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# Environmental and Biotechnological Perspectives on Hydrolases

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## Abstract

Micro-organisms are able to survive in high salt concentrations because they have developed diverse biochemical, structural and physiological modifications, allowing the catalytic synthesis of proteins with interesting physicochemical and structural properties. When opposed to chemical catalysts, enzymes are protein-based catalysts that offer significant advantages. Enzymes are viewed as a greener alternative to conventional chemical catalysis in industrial processes since they are biodegradable, reusable, and do not produce excessive waste products. Enzymes are useful biological instruments for bioremediation that are both environmentally acceptable and bio based. An essential class of enzymes called hydrolases encourages the depolymerisation of macromolecules, which is crucial for the metabolism of nutrients. They have properties that make them desirable for biotechnological applications, in particular for environment protection, where hydrolase-producing organisms can be used in the removal of contaminants through a process known as bioremediation. They are highly effective catalysts, frequently secreted into the surrounding medium, and the majority do not require cofactors. Hydrolases present an opportunity to investigate the conformational variation that underlies the wide range of biological functions that these enzymes perform because they are the most numerous and diverse class of enzymes. The best choice for eliminating pollutants from the environment may be microbial hydrolases. All facets of life contain these enzymes, which are crucial for key biological activities like the breakdown of cellulosic biomass, viral pathogenesis, antibacterial defence, and typical cellular functioning. The fact that certain lysosomal storage illnesses have been linked to deficits in these enzymes, as well as the potential industrial uses of highly effective glycoside hydrolases. The isolation of active enzymes against particular pollutants comes from microorganisms that have been exposed to contaminated locations and those contaminants specifically. For the bioremediation of contaminants, several enzymes extracted from various species have been utilised. A viable approach to finding more effective and affordable instruments for the clean-up of contaminants would be to identify novel enzymes and new subtypes with specified physicochemical properties.

**Keywords:** Enzymes; Hydrolases; Catalyst; Microbes; Applications

**Abbreviations:** BMH: Bleomycin Hydrolase; BLM: Bleomycin; SfSFGH: S-Formylglutathione Hydrolase.

## Hydrolase Enzyme

Hydrolases are a group of hydrolytic enzymes that break chemical bonds with water to split a big molecule into two smaller ones. They are frequently utilised as biochemical catalysts. According to the EC classification system, hydrolases fall under EC 3 and can be further divided into thirteen sub classes based on the bonds they act on. They are essential to the body because they break down large molecules into smaller pieces for synthesis, expel waste, and supply carbon sources for energy production, which results in the monomerization of numerous biopolymers. Some hydrolases have the potential to release energy when they work (Figure 1).

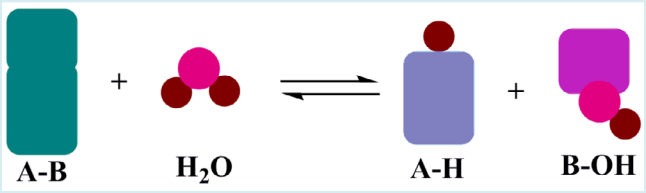


**Figure 1:** Hydrolysis reaction catalyzed by hydrolase [1].

## Hydrolases in Metabolism of Xenobiotics

Our health can be significantly impacted by chemical exposure, some of which are choice (drugs and food) and others of which are involuntary (environmental pollutants). These compounds' metabolism is crucial for their detoxification, elution, and occasionally activation. This focuses on the hydrolase family of enzymes, one of the many enzymes involved in the metabolism of xenobiotics. A diverse set of enzymes, including hydrolytic proteins, catalyse bond cleavages in nature's most prevalent substrate, water, by reacting with it. Hydrolases are involved in the metabolism of many organic and synthetic substances because of their efficiency in adding water to a variety of chemical processes [2].

Ester hydrolases might be helpful for breaking down polymers, medications, and pesticides. Ketoprofen, a non-steroidal anti-inflammatory medication, and polyurethane were both found to be responsive to an esterase isolated from a compost metagenomic library [3]. More recently, in suggested a novel mechanism for the breakdown of the synthetic pyrethroid pesticide cypermethrin by the employment of laccases and esterases in a strain of *Bacillus subtilis* [4].

By condensation and alcoholysis processes, microbial hydrolases break down additives or plasticizers, cyanides, and nitrile-containing chemicals to produce less hazardous byproducts. The copolymers, synthetic polyester, PHA, and parabens were all successfully broken down into different biodegradation products thanks to the substrate specificity and maximum stability of microbial lipase. Thus, it can be concluded that microbial enzymes are environmentally benign, safe, and cost-effective for recovering the biological and physicochemical features of damaged soil during the biodegradation of harmful organic and inorganic contaminants during bioremediation [5].

## Hydrolases in the Human Body

One of the most prevalent hydrolases (cholinesterases) discovered in the human body is acetylcholinesterase. Strong neurotransmitter acetylcholine is necessary for the voluntary contraction of muscles. Cellular lysosomes contain a specific hydrolase called lysosomal hydrolase. It is significant to remember that lipase functions inside of the cell, namely in the lysosomal compartment. Lysosomal lipase's main job is to hydrolyze lipids in the body, including triglycerides and cholesterol.

## Bile Salt Hydrolase

The bile salt hydrolase found in bacteria may help in the creation of new medications for the prevention and treatment of gastrointestinal illnesses as well as in the more deliberate use of living microorganisms as food additives. Human erythrocytes have an enzyme called acyl peptide hydrolase, which may be used as a biomarker for low dosage exposure to organophosphorus in people. Glycoside hydrolases have special functions in many biological processes, including cell wall metabolism, glycan biosynthesis, signalling, plant defence, and the release of stored energy. The purine salvage pathway's key enzyme, nucleoside hydrolase, also serves as a prime target for the development of anti-parasitic medicines. It has been proposed that the activity of bile salt hydrolase, which is frequently found in intestinal microbiota, may increase the hydrogel-forming capacities of some bile salts, the presence of which in the physiological conditions of the human gut is thought to be able to increase bacterial colonisation potential and survival rates in this particular ecological niche. Bile salt hydrolase which is produced by intestinal bacteria, catalyses the deconjugation of glyco- and tauro-conjugated bile acids by hydrolyzing the amide bond and liberating free bile acids (such as cholic acid and chenodeoxycholic acid) and amino acids (glycine and taurine) [6].

## Hydrolases in Biotechnology and Industries

Utilising esterases, it is possible to create optically pure molecules [7,8], a class of chemicals crucial for the creation of enantiopure pharmaceuticals. The recovery of phenolic chemicals from non-wood plants is another application for

esterases. For food, drinks, pharmaceuticals, perfumes, and cosmetics, phenolic substances such ferulic acid, sinapic acid, caffeic acid, coumaric acid, vanillic acid, and vanillin are highly prized [7-9]. Pulp that is produced when the phenolic chemicals from non-wood plants are extracted can subsequently be utilised to make paper. With an added value and cleaner wastes, this method enables the use of agricultural wastes in the manufacturing of paper [9].

Glycoside hydrolases sometimes referred to as glycosidases or glycosyl hydrolases are enzymes that catalyse the hydrolysis of glycosidic bonds in complex sugars. They carry out a variety of tasks in nature, such as the breakdown of cellulose (cellulase), hemicellulose (hemicellulase), and starch (amylase), antibacterial defence mechanisms (like lysozyme), pathogenesis mechanisms (like viral neuraminidases), and regular cellular function (like lysozyme) (e.g., trimming mannosidases involved in N-linked glycoprotein biosynthesis). The synthesis and dissolution of glycosidic connections in the body are carried out by a class of enzymes called glycosidases that collaborate with glycosyltransferases. Screening hydrolase-producing environmental bacteria towards their application in bioremediation [10].

Epoxides can be hydrolyzed asymmetrically by microbial epoxide hydrolases, which have been discovered to be flexible biocatalysts. According to the information currently available from the extensive screening of numerous bacterial and fungal sources, the enantioselectivities of enzymes from some microbial sources can be correlated to the substitutional pattern of substrates. For example, red yeasts, such as *Rhodotorula* or *Rhodosporidium* spp., give the best selectivities with monosubstituted oxiranes, while fun The preferred catalysts for the sterically demanding 2,2- and 2,3-disubstituted oxiranes are bacterial enzymes, particularly those from the Actinomyces family [11].

Cytoplasmic cysteine peptidase with a lengthy evolutionary history is bleomycin hydrolase (BMH). Its biological activity is the hydrolysis of the reactive electrophile homocysteine thiolactone. It also has the ability to metabolically inactivate the glycopeptide bleomycin (BLM), a crucial part of cancer treatment regimens. The protein possesses active site residues typical of the cysteine protease papain superfamily [12].

## Hydrolases from Psychrophiles

For the synthesis of short-chain esters, such as in the creation of flavours in the food sector [13], cold-active esterases from psychrophiles, or microorganisms with optimal growth at 15 °C or lower, may be of interest. The Structural and functional characterization of a novel cold-active S-formylglutathione hydrolase (SfSFGH) homolog from *Shewanella frigidimarina*, a psychrophilic bacterium [14]. A novel cold-active S-formylglutathione hydrolase (SfSFGH) from Shewanella frigidimarina, composed of 279 amino acids with a molecular mass of ~ 31.0 kDa, was characterized. Sequence analysis of SfSFGH revealed a conserved pentapeptide of G-X-S-X-G found in various lipolytic enzymes along with a putative catalytic triad of Ser148-Asp224-His257. Activity analysis showed that SfSFGH was active towards short-chain esters, such as p-nitrophenyl acetate, butyrate, hexanoate, and octanoate. The optimum pH for enzymatic activity was slightly alkaline (pH 8.0). To investigate the active site configuration of SfSFGH, we determined the crystal structure of SfSFGH at 2.32 Å resolution. Structural analysis shows that a Trp182 residue is located at the active site entrance, allowing it to act as a gatekeeper residue to control substrate binding to SfSFGH. Moreover, SfSFGH displayed more than 50% of its initial activity in the presence of various chemicals, including 30% EtOH, 1% Triton X-100, 1% SDS, and 5 M urea.

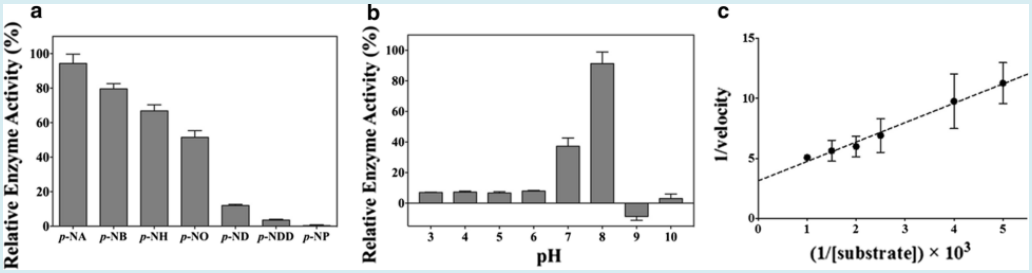


**Figure 2:** Enzymatic activity of SfSFGH. Substrate specificity was investigated using p-nitrophenyl esters with different acyl-chain lengths. b Effects of pH on enzymatic activity were studied from pH 3.0 to 10.0. Activity at the optimal pH was set as 100%. c Lineweaver–Burk plots showing the reciprocal of the velocity of SfSFGH versus the reciprocal of the substrate concentration. All experiments were performed in triplicate [14].

## Hydrolases from Thermophiles

Thermostable esterases can be cloned in *Escherichia coli* or *Pichia pastoris* from a variety of thermophiles, or bacteria having an optimal growth temperature of 45 °C or above. These esterases have highly desirable physical and chemical properties that make them suitable for use in detergent formulations, environmental contaminant degradation, and biotransformation12 (high temperature stability, alkaline pH stability, resistance against denaturant agents, and/or against organic solvents) [15]. Thermophiles are microorganisms with optimum growth temperature of above 60 °C. During the past, several thermophilic organisms have been isolated from geothermal regions and hot environments. The adaptive ability of thermophiles at higher temperatures arrives from thermostabilization of macromolecules like, genome, proteome and other cellular machineries. Although thermophilic and mesophilic proteins exhibit almost similar conformation, an increased occurrence of ion pair networks, hydrogen bonds, hydrophobic interactions, ratio of polar charged to polar uncharged amino acids, aromatic interactions, surface loop deletion and tight packing seem to confer additional degree of stability to thermophilic proteins. Thermophilic enzymes are of considerable interest for industrial applications due to their compatibility to the industrial processes. Biochemical studies on thermophilic enzyme purification and characterization followed by overexpression of the encoding genes in bacterial hosts has allowed their higher level of production for biotechnology industries and also to gain an insight on mechanism of thermophilicity at molecular levels. Moreover, the structural information of thermophilic enzymes has been utilized in engineering of proteins for development of superior enzymes with improved kinetic parameters including thermotolerance. Among the thermophilic enzymes, the thermophilic hydrolases involved in depolymerization of biopolymers, such as xylanases, proteases, cellulases, amylases, and lipases, are of special interest due to their applications in food, pharmaceutical, pulp and paper industries and environmental biotechnology [16].

## Hydrolases from Marine Extremophiles

Nearly 75 percent of the world is covered by the ocean, which is also where evolution began. Numerous extreme marine conditions, including hydrothermal vents, hot springs, salty lakes, and deep-sea floors, support the existence of extremophile microbes. These microorganisms have a high potential for biotechnological activities as evidenced by their capacity to withstand extremes in temperature, salinity, and pressure. For these reasons, hydrolases from hyperthermophiles, psychrophiles, halophiles, and piezophiles have been studied, including amylases, cellulases, peptidases, and lipases. Extremozymes have found increased use in a variety of commercial applications, including the production of biofuel, pharmaceuticals, fine chemicals, and food. Extremozymes are designed to function under challenging physical-chemical circumstances. Understanding the particular elements that give these enzymes the capacity to tolerate harsh environments has grown to be a priority [10].

Comparing extremophile hydrolases to chemical biocatalysts reveals advantages. Their catalysts are eco-friendly, highly precise, and operate under benign reaction circumstances. These hydrolases can function even when organic solvents are present, which is crucial for the production of single-isomer chiral medicines. These hydrolases have been used in numerous applications. They serve as helpful catalysts for inorganic synthesis and have a wide range of industrial uses in the pharmaceutical industry, including as digestive and anti-inflammatory drugs. *Thermatoga maritime*, a hyperthermophilic isolate from a marine geothermal area in Vulcano, Italy, has a homomultimeric peptidase (669 kDa) based on 31 kDa subunits, known as Maritimacin [17]. Peptidases can also be recovered from marine extremophiles. It was discovered that this enzyme resembled bacteriocin, an inhibitor of several Gram-positive bacteria's development from the mesophilic bacterium *Brevibacterium linens*, both structurally and in terms of gene sequence [7]. An intracellular peptidase (PH1704) from the thermophilic *Pyrococcus horikoshii* is remarkably stable. A cysteine peptidase was recently demonstrated to be the first allosteric enzyme to exhibit negative cooperativity with chloride ions (Cl). For the creation of novel pharmaceuticals, finding new allosteric sites is crucial [18]. The extracellular peptidase from *Halobacterium halobium* was utilised for effective peptide synthesis in Water-N′-N′-dimethylformamide as an example of how peptidedases from halophiles have been employed in peptide synthesis [19].

## Hydrolases in Various Processes

Due to the variety of roles, they can play in biological processes; hydrolases can take part in a wide range of biological processes. Large molecules are broken down by hydrolases into smaller pieces that can be employed for synthesis, waste excretion, or as carbon sources for energy production. These include digestive enzymes like cholinesterase, carboxylesterase, lysosomal hydrolases, etc. that are involved in digestion, transport, excretion, control, and signalling activities. In particular, the hydrolase produced by *Lactobacillus* spp. in the human stomach may cause the liver to generate bile salts that aid in meal digestion [20].

The role that hydrolytic enzymes play in many biological processes makes them crucial for human health, but they also

have a wide range of practical uses in industry. Hydrolases are the class of enzymes with the highest proportion of enzymes employed for industrial purposes, outweighing other classes' industrial importance. Hydrolytic enzymes make up around 75% of all industrial enzymes. More than 70% of all enzyme sales are made up of carbohydrases, proteases, and lipases, which control the enzyme market. Hydrolases are essential to many industrial sectors, including the waste management, detergent, leather, textile, pulp and paper, foods and feeds, dairy, biofuel, and leather industries. Due to their widespread use in the dairy and detergent industries, proteases continue to be the most common type of enzyme. The second-largest group consists of various carbohydrases (glycosidases), primarily amylases and cellulases used in the starch, textile, detergent, and baking industries [19,21,22].

## Conclusion

Traditional chemical synthesis methods have been demonstrated to be inferior to hydrolase-based bio-transformations. As hydrolases have an advantageous structural flexibility, these enzymes are also likely to catalyse reactions outside of their "physiological repertoire" as well as the transformation of a broad range of unnatural substrates with highly functionalized structures [23]. These biocatalysts are willingly used in the synthesis of high-value compounds with controlled stereochemical properties, such as pharmaceuticals, agrochemicals, vitamins, flavours, and fragrances, or bulk products, such as nutraceuticals, detergents, cosmetics, biofuels, and biodegradable polymers.

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