Microbial degradation of halogenated aromatics: molecular mechanisms and enzymatic reactions

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Summary

Halogenated aromatics are used widely in various industrial, agricultural and household applications. However, due to their stability, most of these compounds persist for a long time, leading to accumulation in the environment. Biological degradation of halogenated aromatics provides sustainable, low-cost and environmentally friendly technologies for removing these toxicants from the environment. This minireview discusses the molecular mechanisms of the enzymatic reactions for degrading halogenated aromatics which naturally occur in various microorganisms. In general, the biodegradation process (especially for aerobic degradation) can be divided into three main steps: upper, middle and lower metabolic pathways which successively convert the toxic halogenated aromatics to common metabolites in cells. The most difficult step in the degradation of halogenated aromatics is the dehalogenation step in the middle pathway. Although a variety of enzymes are involved in the degradation of halogenated aromatics, these various pathways all share the common feature of eventually generating metabolites for utilizing in the energy-producing metabolic pathways in cells. An in-depth understanding of how microbes employ various enzymes in biodegradation can lead to the development of new biotechnologies via enzyme/cell/metabolic engineering or synthetic biology for sustainable biodegradation processes.

Introduction

Halogenated aromatic compounds are widely used in various applications including agricultural, dye, chemical and pharmaceutical industries (Arora et al., 2012, 2018; Arora and Bae, 2014). Widespread usage and accumulation of these compounds in the environment is a source of great concern, as they can cause harmful and toxic effects on human and animal health. Exposure to these compounds can cause many severe health problems such as DNA damage, organ damage, cancer or even acute death (Olaniran and Igbinosa, 2011; Georgiadis et al., 2018). Thus, technologies for limiting their spread into the environment, such as more effective methods for degrading these compounds are important for human health. In the past, several physical and biological processes have been explored for detoxification or degradation of chlorinated phenols (CPs) (Pera-Titus et al., 2004; Field and Sierra-Alvarez, 2008; Arora and Bae, 2014; Cycon et al., 2017; Huang et al., 2017). In nature, various microbes have evolved metabolic pathways to combat the existence of halogenated aromatics in their habitat. Proper exploitation of microbial biodegradation processes can provide a green and sustainable method for detoxifying these toxic compounds and reduce their harmful levels in freshwater and soil. The purpose of this review is to highlight the current state of knowledge regarding microbial pathways related to halogenated aromatics degradation.

In general, halogenated aromatics commonly used in household or industries can be grouped into three types based on their structural components (Fig. 1). The first...
type is a class of halogenated aromatics which contain only an aromatic skeleton with substituents such as halogenated benzene (HB), halogenated phenol (HP) [such as 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), 2,4,5-trichlorophenol (2,4,5- TCP), 2,4,6-trichlorophenol (2,4,6- TCP), pentachlorophenol (PCP)], halogenated aniline and its derivatives (such as 4-chloroaniline, 2-chloro-4-aminophenol, 4-chloro-2-aminophenol), 4-chlorobenzoic acid and tetrachlorohydroquinone (TCHQ). The second type is a class of compounds in which the aromatic rings are attached to extra moieties which often result in increased pesticide potency, but at the same time are more persistent due to their resistance to degradation (Casida and Quistad, 2004; Huang et al., 2017; Mostafalou and Abdollahi, 2017; Supreeth and Raju, 2017). These include 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its analogues, including 2-(4-chloro-2-methylphenoxy)propionic acid (mecoprop), 2-(2,4-dichlorophenoxy)propionic acid (dichlorprop), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 4-(4-chloro-2-methylphenoxy)butanoic acid (MCPB) and organophosphate herbicides such as chlorpyrifos and profenofos. The third type is a class of compounds containing more than one aromatic ring such as polychlorinated biphenyl (PCBs) and polybrominated biphenyl (PBBs). Most of the compounds shown in Fig. 1 have been listed in the priority pollutants list and/or hazardous substances by the United States Environmental Protection Agency (USEPA) (United States Environmental Protection Agency, 2014) and European List of Notified Chemical Substances (ELINCS) published by the European Commission (EC) (Joaquin et al., 2009). The acceptable levels of these compounds in the environment and food-related samples are tightly regulated.

Although most halogenated aromatic compounds are introduced to the environment via anthropogenic activities with limited biodegradability, some microbes have evolved...
enzymes and metabolic pathways to detoxify and utilize these compounds as their sole carbon sources (Reineke and Knackmuss, 1988; Commandeur and Parsons, 1990; Häggblom, 1992; Fetzner and Lingens, 1994; Copley, 1997; Arora and Bae, 2014). Overall microbial degradation pathways can be simplified and arranged into three stages as shown in Fig. 2. The upper pathway deals mainly with the debranching process to remove additional moieties attached to the aromatic ring such as removing an acetyl group from 2,4-D or dephosphorylation of organophosphate halogenated aromatics. The compounds derived from this stage are mostly derivatives of mono-aromatic ring compounds such as halogenated benzene, halogenated phenol, halogenated aniline and halogenated benzoate derivatives which serve as substrates for enzymes in the next stage (middle pathway). The middle pathway deals mainly with halide removal and activation of aromatic rings by incorporation of oxygen atom (in the case of aerobes). Halogen atom removal is the key step for detoxification of halogenated aromatics because the recalcitrance of substance for degradation depends on the type and number of halogen substituents (Fetzner and Lingens, 1994). The halogen atom removal can occur via reductive and oxidative modes. For the oxidative mode, it generally involves with oxygen incorporation via various pathways yielding hydroxylated aromatic products such as derivatives of catechol, hydroquinone or protocatechuate which also have low cellular toxicity and higher solubility than the original toxic substrates. The final stage (lower pathway) deals with ring-cleavage reactions to convert aromatic into aliphatic molecules which can be further cleaved to form common metabolites of central metabolic pathways such as succinate, pyruvate, acetyl CoA, oxaloacetate and acetaldehyde (Commandeur and Parsons, 1990; Harwood and Parales, 1996; Fuchs et al., 2011; Arora and Bae, 2014; Tinikul et al., 2018; Chenprakhon et al., 2019).

Complete degradation pathways of 2,4-D from Cupriavidus necator JMP134 (Plumeier et al., 2002; Matus et al., 2003; Kumar et al., 2016), chlorpyrifos from Cupriavidus nantongensis X1T (Fang et al., 2019) and polychlorinated biphenyls (PCBs) from Pseudomonas sp. (Furukawa and Miyazaki, 1986; Benning et al., 1996; Copley, 1997; Nováková et al., 2002; Pieper, 2005) are shown in Fig. 3 as common examples of microbial degradation of halogenated aromatics that employ the three-stage reactions mentioned above. It should be noted that most of the enzymes in the lower pathway do not have much reaction variation because they deal with a certain common set of metabolites from the central pathway. In contrast, enzymes in the upper and middle pathways show high reaction variation because they have to deal with various types of compounds. As most of exclusively reviews for molecular mechanisms and enzymatic reactions of halogenated aromatic biodegradation were published around two decades ago during 1980–1990s (Reineke and Knackmuss, 1988; Fetzner and Lingens, 1994), this review aims to highlight more updated information of the field since then and also discusses the enzymatic reactions used by various microbes to degrade specific halogenated aromatics. We also discuss recent technology for detection of these toxic substances which was developed based on basic knowledge of enzymes degrading halogenated aromatics. Enzymatic reactions are arranged based on the three stages of the degradation process and are organized under the following topics:

i. The Upper Metabolic Pathways for Degradation of Halogenated aromatic

a. Debranching of 2,4-dichlorophenoxyacetic acid (2,4-D) and its derivatives
b. Dephosphorylation of organophosphate pesticides
c. Cleavage of the phenyl moiety from polyhalogenated biphenyl derivatives
ii. The Middle Metabolic Pathways for Dehalogenation of Halogenated aromatics

a. Reductive dehalogenation of halogenated aromatics
b. Oxidative dehalogenation of halogenated aromatics by monooxygenases
c. Oxidative dehalogenation of halogenated aromatics by dioxygenases
d. Hydrolytic dehalogenation of halogenated aromatics

iii. Ring Breakage and Generation of Common Metabolites by the Lower Metabolic Pathways

iv. Technologies of Halogenated aromatic-degrading microbes and its Future Perspectives

The upper metabolic pathways for degradation of halogenated aromatics

The upper metabolic pathway generally involves removal of extra moieties to generate simple aromatic compounds that can be easily metabolized by downstream enzymes in the middle and lower pathways. Examples of enzymatic reactions involved in group removals or

Fig. 3. Upper catabolic pathway of halogenated aromatic pesticides. A. Biodegradation pathway of 2,4-dichlorophenoxyacetic acid (2,4-D) from Cupriavidus necator JMP134. The enzymes involved are TfdA, α-ketoglutarate-dependent dioxygenase; TcpA, flavin-dependent chlorophenol monoxygenase; TcpX, flavin reductase; TcpC, hydroquinone-1,2-dioxygenase; and TcpD, maleylacetate reductase (Plumeier et al., 2002; Matus et al., 2003; Kumar et al., 2016). B. Biodegradation pathway of chlorpyrifos from Cupriavidus nantongensis XT1. The enzymes involved are OpdB, organophosphorus hydrolase; TcpA, flavin-dependent monooxygenase; and Fre, flavin reductase (Fang et al., 2019). C. Biodegradation pathway of polychlorinated biphenyls (PCBs) from Pseudomonas sp. The enzymes involved are BphA, biphenyl-2,3-dioxygenase; TcpA, flavin-dependent monooxygenase, and Fre, flavin reductase (Fang et al., 2019). Biodegradation pathway of polychlorinated biphenyls (PCBs) from Pseudomonas sp. The enzymes involved are BphA, biphenyl-2,3-dioxygenase; BphB, biphenyl-dihydrololate-dehydrogenase; BphC, 2,3-dihydroxybiphenyl-1,2-dioxygenase; and BphD, 2-hydroxychloro-6-oxo-6-phenyhexa-2,4-dienoate. Dehalogenase, 4-chlorobenzoyl coenzyme A dehalogenase; and Hydroxylase, 4-hydroxybenzoate hydroxylase (Furukawa and Miyazaki, 1986; Benning et al., 1996; Copley, 1997; Novaková et al., 2002; Pieper, 2005). Green, blue and red reactions indicated the upper, middle and lower pathway respectively.

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debranching of 2,4-D and its derivatives, organophosphate pesticides (chlorpyrifos, profenofos, etc.) and polyhalogenated biphenyls are discussed below. These reactions are critically required prior to the downstream biodegradation process (Fig. 3). However, this upper pathway is not necessary for biodegradation of small halogenated aromatic compounds which can be directly processed by enzymes in the middle pathway.

**Debranching of 2,4-dichlorophenoxyacetic acid (2,4-D) and its derivatives**

Biodegradation of 2,4-D is among the best understood pathways of halogenated aromatic degradation in bacteria. Several phylogenetically diverse bacterial species were reported to be capable of using 2,4-D as a sole carbon source (Tonso et al., 1995; Kamagata et al., 1997; Itoh et al., 2002; Kumar et al., 2016). The 2,4-D biodegradation pathway of *C. necator* JMP 134 was extensively studied (Don et al., 1985; Laemmli et al., 2000, 2004; Plumeier et al., 2002). The debranching step uses the enzyme α-ketoglutarate (α-KG)-dependent dioxygenase (TfdA) encoded by the *tfdA* in the *tfd* operon (*tfdA-tfdF*). The *tfd* operon contains the genes involved in 2,4-D degradation to convert 2,4-D to 2,4-dichlorophenol (2,4-DCP) and other smaller molecules. TfdA catalyses the cleavage of the ether bond that links the acetyl and phenoxyl moieties (green reaction in Fig. 3A). TfdA requires Fe(II) as a cofactor and uses α-KG, 2,4-D and oxygen as substrates to produce carbon dioxide, succinate, 2,4-DCP and glyoxylate as products. Ferrous ion is required for its activity and cannot be replaced by other metals (Hogan et al., 1997).

The product from the TfdA reaction, 2,4-dichlorophenol is a substrate for the next enzyme in the operon, 2,4-dichlorophenol hydroxylase which further hydroxylates 2,4-DCP to generate the hydroxylated product (blue reaction in Fig. 3A) (Fukumori and Hausinger, 1993a,b; Saari and Hausinger, 1998; Hegg et al., 1999; Dunning Hotopp and Hausinger, 2002). Additionally, the *tdf* genes for the debranching of 2,4-D were also found in diverse bacteria that contain 2,4-D degrading abilities such as *Burkholderia* sp. (Baelum et al., 2010), *Aeromonas hydrophila* IBRB-36 4CPA (Markushева et al., 2004) and some aquatic bacteria of the genus *Alcaligenes* (Amy et al., 1985). The bacterial strain RDS-5C2 which is a member of the *Bradyrhizobium-Agromonas-Nitrobacter-Afipia* cluster in the α-proteobacteria was reported to carry *tfdA*-like genes that encode proteins belonging to the α-KG-dependent dioxygenase family (Itoh et al., 2002).

In addition to the simple 2,4-D compound described above, some halogenated aromatic herbicides such as mecoprop and dichlorprop are chiral molecules which require chiral-specific α-KG dioxygenases to catalyse etherolytic cleavage at the initial degradation steps (Lu et al., 2019). Reactions of enantiomer-specific α-KG dioxygenases such as RdpA and SdpA have been investigated (Nickel et al., 1997; Muller et al., 2004, 2006). The results have shown that RdpA dioxygenase is highly specific to the R enantiomer of (R)-2-(4-chloro-2-methylphenoxy) propionic acid (R-mecoprop) and has low activity toward the S enantiomer. In contrast, SdpA is enantioselective to the S enantiomer but can also utilize other phenoxyacetate derivatives such as 2,4-D and MCPA (Westendorf et al., 2003, 2006; Muller et al., 2006).

**Dephosphorylation of organophosphate pesticides**

For degradation of organophosphate pesticides such as chlorpyrifos and profenofos, the upper metabolic pathway involves cleavage of phosphoester bonds by hydrolyase reactions to yield halogenated aromatic compounds in the form of phenol derivatives. Various bacteria including *C. nantognensis* X1T (Fang et al., 2019), *Xanthomonas* sp. 4R3-M1, *Pseudomonas* sp. 4H1-M3 (Rayu et al., 2017), *Bacillus subtilis* (Salunkhe et al., 2013), *Stenotrophomonas* sp. G1 (Deng et al., 2015), several *Pseudomonas* sp. (Siripattanakul-Ratpukdi et al., 2015) and others have been isolated and reported to utilize organophosphate pesticides as their sole carbon sources. Recently, the catabolic pathway of chlorpyrifos degradation in *C. nantognensis* X1T (Fang et al., 2019) was reported. The initial step of chlorpyrifos biodegradation is hydrolysis at the phosphorus–oxygen bond by organophosphorus hydrolase (*opdB* gene) to result in 3,5,6-trichloro-2-pyridinol and diethylphosphate as products (green reaction in Fig. 3B). *OpdB* hydrolase also has a broad spectrum of substrate utilization, allowing it to hydrolyse various organophosphate pesticides including triazophos, parathion, isocarbophos, profenofos, malathion, cadusafos, methidathion and dimethoate. Some of the products, such as 3,5,6-trichloro-2-pyridinol can be further degraded by enzymes in the middle and lower metabolic pathways found in the same bacterial strain.

**Cleavage of the phenyl moiety from polyhalogenated biphenyl derivatives**

Biodegradation of polyhalogenated biphenyl compounds that contain two aromatic rings require a cleavage between the two phenyl rings to generate single aromatic derivatives which can be further metabolized through enzymes in the middle and lower pathways. Various microbes are known for carrying genes encoding for the catabolic pathway of PCBs (Nováková et al., 2002;
Reductive dehalogenation of halogenated aromatic compounds

Reductive dehalogenation is the replacement of halogen with hydrogen atom that can be performed in anaerobes and aerobes. Well-studied anaerobes that are capable of performing reductive dehalogenation are organohalide-respiring bacteria that are also capable of degrading a broad spectrum of halogenated aromatics including halogenated benzenes, halogenated phenols, halogenated dioxin and also polyhalogenated biphenyls (Adrian et al., 2000; Bunge et al., 2003; Wang et al., 2018). In the strict anaerobes, halogenated aromatics serve as a terminal electron acceptor in the electron transport chain of organohalide-respiring bacteria with hydrogen gas ($H_2$) as a sole electron donor (Jugder et al., 2016; Schubert et al., 2018; Wang et al., 2018). The key enzymes in this electron transport chain are membrane-bound reductive dehalogenases (RDs) that receive electrons and pass them through Fe-S clusters and B12 cofactors to halogenated aromatics as their final electron acceptors via a two-step electron transfer (Fig. 4A) (Jugder et al., 2016). Because halogenated aromatics generally have high two-electron reduction potentials, they serve as efficient electron acceptors in the $H_2$-dependent electron transport chain (Dolfing and Novak, 2015).

Organohalide-respiring bacteria such as Dehalobacter sp. strain TeCB1 (Alfan-Guzman et al., 2017), Dehalococcoides mccartyi strain DCMB5 (Poritz et al., 2015), Dehalococcoides mccartyi strain CBDB1 (Adrian et al., 2007; Wagner et al., 2012) were reported to be capable of utilizing halobenzenes. The strain TeCB1 can reductively remove a chlorine atom from 1,2,4,5-tetrachlorobenzene to form 1,2,4,5-tetrachlorobenzene and 1,3- or 1,4-dichlorobenzene respectively (Alfan-Guzman et al., 2017). The strain DCMB5 is capable of dehalogenating various chlorobenzenes including pentachlorobenzene, all types of tetrachlorobenzenes, 1,2,3-trichlorophenol, as well as 1,2,3,4-tetra- and 1,2,4-trichlorodibenzo-p-dioxin, pentachlorophenol, tetrachlorophenol and all types of trichlorophenols (Poritz et al., 2015). Some anaerobes contain RDs that are involved in halogen removal from halogenated phenols. RDs from Dehalococcoides mccartyi strain JNA specifically remove the ortho-chlorine atom from multi-substituted chlorophenols.
(A) 2,6-Dibromophenol biodegradation from *Nitrilirreductor pacificus* pht-3B

(B) 4-Fluorobenzoyl CoA biodegradation from *Thauera aromatica*

(C) Pentachlorophenol biodegradation from *Sphingobium chlorophenolicum*

(D) 2,4,5-Trichlorophenol biodegradation from *Ralstonia pickettii* DTP0602

(E) Chlorobenzene biodegradation from *Pseudomonas putida* strain GJ31

(F) 3,4-Dichloroaniline biodegradation from *Acinetobacter baylyi* GFJ2
Fig. 4. Various reactions in the middle catabolic pathway. A. Reductive dehalogenation of 2,6-dibromophenol from Nitratireductor pacificus pht-3B involves the cobalamin (B12)-dependent reductive dehalogenase, NpRdhA. Two electrons (2e⁻) are transferred from H₂ through the membrane-bound hydrogenase (MΦH), cytochrome b (Cyt b) and the unidentified electron transfer component prior to their split through two, one electron (e⁻) reduction steps in the reductive dehalogenation of NpRdhA (Payne et al., 2015; Jugder et al., 2016; Collins et al., 2018). B. 4-fluorobenzoyl CoA biodegradation from Thuera aromatica involves the reductive dehalogenation by ATP-dependent class I benzoyl CoA reductase (BCR) to catalyse deamortization of 4-fluorobenzoyl CoA to 4-fluoro-1,5-dienoyl-CoA prior to elimination of HF to generate benzoyl CoA as a central intermediate (Tiedt et al., 2016). C. Pentachlorophenol biodegradation pathway from Sphingobium chlorophenolicum is initiated by oxidative dehalogenation involving a single-component flavin-dependent pentachlorophenol hydroxilase. PcpB, prior to reductive dehalogenation of tetrachlorolhydroquinone by glutathione-dependent tetrachlorolhydroquinone dehalogenase, PcpC (Warner and Copley, 2007a; b; Hlouchova et al., 2012). D. Oxidative catabolism of 2,4,5-trichlorophenol from Ralstonia picketii DTP0602 involves Had enzymes. HadA and HadX are the two-component flavin-dependent monoxygenase and reductase, respectively, while HadB is a quinone reductase (Payne et al., 2017; Pimviriyakul and Chaiyen, 2018). E. Chlorobenzene biodegradation pathway from Pseudomonas putida strain GJ31 occurs via a dioxygenation reaction by chlorobenzene dioxygenase (CbbA) and chlorobenzene dihydrodiol dehydrogenase (CbzB) to generate 3-chlorocatechol as a product. F. Branching catabolism of 3,4-dichloroaniline involves both unidentified reductive dehalogenase and aniline dioxygenase respectively (Hongsawat and Vangnai, 2011). The red reaction indicates the dehalogenation via a reductive type reaction, while the blue reaction indicates the dehalogenation via an oxidative type reaction.

(Fricke et al., 2014). A putative chlorophenol RD, cprA, from Dehalobacter sp. strain TCP1 can remove the ortho-chlorine atom from 2,4,6-TCP (Wang et al., 2014). Desulfotobacterium hafniense strain Y51 (Mac Nelly et al., 2014) and NpRdhA from Nitratireductor pacificus pht-3B (Payne et al., 2015) are RDs which use cobalamin as a cofactor to catalyse halide elimination. The recombinant RdhA3 contains a cobalamin cofactor with two iron–sulfur clusters that can reductively dechlorinate 3,5,5-DCP, 2,3-DCP and 2,4-DCP to generate 3-CP, 2-CP and 4-CP as dechlorinated products respectively (Mac Nelly et al., 2014). NpRdhA is also a cobalamin (B12)-dependent enzyme that can catalyse reduction of 2,6-dibromophenol to 2-bromophenol via a halide-cobalt (II) formation. Investigation of the reaction mechanism based on structure determination combined with electron paramagnetic resonance spectroscopy and simulations using 2,6-dibromophenol as a substrate revealed that a bromide–cobalt(II) intermediate forms before the cleavage of the carbon–bromide bond occurs to eliminate a bromide from the organic substrate (red reaction in Fig. 4A) (Payne et al., 2015). As ferredoxin (Fd) is required as an electron donor, the reaction of NpRdhA requires an additional reaction of ferredoxin reductase to generate reduced Fd. It was demonstrated that E. coli ferredoxin reductase (EcFldr) and spinach ferredoxin (SpFd) can provide electrons to the cobalt centre at active site of NpRdhA. Therefore, the overall reductive halogenation with all involved enzymes is NADPH-dependent (Collins et al., 2018).

Another type of reductive dehalogenation is involved in the degradation pathway of halogenated benzoyl CoA which is ATP-dependent (Fuchs et al., 2011; Boll et al., 2014). There are several microbes which contain this ATP-dependent pathway including Thuara aromatica (Boll and Fuchs, 1995; Tiedt et al., 2016), Thuara chlorobenzoica sp. (Kuntze et al., 2011; Song et al., 2001), Pseudomonas stutzeri (Vargas et al., 2000) and Rhodopseudomonas palustris (Egland et al., 2001). The first step usually involves with the conversion of halogenated benzoate to halogenated benzoyl CoA by an ATP-dependent benzoyl-CoA ligase (Wischgoll et al., 2005). In the following step, an ATP-dependent reductive dehalogenase catalyses deamortization of aromatic ring in concomitant with halide elimination (Boll and Fuchs, 1995; Fuchs et al., 2011; Boll et al., 2014). Recently, the enzyme in class I benzoyl CoA reductase (BCR) from T. aromatica was reported to catalyse reductive defluorination of 4-fluorobenzoyl CoA to benzoyl CoA and hydrofluoric (HF) (Tiedt et al., 2016). This enzyme is composed of two main modules which are responsible for electron transfer via ferredoxin and [4Fe-4S] clusters and aromatic ring binding (Fuchs et al., 2011; Boll et al., 2014). In its enzymatic mechanism (red reaction in Fig. 4B), the proposed mechanism was initiated by deamortization of 4-fluorobenzoyl CoA to 4-fluoro-1,5-dienoyl-CoA by receiving two electrons from ferredoxin in concomitant with hydrolysis of ATP to ADP. After that, HF was eliminated and rearomatization occurs to yield benzoyl CoA as a product. The resulting benzoyl CoA central intermediate is metabolized by downstream enzymes in the lower pathway via β-oxidation-like reactions to yield acetyl-CoA and CO₂ as final products (Wischgoll et al., 2005; Fuchs et al., 2011; Boll et al., 2014; Tiedt et al., 2016).

Reductive dehalogenation also occurs in aerobes such as Delftia sp. EOB-17 (Chen et al., 2015) which can reductively dehalogenate 3,5-dibromo-4-hydroxybenzoate (DBHB) to form 4-hydroxybenzoate as a product. The enzymatic reactions of RDs from aerobes are different from those of the anaerobe RDs explained in the previous section. The well-known tetrachlorolhydroquinone dehalogenase or PcpC found in the pentachlorophenol biodegradation pathway of Sphingobium chlorophenolicum (originally called Flavobacterium sp.), is classified as a member of the glutathione...
S-transferase family. PcpC catalyses the dechlorination of tetrachlorohydroquinone to trichlorohydroquinone and then trichlorohydroquinone to dichlorohydroquinone (red reaction in Fig. 4C) (Xun et al., 1992a; McCarthy et al., 1996). The reaction mechanism of PcpC has been extensively investigated (Warner and Copley, 2007a,b). Experiments using transient kinetic approaches (stopped-flow and rapid-quench techniques) with spectroscopic detection of PcpC using trichlorohydroquinone (TriCHQ) as a substrate were carried out. The reaction starts with rapid deprotonation of TriCHQ after binding to PcpC followed by delocalization of the electrons of a hydroxyl group to form 3,5,6-trichloro-4-hydroxycyclohexa-2,4-dienone (TriCHQ*). In the presence of glutathione (GSH), GSH attacks TriCHQ* to form the 3,5-dichloro-2-S-glutathionylhydroquinone intermediate (GSH-DCHO) before chloride elimination occurs. This intermediate decays by reacting with another GSH to result in the final product of 2,6-dichlorohydroquinone (2,6-DCHO) and glutathione disulfide (red reaction in Fig. 4C).

Oxidative dehalogenation of halogenated aromatic compounds by monooxygenases

Oxidative dehalogenation is another means to decrease the toxicity of halogenated aromatic compounds by incorporation of an –OH group to yield more hydrophilic products with lower toxicity. The addition of the extra hydroxyl group prepares the products for ring-cleavage enzymes in the following stage of biodegradation. The concomitant incorporation of a hydroxyl group with halide elimination can be catalysed by monooxygenases.

Monooxygenation is the incorporation of one hydroxyl group (–OH) derived from molecular oxygen into organic compounds. Monooxygenases that catalyse monooxyge
nation are metal-dependent, pterin-dependent and flavin-dependent enzymes (van Berkel et al., 2006; Roberts and Fitzpatrick, 2013; Huijbers et al., 2014; Romero et al., 2018; Banerjee et al., 2019; Chenprakhon et al., 2019). For monooxygenation of halogenated aromatics, the majority of the reactions are catalysed by flavin-dependent monooxygenases (Arora and Bae, 2014). These enzymes catalyse dehalogenation plus incorporation of a hydroxyl group into halogenated phenols which are generated by enzymes in the upper pathway such as phenoxycetic acid and organophosphate debranching enzymes previously discussed in the upper pathway section. These enzymes catalyse concomitant hydroxylation with halide elimination of halogenated phenols using reduced flavin (flavin_red) and molecular oxygen (O₂) as co-substrates.

Flavin-dependent dehalogenases can be divided into two types: single- and two-component flavin-dependent monooxygenases. Generally, all steps of the single-component monooxygenases are catalysed by one polypeptide which binds oxidized flavin (flavin₉) as a cofactor (Ballou et al., 2005; van Berkel et al., 2006; Palfey and McDonald, 2010; Huijbers et al., 2014; Chenprakhon et al., 2019). In contrast, reactions of two-component flavin-dependent monooxygenases are carried out by a separate reductase and oxygenase. Oxidized flavin is first reduced by NADH or NADPH on the reductase component before being transferred via diffusion to the oxygenase component (Huijbers et al., 2014; Sucharitakul et al., 2014; Chenprakhon et al., 2019). The rest of the hydroxylation plus dehalogenation reactions occur in the oxygenase component similar to the reaction of single-component monooxygenases (Fig. 5).

PcpB from the pentachlorophenol degradation pathway of S. chlorophenolicum represents an extensively investigated single-component flavin-dependent monooxygenase (Hlouchova et al., 2012; Rudolph et al., 2014). The enzyme catalyses the conversion of PCp to tetrachlorohydroquinone (blue reaction in Fig. 4C) which can be further reductively dehalogenated by the PcpC discussed in the previous section (red reaction in Fig. 4C) (Hlouchova et al., 2012; Rudolph et al., 2014). In addition, PcpB was also reported to be able to catalyse elimination of other substituents such as bromide, iodide, nitro or cyano from phenolic derivatives (although with very low yield) (Xun et al., 1992b). The reaction mechanism of PcpB has been extensively investigated (Fig. 5). The reaction is initiated by the reduction of a flavin cofactor by NADH or NADPH during a reductive half-reaction to generate the enzyme-bound reduced FAD (FADH²). O₂ then reacts with FADH² to form the C4a-hydroperoxy-flavin intermediate in which its terminal peroxide –OH acts as an electrophile to be incorporated into a phenolic substrate. The hydroxylated product eliminates a halide group in the next step while the resulting C4a-hydroxyflavin eliminates water to yield the resting state of the cofactor, oxidized FAD, to complete the catalytic cycle (Hlouchova et al., 2012; Rudolph et al., 2014). Similar to PcpB, phenol hydroxylase from Trichosporon cutaneum which normally catalyses hydroxylation of the phenol also has promiscuous activities in hydroxylation and elimination of ortho-fluoride from pentafluorophenol to yield tetrafluorocatechol (Peelen et al., 1995).

Two-component monooxygenase-reductase systems found in various aerobic microbes include the HadA-HadX system from Ralstonia picketti DTP006 (blue reaction in Fig. 4D) (Hatta et al., 2012; Pimviriyakul and Chaiyen, 2018), the TcpA-TcpX system from Ralstonia eutropha JMP134 (blue reaction in Fig. 3A) (Matus et al., 2003; Xun and Webster, 2004; Belchik and Xun, 2008), the Trd-Trc system from Burkholderia cepacia AC1100 (Gisi and Xun, 2003; Webb et al., 2010), the

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CphC-I-CphB system from *Arthrobacter chlorophenolicus* A6 (Cho et al., 2017; Kang et al., 2017), the DcmB1-DcmB2 system from *Rhodococcus sp. JT-3* (Zhang et al., 2018) and the TcpA-Fre system from *C. nantongensis* X1T (blue reaction in Fig. 3B) (Fang et al., 2019). These enzymatic systems catalyse the hydroxylation and halide elimination of halogenated phenol derivatives at the para- or ortho-positions to yield either hydroquinone or catechol derivatives as products. It should be mentioned that in addition to dehalogenation, some of these systems can also catalyse elimination reactions of other substituents including denitration of nitrophenol by HadA-HadX (Pimviriyakul and Chaiyen, 2018; Pimviriyakul et al., 2018) and by CphC-I-CphB (Kang et al., 2017).

HadA catalyses biodegradation steps at the para- and ortho-positions to generate 5-chlorohydroxyquinol as a final product (blue reaction in Fig. 4D). Recently, in-depth investigation of HadA-HadX reaction mechanisms using 4-chlorophenol (4-CP) as substrate (Pimviriyakul et al., 2017, 2018; Pimviriyakul and Chaiyen, 2018) has been reported (Fig. 5). Based on transient kinetic studies using stopped-flow and rapid-quench techniques, the first step of the HadA reaction is the binding of reduced flavin supplied from a reductase partner such as HadX via free diffusion. This step is followed by reaction with O₂ to form the C4a-hydroperoxy-flavin reactive intermediate. 4-CP then reacts with the C4a-hydroperoxy-flavin via electrophilic aromatic substitution to result in hydroxylated 4-CP which then eliminates the chloride group to result in the final products of benzoquinone and the C4a-hydroxyflavin adduct which decays to oxidized flavin via dehydration (Pimviriyakul et al., 2017). The overall simple reaction scheme of single and two-component dehalogenases can be summarized as in Fig. 5. Correlation of the rate constants of the individual steps of the HadA reaction with the physicochemical parameters obtained from density functional theory (DFT) analysis indicated that the overall reaction is dictated by the deprotonation ability of the substrates which is likely the step with the highest activation energy (Pimviriyakul et al., 2018). Currently, structures of HadA and its homologous enzymes including TftD and TcpA are only available in the apoform (Webb et al., 2010; Hayes et al., 2012; Pongpamorn et al., 2019). Based on structural modelling of substrate binding, His290 of HadA, H289 of TftD and H290 of TcpA are proposed to be the catalytic base for proton abstraction to facilitate electrophilic aromatic substitution.

**Fig. 5.** Mechanism of flavin-dependent monooxygenase for dehalogenation of halogenated phenol. A simple reaction scheme of halogenated phenol oxidative dehalogenation by a flavin-dependent monooxygenase which occurs via the reaction of the C4a-hydroperoxy-flavin intermediate. For a single-component enzyme like PcpB from *S. chlorophenolicum*, the substrate is pentachlorophenol and the flavin is reduced via the blue pathway (Hlouchova et al., 2012; Rudolph et al., 2014). For two-component enzymes, the HadA-HadX system from *R. picketti*, the substrate is 4-chlorophenol and the flavin is reduced via the red pathway where HadA is a flavin-dependent monooxygenase and HadX is a flavin reductase (Pimviriyakul et al., 2017; Pimviriyakul and Chaiyen, 2018). B represents a catalytic base for proton abstraction to facilitate electrophilic aromatic substitution.
HadA reaction. As the initial hydroxylated product from HadA and other two-component monooxygenases are benzoquinone derivatives, some microbes possess quinone reductases such as HadB in *Ralstonia pickettii* DTP006 (Pimviriyakul and Chaiyen, 2018) and TcpB in *Ralstonia eutropha* JMP134 (Belchik and Xin, 2008) to enzymatically convert benzoquinone to hydroquinone. Hydroquinone is a more stable compound which can be used by downstream enzymes in the lower biodegradation pathway (Fig. 4D). The whole system (HadA monooxygenase, HadX flavin reductase and HadB quinone reductase) can be coupled together as an in vitro enzymatic cascade, resulting in high efficiency of hydroxyl and group elimination (Pimviriyakul and Chaiyen, 2018). The hydroquinone product can be easily metabolized by a downstream HadC 1,2-hydroquinone dioxygenase (Hatta *et al.*, 1999). The whole cascade of HadA can be used as a basis set for applications in bioremediation processes.

**Oxidative dehalogenation of halogenated aromatic compounds by dioxygenases**

Dioxygenation is incorporation of two hydroxyl groups (both oxygen atoms are derived from O$_2$) into organic substrates. Reactions are catalysed by dioxygenase enzymes and they are generally found in the biodegradation of halogenated benzenes. Degradation of chlorobenzene (CB) by *Ralstonia pickettii* strain L2 occurs via two steps of hydroxylation to yield a phenolic intermediate and catecholic product respectively (Zhang *et al.*, 2009). CB is first hydroxylated by chlorobenzene dioxygenase (CzbA) to form chlorobenzenediol which is then further converted to 3-chlorocatechol by another enzyme chlorobenzene dihydrodiol dehydroge-nase (CzbB) (Fig. 4E). Fluorobenzene (FB) can be metabolized by *Burkholderia fungorum* FLU100 (DSM 23736) (Strunk and Engesser, 2013) and *Labrys portulacensis* to result in 3-fluorocatechol as product. Halo- genated catechols can be further cleaved by another enzyme in the pathway, catechol 2,3-dioxygenase (Kunze *et al.*, 2009; Strunk and Engesser, 2013).

Another type of compounds that can be degraded by dioxygenases is halogenated aniline. Chloroanilines are widely used in the industrial production of dyes, cosmetics, pharmaceutical products and herbicides. Many bacteria were reported to have the ability to degrade chloroaniline. The degradation of monochloroaniline can occur via two proposed pathways (Fig. 4F). In the first pathway, the process initiates via dechlorination of monochloroaniline to generate aniline which is then converted to catechol by aniline dioxygenase. In the other pathway, the initial step of monochloroaniline degradation is a dioxygenation which is then followed by deamination of monochloroaniline to form the corresponding chlorocatechol (Hongsawat and Vangnai, 2011; Arora, 2015). Bacterial degradation of dichloroanilines has also been investigated. Based on product analysis by LC/MS, *Bacillus megaterium* IMT21 degrades 2,3-dichloroaniline, 2,4-dichloroaniline and 2,5-dichloroaniline via dichloroaminophenol, and 3,4- and 3,5-dichloroaniline via dichloroacetanilide, although information on the enzymes involved in the degradation process is still unclear (Yao *et al.*, 2011). *Pseudomonas fluorescens* 26-K degrades 3,4-dichloroaniline via 4-amino-2-chlorophenol through initial dehalogenation and hydroxyl (Travkin *et al.*, 2003). A strain of *Pseudomonas* sp. degrades 3,4-dichloroaniline via 4,5-dichlorocatechol, 3,4-dichloroanilic acid, 3-chlorobenzenesulfonfonic acid, 3-chloroaniline and 3-chloro-4-ketoadipate (You and Bartha, 1982). Variation of the degradation pathway of 3,4-dichloroaniline was observed in *Acinetobacter baylyi* strain GFJ2 in which the initial step of degradation involves dehalogenation of 3,4-dichloroaniline to 4-chloroaniline. 4-Chloroaniline can be further degraded via two different routes. In the first route, 4-chloroaniline undergoes dehalogenation to produce aniline that is further degraded via catechol and the ortho-cleavage pathway. In the second route, 4-chloroaniline undergoes dioxygenation to yield 4-chlorocatechol and ammonia (Fig. 4F). However, specific enzymes involved in each degradation step in *A. baylyi* strain GFJ2 have not yet been identified (Hongsawat and Vangnai, 2011; Arora, 2015).

**Hydrolytic dehalogenation of halogenated aromatic compounds**

Hydrolytic dehalogenation is replacement of a halogen by a hydroxyl group derived from H$_2$O. Hydrolytic dehalogenating enzymes catalyse dehalogenation of chlorobenzoate which is part of polychlorinated biphenyls (PCBs) biodegradation pathways (Fig. 3C). Hydrolytic dehalogenation is also found in several organisms such as *Pseudomonas* sp. CBS3 (Klages *et al.*, 1981), *Arthrobacter* sp. 4-CB1 (Crooks and Copley, 1994), *Arthrobacter* sp. (Zhuang *et al.*, 2003; Zhou *et al.*, 2008), *Rhodococcus ruber* P25 (Plotnikova *et al.*, 2012), *Betaproteobacteria* (Chae *et al.*, 2008). 4-Chlorobenzoate can be used as a substrate for ATP-dependent 4-halobenzoate coenzyme A ligase in the cell to generate a reactive metabolite, 4-chlorobenzoyl coenzyme A (Pieper, 2005). 4-Chlorobenzoyl coenzyme A dehalogenase can then carry out the next reaction of hydrolytic dehalogenation (blue reaction in Fig. 3C). The reaction...
mechanism of 4-chlorobenzoyl CoA dehalogenase was extensively investigated (Copley, 1997; Lau and Bruice, 2001; Luo et al., 2001; Zhang et al., 2001; Xu et al., 2004; Wu et al., 2006) and it was found that the key catalytic residue is Asp145, which performs a nucleophilic attack on the para-position of 4-chlorobenzoate CoA where a chlorine atom is present. The chlorine atom was eliminated and replaced by a –OH group derived from H2O (Benning et al., 1996; Copley, 1997). The overall degradation pathway of PCBs, including the reaction of hydrolytic 4-chlorobenzoyl coenzyme A dehalogenase can be summarized in Fig. 3C. The hydroxylated product 4-hydroxybenzoyl-CoA can be hydrolysed to form 4-hydroxybenzoate (4-HB) by 4-hydroxybenzoyl-CoA thioesterase. 4-HB can be further metabolized by 4-hydroxybenzoate hydroxylase and ring-cleavage dioxygenases (Seibold et al., 1996; Palfey and McDonald, 2010; Tinikul et al., 2018; Lubbers et al., 2019).

Ring breakage and generation of common metabolites by the lower metabolic pathways

To achieve full assimilation of aromatic compounds by bacteria, metabolites from the central pathways such as

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**Fig. 6.** Various reactions in the lower catabolic pathways. Red pathway is an intradiol cleavage pathway initiated by 1,2-cleavage via 1,2-dioxygenase for catechol or by 3,4-cleavage via 3,4-dioxygenase for protocatechuate. Blue pathway is an extradiol cleavage pathway initiated by 2,3-cleavage using 2,3-dioxygenase. Green pathway is extradiol cleavage pathway initiated by 4,5-cleavage using 4,5-dioxygenase.

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phenols, hydroquinone, catechol and protocatechuate need to be converted to metabolites commonly found in the central metabolic pathways such as oxo-acids in the tricarboxylic acid (TCA) cycle. Depending on the type of aromatic derivatives, a specific site of ring cleavage, either intradiol or extradiol cleavage can be catalysed by different sets of dioxygenases which result in diverse products (Fig. 6). The intradiol and extradiol dioxygenases are non-heme iron-containing enzymes that have been extensively studied (Fielding et al., 2014; Wang et al., 2017). These enzymes catalyse the incorporation of two oxygen atoms resulting in concomitant C-C bond fission, in which oxygen activation occurs through a substrate-oxygen-metal coordinated complex (Lipscomb, 2008; Fielding et al., 2014; Ponsuwon et al., 2017).

Gene clusters and enzymes from the steps of ring cleavage in the degradation pathways of halogenated aromatics are similar to those found in microbes metabolizing general and non-halogenated aromatic compounds. Enzymes involved in ring cleavage of aromatic compounds have been extensively studied and many good articles and reviews related to this topic are available (Fuchs et al., 2011; Wells and Ragauskas, 2012; Thotsaporn et al., 2016; Kamimura et al., 2017). In general, the reaction pathways can be divided into the intradiol ring-cleavage type (green area in Fig. 6) in which bond cleavage occurs between two hydroxyl groups of catecholic compounds or the extradiol ring-cleavage type in which cleavage occurs outside the dihydroxyl groups (pink area in Fig. 6).

Examples of intradiol dioxygenases (green area in Fig. 6) include catechol-1,2-dioxygenase and protocatechuate-3,4-dioxygenase (red pathway Fig. 6). Genes coding for enzymes in the intradiol ring-cleavage pathway have been identified in various bacteria and fungi (Patel et al., 1975; Bull and Ballou, 1981; Hartnett et al., 1990; Murakami et al., 1997; Iwagami et al., 2000; Wang et al., 2006). The cleavage by intradiol dioxygenases resulted in muconate derivatives such as cis,cis-muconate and β-carboxy-cis,cis-muconate. These compounds can be further lactonized by the enzyme muconate cycloisomerase (Fig. 6). The cleavage reaction pathways of catechol and protocatechuate proceed through a common intermediate at the formation of β-ketoacid enol-lactone step before the lactone ring is hydrolysed by an enzyme enol-lactone hydrolase to generate β-ketoacid (Patel et al., 1975). Further reactions along the pathway include two reactions catalysed by β-ketoacid:succinyl-CoA transferase and β-ketoacyl-CoA thiolase to generate succinyl-CoA and acetyl-CoA, which are common intermediates of the TCA cycle (Harwood and Parales, 1996).

For the extradiol ring-cleavage pathway (pink area in Fig. 6), dioxygenases catalyse C-C bond breaking outside the two hydroxyl groups either between the C2-C3 positions of catechol or the C2-C3 and C4-C5 positions of protocatechuate. The catechol-2,3-dioxygenase cleaves the catecholic ring to yield 2-hydroxymuconic semialdehyde (blue pathways in Fig. 6). This molecule can be further metabolized by the subsequent reactions catalysed by dehydrogenase, isomerase and decarboxylase encoded in the same gene cluster to generate 2-hydroxypenta-2,4-dienoate. With additional reactions of hydratase and aldolase, 2-hydroxypenta-2,4-dienoate is hydroxylated and cleaved to generate pyruvate and acetaldehyde. For the protocatechuate cleavage pathway, two possible cleavage sites can be metabolized by different enzymes to yield either pyruvate and oxaloacetate or pyruvate and acetyl-CoA (blue and green pathways in Fig. 6) (Wolgel et al., 1993; Maruyama et al., 2004) which are common metabolites in the central metabolic pathway. After being cleaved by protocatechuate-4,5-dioxygenase or protocatechuate-2,3-dioxygenase, both paths use similar sets of dehydrogenase, isomerase, hydratase and aldolase to generate the final products. It is also noted that two additional reactions of decarboxylases are required in the protocatechol-2,3-cleavage route (Kasai et al., 2009) (blue pathway in Fig. 6). The organism uses a simpler pathway of protocatechol-4,5-cleavage rather than the protocatechuate-2,3-cleavage, as the gene cluster of the 2,3-cleavage pathway is rarely found in nature. Recently, Paenibacillus sp. was reported to contain a protocatechuate-2,3-position cleavage enzyme (Kasai et al., 2009).

In terms of mechanistic investigation, only ring-cleavage dioxygenases have been extensively explored (Lipscomb, 2008; Fielding et al., 2014). Knowledge related to the reaction mechanisms of the enzymes succeed these dioxygenases is limited and remains an area needing further exploration. More in-depth understanding of these enzymes should be useful for future applications in bioremediation and biocatalysis.

Technologies of halogenated aromatic-degrading microbes and future perspectives

Microbes capable of biodegradation of halogenated aromatics are highly sought after for their ability to perform bioremediation through clean and green processes. Thus, several technologies have been developed to improve their degradation efficiency. The most straightforward approach is to use the microbes directly. However, several parameters related to bioattenuation, bioaugmentation and bioaugmentation processes need to be adjusted to obtain optimal efficiency (Megharaj et al., 2011). For example, improvement of halogenated aromatic degradation by simply mixing microbial consortia that have synergistic effects on the degradation of
halogenated aromatics has been investigated. Reports on pentachlorophenol and chlorobenzene detoxification by mixtures of microbes showed a higher consumption as compared to a single organism (Hechmi et al., 2016; Cheng et al., 2017). On the other hand, bioaugmentation through the induction of native strains was also demonstrated to be useful. The anaerobe *Dehalococcoides mccartyi* was induced by preculture under the condition containing chloroethane as an alternative electron acceptor for 1 week prior to its use in PCBs degradation. This process increased the dechlorination rate of PCBs by the induced microbes by 30-times compared to the non-induced microbes (Chen and He, 2018). In some case, biostimulation or growth of the microbes in favourable environments such as nutrients and its substrate is performed to increase microbe amount before applying the microbes in the treatment process (Megharaj et al., 2011).

More advanced techniques for biodegradation development involve enzyme engineering, metabolic engineering and cell engineering. The use of these techniques for developing powerful halogenated aromatic-degrading microbes has been drawing great interest (Dvorak et al., 2017; Sharma et al., 2018; Sheldon and Woodley, 2018; Bilal et al., 2019; Liu et al., 2019). Based on basic knowledge of the molecular mechanisms of the degradation pathways, selected genes from various organisms are overexpressed in the same host to improve its degradation ability. Recently, the HadA monooxygenase which can catalyse hydroxylation plus halide and nitro group elimination was engineered to increase its thermostability based on computational prediction. A single-point mutation of the G513Y variant resulted in the engineered enzyme that has significantly improved stability relative to the wild-type enzyme and has equivalent activity. G513Y has an activity half-life 72-fold (50°C) and 160-fold (45°C) longer than the wild-type enzyme (Pongpamorn et al., 2019). The engineered *E. coli* strain that contains the constructed pathway (mainly enzymes in the middle and lower pathways) has the ability to degrade aromatic derivatives while the native one cannot (Clarkson et al., 2017; Wang et al., 2019). Other engineering processes such as cell/protein immobilization, enzyme cross-linking and process optimization have also been investigated (Sheldon and Woodley, 2018). As the major purpose of these microbes is for eventual use *in situ* for the decontamination of pollutants mainly in hazardous contaminated sites, most of the development of halogenated aromatic-degrading microbes has been done in whole cell engineering because microbes are more robust than individual enzymes. One of the most efficient approaches is cell surface engineering to localize the enzymes of interested on the cell surface to increase the chances of substrate utilization (Ueda, 2016). The immobilization of laccase to the cell surface combined with the cellular enzymes of *Pseudomonas putida* resulted in an ability to degrade chlorpyrifos without accumulation of its metabolites (Liu et al., 2016).

In addition to biodegradation, biodetection of halogenated aromatics is also important for controlling the level of these compounds in environment. Bio-based detection of toxic halogenated aromatic pesticides based on direct and indirect measurements have been developed (Rao et al., 2014; Nigam and Shukla, 2015; Xu et al., 2018). Enzyme-based biosensors that directly detect the amount of organophosphate pesticides have been invented based on the use of organophosphate hydroxylases from the upper pathway to immobilize the enzyme on an electrode (Gothwal et al., 2014). The contamination of halogenated aromatic pollutants can be determined by the coexpression of genes promoters responsive to specific contaminants and biomarker genes such as luciferase gene (*luc*) or green fluorescent protein gene (*gfp*). The light signal is generated in the presence of contaminants (Kumar et al., 2018). Recently, a novel one-pot chemo-enzymatic cascade based on the reaction of HadA monoxygenase was coupled to a *de novo* synthesis of firefly D-luciferin which is a valuable reagent used in biomedical research. When firefly luciferase was added into the reaction cascade, the glow bioluminescence signal allows the detection of halogenated phenols and nitrophenols in ppb level. This technology can also detect trace amount of halogenated phenols and nitrophenols in biological samples such as urine and serum without requiring sample pretreatment, illustrating its promise as a powerful platform for high throughput biomedical screening to identify farmers or workers that are at risk of occupational hazards from dealing with these toxicants (Watthaisong et al., 2019).

Although a substantial amount of basic knowledge regarding the degradation of halogenated aromatics is available, biotechnological innovations based on this knowledge are still limited. This is largely due to the low efficiency and instability of relevant enzymes and microbes. Further development using enzyme engineering approaches to improve efficiency and stability are needed so that these biomolecules and cells can be used more effectively in biodegradation applications.

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