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Review Article

Dehalogenases for pollutant degradation: A mini review

Sefatullah Zakary [1]*, Habeebat Adekilekun Oyewusi [2], Fahrul Huyop [3]

[1] Department of Botany, Faculty of Biology, Kabul University, Dehburi, Kabul 1006, Afghanistan
[2] Department of Biochemistry, School of Science and Computer Studies, Federal Polytechnic Ado Ekiti, Ado Ekiti PMB 5351, Ekiti State, Nigeria
[3] Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, Johor 81310, Malaysia

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**Corresponding author:*
E-mail: sefatullahz@ku.edu.af

ABSTRACT

Dehalogenases are microbial enzyme catalysed the cleavage of carbon-halogen bond of halogenated organic compounds. It has potential use in the area of biotechnology such as bioremediation and chemical industry. Halogenated organic compounds can be found in a considerable amount in the environment due to utilization in agriculture and industry, such as pesticides and herbicides. The presence of halogenated compound in the environment have been implicated on the health and natural ecosystem. Microbial dehalogenation is a significant method to tackle this problem. This review intends to briefly describe the microbial dehalogenases in relation to the environment where they are isolated. The basic information about dehalogenases in relation to dehalogenation mechanisms, classification, sources and the transportation of these pollutants into bacterial cytoplasm will be described. We also summarised readily available synthetic halogenated organic compound in the environment.

Keywords: Halogenated compounds, Haloacid dehalogenases, L-2-haloacid dehalogenase, Dehalogenation, haloacid dehalogenase type II

Introduction

Halogenated organic compounds are found in a vast amount in the environment, such as lakes, groundwater, drinking water, seawater, and soil. Some are naturally occurring, and some are man-made. However, in the agricultural sector, modern pesticides and herbicides can be harmful to living creatures. The number of these compounds is uprising rapidly from time to time due to demand in the market. Since 1968, less than 50 naturally produced halogen compounds came into existence; however, in 2015, it raised to more than 5,000 and it is still increasing [1]. Halogenated organic compounds that are naturally occurring are useful like antibiotics to treat certain diseases and bacterial infections, but the synthetic one used as solvents, biocides, degreasing agents, pharmaceuticals, and many other industrial applications can be more dangerous if introduced to the environment [2], for example in the potassium salt mining industry annually produces 15,000 tons of $CHCl_3$ and 100- 150 tons of each $CFCl_3$ and CCl_4 [3]. These compounds could spread in water, air, and soil sediments [2]. Halogenated organic compounds can cause severe problems to human health and the environment when present in high concentrations, and some of them are resistant to degradation [4].

Some of the organohalides cause human diseases, including oral infections, reproductive toxicity, digestive disorders, organ damage, poisoning, skin, and respiratory irritations [5, 6]. Fortunately, halogenated organic compounds can be degraded by biotic and abiotic methods. Abiotic methods involve alteration, removal or isolation of the pollutants, which in many cases, change pollutants from one form to another. However, these methods are expensive. The most suitable way is using biological methods since it is economical, safer, and environmentally friendly [7].

Many bacteria exist in nature that degrades

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Table 1. Types and uses of halogenated compound [5].

Type of sample	Active compound	Application
Fluorinated	Fluoropolymers	Stain resistance
Brominated	Polybrominated diphenyl ethers	Flame retardants
Chlorinated	DDT, Lindane, Endosulfan	Pesticides
	Vinyl chloride monomer (VCM)	Polymer production
	Trichloroethylene, perchloroethylene	Solvents

most of the toxic organic compounds and use them as their sole source of energy and carbon. They play a vital role in the bioremediation of environmental pollutants and providing a biological method to solve this issue. In bioremediation of halogenated organic compounds, these recalcitrant are converted into environmentally harmless and friendly chemicals, for instance, *Rhizobium* sp. RC1 produces three different dehalogenases, such as DehL, DehD, and DehE, which breakdown various halogenated compounds. This bacterium can utilize 2,2-dichlopropionate and activate the ingredients of the herbicide Dalapon and use it as the sole source of carbon and energy and convert it into pyruvate through the dehalogenation process [1]. The current review aims to focus on how halogenated compounds are treated in the presence of dehalogenases that are vital for the degradation of these type of chemicals.

Halogenated Organic Compounds

In the course of time, the consumption and production of some organohalides have been reduced after knowing the harmful effect of the products on health and the environment [5]. Most organohalides are dangerous to human's health, environment and food chain. For instance, cleaning the membrane bioreactor (MBR) using a chemical sodium hypochlorite (NaClO). It may produce many dissolved organic matters (DOM) compounds, like haloacetic acids. Some of the haloacetic acids are dibromoacetic acid, tribromoacetic acid, dichloroacetic acid, and trichloroacetic acid. These organohalides may flow to the natural water system, and soil through the discharge of MBR permeates that cause serious environmental, human, and animal health problems. In some countries, the public health and environmental concerns of these acids have not been fully aware and paid sufficient attention [8]. Examples of halogenated products and uses are given in Table 1.

Source of Dehalogenases

Several microbes, such as bacteria and fungi, use halogenated organic compounds as their sole source of carbon and energy, thereby reducing the effects of environmental halogen-related pollution. Dehalogenase was first discovered by Jensen when he isolated bacteria and fungi that grew on the halogenated alkanoic acids containing media [9]. Up to now, several microorganisms have been reported that degrade halogenated compounds (Tables 2 and 3). However, many studies were carried out on dehalogenase producing bacteria and its dehalogenases [1, 10-13]. Among fungal species, Parvizpour *et al.* (2012) reported for the first time *Trichoderma* sp. SP2 and *Mucor* sp. SP1 isolated from UTM agricultural land which were exposed to pesticides and herbicides that could degrade 3-chloropropionic acid (3CP) and use it as the sole source of carbon and energy [14]. *T. Aspere-llum* strain SD1 is another fungus isolated from palm oil plantation soil that can also degrade 3CP. This strain can degrade environmental pollutants in both presence and absence of another carbon source, which makes it more critical since most pollutant-degrading microbes cannot degrade contaminants in the resources-rich environment [15]. Many of these microbes have been isolated from other environments such as extreme and polluted environments [10, 16]. Interestingly, these microbial dehalogenases had gained repeatedly increased attention due to their potential use in bioremediation of organo-halide compounds, to treat polluted environments and industrial applications and site-directed synthesis of isomers.

Dehalogenase Enzyme Classification

Several common types of microbial enzymatic dehalogenation had been reported, including reductive dehalogenation, oxidative dehalogenation, dehydrohalogenation, and substitutive dehalogenation (hydrolytic dehalogenation) [25]. The latter was common and more advantageous compared to others. This is because they are cytosolic protein, use water as the only co-substrate, lack of co-factor stereoselectivity, and can also tolerate the addition of water-miscible solvents. Hydrolytic dehalogenase is classified into aliphatic and haloaromatic dehalogenase. The formal includes haloalkane, haloacid, fluoroacetate and halohydrin dehalogenases catalyze the dehalogenation of aliphatic organohalide compounds.

Table 2. List of selected dehalogenase producing bacteria

Organism	Substrate	Reference
Burkholderia pseudomallei MF2	2,2 dichloropropionic acid (2,2-DCP)	[4]
Bacillus subtilis H1 and *Bacillus thuringiensis* H2	2,2 dichloropropionic acid, L-2-chloropropionic acid, D-2-chloropropionic acid, 3-chloropropionic acid, monochloroacetate, trichloroacetate	[10]
L.boronitolerans MH2	Trichloroacetic acid (TCA)	[11]
Bacillus cereus WH2	3-chloropropionic	[12]
Bacillus megaterium BHS1	2,2-dichloropropionic acid (2,2-DCP)	[13]
Rhizobium sp. RC1	L-2-chloropropionic acid Dichloroacetate, dibromoacetate	[17]
Pseudomonas cepacia MBA4	Monobromoacetate, monochloroacetate, dichloroacetate, L-2-chloropropionate, L-2-bromopropionate	[18]
Pseudomonas sp. strain YL	Monochloroacetate, monoiodoacetate, L-2-chloropropionate, 2,2-dichloropropionate	[19]
Pseudomonas sp. strain CBS3	Monochloroacetate, l-2-chloropropionate	[20]
Xanthobacter autotrophicus GJ10	Monochloroacetate, monobromoacetate, 2-chloropropionate, Dichloroacetate, Dibromoacetate, Chloroacetate	[21]
Pseudomonas putida No. l09	Monochloroacetate, monobromoacetate, l-2-chloropropionate, 2,2-dichloropropionate	[22]
Ancylobacter aquaticus strain UV5	Monochloroacetate, l-2-monochloropropionate	[23]
Klebsiella Pneumoniae ITB1	Monochloroacetic acid	[24]

In contrast, 4-chlorobenzyol-CoA and tetrachloro hydroquinone dehalogenases are reported haloaromatic dehalogenases, which concentrate on carbon-halogen linking a halogen and an arsenic or vinylic carbon atom in haloaromatic compounds.

Substrate Specificities and Mechanism of Bacterial Dehalogenase

Haloalkane dehalogenase catalyzes the hydrolytic cleavage of carbon-halogen bond in many organo-halide compounds. It has a potential practical application in bioremediation, biosensing, molecular imaging, and industrial biocatalysts [26, 27]. Haloalkane dehalogenases are α/β-hydrolase superfamily. Structurally, their active site is hidden in the mostly hydrophobic cavity at the α/β-hydrolase core and helical cap domain interface and tied to the bulk solvent by access tunnels [28]. The active site residues are described as a catalytic pentad and consist of a nucleophilic aspartate, a basic histidine, an aspartate or glutamate moiety, which serves as a general acid and either two tryptophan or tryptophan-asparagine pair that serve to stabilize the leaving halide ion [29]. Dehalogenation reaction is completed in 2 steps via the formation of ester intermediates and subsequently hydrolyzed and liberating the products as well as reinstating the carboxylic acid groups of the enzyme (Figure 1). However, a recent study on the sequence and structure of haloalkane dehalogenase and their homologs classified into three phylogenetic subfamilies, HLD-I, HLD-II, and HLD-III, which differ mainly in the composition of the catalytic pentad and cap domain [29]. HLDs catalyze the hydrolysis of chlorinated, brominated, and iodinated alkanes, alkenes, cycloalkanes, alcohols, carboxylates, esters, ethers, epoxides, amides, and nitriles [30] and thus displaying a wide range of substrate-specificities. Therefore, their substrate-specificity can be described in terms of a quantitative profile of their substrate activities with respect to a set of specific substrates. However, the identification of substrate specificity of haloalkane dehalogenase remains a challenge for further research on these enzymes.

Haloacid dehalogenase (HAD) enzymes belong to a large superfamily of phosphohydrolases involved in the conversion of 2-alkanoic acids to produce 2-hydroxyalkanoic acids (Figure 2). Based on the substrate specificities, 2-haloacid de halogenases are placed in three groups [32]. D-Specific dehalogenase, D-2-haloalkanoic acid dehalogenase (D-DEX) catalyzes the dehalogena-

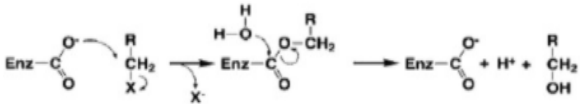

Figure 1. Hydrolytic dehalogenation of haloalkane dehalogenase [31].

tion of D-2-haloalkanoic acids to yield L-2-hydroxyalkanoic acids. D-specific dehalogenase (DehD) belongs to *Rhizobium* sp. RC1 was previously studied further by Huyop and Sudi [33]. The protein model was resolved based on homology modeling studies. L-specific dehalogenase: L-2-haloacid dehalogenases or L-DEX react with L-2-haloalkanoic acids to produce the corresponding D-2-hydroxyalkanoic acid. L-specific dehalogenase (DehL) of *Rhizobium* sp. RC1 was studied extensively, the protein was properly modeled, and important amino acids were detected at its catalytic pocket, together with its stereo-specificity mechanism [1]. Non-stereo-specific dehalogenase: D,L-2-haloacid dehalogenases, also known as D,L-DEX acts on both D- and L-2-haloalkanoic acids, yielding L- and D-2-hydroxyalkanoic acids, respectively [25]. The structure and functions of D, L-2-haloacid dehalogenase belongs to *Rhizobium* sp. RC1 (DehE) was established [34]. Degradation of D- or L-2-haloalkanoic acids or α-Haloacids (αHA) by bacteria, are commonly reported in the literature [35] but not degradation of 3-chloropropionic acid (3-CP) or βHA [36-38].

Apart from above described classification, Hill et al. [39] classified dehalogenases based on genetic approach by investigating the diversity of the dehalogenase genes using phylogenetic classification into groups I and II. The group II is a stereoselective dehalogenase acting only on L-2-haloacids, whereas, group I dehalogenases are specific for D-2-haloacids. Other members of the group I can also act on D and L form substrates producing L and D products respectively or can retain the isomeric form of the product (non-stereospecific) [39].

The dehalogenation process by L-2-haloacid dehalogenases involves two steps. In the first step, an aspartate residue that is conserved among all haloacid dehalogenases or glutamate close to N-terminal is engaged in an S_N2-type nucleophilic substitution with the halogen bearing carbon atom to yield an ester intermediate and free halide ion (Figure 2a). In the second step, the catalytic water molecule is activated by histidine or lysine residue to hydrolysis ester intermediate and release the product [1]. Besides, arginine is another catalytic momentous amino acid that is associated binding with halide to stabilize the substrate and ease the dehalogenation reaction. In the second mechanism, the catalytic residue activates a water molecule that directly attacks the carbon-halogen bond and substitutes the halogen atom (Figure 2b) [1]. The 2-haloacid dehalogenase and haloalkane dehalogenase need water molecules to further breakdown the ester intermediate formed after nucleophile aspartate attacks the alpha carbon (carbon bonds to halogen) of organohalide (Figure 2c) [25].

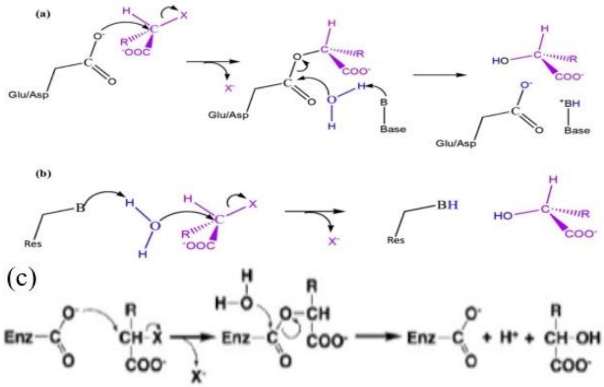

Figure 2. The reaction mechanism of L-2 haloacid dehalogenase. (a) The acidic residue attacks carbon-halogen bond and release halide ion (X^-) and form an ester intermediate, which is then hydrolyzed by an activated water molecule. (b) The single-step mechanism. The active site amino acid of the enzyme activates the water molecule, which hydrolysis the substrate and releases halide ion [1]. (c) reaction mechanism of 2-haloacid dehalogenase [40].

On the other hand, fluoroacetate dehalogenase converts fluoroacetate to non-toxic glycolate by cleaving a strong carbon-fluorine bond. It also degrades many brominated and chlorinated compounds at a slower rate. Defluorination reaction required a halide pocket involved in the stability of hydrogen bonds, the arrangement of fluoride ion, and modification of fluorine hydrogen atom to establish substrate specificity towards fluorinated compounds. This reaction is performed by aspartate nucleophilic of the substrate by fluorine ion injection (Figure 3).

4-chlorobenzyol-CoA and tetrachloro hydroquinone dehalogenases are common examples of haloaromatic dehalogenases. The reaction mechanism involves the displacement of chlorine by nucleophilic attack of aromatic halogens, and the substrate is mainly triggered by reacting with

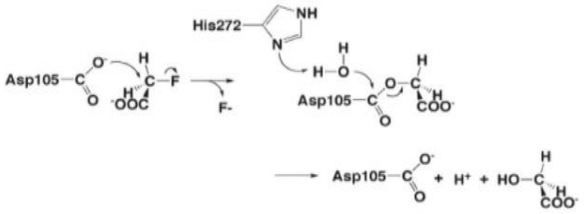

Figure 3. Reaction mechanism of fluoroacetate dehalogenase [40].

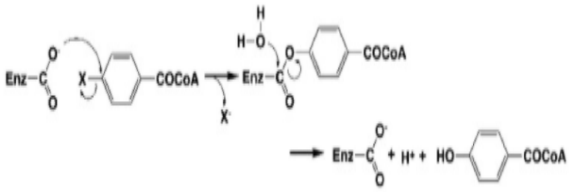

Figure 4. Reaction mechanism of 4-chlorobenzyol-CoA dehalogenase [31].

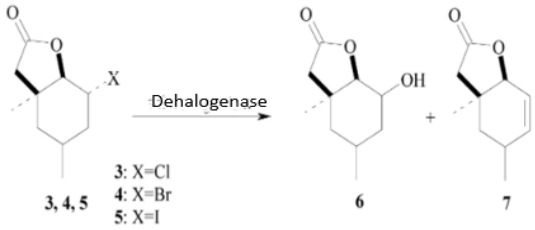

Figure 5. Proposed dehalogenation of halolactones (halolactone 3-5) [51].

CoA, after which hydrolytic dehalogenase belongs to the superfamily of enoyl hydratase cleaves the halogen by nucleophilic addition-elimination reaction mechanism (Figure 4). Although, based on X-ray structural analysis shown no separate halide-binding domain, however a nucleophilic aspartate and histidine were recognized to be involved in catalysis.

Mechanism of Fungal Dehalogenases

More than 3000 halogenated organic compounds are produced abiotically [41, 42] while anthropogenic synthesis had given up to 15,000 compounds. Naturally, the production of organohalides represents the synthesis aspect of a global halogen cycle. The search for microorganisms able to degrade halogenated pollutants had been less focused on fungi. Only few studies focused on the dehalogenation of these pollutants by fungi (Table 3). Fungi such as *Pleurotus ostreatus, Trametes versicolor, Phanerochaete chrysosporium, P. cinnabarinus* [43-45] degrade aro-

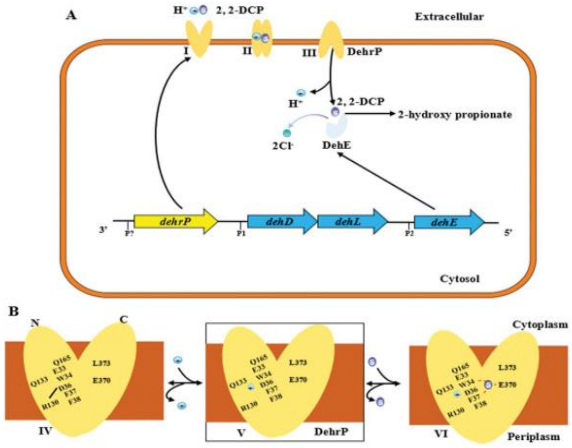

Figure 6. The proposed mechanism of haloacid intake: H^+ Symport. (A) The transport cycle exhibits the proposed steps with the outward facing (I), inward-facing (II), and changes by the occluded intermediate conformation (III). Proton (H^+; +) and substrate (S; 2,2-DCP) are engaged in the process. (B) Salt bridge is formed between the side chains of Asp^{36} ($-COO^-$) and Arg^{130} ($-NH3^+$) in the absence of H^+ (IV). Protonated D^{36} becomes closer to the Asp^{36} binding cavity (V). The binding amino acids coordinate substrate and H^+ in the cavity (VI). The *dehrP* gene is located at the upstream region of dehalogenase *dehD*, *dehL* and *dehE* genes within the genomic DNA [55].

tic halogenated compounds. Many dehalogenase dehalogenate aromatic pollutants like *Pchrysosporium* and *Candida maltosa* are reported for dehalogenation of pentachlorophenol, methoxychlor, and monochlorophenols [46, 47]. Similarly, several fungi strains had been used for biodegradation of aromatic containing organohalide compounds [48, 49]. For example, many strains degrade halogenated compounds such as terpenoid halolactones [50-52] by introducing a hydroxyl group in the place of the halogens atom or eliminating the halogen via the formation of the double bond (Figure 5).

Microbial physiology: The mechanism of uptake system

The degradation of haloacid pollutants inbacteria depend on haloacid uptake into the cytoplasm of the bacteria. Every haloacid uptake system has specific substrate specificities and induction patterns [54]. It has been reported that transportation occurs by dehalogenase-associated inducible haloacid transport protein. The 3D structure and uptake mechanism of permease was

Table 3. Selection of fungal species degrading halogenated compounds

Organism	Compounds	Reference
Mucor sp. SP1 *Trichoderma* sp. SP2	3-chloropropionic acid	[14]
Trichoderma asperellum	3-chloropropionic acid	[15]
Pycnoporus cinnabarinus	2-hydroxy-5-chlorobiphenyl, 3-chloro-4-hydroxybiphenyl	[43]
Phanerochaete chrysosporium	4,4- dichlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, 2,2',4,4',5,5'-hexachlorobiphenyl	[44]
Pleurotus ostreatus *Trametes versicolor*	Monochlorobiphenyl, Dichlorobiphenyl, Tricholorobiphenyl, Tetrachlorobiphenyl, Pentachlorobiphenyl	[45]
Phanerochaete chrysosporium	1,1-dichloro-2,2-bis(4-methoxyphenyl) ethane 2-hydroxy derivative 2,2,2-trichloro-1,1-bis (4 methoxyphenyl) ethanol 2,2-dichloro-1,1-bis (4 methoxyphenyl) ethanol	[46]
Candida maltosa	2-Chlorophenol, 3-Chlorophenol, 4-Chlorophenol	[47]
Irpex lacteus, *Pycnoporus cinnabarinus* *Dichomitus squalens*	chlorobenzoic acids	[48]
Phanerochaete chrysosporium	chlorobenzene	[49]
Botrytis cinerea	iodolactones	[50]
	(R)- (+)-1-(4′-chlorophenyl) propan-1-ol	[52]
Fusarium sp. HJ01	4-chlorophenol (4-CP)	[53]

unknown until Musa *et al.* (2018) studied haloacid permease (DehrP) of *Rhizobium* sp. RC1. DehrP is a Major Facilitator Superfamily protein that belongs to the Metabolite: H^+ Symporter protein. In the proposed mechanism of DehrP, H^+-binding sites and sugar$^-$ are located at each side of the membrane (Figure 6(A) I-III). The chains that are significant for H^+- and haloacid-binding in DehrP usually are located in the N-terminal helix bundle. When H^+ is not present, a salt bridge is formed by Asp^{36}, and Arg^{130} results in the widening of the binding cavity following the changes in the location of transmembrane helices (TMs) (Figure 6(B) IV). When H^+ is available, Asp^{36} is protonated, and the salt bridge is broken, which causes TMs to undergo rearrangement resulting in reduces the size of the binding cavity (Figure 6(B) V). Most importantly, the smaller size of the binding cavity results in a concomitant lowering of the energetic barrier of the transporter's conformations. This leads to allowing the expeditious haloacid translocation (Figure 6(B) VI). When the haloacid and residues between the two domains interact, the N-and C domains become closer (Figure 6(B) VI) [55, 56].

Conclusion

Halogenated organic compounds can be naturally occurring or man-made. The latter can cause pollution and toxic to the environment. Fortunately, some fungi and many bacterial species produce dehalogenases that allow them to reduce these toxic compounds into non-hazardous materials. The search for microorganisms able to degrade halogenated pollutants were focused on both bacteria and fungi. The degradation of haloacids depends on the transportation or uptake of these components into the cytoplasm. Various dehalogenation process occurs via various mechanisms. Finally, the discovery of new isolation and characterizing new dehalogenating microbial species can be seen as an important way of microbial treatment of polluted area.

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