorganisms are the major group of living organisms in the environment, existing as free-living cells or biofilm. They play roles in many natural/environmental processes including bioremediation. Due to their high diversity and adaptability, microorganisms show great potential for POPs degradation. The successful POPs degradation by bacteria, fungi, and yeasts have been reported (Oyetibo et al., 2017). POPs can be degraded by being a direct substrate/carbon for microbial growth or getting destroyed through other biochemical pathways in a co-metabolism process (Oyetibo et al., 2017). POPs can be transformed by chemoorganotrophs through several pathways; for example, oxidation, reduction, hydrolysis and dehalogenation under both aerobic and anaerobic conditions (Chakraborty & Das, 2016; Ewald et al., 2019; Tripathi et al., 2015; Watanabe & Yoshikawa, 2008). The dehalorespiration of POPs by anaerobes yield the ATP and also the less halogenated congeners which are likely less toxic (Jeon et al., 2016; Sharma et al., 2018). However, the aerobic degradation of POPs appears to be a more effective alternative for POPs removal due to higher respiration-mediated energy yield since it could support microbial growth and POPs transformation (Jeon et al., 2016). Beside free-living cells, biofilms, the form of microorganisms living as a community within the extracellular polymers, have been found effective to degrade POPs as they have their biofilm structure to tolerate toxicity and create suitable conditions for growth (Gaur et al., 2018).

To ensure that biodegradation works successfully in the field, the survivability of the working bacteria in the environment, which needs to tolerate not only the toxicity from POPs but also harsh environmental conditions must be taken into account. Methanotrophs, for example, are one group of bacteria that can be isolated in a wide variety of habitats that can oxidize target pollutants including POPs by using the wide-range methane monooxygenase enzymes (Pandey et al., 2014). However, more research on using this group of bacteria is still ongoing whether they can adapt to various stresses in the field including pH, temperature, salinity, drought, and different chemicals (Jiang et al., 2010). Research has developed various methods in order to successfully apply bioremediation, which can be summarized as the possible alternatives for bioremediation of POPs shown in Figure 2. It should be noted that the ex-situ treatments such as bioreactor or slurry reactor are not discussed in this review. This is because despite having high removal rate in a short treatment time, ex-situ treatment requires the excavation or removal of soil and water, which increases the cost of treatment and needs specific adjustment varying among the contaminated sites (Eibes et al., 2015).

The genetic modification is also a potent approach for enhancing the efficiency of bioremediation (Singh et al., 2011). The biodegradation does not only depend on whether the microbes can produce degradative enzymes but they also need to endure the unfavorable conditions and grow fast enough to compete with other native microbes. Accordingly, unlike the laboratory or other foreign strains, the indigenous microbes appears to be a better choice for genetic engineering, e.g., introduction of the degradative enzyme, due to their higher chance of survival in the local environment (Singh et al., 2011). To construct a strain of genetically engineered microbes, the involved enyzyme(s), mechanisms or pathway need to be well laid. For examples, the isolate *Rhodococcus* sp. strain p52, is able to degrade wide ranges of contaminants including dioxins owing to two dioxygenases encoded by *dbfA* and *dfdA* (Peng et al., 2012). These two dioxygenases are involved in the important ring dihydroxylation during dioxin degradation. The two genes are located on the two different self-transmissible plasmids of the strain p52 thus they can be transferred to other bacteria including *B. cereus* (Peng et al., 2012), *Pseudomonas aeruginosa* and activated sludge bacteria (Ren et al., 2018a; Sun et al., 2017). After mated with the strain p52, the activated slude bacteria showed the almost complete degradation of 300 mg/L dibenzofuran, a model compound for dioxin degradation, within 50 hours whereas only 50 and 150 mg/L of dibenzofuran were degraded by the unmated activated sludge bacteria and the strain p52, respective (Sun et al., 2017). After 96 days, the activated sludge in the laboratory-scale sequenctial batch reactor (SBR) bioaugmented with strain p52 could completely degrade dibenzofuran whereas only 53% of dibenzofuran was removed in the nonbioaugmented SBR (Ren et al., 2018a).

Instead of using the whole-cell biocatalyst for biodegradation, applying only the isolated enzymes provides many advantages over the whole cells including no requirement for in-situ growth, easier handling and storage, and the comparable activity for biodegradation compared with cells (Eibes et al., 2015). Furthermore, using enzymes is less stringent than using the genetically engineered cells in many countries. Enzymes can be applied as a free form or immobilized with media, which is to prevent the loss of enzymatic activity. Normally in environment, enzymes tend to bind with the mineral and organic part of soil particles (Zimmerman & Ahn, 2011); however, the enzyme-organo-mineral relationships and interactions that contribute to the biodegradation of POPs still need to be elucidated.

Plants also play roles in POPs removal via several mechanisms. Some POPs, for example, PCBs can be adsorbed to plant root (phytostabilization/rhizofiltration) or taken up into plant tissue (phytoextraction) then volatilized into the atmosphere (phytovolatilization) or transformed (phytotransformation) (Aken et al., 2010). The typical pathway for phytotransformation of xenobiotics is as shown in Fig. 1. Plants appear to be a promising alternative for POPs remediation; however, its slow remediation rate is still a major challenge and that the accumulation of POPs may be toxic to plant cells. The use of plants and certain microorganisms has been found useful and could accelerate the remediation of POPs through their symbiotic relationship. Plants and their associated bacteria include ryegrass with *Pseudomonas* sp. and *Rhodococcus* sp.; Italian ryegrass, burdsfoot trefoil, and alfalfa with *Enterobacter ludwigii;* and corn and wheat with *Burkholderia cepacia* (Andria et al., 2009; Wang et al., 2010; Yousaf et al., 2011). Microorganisms can either reside inside plant tissue (endophytic microorganisms) or localize around plant roots (rhizospheric microorganisms). While plants provide nutrients and space for microbial colonization (Nanasato & Tabei, 2018; Zhu et al., 2014), these microorganisms, in return, could promote plant growth, help plant tolerate abiotic stress, preventing plant pathogen and degrade the xenobiotics including POPs in soil/water (Chakraborty & Das, 2016; Dimkpa et al., 2009; McGuinness & Dowling, 2009). Besides acting as a carbon and energy sources for rhizospheric microbes, root exudates can also act as an inducer for some enzymes in microbial degradative pathways (Jha et al., 2015). Growing the rhizospheric *Rhodococcus erythropolis* in the presence of a non-carbon source flavanone, which is a major component in root exudate of *Arabisopsis thaliana,* together with sodium acetate could enhance the degradation of 4-chlorobiphenyl (Toussaint et al., 2011). Pham et al. (2015) further reported the similar effect of other plant flavonoids on 4-chlorobiphenyl degradation and the 13-fold up-regulation of *bphA* (encoding for large subunit of biphenyl 2,3-dioxygenase) induced by isoflavone (Pham et al., 2015). Microbial-mediated process is the mojar POPs removal in the environment (Zhu et al., 2014). In addition to the plant and microbe-assisted remediation, the transgenic plants containing microbial catabolic enzymes have also been studied for POPs removal (Rylott et al., 2015). This plant-based remediation technologies can also provide products for further uses such as biofuels, biomass, and the chemicals extracted from the biomass (Tripathi et al., 2015).

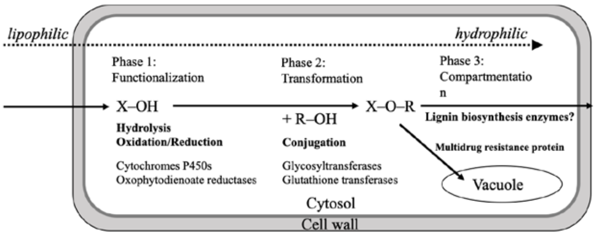

Fig. 1 The typical transformation/degradation pathway of xenobiotics in plant cell (Rylott et al., 2015).

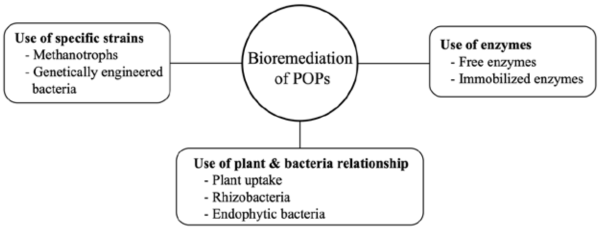

Fig. 2 Possible alternatives for bioremediation of POPs.

Factors limiting bioremediation of POPs

Generally, bioremediation of POPs can be applied using two methods: bioaugmentation and biostimulation. These two different techniques require different considerations. If non-native microorganisms are introduced to environment (bioaugmentation), the survivability of them in the field needs to be ensured; however, if the native species are induced for biodegradation (biostimulation), the condition adjustment is required. Either way, it is clear that environmental conditions and parameters are crucial for bioremediation of POPs. This is because environmental bioremediation of POPs by microorganisms depend on the conditions to allow microbial growth and metabolism of POPs (Pariatamby & Kee, 2016). It is difficult to achieve the same conditions for bioremediation in the contaminated sites as in laboratories, and the results are usually different between the lab-scale and pilot-scale treatments (Varjani et al., 2017).

Although the indigenous bacteria can be genetically engineered to increase the ability to withstand harsh conditions or to effectively degrade POPs (Chakraborty & Das, 2016), it is not always the case that the genetically engineered bacteria will be able to survive and work for a long period of time. Also, low public acceptability for using genetically engineered organisms can reduce the chance of in-situ application despite their high degradation efficiency (Singh et al., 2011). Even though enzymes are more easy-to-use than whole bacterial cells, the low stability of certain enzymes still needs to be improved. Studies have shown that immobilization of enzymes on nanoclay, metal minerals, and organic acids, which serve as the enzyme carriers, might be required in order to protect the denaturation of enzymes and ensure the effectiveness of POP degradation (Eibes et al., 2015).

In the presence of too many types of POPs, bioremediation using plants and microorganisms does not likely to give the desired degradation. Also, climate conditions are likely to affect the interactions between plant and microbes as well as the fate and transport of pollutants, all of which result in different degree of bioremediation (Tripathi et al., 2015). Factors affecting bioremediation of POPs by plants and plant-microbe interactions are bioavailability of POPs to bacteria, plant and bacteria tolerance to the toxicity of POPs, and the contribution of each bacteria survivability and detoxification ability to the whole plant-microbe community (Arslan et al., 2017).

Lastly, despite being the inexpensive and environmentally friendly techniques, bioremediation still has a challenge to overcome the treatment time (Ashraf, 2017). The biological treatment has been proven efficient for removal of hazardous substances, but more research should focus on developing a process that is less time-consuming as well.

Conclusion

From the cost and effectiveness point-of-view, bioremediation is among the most preferable techniques for remediation of POPs. Even though it seems promising, it is not a quick tool to use since it needs optimization. The options for bioremediation of POPs do not limit to building the treatment system or reactor but include bioaugmentation and biostimulation. Therefore, research in this area is still required to develop a stable system that can be applicable in the field. Using the native soil and aquatic bacteria will eliminate the problems of losing the specific species to the indigenous species. In summary, the isolation of indigenous microorganisms capable of degrading these emerging pollutants should still be going on along with the novel methods to control the environmental parameters important to biodegradation during the application of bioremediation.

Table 1 Initial and new POPs with their sources and health risks

Compounds	Sources	Health risks	Annex
Initial 12 POPs			
Aldrin	Pesticide	Nervous system and kidney effects	A: Elimination
Chlordane	Pesticide	Nervous system effects	A: Elimination
DDT	Pesticide	Liver and reproduction effects	B: Restriction
Dieldrin	Pesticide	Nervous system and kidney effects	A: Elimination
Endrin	Pesticide	Central nervous system effects on high dose	A: Elimination
Heptachlor	Pesticide	Effects on liver and fertility	A: Elimination
Hexachlorobenzene	Industrial compound	Nervous system and liver effects	A: Elimination
Mirex	Pesticide	Effects on eyes, thyroid, nervous and reproductive systems	A: Elimination
Toxaphene	Pesticide	Liver effects	A: Elimination
PCBs	Industrial compound	Skin and liver effects, probable human carcinogens	A: Elimination
Polychlorinated dibenzo-p-dioxins	Unintentional by-product	Liver, gastrointestinal, and endocrine system effects	C: Reduce the unintentional release
Polychlorinated dibenzofurans	Unintentional by-product effects	Liver, gastrointestinal, and endocrine system	C: Reduce the unintentional release
The new POPs (according to the Stockholm Convention)			
Alpha hexachlorocyclohexane	Pesticide and unintentional by-product of lindane	Potential carcinogen	A: Elimination
Beta hexachlorocyclohexane	Pesticide and unintentional by-product of lindane	Potential carcinogen	A: Elimination
Chlordecone	Pesticide	Effects on liver, nervous, and reproductive systems	A: Elimination
Decabromodiphenyl ether (commercial mixture, c-decaBDE)	Industrial compound	Endocrine toxic effects	A: Elimination
Hexabromobiphenyl	Industrial compound	Endocrine and reproductive system effects	A: Elimination
Hexabromocyclododecane	Industrial compound	Potential effects of respiratory and gastrointestinal systems	A: Elimination
Hexabromodiphenyl ether and heptabromodiphenyl ether (commercial octabromodiphenyl ether)	Industrial compound	Neurotoxic effects	A: Elimination
Hexachlorobutadiene	Industrial compound, unintentional by-product	Effects on kidney and liver	A: Elimination, and C: Reduce the unintentional release
Lindane	Pesticide	Moderately toxic and effects on nervous system	A: Elimination
Pentachlorobenzene	Pesticide, industrial compound, unintentional by-product	Central nervous system effect	A: Elimination, and C: Reduce the unintentional release
Pentachlorophenol and its salts and esters	Pesticides	Effects on liver, blood and nervous systems	A: Elimination
Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride	Industrial compound	Effects onthyroid function and fertility	B: Restriction
Polychlorinated naphthalenes	Industrial compound, Unintentional by-product	Skin and liver effects	A: Elimination, and C: Reduce the unintentional release
Short-chain chlorinated paraffins (SCCPs)	Industrial compound	Skin and developmental effects	A: Elimination
Technical endosulfan and its related isomers	Pesticides	Respiratory and skin effects	A: Elimination
Tetrabromodiphenyl ether and pentabromodiphenyl ether (com-mercial pentabromodiphenyl ether)	Industrial compound	Neurotoxic effects	A: Elimination

Sources: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services (2019); United States Environmental Protection Agency (2009)

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