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Analytics of Dehalogenases: Structural, Functional, and In *Silico* Perspectives

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AB S T RAC T

Halogenated organic compounds are widely recognized as persistent environmental pollutants that arise from industrial, agricultural, pharmaceutical, and medical sources. Their chemical stability and toxicity make them resistant to natural degradation, creating serious risks not only to ecosystems but also to human health. Dehalogenases a diverse group of enzymes, catalyse the cleavage of carbon halogen bonds and play a crucial role in the detoxification and breakdown of these compounds. Beyond their ecological relevance, dehalogenases are gaining importance in biomedical and healthcare contexts, such as in drug metabolism, detoxification of halogenated pharmaceuticals and the design of novel biocatalysts for therapeutic applications. This review highlights the major classes of hydrolytic, reductive, and oxidative dehalogenases and provides mechanistic insights into their catalytic strategies. Emphasis is placed on traditional characterization methods, such as enzyme assays, spectroscopy, chromatography, and crystallography, along with the increasing use of *in silico* tools, including sequence analysis, structural modeling, molecular docking, and molecular dynamics simulations, to predict enzyme behavior and guide protein engineering. The ecological importance of dehalogenases in biodegradation, organohalide respiration, and pollutant cycling is also discussed, alongside their emerging roles in precision medicine, enzyme-based diagnostics, and synthetic biology. In addition, the role of genomics and metagenomics has been explored as a means to uncover novel dehalogenase genes, monitor their roles in bioremediation, and identify medically relevant variants.

In this review, we have explored how combinations of experimental methods with computational approaches have deepened our understanding of dehalogenases. By bridging biochemical evidence with modern bioinformatics and healthcare applications, this review highlights the promise of dehalogenases not only as effective agents for environmental detoxification but also as valuable tools in biotechnology, drug discovery, and therapeutic interventions.

INTRODUCTION

Halogenated organic pollutants are one of the most pressing environmental challenges of the 21st century. These compounds are widely employed in agriculture, pharmaceuticals, and industrial processes, where they are used in pesticides, solvents, flame retardants, refrigerants, and plastics [1,2]**.** Their popularity stems from their high chemical stability, resistance to degradation, and broad-spectrum

biological activity. Unfortunately, the features that make these compounds useful also contribute to their persistence in the environment. Once released, halogenated organics often resist natural degradation processes and accumulate in soil, sediments, groundwater, and living organisms [3]. Numerous studies have linked chronic exposure to such pollutants to severe health problems, including endocrine disruption, neurotoxicity, immune suppression, reproductive issues, and carcinogenicity [4]. Thus, development of strategies for their effective and sustainable removal remains a global priority.

Traditional remediation strategies, such as high-temperature incineration, chemical oxidation, and solvent extraction, have been employed to mitigate halogenated pollution. However, these methods are not only expensive and energy-intensive, but they may also produce hazardous by-products, leading to secondary pollution concerns [5]. In contrast, bioremediation, which involves the use of microorganisms or their enzymes to degrade pollutants into harmless products, offers a promising, eco-friendly, and cost-effective alternative [1,6]. Dehalogenases, a specialized group of enzymes that catalyze the cleavage of carbon–halogen bonds, are central to this process and often represent the rate-limiting and essential first step in the detoxification pathway [1,5].

Dehalogenases have been extensively studied in terrestrial bacteria, such as *Pseudomonas*, *Xanthobacter*, and *Dehalococcoides*, where they play critical roles in breaking down chlorinated solvents, haloalkanes, and haloacids[5]. These studies have revealed the diversity of hydrolytic, reductive, and oxidative dehalogenases and provided important insights into their structure–function relationships, substrate specificity, and potential biotechnological applications [5]. However, relatively little attention has been directed towards dehalogenases in cyanobacteria, especially filamentous species such as *Nostoc* sp. PCC 7120 [6,7,].

Cyanobacteria are ancient photosynthetic microorganisms that contribute significantly to global primary production and nitrogen cycling. Their remarkable ability to thrive in diverse and often extreme habitats, ranging from deserts to freshwater lakes, suggests a highly adaptable metabolic repertoire [6]. Emerging genomic and transcriptomic data indicate that cyanobacteria encode dehalogenases with potential ecological and biotechnological importance, although these enzymes remain largely unexplored compared to their bacterial counterparts [6,7,8]. In particular, Nostoc 7120, a model organism for cyanobacterial genetics and physiology, has recently been highlighted as a potential source of novel dehalogenases that could contribute to the biodegradation of halogenated pollutants [7,8].

The growing field of *in silico* enzyme analysis offers powerful tools for accelerating the discovery and characterization of such enzymes. Advances in bioinformatics, molecular docking, and protein–ligand interaction studies have enabled researchers to predict enzyme structure, active site architecture, and substrate-binding specificity without relying solely on labor-intensive laboratory experiments. Programs such as AutoDockVina enable the virtual screening of halogenated pollutants against putative dehalogenases, providing insights into binding affinities and catalytic residues [9]. Likewise, visualization tools such as LigPlot+ map hydrogen bonding and hydrophobic interactions, clarifying the molecular basis of substrate recognition [10]. These computational approaches are especially valuable in underexplored microbial systems, such as cyanobacteria, where experimental data are limited [6,7]. In recent years, molecular docking studies on *Nostoc* 7120 proteins have provided early evidence of dehalogenase–substrate interactions, revealing candidate enzymes capable of binding and potentially transforming halogenated pollutants [7,10]. These findings highlight the untapped potential of cyanobacteria in environmental biotechnology and demonstrate how computational strategies are reshaping our understanding of microbial enzymology. This review aims to provide a comprehensive overview of dehalogenases, with a particular focus on *Nostoc* 7120 dehalogenases. This review summarizes the current knowledge on enzyme diversity, sources, and mechanisms, while emphasizing the role of *in silico* approaches in identifying and characterizing novel cyanobacterial dehalogenases. By integrating bioinformatics with environmental microbiology, this study underscores the potential of cyanobacterial enzymes in developing sustainable solutions for halogenated pollutant detoxification.

DEHALOGENASE AND THEIR MECHANISM

Dehalogenases are a diverse group of enzymes that catalyze the cleavage of carbon–halogen bonds, thereby detoxifying halogenated organic compounds that often accumulate as environmental pollutants. These enzymes play a pivotal role in the global halogen cycle, enabling microorganisms to survive in habitats contaminated with chlorinated, brominated and fluorinated compounds. Based on their catalytic mechanisms, dehalogenases are classified into three major categories: hydrolytic, reductive, and oxidative dehalogenases [9]. Recent advances have highlighted their mechanistic diversity, evolutionary plasticity, and potential for biotechnological engineering [1,11].

2.1 Hydrolytic Dehalogenases

Hydrolytic dehalogenases are predominantly active under aerobic conditions and utilize water as a nucleophile to displace halogen atoms from substrates, producing less toxic hydroxylated compounds than the original substrate].Two important classes within this group are Haloalkane Dehalogenases (HLDs) and Haloacetate Dehalogenases (HADs) [9].

2.1.1 Haloalkane Dehalogenases (HLDs)

HLDs act on halogenated alkanes and belong to the α/β-hydrolase fold superfamily of enzymes [1]. They possess a well-conserved catalytic triad (typically Asp–His–Glu/Asp) that drives a two-step SN2-like reaction. In the first step, a nucleophilic attack by an aspartate residue forms a covalent ester intermediate, and in the second step, a water molecule hydrolyzes the intermediate, releasing the halide ion and generating the hydroxylated product [1]. Owing to their broad substrate specificity, HLDs have been widely investigated for the bioremediation of chlorinated solvents, pesticides, and industrial by-products [9]. Recent studies have shown that HLDs display structural plasticity and polyphyletic evolutionary origins, suggesting multiple independent adaptations to these halogenated pollutants [11]. Novel structural findings have revealed unusual dimerization and enantioselectivity in the HLD DmmarA from *Mycobacterium marinum*, providing new insights into its catalytic diversity [12].

In addition, AI-driven protein engineering has been successfully used to design hyperstable and more efficient HLD variants, such as DhaA223 [13] and MaxEnt-engineered mutants [13], demonstrating the future of computational approaches in enzyme design.

2.1.2 Haloacetate Dehalogenases (HADs)

In contrast, HADs are specialized for short-chain haloacetic acids, such as monochloroacetate and monobromoacetate. Their catalytic mechanism is simpler than that of HLDs, involving the direct displacement of the halogen atom by a hydroxyl group in a single-step reaction, yielding harmless products such as glycolate HADs are ecologically important in soils contaminated with herbicides, such as 2-chloroacetate, where they confer a selective advantage to microorganisms capable of metabolizing these xenobiotics [9].

2.2 Reductive Dehalogenases

Reductive dehalogenases are typically found in anaerobic bacteria, where they catalyze the replacement of halogen atoms with hydrogen atoms.These enzymes are crucial in anaerobic niches, such as sediments and groundwater aquifers, where halogenated compounds serve as terminal electron acceptors in respiration. The catalytic process involves electron transfer from cofactors, such as corrinoids (vitamin B_{12} derivatives) and iron–sulfur clusters, enabling the reductive cleavage of carbon–halogen bonds [18]. A classic example is *Dehalococcoidesmccartyi*, which uses reductive dehalogenases to dechlorinate highly toxic compounds, such as tetrachloroethene (PCE) and trichloroethene (TCE), converting them into ethene, a non-toxic hydrocarbon .This process, termed organohalide respiration, has immense implications for the *in situ* bioremediation of chlorinated solvents in contaminated groundwater [18]. Recent advances include the discovery of an oxygen-tolerant, self-sufficient reductive dehalogenase from *Jhaorihellathermophila*, which is capable of functioning under both aerobic and anaerobic conditions, thereby expanding the known ecological range of such enzymes [14]. Mechanistic studies using spectroscopy and computational methods have revealed that [4Fe–4S] clusters mediate proton-coupled electron transfer, providing deeper insights

into the catalytic pathways [15]. Moreover, new *Dehalococcoides* strains have been shown to completely debrominatepolybrominated diphenyl ethers (PBDEs), which are persistent flame retardants that are otherwise difficult to degrade [16].

2.3 Oxidative Dehalogenases

Oxidative dehalogenases function mainly in aerobic environments, where they catalyze halogen removal through oxidative reactions. These enzymes are often flavin- or heme-dependent and couple halogen removal with oxygen insertion. For example, fluoroacetate dehalogenase oxidativelydefluorinates fluoroacetate, one of the most toxic natural halogenated compounds, and converts it into glycolate and fluoride ions. Similarly, heme-dependent peroxidases and monooxygenases can catalyze the oxidative dehalogenation of aromatic compounds, breaking down recalcitrant pollutants such as chlorophenols and brominated aromatics. Although less studied than their hydrolytic and reductive counterparts, oxidative dehalogenases are recognized for their role in the microbial degradation of complex, highly substituted halogenated pollutants. Recent computational enzyme-mining studies suggest that oxidative dehalogenases remain an underexplored reservoir of biocatalysts with potential roles in defluorination and the degradation of aromatic pollutants [17].

Table 1: Key Features of Dehalogenase

Type	Typical Substrates	Catalytic Features	References
Oxidative	Fluoroacetate, chlorophenols, brominated aromatics	Flavin (e.g., FAD), heme groups	Harris *et al.* 2023);Sharma *et al.* (2020);
Reductive	Chlorinated ethenes (chlorinated benzenes	Corrinoid (B_{12} derivatives), Fe–S clusters	Kruse *et al.* (2022); Jugder *et al.* (2016
Hydrolytic	Haloalkanes alcohols; haloacetates; aromatic substrates	Catalytic triad/pentad (Asp–His–Asp/Glu + stabilizing residues); CoA and ATP in aromatic cases	Janssen (2019); Rohwerder & Müller (2018);

SOURSES OF DEHALOGENASE

Dehalogenases are widely distributed enzymes that play crucial roles in the degradation and recycling of halogenated compounds in diverse ecological niches. These enzymes have evolved independently in multiple domains of life, reflecting the widespread presence of halogenated compounds in natural and anthropogenic environments. Dehalogenases, found in bacteria, fungi, archaea, cyanobacteria, and marine microorganisms, exhibit remarkable biochemical diversity, substrate specificity, and adaptive features, underscoring their ecological significance and biotechnological potential.

3.1 Bacteria: The Primary Reservoir

Bacteria are the most extensively characterized source of dehalogenases and serve as natural biocatalysts for the detoxification of xenobiotic pollutants. Hydrolytic dehalogenases are widely distributed in soil and aquatic bacteria, such as *Pseudomonas*, *Xanthobacter*, and *Rhodococcus*, where they catalyze the cleavage of carbon–halogen bonds in haloalkanes and haloacids through nucleophilic substitution mechanisms [1]. These enzymes often participate in central carbon metabolism, enabling bacteria to use halogenated substrates as the sole carbon and energy sources.

Reductive dehalogenases (RDases), primarily encoded by obligate organohalide-respiring bacteria such as *Dehalococcoides mccartyi*, *Desulfitobacterium hafniense*, and *Sulfurospirillum multivorans*, play a pivotal role in anaerobic bioremediation. RDases enable bacteria to couple the reductive dechlorination of persistent halogenated solvents such as trichloroethene (TCE), tetrachloroethene (PCE), and polychlorinated biphenyls (PCBs) to energy conservation and growth [2,4]. These enzymes are often corrinoid-dependent and membrane-associated, reflecting their specialized roles in respiratory metabolism.

Oxidative dehalogenases, although less widespread, have been reported in aerobic bacteria capable of degrading chlorinated aromatics, such as chlorophenols and halobenzoates. For example, oxygen-dependent halogenated aromatic

dioxygenases in *Sphingomonas* contribute to the breakdown of industrially derived halophenols [17]. Collectively, the bacterial domain remains the richest reservoir of biochemically diverse dehalogenases, underpinning most bioremediation applications in soil and groundwater.

3.2 Fungi: Aerobic Degraders of Aromatic Compounds

Fungi are another important reservoir of dehalogenases, particularly oxidative enzymes. White-rot fungi, such as *Phanerochaete chrysosporium* and *Trametes versicolor*, are well known for their ligninolytic enzyme systems, which include extracellular oxidative dehalogenases, laccases, and peroxidases that facilitate the degradation of chlorophenols, halogenated biphenyls, and other recalcitrant aromatics .Unlike bacteria, fungi often act extracellularly, producing enzymes capable of degrading polymeric and insoluble halogenated pollutants in soil and decaying wood.

Recent metatranscriptomic analyses have shown that fungal dehalogenases are actively expressed in forest and agricultural soils contaminated with halogenated pesticides, indicating their ecological role in detoxification processes [19]. Moreover, fungi can undergo cometabolic transformation, breaking down halogenated compounds not as primary substrates but through secondary enzymatic pathways. This highlights their importance as ecological “cleaners” of complex organic pollutants in natural environments.

3.3 Archaea: Extremophilic Sources of Novel Enzymes

Archaea, though less explored than bacteria and fungi, are emerging as promising reservoirs of dehalogenases with unique biochemical properties. Genomic surveys of halophilic and thermophilic archaea have revealed the presence of hydrolytic and oxidative dehalogenase homologs adapted to extreme conditions, such as hypersaline lakes, hydrothermal vents, and acidic hot springs [22]. These archaeal enzymes often display exceptional thermostability, halotolerance, and pH resilience, making them attractive candidates for industrial bioprocesses that operate under harsh conditions.

For example, halophilic archaea isolated from hypersaline environments have been shown to degrade halogenated alkanes in brine-rich wastewater streams, offering potential solutions for industrial effluents where conventional bacterial enzymes are unstable [23]. With the rise of archaeal metagenomics and single-cell sequencing technologies, many novel dehalogenase families are expected to be uncovered, thereby expanding the catalog of extremozymes for biotechnological applications [22,23].

3.4 Marine Microorganisms: Guardians of the Halogen Cycle

Marine ecosystems are unique environments where halogenated compounds are abundant due to biosynthesis by algae, actinomycetes, sponges, and other marine organisms. Marine bacteria such as *Marinobacter*, *Pseudoalteromonas*, and *Alteromonas* encode hydrolytic and oxidative dehalogenases that metabolize brominated and chlorinated compounds, playing an essential role in global halogen cycling .

Marine-derived dehalogenases often exhibit cold activity and salt tolerance, reflecting their adaptation to oceanic environments. Such traits are advantageous for industrial processes, where low-temperature catalysis reduces energy costs and prevents unwanted side reactions [24]. A 2023 metagenomic study uncovered several novel halogen-metabolizing enzyme families from marine sediments, highlighting the ecological and applied significance of marine microorganisms as biocatalyst reservoirs [25].

3.5 Cyanobacteria: Emerging but Underexplored Sources

Cyanobacteria are photosynthetic microorganisms that are increasingly recognized as potential dehalogenase producers. Genomic and transcriptomic analyses of *Nostoc sp.* PCC 7120 and *Synechococcuselongatus* have identified putative hydrolytic and oxidative dehalogenases, supported by in silico structural predictions and docking studies [6,7,8]. These enzymes may allow

cyanobacteria to detoxify halogenated metabolites in aquatic environments, providing them with an ecological advantage in polluted waters.

Although experimental validation of cyanobacterial dehalogenases remains limited, computational and synthetic biology approaches have begun to shed light on their structural and catalytic diversity [8,26]. Given their ability to fix carbon and nitrogen, cyanobacteria represent sustainable microbial platforms for engineering dehalogenase-based bioremediation systems in aquatic ecosystems.

SIGNIFICANCE OF DEHALOGENASE

Dehalogenases are enzymes that catalyze the cleavage of carbon–halogen bonds, effectively removing halogen atoms, such as chlorine, bromine, or fluorine, from organic molecules. These enzymes are found in a wide variety of microorganisms and play a pivotal role in detoxifying halogenated compounds, which are often xenobiotic, persistent, and toxic in nature. Dehalogenase enzymes are significant in several domains, including environmental detoxification, industrial biocatalysis, green chemistry, and synthetic biology, making them indispensable in modern biotechnology [24].

4.1. Environmental Detoxification and Bioremediation

One of the most impactful applications of dehalogenases is their ability to detoxify halogenated environmental pollutants. Industrialization and agricultural expansion have led to the widespread release of halogenated compounds, such as polychlorinated biphenyls (PCBs), chlorinated solvents (e.g., trichloroethylene), brominated flame retardants, and organochlorine pesticides (e.g., DDT), into the environment. These compounds are highly recalcitrant, meaning they resist natural degradation, accumulate in ecosystems, and pose serious risks to human and ecological health[24].

Dehalogenase-producing microorganisms can break down these toxic substances by cleaving the carbon–halogen bond, thereby converting them into less harmful or readily degradable compounds. For instance, haloalkane dehalogenases catalyze the hydrolytic cleavage of halogenated alkanes, whereas reductive dehalogenases catalyze the anaerobic reductive dechlorination of chlorinated ethenes, ultimately converting them into ethane, a non-toxic product. This process, known as halorespiration, is crucial in anaerobic environments, such as groundwater aquifers, where aerobic degradation is limited [24].

Numerous field applications have successfully used dehalogenase-expressing microbial consortia for in situ remediation of contaminated soils and aquifers. Bacteria such as *Dehalococcoides mccartyi*, *Desulfitobacterium*, and *Sulfurospirillum* spp. have been extensively studied and deployed in bioremediation settings. They derive energy from the reductive dehalogenation of chlorinated compounds and play an essential role in detoxifying environments polluted with compounds such as perchloroethylene (PCE) and trichloroethylene (TCE) [25].

Moreover, recent studies have demonstrated that immobilizing dehalogenases on nanomaterials or engineering more stable variants significantly improves their activity, longevity, and reusability under harsh environmental conditions [26]. Such enhancements make them even more practical for large-scale environmental clean-up strategies.

4.2. Enzymatic Specificity and Stereoselectivity

Beyond environmental applications, dehalogenases are of great value in the synthesis of fine chemicals and pharmaceutical products. A unique property of many dehalogenases is their high degree of regioselectivity and stereoselectivity, which means that they can distinguish between different chemical environments within a molecule and preferentially act on specific isomers or enantiomers. This specificity is particularly advantageous in pharmaceutical manufacturing, where the biological activity of a drug can differ dramatically between enantiomers[27].

Haloacid dehalogenases can resolve racemic mixtures of halo-carboxylic acids into enantiomerically pure products at ambient temperature. This makes them ideal for the

production of optically active compounds, which are critical intermediates in the synthesis of drugs. A well-known example is the resolution of 2-chloropropionic acid, a common chiral building block, by D- and L-specific 2-haloacid dehalogenases. These enzymes selectively convert one enantiomer into its corresponding hydroxy acid while leaving the other enantiomer untouched, thereby enabling the separation of the desired enantiomer with high optical purity [27].

Furthermore, halohydrin dehalogenases (HHDHs) are used for asymmetric synthesis, catalyzing not only dehalogenation but also epoxide ring-opening and C–C and C–N bond formation. Their broad catalytic versatility and capacity for engineering make them attractive platforms for green-synthesis routes [28].

4.3. Biological and Metabolic Functions

Dehalogenases also play important roles in microbial metabolism, allowing certain organisms to survive in toxic halogen-rich environments. In these cases, dehalogenases are not only detoxifying agents but also metabolic enzymes that contribute to the energy conservation and carbon assimilation of the host organism[27]

For instance, bacteria such as *Dehalococcoides ethenogenes* rely almost exclusively on reductive dehalogenation for energy generation under anaerobic conditions. These organisms couple the dehalogenation of chlorinated ethenes with proton motive force generation, essentially using halogenated compounds as terminal electron acceptors, a process unique to organohalide-respiring bacteria (OHRB) [29].

In addition to their role in energy metabolism, some dehalogenases are involved in the degradation of natural halogenated compounds produced by marine organisms, algae, and halogenase-expressing actinomycetes. Thus, dehalogenases contribute to the natural halogen cycle and help maintain the ecological balance in halogen-rich environments.

4.4. Genetic Engineering and Synthetic Biology Applications

With the rapid development of synthetic biology, dehalogenase enzymes have been engineered to improve their performance and novel functionalities. Rational design, directed evolution, and computational modeling techniques have been employed to enhance enzyme stability, broaden substrate specificity, and increase the catalytic efficiency.

For instance, engineered variants of haloalkane dehalogenase (DhaA) have been developed with significantly increased thermostability, enabling their application in industrial settings where harsh conditions prevail. Immobilization techniques, such as cross-linked enzyme aggregates (CLEAs) or nano-biomineralization, further enhance the operational stability and reusability of these enzymes in reactors or bioremediation filters [18].

Synthetic biology has also enabled the construction of biosensors using dehalogenase genes coupled with reporter elements, allowing the detection of environmental pollutants, such as halogenated solvents. For example, a dehalogenase-based whole-cell biosensor was engineered to detect 1,2-dichloroethane in groundwater, offering a low-cost, on-site diagnostic tool for environmental monitoring [30].

Furthermore, dehalogenases have been incorporated into metabolic pathways in synthetic microbial consortia to process halogenated industrial waste into usable intermediates. The integration of dehalogenases into broader microbial production platforms opens new avenues for bio-based recycling and waste valorization [30]

ROLES OF DEHALOGENASE ENZYMES IN THE ENVIRONMENT

Dehalogenase enzymes are central to the breakdown and detoxification of halogenated organic compounds in diverse environmental settings. These hydrolytic, reductive, and oxidative enzymes play key roles in microbial metabolism, pollutant degradation, biogeochemical cycling, and environmental biotechnology. Their ecological importance continues to grow with new discoveries and technological advances.

5.1 Biodegradation of Halogenated Pollutants

Halogenated organic pollutants, such as chlorinated solvents, polychlorinated biphenyls (PCBs), and fluorinated compounds, are persistent and toxic, often accumulating in soil and groundwater. Dehalogenases, especially reductive dehalogenases (RDases), drive the anaerobic breakdown of these pollutants, converting them into less harmful substances. Organohalide-respiring bacteria (OHRB), such as *Dehalococcoides, Dehalogenimonas,* and *Dehalobacter,* use halogenated compounds as electron acceptors during anaerobic respiration, enabling complete dechlorination to ethene or other benign products [31]. These RDases are membrane-associated enzymes equipped with iron–sulfur clusters and cobalamin cofactors, and are essential for transforming environmental pollutants through energy-conserving respiratory pathways [32]. For example, *Dehalococcoides* uniquely transforms PCE, TCE, PCBs, and chlorinated dioxins into non-toxic end products, making them invaluable in bioremediation efforts [33].

5.2 Facilitating Organohalide Respiration (Halorespiration)

Halorespiration, the use of halogenated compounds as terminal electron acceptors, is a vital metabolic process in certain anaerobic bacteria for energy production, enabling them to thrive in oxygen-depleted environments, such as aquifers and sediments. This respiratory mechanism supports the natural attenuation of pollutants and biogeochemical halogen cycling, especially in settings where anaerobic conditions prevail [34]. In engineered bioremediation strategies, enhanced reductive dechlorination (ERD) involves injection of OHRB and electron donors to accelerate the breakdown of contaminants such as PCE and TCE [35].

5.3 Expanding Roles in Diverse and Extreme Environments

Emerging studies have revealed the environmental roles of dehalogenase-producing microorganisms in unique habitats. Hypersaline environments, such as salt lakes, are important yet underexplored sinks for halogenated pollutants. Halophilic and halotolerant bacteria possessing dehalogenase enzymes contribute to biodegradation under high-salinity stress and have significant biotechnological implications [36]. For instance, *Pseudomonas halophila* from Turkey's TuzGölü Lake produces a halotolerant dehalogenase (DehHX) capable of functioning under highly saline conditions, highlighting adaptation-based enzyme resilience [37]. Similarly, other isolates from TuzGölü have demonstrated the biodegradation of haloacids, haloacetates, and pesticides such as chlorpyrifos, reinforcing the capacity of saline microbial communities to remediate contaminants [38].

5.4 Structural Insights and Enzyme Engineering

Understanding the molecular structure of dehalogenases enables the rational design of enzymes with enhanced activity and substrate range. Structural studies of RDases have provided insights into substrate binding, enabling the repurposing of enzyme specificity for broader pollutant degradation [39].

5.5Enhancing Enzyme Stability for Environmental Applications

One of the constraints in deploying dehalogenase enzymes in field applications is their sensitivity to environmental factors. Advances in stabilization and immobilization offer potential solutions. For example, immobilizing haloalkane dehalogenase (DhaA) into an iron–phosphate nano-hybrid resulted in boosted activity (over 138%) and improved tolerance to pH extremes, with reusability over multiple reaction cycles making it an efficient biocatalyst for pollutant decontamination [40].

5.6 Met genomics, Genomics and Bioremediation Monitoring

Genomic approaches are increasingly critical for revealing the prevalence and functionality of dehalogenase enzymes in the environment. Whole-genome sequencing (WGS), metagenomics, and single-cell genomics enable the identification of dehalogenase genes and their regulatory networks, offering a culture-independent window into microbial dehalogenation potential[41]. Furthermore, molecular markers for RDases and

related genes serve as biomarkers for assessing the intrinsic or enhanced bioremediation potential at contaminated sites [42].

PHYSIOCHEMICAL ANALYSIS OF DEHALOGENASE

Before the widespread application of computational tools, physical and chemical analysis methods were the primary approaches used to study dehalogenases. These techniques remain indispensable as they provide direct and experimentally validated evidence of enzyme activity, structure, stability, and suitability for applied purposes.

6.1Enzyme Activity Assay

The fundamental measure of dehalogenase function is its ability to catalyze the cleavage of carbon–halogen bonds in the substrate. Enzyme activity is often quantified by monitoring the release of halide ions (Cl−, Br−, and F−) using ion-selective electrodes, colorimetric assays (e.g., mercuric thiocyanate method), or ion chromatography [1,2]. From these assays, kinetic parameters such as Km (Michaelis constant), Vmax (maximum velocity), and kcat (turnover number) can be determined, which are essential for comparing enzyme efficiencies across species and engineered variants [3,38].

6.2 Spectroscopic Techniques

Spectroscopy provides critical insights into the structure and reaction mechanisms of enzymes. UV spectroscopy can monitor absorbance changes during substrate turnover or cofactor involvement (e.g., flavins and hemes). Fluorescence spectroscopy detects conformational changes or ligand-binding events, whereas Circular Dichroism{CD}spectroscopy provides secondary structural information (α-helices and β-sheets), helping to evaluate enzyme folding and thermal stability [39].

6.3 Chromatographic and Mass Spectrometric Techniques

Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC) are widely used to separate and quantify reaction products and confirm substrate conversion [40]. When coupled with Mass Spectrometry (MS), these methods allow for the precise identification of dehalogenation products and intermediates, distinguishing between complete detoxification and partial transformation [41]. Such analyses are especially relevant in environmental studies, where incomplete degradation can generate toxic byproducts.

6.4 X-ray Crystallography and NMR Spectroscopy

X-ray crystallography remains the gold standard for high-resolution structural determination of dehalogenases, revealing their catalytic residues, substrate-binding pockets, and overall folding [42]. However, crystallization challenges mean that complementary techniques such as Nuclear Magnetic Resonance (NMR) spectroscopy are valuable for studying enzyme structure and dynamics in solution [43]. These methods have significantly advanced our understanding of dehalogenase catalytic mechanism.

6.5 Physicochemical Property Assessment

The stability of dehalogenases under different environmental conditions is a key factor in their potential application. Thermal stability (Tm) can be determined using Differential Scanning Calorimetry (DSC) or thermal shift assays, whereas pH profiles are obtained by assaying enzyme activity across a range of pH values [44]. These analyses help evaluate whether enzymes are suitable for harsh industrial or environmental conditions, such as the bioremediation of halogenated pollutants in extreme environments.

IN SIILICO ANALYSIS OF DEHALOGENASE

Computational biology provides a wide spectrum of tools that can be broadly grouped into sequence analysis tools, structure prediction and modelling tools, molecular docking and interaction studies, molecular dynamics (MD) simulations, and visualization platforms. Each tool class plays a complementary role in studying the catalytic

potential, substrate specificity, and stability of dehalogenases.

7.1 Sequence Analysis Tools

Sequence-based analyses form the foundation for enzyme characterization. One of the most widely used tools is the Basic Local Alignment Search Tool (BLAST), which helps identify homologous sequences in large genomic databases Using BLAST, researchers have uncovered dehalogenase-like genes in a wide range of microorganisms. Interestingly, searches in cyanobacteria, such as *Nostoc* sp. PCC 7120, have revealed several putative dehalogenases, expanding our view of where these enzymes might occur in nature [44].

To go beyond simple identification, scientists often rely on multiple sequence alignment tools, such as Clustal Omega [45]. These programs highlight conserved motifs that define catalytic activity, such as the well-known Asp–His–Glu catalytic triad in haloalkane dehalogenases or the nucleophilic Asp residue characteristic of haloacid dehalogenases (HADs). By comparing sequences from different organisms, alignments help map evolutionary relationships and distinguish between hydrolytic, reductive, and oxidative enzyme families.

Functional annotation tools provide deeper insights into enzyme architecture: Pfam classifies dehalogenases into protein families, such as the α/β-hydrolase fold and HAD superfamily, providing clues to their structural fold and likely function[46] . InterPro combines data from multiple classification systems, making it easier to pinpoint structural domains, such as the “nucleophilic elbow” and cap regions, which often determine substrate binding [47] . The Conserved Domain Database (CDD) goes a step further by linking sequences to known structural templates [48]. For instance, CDD analysis of cyanobacterial genomes has shown that some *Nostoc* dehalogenase-like proteins share significant similarities with well-characterized bacterial enzymes, suggesting that they may perform similar functions despite being found in very different organisms.

Taken together, these tools form a powerful workflow. BLAST helps locate candidate genes, Clustal Omega reveals conserved motifs and evolutionary trends, and Pfam, InterPro, and CDD confirm the presence of catalytic domains in the encoded proteins. When applied to cyanobacteria, this integrated approach has uncovered an intriguing set of haloacid dehalogenase-like and reductive dehalogenase-like proteins in *Nostoc* PCC 7120, pointing to a hidden enzymatic toolkit with exciting potential for environmental detoxification [44–48].

7.2 Structure Prediction and Modeling Tools

Because only a limited number of dehalogenases have been crystallized, homology modelling and other computational approaches play vital roles in understanding their structural features. Homology modelling and other structure-prediction approaches have become essential for visualizing the 3D architecture, providing insights into the catalytic residues, active-site geometry, and substrate-binding sites that govern specificity.

One of the most widely used platforms is SWISS-MODEL which builds comparative protein models using homologous templates[49]. For dehalogenases, it has been used to predict the folding of haloalkane dehalogenases (HLDs), revealing the α/β-hydrolase fold and access tunnels that guide halogenated substrates to the Asp–His–Glu catalytic triad [46].

Phyre2 is another widely used platform that relies on fold-recognition algorithms to generate structural predictions when close templates are unavailable [50]. This has proven especially useful for reductive dehalogenases, which are less conserved and underrepresented in the crystallographic databases. For instance, Phyre2-based modeling of cyanobacterial HAD-like dehalogenases has provided insights into the structural flexibility of cap domains, which are critical for substrate specificity [50]

More recently, AlphaFold2 which uses deep learning to produce remarkably accurate structural predictions, has been developed. Unlike template-based methods, AlphaFold2 can model proteins even when no close crystallized homologs exist, providing reliable active-site architectures and

enabling downstream molecular docking studies [51].

Predicted structures must be validated before they can be used for docking or dynamic simulations. Ramachandran plots provide a stereochemical check by evaluating backbone dihedral angles to ensure residues fall within allowed regions [52]. Similarly, PROCHECK offers a more detailed stereochemical assessment by evaluating bond lengths, angles, and torsion geometries [53].

Taken together, these tools form a clear workflow: starting with template-based or AI-driven structure prediction (SWISS-MODEL, Phyre2,AlphaFold2) followed by rigorous structural validation (Ramachandran plots, PROCHECK). This combined approach has been key to bridging the gap between sequence data and functional understanding of dehalogenases, particularly in organisms such as cyanobacteria, where experimental structures are scarce but computational tools can reveal a wealth of insight.

7.3 Molecular Docking Tools

Molecular docking has become one of the most powerful approaches for studying the interaction between dehalogenases and their substrates. By simulating the binding of halogenated compounds to enzyme active sites, docking allows researchers to predict the binding conformations, affinities, and possible reaction pathways.

Among the most widely used tools is AutoDockVina which applies a scoring function to estimate binding energies [54]. Another widely used program is GOLD which relies on genetic algorithms to optimize docking poses[55]. Schrödinger Glide offers high-accuracy docking, applied to model interactions of haloacid dehalogenases (HADs) with herbicide-like haloacetates [56] .

Together, docking tools such as AutoDockVina, GOLD, and Glide provide a framework for linking enzyme structure and function.

7.4 Molecular Dynamics (MD) Simulations

Although docking provides a valuable first glimpse of enzyme–substrate interactions, it is ultimately a static snapshot. MD simulations are indispensable in this regard, as they allow researchers to follow the motions of enzyme–ligand complexes, revealing how flexible regions of dehalogenases contribute to substrate binding.

Among the most widely used software is GROMACS known for its efficiency in simulating biomolecular systems[57]. AMBER offers another platform with force fields optimized for proteins and ligands[58] . Post-processing methods such as MM-PBSA and MM-GBSA are commonly used to estimate the free energies of binding [59].

Together, MD simulations using GROMACS and AMBER, combined with free-energy analyses, provide a dynamic view of dehalogenase function.

7.5 Visualization and Interaction Analysis Tools

One of the biggest challenges after modeling, docking, simulating enzyme–substrate complexes is determining the meaning of the raw data. To convert these into meaningful insights, researchers rely on visualization and interaction analysis tools.

PyMOL is one of the most widely used molecular graphics systems for biomolecules[60]. UCSF Chimera and ChimeraX provide advanced visualization and trajectory analysis capabilities[61]. LigPlot+ generates 2D diagrams of hydrogen bonds and hydrophobic interactions (already cited in your earlier sections) [10]. Discovery Studio Visualizer has been applied to highlight substrate conformations within dehalogenase active sites [62].

APPLICATION OF DEHALOGENASE ENZYME

Dehalogenase enzymes, which catalyze the cleavage of carbon–halogen bonds, are invaluable in environmental science, industrial catalysis, biotechnology, and diagnostics. Their exceptional selectivity, adaptability, and catalytic versatility make them foundational tools in pollutant

remediation, synthetic chemistry, environmental monitoring, and advanced bioengineering.

8.1 Bioremediation of Halogenated Pollutants

8.1.1 Anaerobic Reductive Dehalogenation (Organohalide Respiration)

Reductive dehalogenases (RDases), found in organohalide-respiring bacteria, are instrumental in transforming toxic, persistent halogenated compounds, such as polychlorinated biphenyls (PCBs), perchloroethene (PCE), and trichloroethene (TCE), into benign products such as ethene. This process is fundamental to both *in situ* and *ex situ* bioremediation strategies [2, 16, 29, 31].

8.1.2 Enhanced Field Applications

Although enzyme-based remediation offers specificity and environmental safety, limitations such as enzyme instability, cofactor dependency, and cost hinder its field-scale use. Advancements in enzyme immobilization, nanomaterials, and bioreactor engineering have enhanced their applicability and cost-effectiveness [26, 28, 59].

8.2 Detoxification of Fluorinated Contaminants

Defluorination of organofluorine compounds and polyfluoroalkyl substances (PFAS) is critical due to their extreme stability and toxicity. Recent studies have identified fluoroacetate dehalogenases from *Delftiaacidovorans* (DeHa2 and DeHa4) that catalyzethe defluorination of mono- and difluoroacetate, demonstrating sustained activity even under acidic conditions [60]. These enzymes show promise for the remediation of challenging fluorinated compounds that resist conventional treatments.

8.3 Industrial Biocatalysis and Green Synthesis

Hydrolytic dehalogenases, such as haloalkane and haloaciddehalogenases, are increasingly used to produce optically pure intermediates for pharmaceuticals and agrochemicals. Their regioselectivity and stereospecificity, along with their compatibility with mild and eco-friendly reaction conditions, render them critical tools in green chemistry.

Halohydrin dehalogenases (HHDHs) catalyze epoxide formation and enable diverse bond-forming reactions, including C–C, C–O, and C–N. Through protein engineering and immobilization strategies, these enzymes have been optimized for industrial applications requiring stability, high turnover, and substrate diversity [62].

8.4 Enhancing Enzyme Stability via Nanobiotechnology

In field settings, dehalogenases often encounter fluctuating pH, temperatures, and ionic strengths. A recent study developed an iron phosphate nano-hybrid version of DhaA (a haloalkane dehalogenase), which showed significant improvements in catalytic efficiency, reusability, and tolerance to environmental stressors [63, 28].

8.5 Biosensing and Environmental Monitoring

Dehalogenases are integrated into biosensor platforms to detect halogenated compounds in contaminated environments. These biosensors operate through halide or proton release, monitored via optical or electrochemical methods, offering a rapid, sensitive, and cost-effective detection method compared to traditional chromatography-based approaches [64].

8.6 Enzyme Engineering and Directed Evolution

Dehalogenases are integrated into biosensor platforms to detect halogenated compounds in contaminated environments. These biosensors operate through halide or proton release, monitored via optical or electrochemical methods, offering a rapid, sensitive, and cost-effective detection method compared to traditional chromatography-based approaches [64].

8.7 Structure-Guided Mutagenesis and Functional Enhancement

Recent structural studies on dehalogenases have enabled rational mutations to expand substrate scope and catalytic efficiency. For instance, modifying access tunnels or key residues in the active site has allowed the processing of previously inaccessible halogenated substrates [65].

CHALLENGES AND FUTURE DIRECTION

Dehalogenases from *Nostoc* sp. PCC 7120 show exciting potential, but several important hurdles must be overcome before it can be widely applied in environmental or industrial settings, one of which is the lack of experimental validation. Much of our current understanding of these enzymes is based on in silico predictions. Although these tools provide powerful insights, they cannot replace the laboratory experiments. It remains essential to express, purify, and characterize these enzymes under real-world conditions to confirm their activity, stability, and substrate specificity [64].

Another limitation is the slow progress of structural genomics and protein expression. Although genomic studies have confirmed the presence of dehalogenase-like genes in cyanobacteria, such as *Nostoc*, most of these proteins have not yet been expressed or structurally characterized. Determining their 3D structures and successfully producing them in lab-friendly hosts, such as *E. coli*, is vital for understanding their catalytic mechanisms and engineering them for targeted applications [8, 15].

The integration of artificial intelligence (AI) and machine learning (ML) could significantly accelerate progress in this field. These technologies are already helping to predict enzyme–substrate interactions, identify beneficial mutations, and simulate environmental conditions for enzyme function. By modeling enzyme performance in different scenarios, AI can help researchers prioritize the most promising candidates for development and testing [68].

In summary, bridging the gap between computational predictions and experimental validation, along with leveraging AI tools, will be essential to unlock the full potential of these promising cyanobacterial dehalogenases. Addressing these challenges will pave the way for their use in sustainable bioremediation, industrial biotechnology, and environmental monitoring applications.

CONCLUSION

Haloalkane dehalogenases represent a unique and versatile class of enzymes with significant environmental, industrial, and biomedical importance. Their ability to catalyse the hydrolytic cleavage of carbon–halogen bonds makes them vital biocatalysts for the detoxification of recalcitrant halogenated compounds, many of which pose serious ecological and health risks. Advances in structural biology, protein engineering, and *in silico* approaches such as molecular modeling and dynamics simulations have deepened our understanding of their catalytic mechanisms and substrate specificities, offering valuable insights for enzyme redesign.

Despite remarkable progress, several challenges remain, including the need to enhance catalytic efficiency toward a broader range of halogenated pollutants and to improve enzyme stability under harsh environmental and physiological conditions. The integration of computational modeling with experimental validation, along with directed evolution and synthetic biology approaches, holds promise for generating optimized dehalogenase variants.

Overall, haloalkane dehalogenases continue to emerge as powerful tools in bioremediation, healthcare biotechnology, and green chemistry. By combining molecular insights with applied research, these enzymes can be harnessed more effectively for environmental detoxification, sustainable industrial processes, and potential biomedical applications.

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