

Minireview

Microbial degradation of halogenated aromatics: molecular mechanisms and enzymatic reactions

Panu Pimviriyakul,¹  Thanyaporn Wongnate,² 
Ruchanok Tinikul³ and Pimchai Chaiyen^{2,*} 

¹Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakhon Pathom, 73000, Thailand.

²School of Biomolecular Science and Engineering, Vidyasirimedhi Institute of Science and Technology (VISTEC), Wangchan Valley, Rayong, 21210, Thailand.

³Department of Biochemistry and Center for Excellence in Protein and Enzyme Technology, Faculty of Science, Mahidol University, Bangkok, 10400, Thailand.

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 Igbiosa, 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

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*For correspondence. E-mail pimchai.chaiyen@vistec.ac.th; Tel. +66896695410; Fax +6633014445.

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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-chlorophenoxy acetic 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 environment *via* anthropogenic activities with limited biodegradability, some microbes have evolved

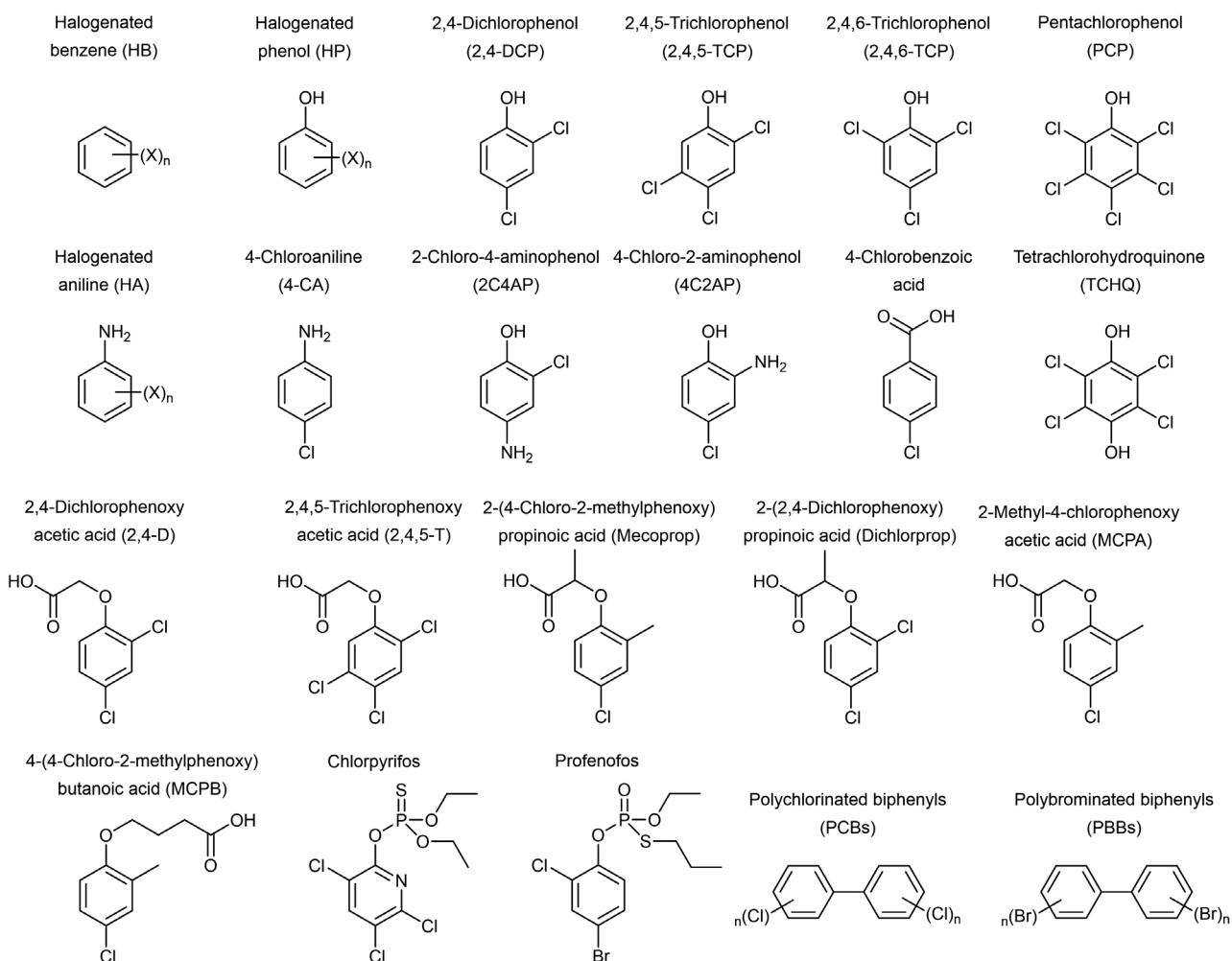


Fig. 1. Structures of toxic halogenated aromatics generally found as pollutants.

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* X1^T (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

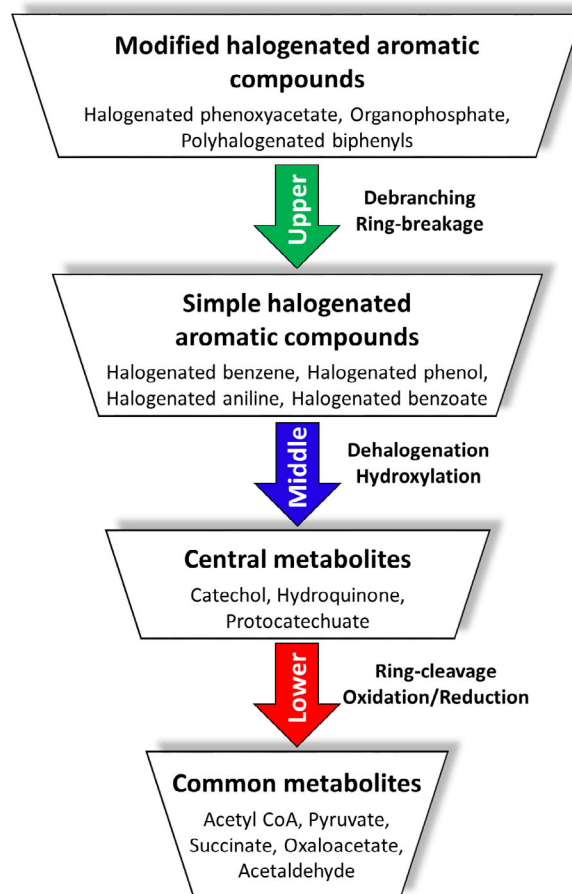


Fig. 2. General microbial pathways of halogenated aromatics degradations. Three main catabolic pathways (upper, middle and lower pathways) are involved in overall biodegradation.

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

- Reductive dehalogenation of halogenated aromatics
- Oxidative dehalogenation of halogenated aromatics by monooxygenases
- Oxidative dehalogenation of halogenated aromatics by dioxygenases
- 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

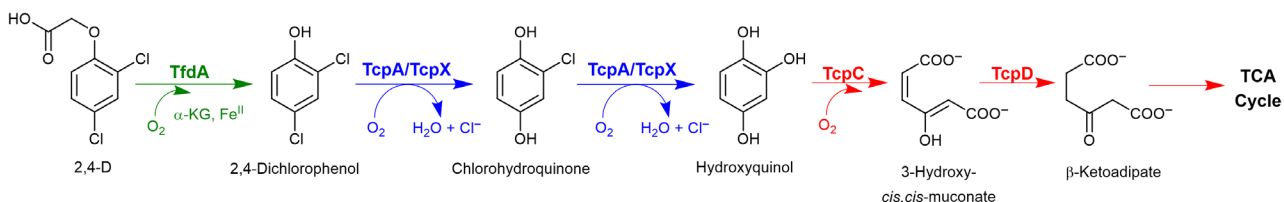
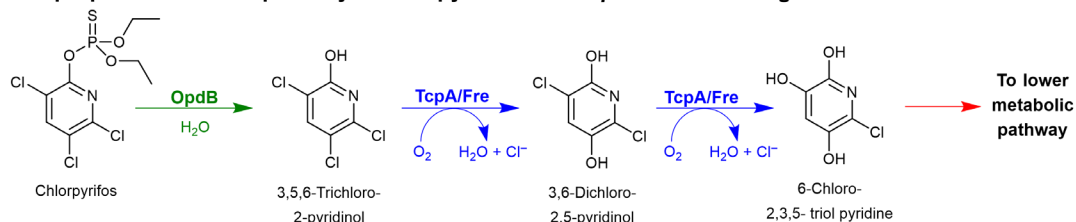
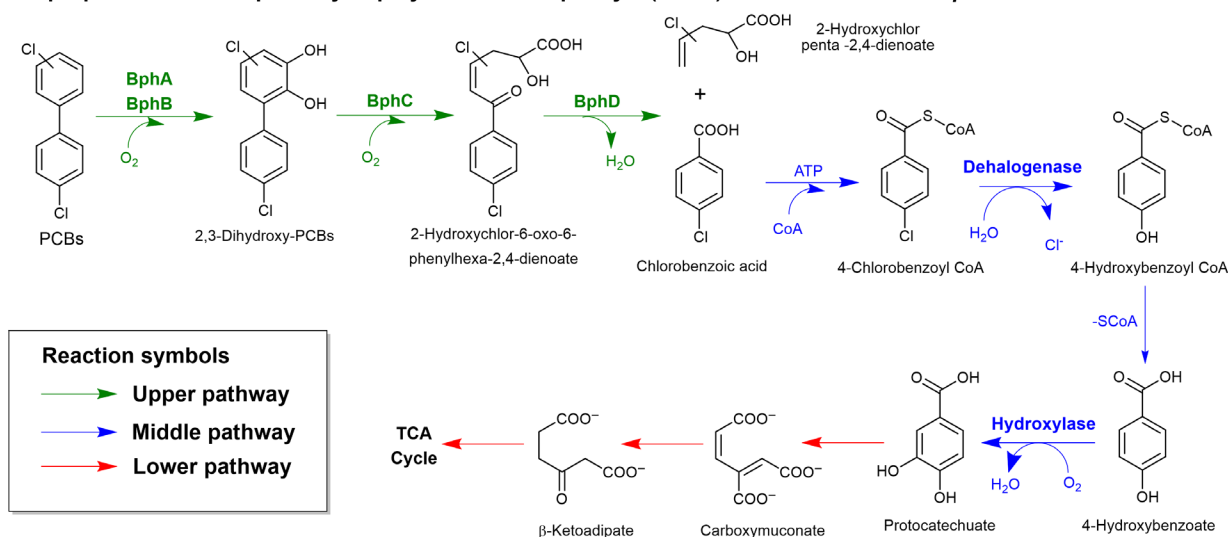
(A) The proposed catabolic pathway of 2,4-dichlorophenoxyacetic acid from *Cupriavidus necator* JMP134**(B) The proposed catabolic pathway of chlorpyrifos from *Cupriavidus nantongensis* X1^T****(C) The proposed catabolic pathway of polychlorinated biphenyls (PCBs) from *Pseudomonas* sp.**

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 monooxygenase; TcpX, flavin reductase; TcpC, hydroxyquinol-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* X1^T. 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; BphB, biphenyl-dihydrodiol-dehydrogenase; BphC, 2,3-dihydroxy-biphenyl-1,2-dioxygenase; BphD, 2-hydroxychlor-6-oxo-6-phenylhexa-2,4-dienoate hydroxylase; Dehalogenase, 4-chlorobenzoyl coenzyme A dehalogenase; and Hydroxylase, *p*-hydroxybenzoate hydroxylase (Furukawa and Miyazaki, 1986; Benning *et al.*, 1996; Copley, 1997; Nováková *et al.*, 2002; Pieper, 2005). Green, blue and red reactions indicated the upper, middle and lower pathway respectively.

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; Laemli *et al.*, 2000, 2004; Plumeier *et al.*, 2002). The debranching step uses the enzyme α -ketoglutarate (α -KG)-dependent dioxygenase (TfdA) encoded by the gene *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 phenoxy 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 *tfd* genes for the degradation 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 (Markusheva *et al.*, 2004) and some aquatic bacteria of the genus *Alcaligenes* (Amy *et al.*, 1985). The bacterial strain RD5-C2 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 hydrolase reactions to yield halogenated aromatic compounds in the form of phenol derivatives. Various bacteria including *C. nantognensis* X1^T (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* X1^T (Fang *et al.*, 2019) was reported. The initial step of chlorpyrifos biodegradation is hydrolysis at the phosphorus–oxygen bond by organophosphorus hydrolase (OpdB) encoded by the *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;

Pieper, 2005). *Pseudomonas pseudoalcaligenes* KF707 (Furukawa and Miyazaki, 1986) and *Pseudomonas sp.* LB400 (Mondello, 1989) have the gene cluster, *bphABCD*, which encodes enzymes for biphenyl ring breakage. The main enzymes in the upper catabolic pathway that can catalyse biphenyl ring cleavage of PCBs are two dioxygenases, biphenyl-2,3-dioxygenase (BphA) and 2,3-dihydroxy-biphenyl-1,2-dioxygenase (BphC). BphA is the first enzyme to incorporate two hydroxyl groups into a biphenyl ring, while BphC uses its dioxygenation activity to catalyse a ring-cleavage reaction to generate 2-hydroxychlor-6-oxo-6-phenylhexa-2,4-dienoate. Another enzyme, 2-hydroxychlor-6-oxo-6-phenylhexa-2,4-dienoate hydrolase (BphD), then hydrolyses this compound to yield chlorobenzoic acid and 2-hydroxychloro-penta-2,4-dienoate (green reaction in Fig. 3C) which will then be further metabolized by enzymes from the middle pathway as explained below.

It can be seen that enzymatic reactions in the upper metabolic pathway are diversified, depending of the types of halogenated aromatics and bacterial species. We have shown above three major pathways including debranching by etherolytic of halophenoxyacetate derivatives, phosphoester bond hydrolysis of organophosphate and ring breakage of PCBs. Although these enzymatic reactions are different, the resulting products from these different reactions narrow down to a few types of small halogenated aromatic compounds such as halogenated benzene, halogenated phenol, halogenated benzoate or halogenated aniline. These common compounds can be further degraded by enzymes in the middle pathway.

The middle metabolic pathways for dehalogenation of halogenated aromatics

At the middle metabolic biodegradation pathway, small halogenated aromatics are converted into less toxic and more soluble compounds such as catechol, hydroquinone or protocatechuate by halide removal (dehalogenation) in conjunction with or without group addition reactions (Fig. 2). In oxidative dehalogenation, the concomitant group addition can be catalysed by oxygenases to incorporate molecular oxygen or by hydrolases to incorporate water into the substrates. Because halogen substituents mainly affect the recalcitrance of halogenated aromatic degradation (Fetzner and Lings, 1994) and they also have electronic and steric effects which reduce electron delocalization efficiency and hinder dioxygenase ring-cleavage activities in the lower pathway (Copley, 1997), dehalogenation in the form of group removal generally occurs prior to the process catalysed by further enzymes. Therefore, the group addition (especially hydroxylation) in conjunction with dehalogenation is required to prepare the compounds to be

ready for ring-cleavage dioxygenases to convert the aromatic compounds to aliphatic compounds. However, dehalogenation without involvement of oxygen (reductive dehalogenation) was also reported (Fuchs *et al.*, 2011; Boll *et al.*, 2014). The following discussion will highlight the nature of the reaction and the mechanistic features of the dehalogenases that catalyse group removal *via* reductive, oxidative and hydrolytic reactions.

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 (H₂) 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 H₂-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-trichlorobenzene 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

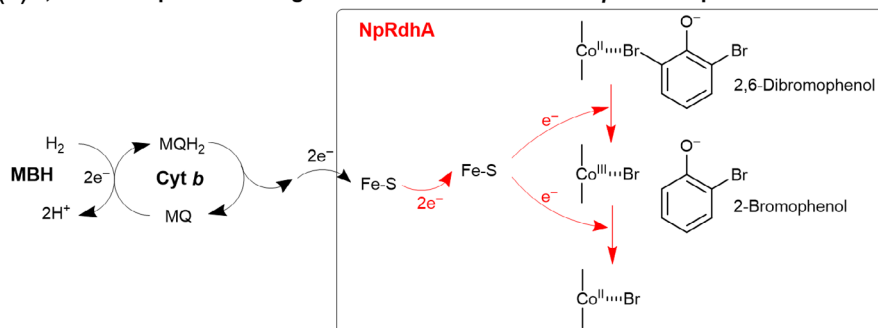
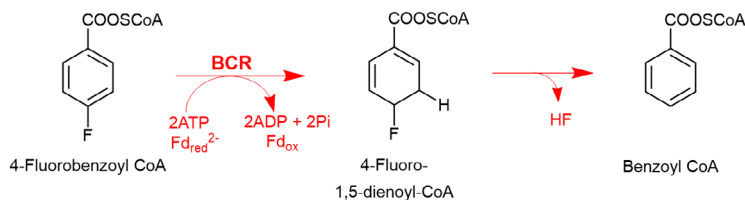
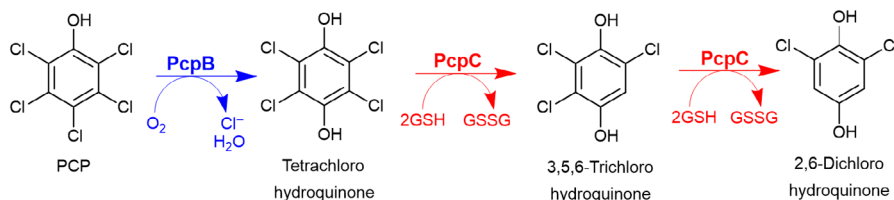
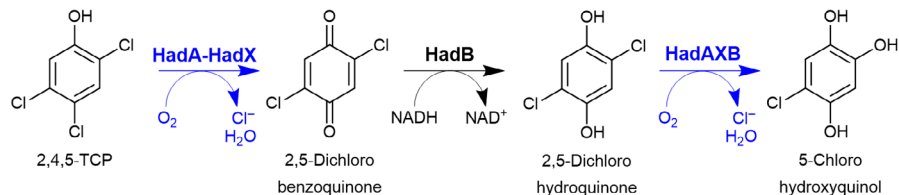
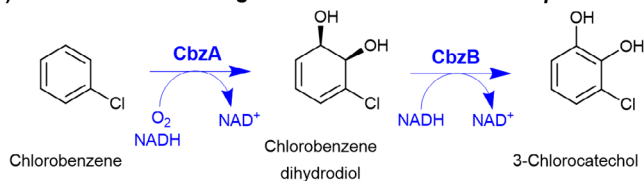
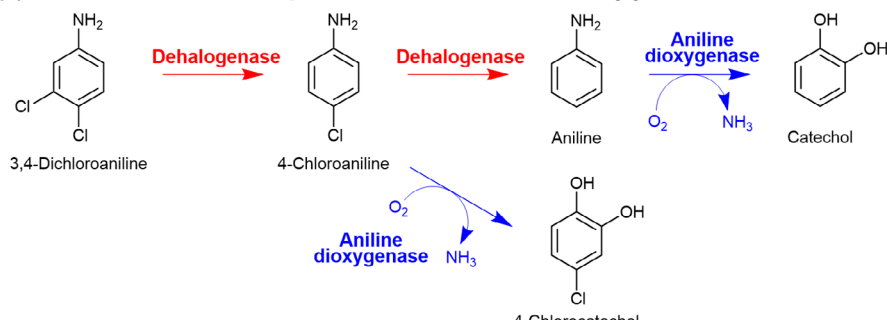
(A) 2,6-Dibromophenol biodegradation from *Nitratireductor pacificus* pht-3B

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

(C) Pentachlorophenol biodegradation from *Shingobium chlorophenicum*

(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 H_2 through the membrane-bound hydrogenase (MPH), 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 *Thauera aromatica* involves the reductive dehalogenation by ATP-dependent class I benzoyl CoA reductase (BCR) to catalyse dearomatization 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 hydroxylase, PcpB, prior to reductive dehalogenation of tetrachlorohydroquinone by glutathione-dependent tetrachlorohydroquinone dehalogenase, PcpC (Warner and Copley, 2007a,b; Hlouchova *et al.*, 2012). D. Oxidative catabolism of 2,4,5-trichlorophenol from *Ralstonia pickettii* DTP0602 involves Had enzymes. HadA and HadX are the two-component flavin-dependent monooxygenase and reductase, respectively, while HadB is a quinone reductase (Pimviriyakul *et al.*, 2017; Pimviriyakul and Chaiyen, 2018). E. Chlorobenzene biodegradation pathway from *Pseudomonas putida* strain GJ31 occurs via a dioxygenation reaction by chlorobenzene dioxygenase (CbzA) 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.

(Fricker *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). *Desulfitobacterium hafniense* strain PCP-1 can dehalogenate and convert PCP to 3-CP (Bisaillon *et al.*, 2010).

RdhA3 from *Desulfitobacterium 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-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 *Thauera aromatica* (Boll and Fuchs, 1995; Tiedt *et al.*, 2016), *Thauera chlorobenzoica* sp. (Kuntze *et al.*, 2011; Song *et al.*,

2001), *Pseudomonas stutzeri* (Vargas *et al.*, 2000) and *Rhodospseudomonas palustris* (Egland *et al.*, 2001). The first step usually involves with the conversion of halogenated benzoate to halogenated benzoyl CoA by an ATP-dependent benzoate-CoA ligase (Wischgoll *et al.*, 2005). In the following step, an ATP-dependent reductive dehalogenase catalyses dearomatization 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 dearomatization 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 CO_2 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 tetrachlorohydroquinone 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 (GS-DCHQ^{*}) before chloride elimination occurs. This intermediate decays by reacting with another GSH to result in the final product of 2,6-dichlorohydroquinone (2,6-DCHQ) 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 monooxygenation 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 phenoxyacetic 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 (flavin_{ox}) 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 (FADH[–]). O₂ then reacts with FADH[–] 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 pickettii* 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 TftD-TftC system from *Burkholderia cepacia* AC1100 (Gisi and Xun, 2003; Webb *et al.*, 2010), the

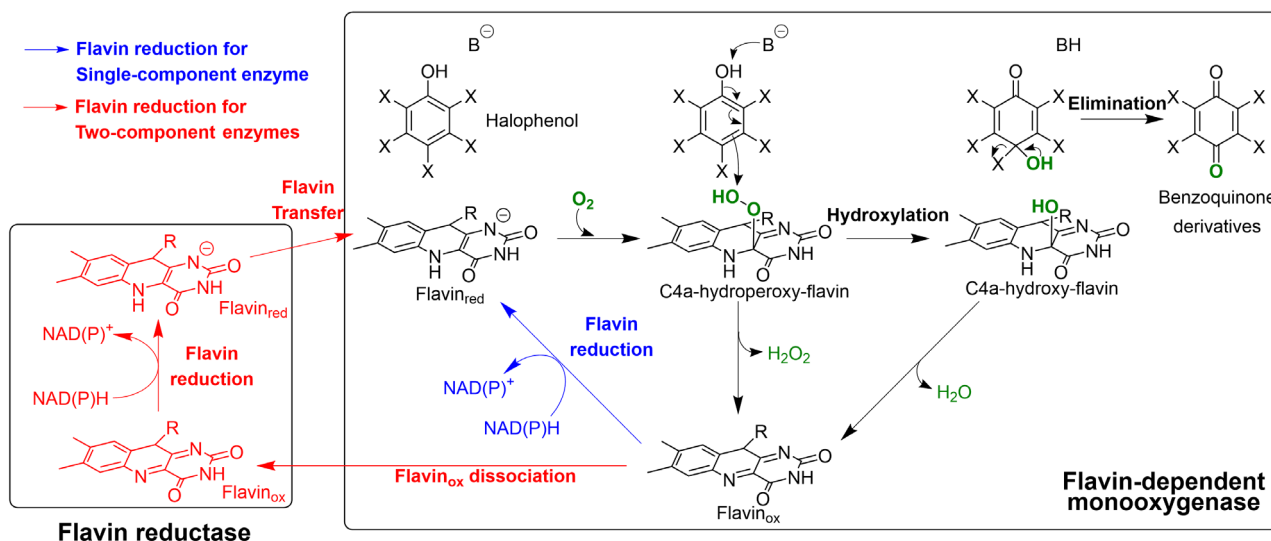


Fig. 5. Mechanism of flavin-dependent monooxygenase for dehalogenation of halogenated phenol. A simple reaction scheme of halogenated phenol oxidative dehalogenation by a flavin-dependent monooxygenase which occurs *via* the reaction of the C4a-hydroperoxy-flavin intermediate. For a single-component enzyme like PcpB from *S. chlorophenolicus*, 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. pickettii*, 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.

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* X1^T (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 biotransformation of 2,4,5-TCP by two consecutive dechlorination 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) have 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 *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 that catalyses proton abstraction from the -OH group of a phenol substrate which is a crucial step for initiating the hydroxylation (B in Fig. 5) (Webb *et al.*, 2010; Hayes *et al.*, 2012; Pimviriyakul *et al.*, 2018; Pongpamorn *et al.*, 2019).

Recent investigations also indicate that two additional reductases are required for completion of HadA activity. HadX, a reductase encoded in the same operon as HadA was shown to be the most efficient and physiologically relevant reductase to provide reduced FAD for the

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 TcbB in *Ralstonia eutropha* JMP134 (Belchik and Xun, 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 hydroxylation 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 O₂) 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.*, 2011). The whole biodegradation pathway for CB in *Pseudomonas putida* strain GJ31 by the enzymes encoded in the *cbz* cluster has been proposed (Kunze *et al.*, 2009). CB is first hydroxylated by chlorobenzene dioxygenase (CbzA) to form chlorobenzene dihydrodiol which is then further converted to 3-chlorocatechol by another enzyme chlorobenzene dihydrodiol dehydrogenase (CbzB) (Fig. 4E). Fluorobenzene (FB) can be metabolized by *Burkholderia fungorum* FLU100 (DSM 23736) (Strunk and Engesser, 2013) and *Labrys portucalensis* to result in 3-fluorocatechol as product. Halogenated 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 hydroxylation (Travkin *et al.*, 2003). A strain of *Pseudomonas* sp. degrades 3,4-dichloroaniline *via* 4,5-dichlorocatechol, 3,4-dichloromuconate, 3-chlorobutenolide, 3-chloromaleylacetate 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 H₂O. 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 Bruce, 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

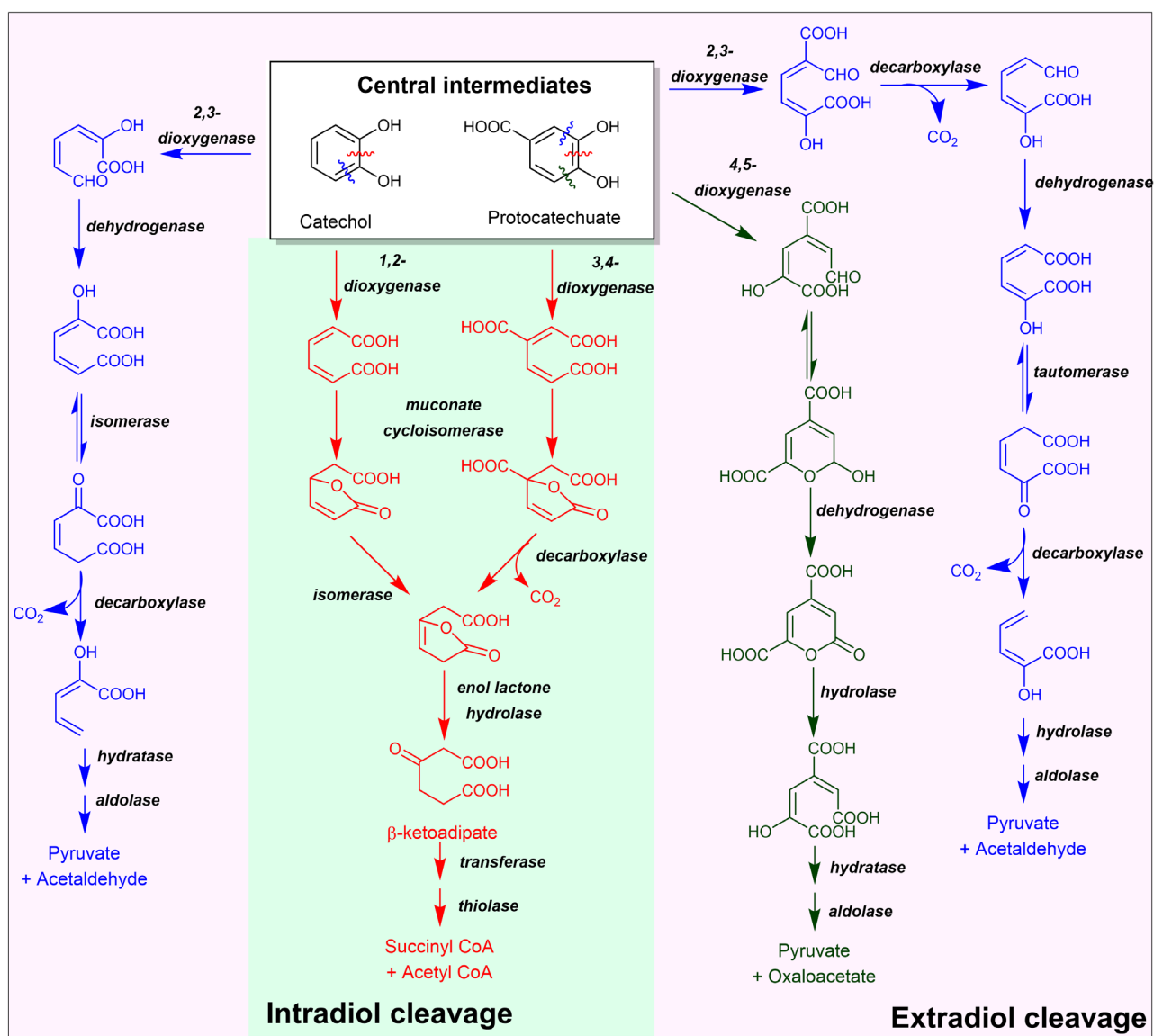


Fig. 6. Various reactions in the lower catabolic pathways. Red pathway is an intradiol cleavage pathway initiated by 1,2-cleavage via 1,2-dioxygenase for catechol or by 3,4-cleavage via 3,4-dioxygenase for protocatechuate. Blue pathway is an extradiol cleavage pathway initiated by 2,3-cleavage using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

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; Pornsuwan *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 -keto adipate enol-lactone step before the lactone ring is hydrolysed by an enzyme enol-lactone hydrolase to generate β -keto adipate (Patel *et al.*, 1975). Further reactions along the pathway include two reactions catalysed by β -keto adipate:succinyl-CoA transferase and β -keto adipyl-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 straight forward approach is to use the microbe directly. However, several parameters related to bioattenuation, biostimulation 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 synergetic 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 monooxygenase 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 (Wattthaisong *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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Conflict of interest

None declared.

References

- Adrian, L., Szewzyk, U., Wecke, J., and Görisch, H. (2000) Bacterial dehalorespiration with chlorinated benzenes. *Nature* **408**: 580–583.
- Adrian, L., Rahnenfuhrer, J., Gobom, J., and Holscher, T. (2007) Identification of a chlorobenzene reductive dehalogenase in *Dehalococcoides* sp. strain CBDB1. *Appl Environ Microbiol* **73**: 7717–7724.
- Alfan-Guzman, R., Ertan, H., Manefield, M., and Lee, M. (2017) Isolation and characterization of *Dehalobacter* sp. strain TeCB1 including identification of TcbA: a novel tetra- and trichlorobenzene reductive dehalogenase. *Front Microbiol* **8**: 558.
- Amy, P.S., Schulke, J.W., Frazier, L.M., and Seidler, R.M. (1985) Characterization of aquatic bacteria and cloning of genes specifying partial degradation of 2,4-dichlorophenoxyacetic acid. *Appl Environ Microbiol* **49**: 1237–1245.
- Arora, P.K. (2015) Bacterial degradation of monocyclic aromatic amines. *Front Microbiol* **6**: 820.
- Arora, P.K., and Bae, H. (2014) Bacterial degradation of chlorophenols and their derivatives. *Microb Cell Fact* **13**: 31.
- Arora, P.K., Sasikala, C., and Ramana, C.V. (2012) Degradation of chlorinated nitroaromatic compounds. *Appl Microbiol Biotechnol* **93**: 2265–2277.
- Arora, P.K., Srivastava, A., Garg, S.K., and Singh, V.P. (2018) Recent advances in degradation of chloronitrophenols. *Bioresour Technol* **250**: 902–909.
- Baelum, J., Jacobsen, C.S., and Holben, W.E. (2010) Comparison of 16S rRNA gene phylogeny and functional *tfdA* gene distribution in thirty-one different 2,4-dichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid degraders. *Syst Appl Microbiol* **33**: 67–70.
- Ballou, D.P., Entsch, B., and Cole, L.J. (2005) Dynamics involved in catalysis by single-component and two-component flavin-dependent aromatic hydroxylases. *Biochem Biophys Res Commun* **338**: 590–598.
- Banerjee, R., Jones, J.C., and Lipscomb, J.D. (2019) Soluble methane monooxygenase. *Annu Rev Biochem* **88**: 409–431.
- Belchik, S.M., and Xun, L. (2008) Functions of flavin reductase and quinone reductase in 2,4,6-trichlorophenol degradation by *Cupriavidus necator* JMP134. *J Bacteriol* **190**: 1615–1619.
- Benning, M.M., Taylor, K.L., Liu, R.Q., Yang, G., Xiang, H., Wesenberg, G., et al. (1996) Structure of 4-chlorobenzoyl coenzyme A dehalogenase determined to 1.8 Å resolution: an enzyme catalyst generated via adaptive mutation. *Biochemistry* **35**: 8103–8109.
- van Berkel, W.J., Kamerbeek, N.M., and Fraaije, M.W. (2006) Flavoprotein monooxygenases, a diverse class of oxidative biocatalysts. *J Biotechnol* **124**: 670–689.
- Bilal, M., Asgher, M., Cheng, H., Yan, Y., and Iqbal, H.M.N. (2019) Multi-point enzyme immobilization, surface chemistry, and novel platforms: a paradigm shift in biocatalyst design. *Crit Rev Biotechnol* **39**: 202–219.
- Bisaillon, A., Beaudet, R., Lepine, F., Deziel, E., and Villemur, R. (2010) Identification and characterization of a novel CprA reductive dehalogenase specific to highly chlorinated phenols from *Desulfitobacterium hafniense* strain PCP-1. *Appl Environ Microbiol* **76**: 7536–7540.
- Boll, M., and Fuchs, G. (1995) Benzoyl-coenzyme A reductase (dearomatizing), a key enzyme of anaerobic aromatic metabolism. ATP dependence of the reaction, purification and some properties of the enzyme from *Thauera aromatica* strain K172. *Eur J Biochem* **234**: 921–933.
- Boll, M., Löffler, C., Morris, B.E., and Kung, J.W. (2014) Anaerobic degradation of homocyclic aromatic compounds via arylcarboxyl-coenzyme A esters: organisms, strategies and key enzymes. *Environ Microbiol* **16**: 612–627.
- Bull, C., and Ballou, D.P. (1981) Purification and properties of protocatechuate 3,4-dioxygenase from *Pseudomonas putida*. A new iron to subunit stoichiometry. *J Biol Chem* **256**: 12673–12680.
- Bunge, M., Adrian, L., Kraus, A., Opel, M., Lorenz, W.G., Andreesen, J.R., et al. (2003) Reductive dehalogenation of chlorinated dioxins by an anaerobic bacterium. *Nature* **421**: 357.
- Casida, J.E., and Quistad, G.B. (2004) Organophosphate toxicology: safety aspects of nonacetylcholinesterase secondary targets. *Chem Res Toxicol* **17**: 983–998.
- Chae, J.C., Song, B., and Zylstra, G.J. (2008) Identification of genes coding for hydrolytic dehalogenation in the metagenome derived from a denitrifying 4-chlorobenzoate degrading consortium. *FEMS Microbiol Lett* **281**: 203–209.
- Chen, C., and He, J. (2018) Strategy for the rapid dechlorination of polychlorinated biphenyls (PCBs) by *Dehalococcoides mccartyi* strains. *Environ Sci Technol* **52**: 13854–13862.
- Chen, K., Jian, S., Huang, L., Ruan, Z., Li, S., and Jiang, J. (2015) Reductive dehalogenation of 3,5-dibromo-4-hydroxybenzoate by an aerobic strain of *Delftia* sp. EOB-17. *Biotechnol Lett* **37**: 2395–2401.
- Cheng, Z.W., Li, C., Kennes, C.T., Ye, J.X., Chen, D.Z., Zhang, S.H., et al. (2017) Improved biodegradation potential of chlorobenzene by a mixed fungal-bacterial consortium. *Int Biodeterior Biodegradation* **123**: 276–285.
- Chenprakhon, P., Wongnate, T., and Chaiyen, P. (2019) Monooxygenation of aromatic compounds by flavin-dependent monooxygenases. *Protein Sci* **28**: 8–29.
- Cho, S.Y., Kwean, O.S., Yang, J.W., Cho, W., Kwak, S., Park, S., et al. (2017) Identification of the upstream 4-chlorophenol biodegradation pathway using a recombinant monooxygenase from *Arthrobacter chlorophenolicus* A6. *Bioresour Technol* **245**: 1800–1807.
- Clarkson, S.M., Giannone, R.J., Kridelbaugh, D.M., Elkins, J.G., Guss, A.M., and Michener, J.K. (2017) Construction and optimization of a heterologous pathway for protocatechuate catabolism in *Escherichia coli* enables bioconversion of model aromatic compounds. *Appl Environ Microbiol* **83**: 1–13.
- Collins, F.A., Fisher, K., Payne, K.A.P., Gaytan Mondragon, S., Rigby, S.E.J., and Leys, D. (2018) NADPH-driven organohalide reduction by a nonrespiratory reductive dehalogenase. *Biochemistry* **57**: 3493–3502.

- Commandeur, L.C., and Parsons, J.R. (1990) Degradation of halogenated aromatic compounds. *Biodegradation* **1**: 207–220.
- Copley, S.D. (1997) Diverse mechanistic approaches to difficult chemical transformations: microbial dehalogenation of chlorinated aromatic compounds. *Chem Biol* **4**: 169–174.
- Crooks, G.P., and Copley, S.D. (1994) Purification and characterization of 4-chlorobenzoyl CoA dehalogenase from *Arthrobacter* sp. strain 4-CB1. *Biochemistry* **33**: 11645–11649.
- Cycon, M., Mrozik, A., and Piotrowska-Seget, Z. (2017) Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: a review. *Chemosphere* **172**: 52–71.
- Deng, S., Chen, Y., Wang, D., Shi, T., Wu, X., Ma, X., et al. (2015) Rapid biodegradation of organophosphorus pesticides by *Stenotrophomonas* sp. G1. *J Hazard Mater* **297**: 17–24.
- Dolfing, J., and Novak, I. (2015) The Gibbs free energy of formation of halogenated benzenes, benzoates and phenols and their potential role as electron acceptors in anaerobic environments. *Biodegradation* **26**: 15–27.
- Don, R.H., Weightman, A.J., Knackmuss, H.J., and Timmis, K.N. (1985) Transposon mutagenesis and cloning analysis of the pathways for degradation of 2,4-dichlorophenoxyacetic acid and 3-chlorobenzoate in *Alcaligenes eutrophus* JMP134(pJP4). *J Bacteriol* **161**: 85–90.
- Dunning Hotopp, J.C., and Hausinger, R.P. (2002) Probing the 2,4-dichlorophenoxyacetate/alpha-ketoglutarate dioxygenase substrate-binding site by site-directed mutagenesis and mechanism-based inactivation. *Biochemistry* **41**: 9787–9794.
- Dvorak, P., Nikel, P.I., Damborsky, J., and de Lorenzo, V. (2017) Bioremediation 3.0: engineering pollutant-removing bacteria in the times of systemic biology. *Biotechnol Adv* **35**: 845–866.
- Egland, P.G., Gibson, J., and Harwood, C.S. (2001) Reductive, coenzyme A-mediated pathway for 3-chlorobenzoate degradation in the phototrophic bacterium *Rhodospseudomonas palustris*. *Appl Environ Microbiol* **67**: 1396–1399.
- Fang, L., Shi, T., Chen, Y., Wu, X., Zhang, C., Tang, X., et al. (2019) Kinetics and catabolic pathways of the insecticide chlorpyrifos, annotation of the degradation genes, and characterization of enzymes TcpA and Fre in *Cupriavidus nantongensis* X1^T. *J Agric Food Chem* **67**: 2245–2254.
- Fetzner, S., and Lingens, F. (1994) Bacterial dehalogenases: biochemistry, genetics, and biotechnological applications. *Microbiol Rev* **58**: 641–685.
- Field, J.A., and Sierra-Alvarez, R. (2008) Microbial degradation of chlorinated phenols. *Rev Environ Sci Bio* **7**: 211–241.
- Fielding, A.J., Lipscomb, J.D., and Que, L. Jr (2014) A two-electron-shell game: intermediates of the extradiol-cleaving catechol dioxygenases. *J Biol Inorg Chem* **19**: 491–504.
- Fricker, A.D., LaRoe, S.L., Shea, M.E., and Bedard, D.L. (2014) *Dehalococcoides mccartyi* strain JNA dechlorinates multiple chlorinated phenols including pentachlorophenol and harbors at least 19 reductive dehalogenase homologous genes. *Environ Sci Technol* **48**: 14300–14308.
- Fuchs, G., Boll, M., and Heider, J. (2011) Microbial degradation of aromatic compounds – from one strategy to four. *Nat Rev Microbiol* **9**: 803–816.
- Fukumori, F., and Hausinger, R.P. (1993a) *Alcaligenes eutrophus* JMP134 2,4-dichlorophenoxyacetate monooxygenase is an alpha-ketoglutarate-dependent dioxygenase. *J Bacteriol* **175**: 2083–2086.
- Fukumori, F., and Hausinger, R.P. (1993b) Purification and characterization of 2,4-dichlorophenoxyacetate/alpha-ketoglutarate dioxygenase. *J Biol Chem* **268**: 24311–24317.
- Furukawa, K., and Miyazaki, T. (1986) Cloning of a gene cluster encoding biphenyl and chlorobiphenyl degradation in *Pseudomonas pseudoalcaligenes*. *J Bacteriol* **166**: 392–398.
- Georgiadis, N., Tsarouhas, K., Tsitsimpikou, C., Vardavas, A., Rezaee, R., Germanakis, I., et al. (2018) Pesticides and cardiotoxicity. Where do we stand? *Toxicol Appl Pharmacol* **353**: 1–14.
- Gisi, M.R., and Xun, L. (2003) Characterization of chlorophenol 4-monooxygenase (TftD) and NADH:flavin adenine dinucleotide oxidoreductase (TftC) of *Burkholderia cepacia* AC1100. *J Bacteriol* **185**: 2786–2792.
- Gothwal, A., Beniwal, P., Dhull, V., and Hooda, V. (2014) Preparation of electrochemical biosensor for detection of organophosphorus pesticides. *Int J Anal Chem* **2014**: 303641.
- Hägglblom, M.M. (1992) Microbial breakdown of halogenated aromatic pesticides and related compounds. *FEMS Microbiol Rev* **9**: 29–71.
- Hartnett, C., Neidle, E.L., Ngai, K.L., and Ormston, L.N. (1990) DNA sequences of genes encoding *Acinetobacter calcoaceticus* protocatechuate 3,4-dioxygenase: evidence indicating shuffling of genes and of DNA sequences within genes during their evolutionary divergence. *J Bacteriol* **172**: 956–966.
- Harwood, C.S., and Parales, R.E. (1996) The beta-ketoadipate pathway and the biology of self-identity. *Annu Rev Microbiol* **50**: 553–590.
- Hatta, T., Nakano, O., Imai, N., Takizawa, N., and Kiyohara, H. (1999) Cloning and sequence analysis of hydroxyquinol 1,2-dioxygenase gene in 2,4,6-trichlorophenol-degrading *Ralstonia pickettii* DTP0602 and characterization of its product. *J Biosci Bioeng* **87**: 267–272.
- Hatta, T., Fujii, E., and Takizawa, N. (2012) Analysis of two gene clusters involved in 2,4,6-trichlorophenol degradation by *Ralstonia pickettii* DTP0602. *Biosci Biotechnol Biochem* **76**: 892–899.
- Hayes, R.P., Webb, B.N., Subramanian, A.K., Nissen, M., Popchok, A., Xun, L., and Kang, C. (2012) Structural and catalytic differences between two FADH₂-dependent monooxygenases: 2,4,5-TCP 4-monooxygenase (TftD) from *Burkholderia cepacia* AC1100 and 2,4,6-TCP 4-monooxygenase (TcpA) from *Cupriavidus necator* JMP134. *Int J Mol Sci* **13**: 9769–9784.
- Hechmi, N., Bosso, L., El-Bassi, L., Scelza, R., Testa, A., Jedidi, N., and Rao, M.A. (2016) Depletion of pentachlorophenol in soil microcosms with *Byssoschlamys nivea* and *Scopulariopsis brumptii* as detoxification agents. *Chemosphere* **165**: 547–554.

- Hegg, E.L., Whiting, A.K., Saari, R.E., McCracken, J., Hausinger, R.P., and Que, L. Jr (1999) Herbicide-degrading alpha-keto acid-dependent enzyme TfdA: metal coordination environment and mechanistic insights. *Biochemistry* **38**: 16714–16726.
- Hlouchova, K., Rudolph, J., Pietari, J.M., Behlen, L.S., and Copley, S.D. (2012) Pentachlorophenol hydroxylase, a poorly functioning enzyme required for degradation of pentachlorophenol by *Sphingobium chlorophenolicum*. *Biochemistry* **51**: 3848–3860.
- Hogan, D.A., Buckley, D.H., Nakatsu, C.H., Schmidt, T.M., and Hausinger, R.P. (1997) Distribution of the *tfdA* gene in soil bacteria that do not degrade 2,4-dichlorophenoxyacetic acid (2,4-d). *Microb Ecol* **34**: 90–96.
- Hongsawat, P., and Vangnai, A.S. (2011) Biodegradation pathways of chloroanilines by *Acinetobacter baylyi* strain GFJ2. *J Hazard Mater* **186**: 1300–1307.
- Huang, X., He, J., Yan, X., Hong, Q., Chen, K., He, Q., et al. (2017) Microbial catabolism of chemical herbicides: microbial resources, metabolic pathways and catabolic genes. *Pestic Biochem Physiol* **143**: 272–297.
- Huijbers, M.M., Montersino, S., Westphal, A.H., Tischler, D., and van Berkel, W.J. (2014) Flavin dependent monooxygenases. *Arch Biochem Biophys* **544**: 2–17.
- Itoh, K., Kanda, R., Sumita, Y., Kim, H., Kamagata, Y., Suyama, K., et al. (2002) *tfdA*-like genes in 2,4-dichlorophenoxyacetic acid-degrading bacteria belonging to the *Bradyrhizobium-Agromonas-Nitrobacter-Atipia* cluster in alpha-Proteobacteria. *Appl Environ Microbiol* **68**: 3449–3454.
- Iwagami, S.G., Yang, K., and Davies, J. (2000) Characterization of the protocatechuic acid catabolic gene cluster from *Streptomyces* sp. strain 2065. *Appl Environ Microbiol* **66**: 1499–1508.
- Joaquin, B.F., Heidi, O. and Birgit, S.K. (2009) JRC Scientific and Technical report : European list of notified chemical substances (ELINCS). URL http://publications.jrc.ec.europa.eu/repository/bitstream/JRC52455/reqno_jrc52455.pdf.
- Jugder, B.E., Ertan, H., Bohl, S., Lee, M., Marquis, C.P., and Manefield, M. (2016) Organohalide respiring bacteria and reductive dehalogenases: key tools in organohalide bioremediation. *Front Microbiol* **7**: 249.
- Kamagata, Y., Fulthorpe, R.R., Tamura, K., Takami, H., Forney, L.J., and Tiedje, J.M. (1997) Pristine environments harbor a new group of oligotrophic 2,4-dichlorophenoxyacetic acid-degrading bacteria. *Appl Environ Microbiol* **63**: 2266–2272.
- Kamimura, N., Takahashi, K., Mori, K., Araki, T., Fujita, M., Higuchi, Y., et al. (2017) Bacterial catabolism of lignin-derived aromatics: new findings in a recent decade: update on bacterial lignin catabolism. *Environ Microbiol Rep* **9**: 679–705.
- Kang, C., Yang, J.W., Cho, W., Kwak, S., Park, S., Lim, Y., et al. (2017) Oxidative biodegradation of 4-chlorophenol by using recombinant monooxygenase cloned and over-expressed from *Arthrobacter chlorophenolicus* A6. *Biore-sour Technol* **240**: 123–129.
- Kasai, D., Fujinami, T., Abe, T., Mase, K., Katayama, Y., Fukuda, M., et al. (2009) Uncovering the protocatechuic acid 2,3-cleavage pathway genes. *J Bacteriol* **191**: 6758–6768.
- Klages, U., Markus, A., and Lingens, F. (1981) Degradation of 4-chlorophenoxyacetic acid by a *Pseudomonas* species. *J Bacteriol* **146**: 64–68.
- Kumar, A., Trefault, N., and Olaniran, A.O. (2016) Microbial degradation of 2,4-dichlorophenoxyacetic acid: Insight into the enzymes and catabolic genes involved, their regulation and biotechnological implications. *Crit Rev Microbiol* **42**: 194–208.
- Kumar, V., Shahi, S.K., and Singh, S. (2018) Bioremediation: An eco-sustainable approach for restoration of contaminated sites. In *Microbial Bioprospecting for Sustainable Development*. Singh, J., Sharma, D., Kumar, G., and Sharma, N.R. (eds). Singapore: Springer Singapore, pp. 115–136.
- Kuntze, K., Kiefer, P., Baumann, S., Seifert, J., von Bergen, M., Vorholt, J.A., et al. (2011) Enzymes involved in the anaerobic degradation of meta-substituted halobenzoates. *Mol Microbiol* **82**: 758–769.
- Kunze, M., Zerlin, K.F., Retzlaff, A., Pohl, J.O., Schmidt, E., Janssen, D.B., et al. (2009) Degradation of chloroaromatics by *Pseudomonas putida* GJ31: assembled route for chlorobenzene degradation encoded by clusters on plasmid pKW1 and the chromosome. *Microbiology* **155**: 4069–4083.
- Laemmli, C.M., Leveau, J.H., Zehnder, A.J., and van der Meer, J.R. (2000) Characterization of a second *tfd* gene cluster for chlorophenol and chlorocatechol metabolism on plasmid pJP4 in *Ralstonia eutropha* JMP134(pJP4). *J Bacteriol* **182**: 4165–4172.
- Laemmli, C., Werlen, C., and van der Meer, J.R. (2004) Mutation analysis of the different *tfd* genes for degradation of chloroaromatic compounds in *Ralstonia eutropha* JMP134. *Arch Microbiol* **181**: 112–121.
- Lau, E.Y., and Bruce, T.C. (2001) The active site dynamics of 4-chlorobenzoyl-CoA dehalogenase. *Proc Natl Acad Sci USA* **98**: 9527–9532.
- Lipscomb, J.D. (2008) Mechanism of extradiol aromatic ring-cleaving dioxygenases. *Curr Opin Struct Biol* **18**: 644–649.
- Liu, J., Tan, L., Wang, J., Wang, Z., Ni, H., and Li, L. (2016) Complete biodegradation of chlorpyrifos by engineered *Pseudomonas putida* cells expressing surface-immobilized laccases. *Chemosphere* **157**: 200–207.
- Liu, L., Bilal, M., Duan, X., and Iqbal, H.M.N. (2019) Mitigation of environmental pollution by genetically engineered bacteria - current challenges and future perspectives. *Sci Total Environ* **667**: 444–454.
- Lu, Q., Qiu, L., Yu, L., Zhang, S., de Toledo, R.A., Shim, H., et al. (2019) Microbial transformation of chiral organohalides: distribution, microorganisms and mechanisms. *J Hazard Mater* **368**: 849–861.
- Lubbers, R.J.M., Dilokpimol, A., Visser, J., Mäkelä, M.R., Hildén, K.S. and de Vries, R.P. (2019) A comparison between the homocyclic aromatic metabolic pathways from plant-derived compounds by bacteria and fungi. *Biotechnol Adv*, in press.
- Luo, L., Taylor, K.L., Xiang, H., Wei, Y., Zhang, W., and Dunaway-Mariano, D. (2001) Role of active site binding interactions in 4-chlorobenzoyl-coenzyme A dehalogenase catalysis. *Biochemistry* **40**: 15684–15692.
- Mac Nelly, A., Kai, M., Svatos, A., Diekert, G., and Schubert, T. (2014) Functional heterologous production of

- reductive dehalogenases from *Desulfitobacterium hafniense* strains. *Appl Environ Microbiol* **80**: 4313–4322.
- Markusheva, T.V., Zhurenko, E., Galkin, E.G., Korobov, V.V., Zharikova, N.V., and Gafiatova, L.R. (2004) Identification and characterization of a plasmid in strain *Aeromonas hydrophila* IBRB-36 4CPA carrying genes for catabolism of chlorophenoxyacetic acids. *Genetika* **40**: 1469–1474.
- Maruyama, K., Shibayama, T., Ichikawa, A., Sakou, Y., Yamada, S., and Sugisaki, H. (2004) Cloning and characterization of the genes encoding enzymes for the protocatechuate meta-degradation pathway of *Pseudomonas ochraceae* NGJ1. *Biosci Biotechnol Biochem* **68**: 1434–1441.
- Matus, V., Sanchez, M.A., Martinez, M., and Gonzalez, B. (2003) Efficient degradation of 2,4,6-trichlorophenol requires a set of catabolic genes related to *tcp* genes from *Ralstonia eutropha* JMP134(pJP4). *Appl Environ Microbiol* **69**: 7108–7115.
- McCarthy, D.L., Navarrete, S., Willett, W.S., Babbitt, P.C., and Copley, S.D. (1996) Exploration of the relationship between tetrachlorohydroquinone dehalogenase and the glutathione S-transferase superfamily. *Biochemistry* **35**: 14634–14642.
- Megharaj, M., Ramakrishnan, B., Venkateswarlu, K., Sethunathan, N., and Naidu, R. (2011) Bioremediation approaches for organic pollutants: a critical perspective. *Environ Int* **37**: 1362–1375.
- Mondello, F.J. (1989) Cloning and expression in *Escherichia coli* of *Pseudomonas* strain LB400 genes encoding polychlorinated biphenyl degradation. *J Bacteriol* **171**: 1725–1732.
- Mostafalou, S., and Abdollahi, M. (2017) Pesticides: an update of human exposure and toxicity. *Arch Toxicol* **91**: 549–599.
- Muller, T.A., Byrde, S.M., Werlen, C., van der Meer, J.R., and Kohler, H.P. (2004) Genetic analysis of phenoxyalkanoic acid degradation in *Sphingomonas herbicidovorans* MH. *Appl Environ Microbiol* **70**: 6066–6075.
- Muller, T.A., Fleischmann, T., van der Meer, J.R., and Kohler, H.P. (2006) Purification and characterization of two enantioselective alpha-ketoglutarate-dependent dioxygenases, RdpA and SdpA, from *Sphingomonas herbicidovorans* MH. *Appl Environ Microbiol* **72**: 4853–4861.
- Murakami, S., Kodama, N., Shinke, R., and Aoki, K. (1997) Classification of catechol 1,2-dioxygenase family: sequence analysis of a gene for the catechol 1,2-dioxygenase showing high specificity for methylcatechols from Gram+ aniline-assimilating *Rhodococcus erythropolis* AN-13. *Gene* **185**: 49–54.
- Nickel, K., Suter, M.J., and Kohler, H.P. (1997) Involvement of two alpha-ketoglutarate-dependent dioxygenases in enantioselective degradation of (*R*)- and (*S*)-mecoprop by *Sphingomonas herbicidovorans* MH. *J Bacteriol* **179**: 6674–6679.
- Nigam, V.K., and Shukla, P. (2015) Enzyme based biosensors for detection of environmental pollutants—a review. *J Microbiol Biotechnol* **25**: 1773–1781.
- Nováková, H., Vošahlíková, M., Pazlarová, J., Macková, M., Burkhard, J. and Demnerová, K. (2002) PCB metabolism by *Pseudomonas* sp. P2. *Int Biodeterior Biodegradation* **50**: 47–54.
- Olaniran, A.O., and Igbinsa, E.O. (2011) Chlorophenols and other related derivatives of environmental concern: properties, distribution and microbial degradation processes. *Chemosphere* **83**: 1297–1306.
- Palfey, B.A., and McDonald, C.A. (2010) Control of catalysis in flavin-dependent monooxygenases. *Arch Biochem Biophys* **493**: 26–36.
- Patel, R.N., Mazumdar, S., and Ornston, L.N. (1975) Beta-ketoadipate enol-lactone hydrolases I and II from *Acinetobacter calcoaceticus*. *J Biol Chem* **250**: 6567.
- Payne, K.A., Quezada, C.P., Fisher, K., Dunstan, M.S., Collins, F.A., Sjuts, H., et al. (2015) Reductive dehalogenase structure suggests a mechanism for B12-dependent dehalogenation. *Nature* **517**: 513–516.
- Peelen, S., Rietjens, I.M., Boersma, M.G., and Vervoort, J. (1995) Conversion of phenol derivatives to hydroxylated products by phenol hydroxylase from *Trichosporon cutaneum*. A comparison of regioselectivity and rate of conversion with calculated molecular orbital substrate characteristics. *Eur J Biochem* **227**: 284–291.
- Pera-Titus, M., Garcia-Molina, V., Banos, M.A., Gimenez, J., and Esplugas, S. (2004) Degradation of chlorophenols by means of advanced oxidation processes: a general review. *Appl Catal B* **47**: 219–256.
- Pieper, D.H. (2005) Aerobic degradation of polychlorinated biphenyls. *Appl Microbiol Biotechnol* **67**: 170–191.
- Pimviriyakul, P., and Chaiyen, P. (2018) A complete bioconversion cascade for dehalogenation and denitration by bacterial flavin-dependent enzymes. *J Biol Chem* **293**: 18525–18539.
- Pimviriyakul, P., Thotsaporn, K., Sucharitakul, J., and Chaiyen, P. (2017) Kinetic mechanism of the dechlorinating flavin-dependent monooxygenase HadA. *J Biol Chem* **292**: 4818–4832.
- Pimviriyakul, P., Surawatanawong, P., and Chaiyen, P. (2018) Oxidative dehalogenation and denitration by a flavin-dependent monooxygenase is controlled by substrate deprotonation. *Chem Sci* **9**: 7468–7482.
- Plotnikova, E.G., Solyanikova, I.P., Egorova, D.O., Shumkova, E.S., and Golovleva, L.A. (2012) Degradation of 4-chlorobiphenyl and 4-chlorobenzoic acid by the strain *Rhodococcus ruber* P25. *Microbiology* **81**: 143–153.
- Plumeier, I., Perez-Pantoja, D., Heim, S., Gonzalez, B., and Pieper, D.H. (2002) Importance of different *tfd* genes for degradation of chloroaromatics by *Ralstonia eutropha* JMP134. *J Bacteriol* **184**: 4054–4064.
- Pongpamorn, P., Watthaisong, P., Pimviriyakul, P., Jaruwat, A., Lawan, N., Chitnumsub, P., et al. (2019) Identification of a hotspot residue for improving the thermostability of a flavin-dependent monooxygenase. *ChemBioChem*, in press. <https://doi.org/10.1002/cbic.201900413>
- Poritz, M., Schiffmann, C.L., Hause, G., Heinemann, U., Seifert, J., Jehmlich, N., et al. (2015) *Dehalococcoides mccartyi* strain DCMB5 respire a broad spectrum of chlorinated aromatic compounds. *Appl Environ Microbiol* **81**: 587–596.
- Ponsuwan, S., Maenpuen, S., Kamutira, P., Watthaisong, P., Thotsaporn, K., Tongsook, C., et al. (2017) 3,4-Dihydroxyphenylacetate 2,3-dioxygenase from *Pseudomonas aeruginosa*: An Fe(II)-containing enzyme with fast turnover. *PLoS ONE* **12**: e0171135.

- Rao, M.A., Scelza, R., Acevedo, F., Diez, M.C., and Gianfreda, L. (2014) Enzymes as useful tools for environmental purposes. *Chemosphere* **107**: 145–162.
- Rayu, S., Nielsen, U.N., Nazaries, L., and Singh, B.K. (2017) Isolation and molecular characterization of novel chlorpyrifos and 3,5,6-trichloro-2-pyridinol-degrading bacteria from sugarcane farm soils. *Front Microbiol* **8**: 518.
- Reineke, W., and Knackmuss, H.J. (1988) Microbial degradation of haloaromatics. *Ann Rev Microbiol* **42**: 263–287.
- Roberts, K.M., and Fitzpatrick, P.F. (2013) Mechanisms of tryptophan and tyrosine hydroxylase. *IUBMB Life* **65**: 350–357.
- Romero, E., Gomez Castellanos, J.R., Gadda, G., Fraaije, M.W., and Mattevi, A. (2018) Same substrate, many Reactions: oxygen activation in flavoenzymes. *Chem Rev* **118**: 1742–1769.
- Rudolph, J., Erbse, A.H., Behlen, L.S., and Copley, S.D. (2014) A radical intermediate in the conversion of pentachlorophenol to tetrachlorohydroquinone by *Sphingobium chlorophenolicum*. *Biochemistry* **53**: 6539–6549.
- Saari, R.E., and Hausinger, R.P. (1998) Ascorbic acid-dependent turnover and reactivation of 2,4-dichlorophenoxyacetic acid/alpha-ketoglutarate dioxygenase using thiophenoxyacetic acid. *Biochemistry* **37**: 3035–3042.
- Salunkhe, V.P., Sawant, I.S., Banerjee, K., Rajguru, Y.R., Wadkar, P.N., Oulkar, D.P., et al. (2013) Biodegradation of profenofos by *Bacillus subtilis* isolated from grapevines (*Vitis vinifera*). *J Agric Food Chem* **61**: 7195–7202.
- Schubert, T., Adrian, L., Sawers, R.G., and Diekert, G. (2018) Organohalide respiratory chains: composition, topology and key enzymes. *FEMS Microbiol Ecol* **94**: 1–17.
- Seibold, B., Matthes, M., Eppink, M.H., Lings, F., Van Berkel, W.J., and Muller, R. (1996) 4-Hydroxybenzoate hydroxylase from *Pseudomonas* sp. CBS3: purification, characterization, gene cloning, sequence analysis and assignment of structural features determining the coenzyme specificity. *Eur J Biochem* **239**: 469–478.
- Sharma, B., Dangi, A.K., and Shukla, P. (2018) Contemporary enzyme based technologies for bioremediation: a review. *J Environ Manage* **210**: 10–22.
- Sheldon, R.A., and Woodley, J.M. (2018) Role of biocatalysis in sustainable chemistry. *Chem Rev* **118**: 801–838.
- Siripattanakul-Ratpukdi, S., Vangnai, A.S., Sangthean, P., and Singkibut, S. (2015) Profenofos insecticide degradation by novel microbial consortium and isolates enriched from contaminated chili farm soil. *Environ Sci Pollut Res Int* **22**: 320–328.
- Song, B., Palleroni, N.J., Kerkhof, L.J., and Häggblom, M.M. (2001) Characterization of halobenzoate-degrading, denitrifying *Azoarcus* and *Thauera* isolates and description of *Thauera chlorobenzoica* sp. nov. *Int J Syst Evol Microbiol* **51**: 589–602.
- Strunk, N., and Engesser, K.H. (2013) Degradation of fluorobenzene and its central metabolites 3-fluorocatechol and 2-fluoromuconate by *Burkholderia fungorum* FLU100. *Appl Microbiol Biotechnol* **97**: 5605–5614.
- Sucharitakul, J., Tinikul, R., and Chaiyen, P. (2014) Mechanisms of reduced flavin transfer in the two-component flavin-dependent monooxygenases. *Arch Biochem Biophys* **555–556**: 33–46.
- Supreeth, M., and Raju, N.S. (2017) Biotransformation of chlorpyrifos and endosulfan by bacteria and fungi. *Appl Microbiol Biotechnol* **101**: 5961–5971.
- Thotsaporn, K., Tinikul, R., Maenpuen, S., Phonbuppha, J., Wathaisong, P., Chenprakhon, P., et al. (2016) Enzymes in the p-hydroxyphenylacetate degradation pathway of *Acinetobacter baumannii*. *J Mol Catal B Enzym* **134**: 353–366.
- Tiedt, O., Mergelsberg, M., Boll, K., Muller, M., Adrian, L., Jehmlich, N., et al. (2016) ATP-dependent C-F bond cleavage allows the complete degradation of 4-fluoroaromatics without oxygen. *MBio* **7**: 1–9.
- Tinikul, R., Chenprakhon, P., Maenpuen, S., and Chaiyen, P. (2018) Biotransformation of plant-derived phenolic acids. *Biotechnol J* **13**: e1700632.
- Tonso, N.L., Matheson, V.G., and Holben, W.E. (1995) Polyphasic characterization of a suite of bacterial isolates capable of degrading 2,4-D. *Microb Ecol* **30**: 3–24.
- Travkin, V.M., Solyanikova, I.P., Rietjens, I.M., Vervoort, J., van Berkel, W.J., and Golovleva, L.A. (2003) Degradation of 3,4-dichloro- and 3,4-difluoroaniline by *Pseudomonas fluorescens* 26-K. *J Environ Sci Health B* **38**: 121–132.
- Ueda, M. (2016) Establishment of cell surface engineering and its development. *Biosci Biotechnol Biochem* **80**: 1243–1253.
- United States Environmental Protection Agency. (2014) Priority pollutant list. URL <https://www.epa.gov/eg/toxic-and-priority-pollutants-under-clean-water-act#priority>.
- Vargas, C., Song, B., Camps, M., and Häggblom, M.M. (2000) Anaerobic degradation of fluorinated aromatic compounds. *Appl Microbiol Biotechnol* **53**: 342–347.
- Wagner, A., Cooper, M., Ferdi, S., Seifert, J., and Adrian, L. (2012) Growth of *Dehalococcoides mccartyi* strain CBDB1 by reductive dehalogenation of brominated benzenes to benzene. *Environ Sci Technol* **46**: 8960–8968.
- Wang, C.L., You, S.L., and Wang, S.L. (2006) Purification and characterization of a novel catechol 1,2-dioxygenase from *Pseudomonas aeruginosa* with benzoic acid as a carbon source. *Process Biochem* **41**: 1594–1601.
- Wang, S., Zhang, W., Yang, K.L., and He, J. (2014) Isolation and characterization of a novel *Dehalobacter* species strain TCP1 that reductively dechlorinates 2,4,6-trichlorophenol. *Biodegradation* **25**: 313–323.
- Wang, Y., Li, J., and Liu, A. (2017) Oxygen activation by mononuclear nonheme iron dioxygenases involved in the degradation of aromatics. *J Biol Inorg Chem* **22**: 395–405.
- Wang, S., Qiu, L., Liu, X., Xu, G., Siegert, M., Lu, Q., et al. (2018) Electron transport chains in organohalide-respiring bacteria and bioremediation implications. *Biotechnol Adv* **36**: 1194–1206.
- Wang, B., Xu, J., Gao, J., Fu, X., Han, H., Li, Z., et al. (2019) Construction of an *Escherichia coli* strain to degrade phenol completely with two modified metabolic modules. *J Hazard Mater* **373**: 29–38.
- Warner, J.R., and Copley, S.D. (2007a) Mechanism of the severe inhibition of tetrachlorohydroquinone dehalogenase by its aromatic substrates. *Biochemistry* **46**: 4438–4447.

- Warner, J.R., and Copley, S.D. (2007b) Pre-steady-state kinetic studies of the reductive dehalogenation catalyzed by tetrachlorohydroquinone dehalogenase. *Biochemistry* **46**: 13211–13222.
- Watthaisong, P., Pongpamorn, P., Pimviriyakul, P., Maenpuen, S., Ohmiya, Y. and Chaiyen, P. (2019) A chemoenzymatic cascade for smart detection of nitro and halogenated phenols. *Angew Chem Inter Ed Engl*, in press. <https://doi.org/10.1002/anie.201904923>
- Webb, B.N., Ballinger, J.W., Kim, E., Belchik, S.M., Lam, K.S., Youn, B., et al. (2010) Characterization of chlorophenol 4-monooxygenase (TftD) and NADH:FAD oxidoreductase (TftC) of *Burkholderia cepacia* AC1100. *J Biol Chem* **285**: 2014–2027.
- Wells, T. Jr, and Ragauskas, A.J. (2012) Biotechnological opportunities with the beta-ketoadipate pathway. *Trends Biotechnol* **30**: 627–637.
- Westendorf, A., Müller, R.H., and Babel, W. (2003) Purification and characterisation of the enantiospecific dioxygenases from *Delftia acidovorans* MC1 initiating the degradation of phenoxypropionate and phenoxyacetate herbicides. *Acta Biotechnol* **23**: 3–17.
- Westendorf, A., Benndorf, D., Pribyl, T., Harms, H., and Müller, R.H. (2006) Kinetic traits and enzyme form patterns of (*R*)-2-(2,4-dichlorophenoxy)propionate/ α -ketoglutarate dioxygenase (RdpA) after expression in different bacterial strains. *Eng Life Sci* **6**: 552–559.
- Wischgoll, S., Heintz, D., Peters, F., Erleben, A., Samighausen, E., Reski, R., et al. (2005) Gene clusters involved in anaerobic benzoate degradation of *Geobacter metallireducens*. *Mol Microbiol* **58**: 1238–1252.
- Wolgel, S.A., Dege, J.E., Perkins-Olson, P.E., Jaurez-Garcia, C.H., Crawford, R.L., Munck, E., et al. (1993) Purification and characterization of protocatechuate 2,3-dioxygenase from *Bacillus macerans*: a new extradiol catecholic dioxygenase. *J Bacteriol* **175**: 4414–4426.
- Wu, J., Xu, D., Lu, X., Wang, C., Guo, H., and Dunaway-Mariano, D. (2006) Contributions of long-range electrostatic interactions to 4-chlorobenzoyl-CoA dehalogenase catalysis: a combined theoretical and experimental study. *Biochemistry* **45**: 102–112.
- Xu, D., Wei, Y., Wu, J., Dunaway-Mariano, D., Guo, H., Cui, Q., et al. (2004) QM/MM studies of the enzyme-catalyzed dechlorination of 4-chlorobenzoyl-CoA provide insight into reaction energetics. *J Am Chem Soc* **126**: 13649–13658.
- Xu, Y.-L., Li, F.-Y., Ndikuryayo, F., Yang, W.-C., and Wang, H.-M. (2018) Cholinesterases and engineered mutants for the detection of organophosphorus pesticide residues. *Sensors* **18**: 4281–4295.
- Xun, L., and Webster, C.M. (2004) A monooxygenase catalyzes sequential dechlorinations of 2,4,6-trichlorophenol by oxidative and hydrolytic reactions. *J Biol Chem* **279**: 6696–6700.
- Xun, L., Topp, E., and Orser, C.S. (1992a) Diverse substrate range of a *Flavobacterium* pentachlorophenol hydroxylase and reaction stoichiometries. *J Bacteriol* **174**: 2898–2902.
- Xun, L., Topp, E., and Orser, C.S. (1992b) Purification and characterization of a tetrachloro-*p*-hydroquinone reductive dehalogenase from a *Flavobacterium* sp. *J Bacteriol* **174**: 8003–8007.
- Yao, X.F., Khan, F., Pandey, R., Pandey, J., Mourant, R.G., Jain, R.K., et al. (2011) Degradation of dichloroaniline isomers by a newly isolated strain, *Bacillus megaterium* IMT21. *Microbiology* **157**: 721–726.
- You, I.S., and Bartha, R. (1982) Stimulation of 3,4-dichloroaniline mineralization by aniline. *Appl Environ Microbiol* **44**: 678–681.
- Zhang, W., Wei, Y., Luo, L., Taylor, K.L., Yang, G., Dunaway-Mariano, D., et al. (2001) Histidine 90 function in 4-chlorobenzoyl-coenzyme A dehalogenase catalysis. *Biochemistry* **40**: 13474–13482.
- Zhang, L.L., Leng, A.Q., Zhu, R.Y., and Chen, J.M. (2011) Degradation of chlorobenzene by strain *Ralstonia pickettii* L2 isolated from a biotrickling filter treating a chlorobenzene-contaminated gas stream. *Appl Microbiol Biotechnol* **91**: 407–415.
- Zhang, H., Yu, T., Li, J., Wang, Y.R., Wang, G.L., Li, F., et al. (2018) Two *dcm* gene clusters essential for the degradation of diclofop-methyl in a microbial consortium of *Rhodococcus* sp. JT-3 and *Brevundimonas* sp. JT-9. *J Agric Food Chem* **66**: 12217–12226.
- Zhou, L., Poh, R.P., Marks, T.S., Chowdhry, B.Z., and Smith, A.R. (2008) Structure and denaturation of 4-chlorobenzoyl coenzyme A dehalogenase from *Arthrobacter* sp. strain TM-1. *Biodegradation* **19**: 65–75.
- Zhuang, Z., Gartemann, K.H., Eichenlaub, R., and Dunaway-Mariano, D. (2003) Characterization of the 4-hydroxybenzoyl-coenzyme A thioesterase from *Arthrobacter* sp. strain SU. *Appl Environ Microbiol* **69**: 2707–2711.