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Impact of extremozymes on the removal of pollutants for industrial wastewater treatment

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The discharge of effluent-containing pollutants from various industries into soil and water requires wastewater treatment. These harmful by-products are difficult to degrade and are poisonous. They cause serious problems to aquatic habitats and organisms. Wastewater treatment methods like physical and chemical procedures have drawbacks such as high costs, toxic by-products, and low removal effectiveness. As a result, it is becoming increasingly important to create effective biological wastewater treatment technology using microbes. Extremophiles have recently been extensively investigated for their ability to degrade contaminants under extreme conditions of temperature, pH, and salinity. Extremophilic microbes are repositories for novel extremozymes offering several advantages over conventional chemical catalysts, including highly specific biocatalysis, reproducibility, and stability at severe industrial wastewater conditions. They aid to generate the least toxic, and biodegradable industrial waste. The choice for the use of these extremophiles containing specific extremozymes needs extensive study considering their adaptability for the removal of pollutants from industrial wastewater. The alteration of extremozyme protein structure for extremophilic bacteria survival in various environmental circumstances, their usage in wastewater treatment, and the mode of action for biodegradation of various contaminants during bioremediation are all discussed in this paper.

Keywords*:* Biodegradation, extremophiles, extremozymes, effluent, wastewater, treatment

INTRODUCTION

Despite the fact that water is one of the world's most abundant natural resources, just 1% of it is currently feasible for human consumption [1]**.** Waste water generation is increasing on a global scale as a result of increased urbanization and industrialization [2] . Global water consumption has increased in the twenty-first century, necessitating waste water management; a variety of biological treatments are required to recycle wastewater and make it appropriate for drinking, washing, and industrial [3]. The treatment of waste water is a significant concern for water treatment enterprises all over the world [4] . Water pollution is caused by the generation and discharge of industrial effluents all over the world, particularly in developing countries [1]. Pollutants and heavy metals cause ill effect on health and environment. They are found in industrial wastes such as metal, refinery, automobile, textile, printing, and pharmaceutical industries [2]. Wastewater treatment has become increasingly important in order to recover clean water from effluent treatment. The key problem for industries in this context is to implement the most efficient, cost-effective, and ecofriendly technologies to ensure reusability of this water in a sustainable manner [1]. Several bioremediation approaches based on enzyme-driven treatment systems are useful for polluted wastewater treatment [5].

Enzymes have recently used as a useful technique for improving pollution degradation. As a high-efficiency biocatalyst, enzymes offer remediation of these recalcitrant pollutants. It covers enzyme types and suppliers, enzymatic procedures in the remediation of resistant contaminants, enzymatic conversion product identification and ecotoxicological testing, and common enzymatic wastewater treatment systems [6]. Some of the potential advantages of extremozyme remediation over conventional therapy include: its industrial usability; manoeuvrability across a considerable pH, temperature, and salinity range; and a decrease in sludge volume. As a consequence, potential solutions to remove industrial contaminants from water are becoming more popular, and extremophilic extremozymes-based strategies are gaining popularity in this connection. The current state of the industrial waste water market for the various technologies that utilize extremozymes in the cleaning process. Extremozymes produced from extremophilic microbes can thrive at extreme environmental conditions [7] such as temperature stability, high stereo-selectivity, long half-life, solvent-tolerance, affinity to the substrate. They feature heat-stable and solvent-tolerant biocatalytic properties, as well as being environmentally acceptable [6]. Recently, extremozymes are proclaimed as a biological source of waste water treatment due to their high reproduction rate,

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resistance to catalyze reaction under extreme environmental conditions and significant potential [8].

With an annual increase of 4.9 percent, the worldwide economy for enzymes for various industrial applications was estimated to be worth 5.5 billion US dollars in 2018 [9]. The international enzyme industry is valued at $5.5 billion, with a forecasted value of $7.0 billion by 2023. With a combined market share of 35%, Europe and North America are the two most significant continents. between 30% and 40%, are the biggest customers [10]. Japan is also a leading enzyme manufacturer. Novozymes, Danisco, Genencor, DSM, and BASF occupy the top positions in the global market [11]. Among the enzymes, proteases, carbohydrase and lipases occupy the most sought forward category in the market [10, 12]. In this review, the significance of many enzymes from numerous extremophiles, as well as their activity in mitigating pollutants in effluent water, is reviewed.

Extremophiles and their extreme abilities

Extremophiles are microorganisms that can perish in extreme environments [13]. Extremophiles represent a diverse variety according to their area of occurrence and functioning such as hot spring, thermal vents with high temperature, Arctic and Antarctic regions with low temperature, acidic lakes, and high saline lakes. Thermophiles can thrive at high temperatures (> 80 °C up to > 110 °C), psychrophiles may sustain at low temperatures (-2 °C to 20 °C), acidophiles (pH 4), alkaliphiles (pH > 9) and halophiles can flourish at cold temperatures (-2 °C to 20 °C) (high salinity such as 2-5 M NaCl) [14]. Due to their cost-effectiveness, environmentally friendly character, and ability to create less sludge in extreme conditions, extremophiles were utilized as a plausible alternative source to remove contaminants (Fig. 1) in many industries [14, 15]. Microbially generated enzymes have been used to treat wastewater, with benefits such as cheaper costs, reduced environmental toxicity, simplicity of application, and flexibility.

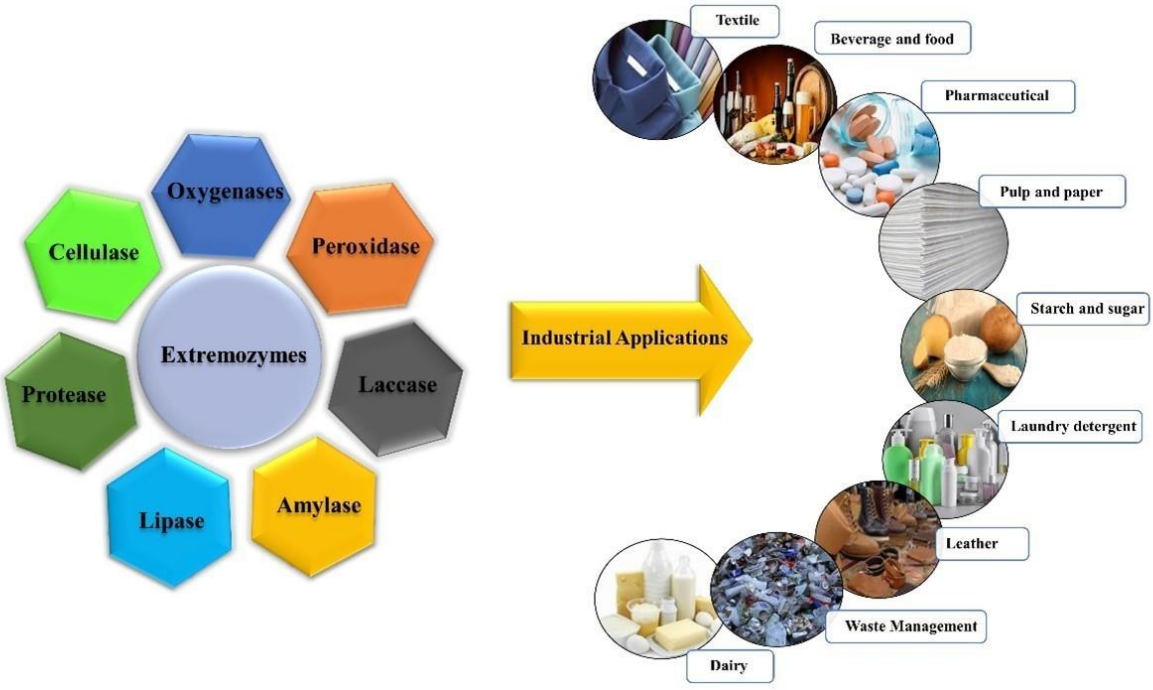

Fig. 1. Extremozymes and their application in different industries

Cold-adapted microorganisms produce psychrophilic enzymes with a high catalytic efficiency, which have important implications for the biotechnology sector [17]. Some cold active extremozyme producing strains, such as *Bacillus cereus* HSS [18] *Pseudomonas palleroniana* GBPI-508 [19], *Pseudomonas marinensis* gcc21 [20], are now gaining interest by industries such as textile and detergent due to their low temperature stability [21]. They can catalyze their respective processes in aqueous and nonaqueous environments, as well as in water/solvent combinations and at freezing temperatures. The features that are developed in synthetically designed enzymes through genetic manipulation, genome shuffling, genetic blending, truncation, or rational protein synthesis, are already existent in these enzymes [22].

Saltpans, salt mines and highly salted fermented

foods all provide a suitable environment for the halophilic enzymes that can live at high salt concentrations [23]. Halophilic bacteria *Hortaea* sp. was able to produce 1,2-dioxygenase and laccase and also degrade solvent green 3 into phthalic acid and 4-hydroxybenzoic acid [24]. FMN-dependent NADH azoreductase was produced by halophilic bacteria *Salinivibrio kushneri HTSP*, which is able to degrade and decolorize three pollutant dyes including safranin and congo red [25] The halophilic bacteria adapted to high salt concentrations have a large proportion of acidic amino acids and low percentage of lysine and aliphatic chains [26]. So, these halophilic extremozymes have industrial potential for textile, laundry detergent, leather, pharmaceutical and food industry because they able to degrade heavy metals, petroleum hydrocarbons, as well as decolorize industrial waste water [26, 27].

Thermophilic bacteria are found mostly in hot springs and can withstand temperatures of 45–80 degrees Celsius. Due to their unique qualities like high-temperature growth and different macromolecular characteristics, thermophiles can have a robust metabolism, chemically and physically stable enzymes, and lower growth but higher productivity than equivalent mesophilic species. Thermophilic enzymes are suitable for biotechnological procedures that need high temperatures, moreover, they are resistant to detergents, solvents, and acidic and alkaline pH [28]. *Brevibacillus borstelensis* strains UE10 and UE27, as well as *Aneurinibacillus thermoaerophilus* strain UE1, were found to be capable of producing cellulase, which was used in carbohydrate fermentation [29]. *Paenibacillus validus*, *Paenibacillus koreensis*, and *Bacillus nealsonii*, among other thermophilic lignocellulolytic bacteria isolates, produced ligninase, xylanase, protease, and urease, and were used to improve composting indices [30]. Protease was produced by the thermophilic bacterium *Thermomonas hydrothermalis*. This makes it a desirable source for thermostable enzymes with commercial applications in biotechnological and environmental applications [31].

Alkaliphile/acidophile enzymes from micro-organisms typically share other extremophilic habitat features like resistance to extreme pH levels. They are active chelators, thus can be very beneficial for application in detergent manufacture [23]. Acidophiles have a place in the bioprocessing of minerals because they share other extreme habitat features like thermophilicity, halophilicity, or heavy metal tolerance. Alkaline proteases are the significant enzymes of alkaliphiles with substantial impact as additives for biological detergents [17].

Extremophiles produce hydrolases, which are widely used in industry for a variety of reasons, including their resilience to organic solvents and severe temperatures [31]. As a result of their environmentally favorable characteristics, they can be used in bioremediation and waste treatment. as peroxidase [16]. In the treatment of industrial wastewater, these extremozymes play an essential function (Fig. 1) [32]. They can degrade dyes, heavy metal, plastics, polyhydroxyalkanoates, pesticides and many more pollutants (Fig. 2). These extremozymes are utilized in various industries such as biofuel, detergent, pharmaceutical, food, agricultural, bioenergy, textile and cosmetic industries [33].

Extremozymes in bioremediation of different pollutants

Several bioremediation approaches based on an enzyme-driven treatment system have been studied as an effective method for polluted wastewater treatment as they require low cost, less time and labor [5]. Extremophiles have hydrolase activity that is widely used in industries because they have resistance to organic solvents and extreme temperatures [31]. So, they can be applied in bioremediation and waste treatments due to their eco-friendly nature. Hydrolytic enzymes and oxidoreductases, are two types of extremozymes beneficial for waste water treatment [16]. These extremozymes are crucial in the treatment of industrial effluents. They can degrade dyes, heavy metals, plastics, polyhydroxyalkanoates, pesticides and many more pollutants (Fig. 2) [32]. These extremozymes are utilized in various industries such as biofuel, detergent, pharmaceutical, food, agricultural, bioenergy, textile and cosmetic industries [33].

The activity of these enzymes produced by extremophilic bacteria on various pollutants is shown in Table 1. Treatments with oxidoreductases and hydrolases of many types of synthetic and recalcitrant compounds, among other things, leads to simpler or covalently connected and less reactive components [34]. The principal extremozymes involved in contaminant bioremediation, including oxygenases, peroxidases, hydrolases, lipases, phosphodiesterase, and many others [35], will be discussed in this context for use in waste water treatment of hazardous substances.

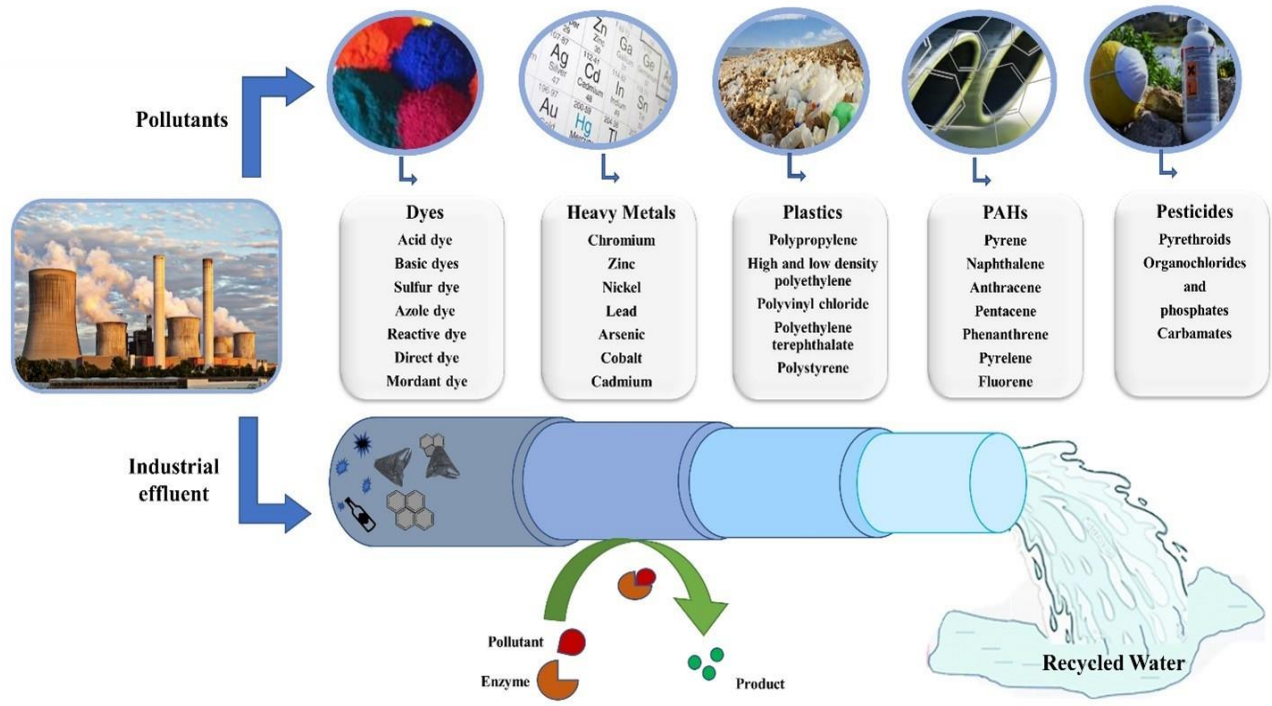

Fig. 2. Schematic diagram of waste water containing toxic industrial pollutants and their treatment using extremozymes produced from extremophiles

Oxidoreductase

Several microorganisms, fungi, and multicellular organisms produce and release oxidoreductases to eliminate compounds by chemical interactions, which entails the flow of energy from reducing agents to oxidizing agents, producing chloride, carbon dioxide, and methanol [36]. Resistant pollutants such as aromatic chemicals, textile dyes, petrochemical pollutants, and phenolics in waste water are biodegraded by the action of oxidoreductase [16]. At 100 degrees Celsius, the hyperthermophile archaeon *Pyrococcus furiosus* includes production *via* a new mechanism involving AdhA and the native enzyme aldehyde oxidoreductase [37].

Oxygenases. Oxygenases are enzymes that belong to the oxidoreductase family wherein the co-substrate FAD/NADH/NADPH contributes to the oxidation of reduced substrates [38]. Glyphosate oxidase (goxB), a FAD-dependent enzyme isolated from *Bacillus aryabhattai* FACU3, is engaged in glyphosate herbicide bioremediation. Glyphosate is converted to aminomethylphosphonate (AMPA) by goxB cleaving the C-N link and releasing the keto acid glyoxylate [39].

Peroxidases. Peroxidases catalyze the conversion of lignin and other phenolic compounds in the presence of hydrogen peroxide (H_2O_2). Lignin peroxidase and manganese-dependent peroxidase have been studied the most among peroxidases because of their remarkable potential to break down hazardous chemicals in nature [40]. Peroxidases are involved in the color removal and detoxification of diverse contaminants in wastewater, as well as the decomposition of polyphenols. *Bacillus albus* MW407057 is involved in the breakdown of methylene blue dye *via* lignin peroxidase [41]. *Bacillus velezensis* Al-Dhabi 140 produces manganese peroxidase, which is capable of degrading tetracycline [42].

Laccases. Laccases are multicopper oxidases, which are redox metalloenzymes. Copper ions in these glycoproteins have a high redox capacity, which enables them to facilitate the oxidation of a wide spectrum of aromatic substrates while also generating water as a by-product of molecular oxygen reduction [43]. The laccase producing thermophilic *Thermus* sp. 2.9 was shown to be resistant to a variety of pH levels, hazardous heavy metals, and has ability to decolorize and breakdown azo dyes such as xylidine and methyl orange found in wastewaters [44].

Hydrolases

Hydrolytic enzymes break down esters, peptide bonds, carbon-halide bonds, and other essential chemical molecules. When compared to physico-chemical remediation for the breakdown of toxic

organic compounds, bioremediation is both safe and cost effective [38].

Table 1. Extremophilic enzymes and their pollutant degradation efficiencies

S.No	Microorganisms	Enzymes	Pollutants	Ref.
1	*Anoxybacillus rupiensis 19S*	Oxidoreductases and hydrolytic enzymes	Phenol	[16]
2	*Novosphingobium* sp. ES2	Monooxygenase	*β*-Estradiol	[45]
3	*Pseudomonas synxantha* S2TR-26 *Pseudomonas mandelii* S2TR-08	Xylene monooxygenase; catechol 2, 3-dioxygenase	p-Xylene	[46]
4	*Pseudomonas stutzeri* OX1	Toluene/*o*-xylene monooxygenase	Toluene; benzene	[47]
5	*Pseudomonas* S2TR-1	Toluene/o-xylene monooxygenase	Benzene, toluene, ethylbenzene, and isomers of xylenes	[48]
6	*Pseudomonas aeruginosa* SJTD-1	AlkB monooxygenases	*n*-Alkanes	[49]
7	*Bacillus albus MW407057,*	Lignin peroxidase	Decolorize and detoxify MB dye for environmental safety.	[41]
8	*Bacillus velezensis* Al-Dhabi 140	Manganese peroxidase	Tetracycline	[42]
9	*Bacillus albus* MW407057	Lignin peroxidase	Methylene blue and removed COD	[41]
10	*Geobacillus stearothermophilus* ATCC 10149	Laccase	Remazol brilliant blue R dye	[50]
11	*Bacillus cohnni* (RKS9)	Laccase, azoreductase, lignin peroxidase and manganese peroxidase	Congo red dye and heavy metal removed cadmium, chromium, lead and nickel	[51]
12	*Bacillus sp. GZB*	Laccases	Bisphenol A, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate) and syringaldazine	[52]
13	*Bacillus* sp. CF96	Laccases	Indigo dye	[53]
14	*Thermus* sp. 2.9	Laccases	Xylidine, Methyl orange	[44]
15	*Bacillus vietnamensis* sp. MSB17	Tyrosinase , laccase, and manganese peroxidase	Malachite green (MG)	[54]
16.	*Geobacillus* sp. G27	Dioxygenase	Naphthalene	[55]
17	*Planococcus maritimus* (MSB2 and MSB16), *Bacillus pumilus* (MSB6 and MSB8) and *Bacillus vietnamensis* (MSB10 and MSB17	Hydrolases (amylases, caseinases, cellulases, gelatinases, lipases, ligninases, and Malachite green dye degraders)	Malachite green dye degraders	[56]
18	*Bacillus cereus* KM201428	Proteases	Detoxification and degradation of Malachite green dye	[57]
19	*Geobacillus* sp. D4, *Geobacillus* sp. D7,	Lipase	Alkanes, toxic poly-aromatic hydrocarbons (PAHs), organosulfur, carboxylic acids,	[58]

	Anoxybacillus geothermalis D9		alkene, resins, organosilicon, alcohol, organochlorine, and ester	
20	*Pseudomonas nitroreducens* AR-3	Organophosphate hydrolase (OPH),	Chloropyrifos	[59]
21	*Bacillus licheniformis* HULUB1 and *Bacillus subtilis* SUNGB2	Amylase	Degradation of food waste	[60]
22	*Escherichia coli* IES-02	Carboxyesterases	Malathion	[61]
23	*Lysinibacillus sphaericus* YMM	Carboxyesterases	Malathion	[62]
24	*Pseudomonas aeruginosa* PA06 and *Achromobacter* sp. AC15	Catechol 1,2-dioxygenase (C12O) and 2,3-dioxygenase activities (C23O) enzyme	Pyrene	[63]
25	*Pseudomonas* sp. strain phDV1	PHA synthase	Degrades phenol and produces polyhydroxyalkanoates	[64]
26	*Comamonas testosterone*	PHB depolymerase (PhaZ)	Polyhydroxybutyrate (PHB)	[65]

Hydrolytic enzyme is highly successful in bioremediation of oil spills, organophosphate, and carbamate pesticides. The carbendazim hydrolysing enzyme obtained from *Klebsiella*, *Flavobacterium*, and *Stenotrophomonas* was recently found to be capable of breakdown of carbendazim into nontoxic forms [66]. Bacillus sp. strain TSCVKK produced extracellular detergent-stable alpha-amylase in the presence of halophilic alkali. As a result, these amylases could be feasible options for bioremediation of waste from paper and other sources [16, 67]. These well-known and fully characterized α-amylases elect this enzyme as a prime candidate for application on bioremediation. A halotolerant and thermostable lipase from *Oceanobacillus* sp. PUMB02 bacterium has a high degree of stability over a wide range of pH, salinity and temperature and is stable at 50-70°C and alkaline pH [68]. An halophilic strain *Halobacillus* sp. strain EG1HPQL degrades n-alkanes and has resistance to heavy metals including Cu, Pb, and Ni [69]. Furthermore, the thermophilic bacterium *Geobacillus sp. GS53* has high stability in the presence of organic solvents and detergent constituents [70]. Protease is used in waste management, food and feed, detergent industry, medicine, leather industry and protease engineering [71]. The bacterium *Bacillus amyloliquefaciens-ASK11* isolated from a tannery waste was found to produce cellulases [72]. Dicofol is an organic pollutant which is degraded by cellulase into 4,4'-dichloro-dibenzophenone by an oxidative process [73]. The mode of action of all these enzymes on different pollutants is explained in Table 2.

ADAPTATION OF EXTREMOZYMES

Extremozymes, or extremophilic microbe proteins, have been biochemically and molecularly modified to tolerate harsh industrial environments. Despite the fact that no single factor has been identified as creating extremozyme stability in the severe environments in which they must survive, a new study on extremophilic enzymes has aided in the understanding of the types of general patterns that influence extremophilic enzyme stability [74]. As the amino acid sequence changes, the structure, flexibility, charge, and/or hydrophobicity of extremozymes changes, resulting in changes in the enzymes. Extremophilic proteins adapt to similar harsh physical or chemical circumstances in a number of ways [75]. We'll look at the adaptations that extremophiles have made to survive in severe settings in the presence of enzymes farther down.

Changes in specific amino acid content on the protein surface

A protein's stability is largely determined by its amino acid composition. According to a study conducted by Enache *et al.,* 2010, labile amino acids are easily modified, especially when exposed to extremes such as extremely high or low temperatures, pH, or pressure. The denaturement of a protein can be caused by covalent alteration of these labile amino acids. The concentration of amino

acid residues such as glutamic acid, cysteine, and aspartic acid on the membrane of proteins is quite low, which contributes significantly to protein stability and flexibility in harsh environments [76].

Table 2. Mode of action of extremozymes on different pollutants

S.No.	Class of enzyme	Type of enzymes	Substrate/ pollutants	Mode of action	Ref.
1	Oxido-reductases	Oxygenases	Aromatic compounds such as PAHs, naphthalene, anthracene and phenanthrene, chlorinated biphenyls, aliphatic olefins, phenols, organic halogen compounds (herbicides, fungicides, and pesticides), aliphatic hydrocarbons	Coenzymes FAD, NADH, or NADPH are used to transport oxygen to organic or inorganic molecules.	[34], [77]
1.1		Monooxygenases (flavin and P450 monooxygenases)	Degrade chlorinated pesticides, hydrocarbons, xenobiotics and heme prosthetic group	Hydrolases are enzymes that transfer one oxygen atom to the substrate while the electrons of the coenzymes NADH or NADPH decrease the other oxygen to water.	[34], [77]
1.2		Dioxygenases	Naphthalene	Molecular oxygen transferred and incorporated into the substrate.	[55]
2		Peroxidases	Phenols, cresols, chlorinated phenolic compounds, polyphenols, amines, and heterocyclic amine polyamines, as well as nitroaromatic compounds such as TNT (2,4,6-trinitrotoluene), lignin, and dyes	Peroxides	[34]
2.1		Lip	Anthracene, pyrene, acenaphthene		[78]
2.2		MnP	Anthracene; fluorene; phenanthrene; dibenzothiophene		[78]
3		Laccases	PHAs, paints, plastics, dyes, estrogenic compounds, paper, and cellulose acenaphthene, anthracene, fluorene, and benzo[a]pyrene		[13], [34], [78]
3.1	Hydrolase	Amylases	Paper, food industries, textile industries, and fossil and fuel products; degradation of petroleum-derived compounds such as polyethylene; n-alkane; paraffin hydrocarbons	Starch and carbohydrate components, catalyse the hydrolysis of 1,4--D-glycosidic linkages.	[34], [60], [79]
3.2		Proteases	Phenols and proteins	Hydrolysis of peptides bonds in presence of water molecules	[16, [69], [80]
3.3		Lipases	Oil residues, petroleum contaminants, effluents, and soil recovery, kitchen waste	Hydrolysis, esterification, alcoholysis, and interesterification of fatty acids, esters, and glycerides	[81], [82]
3.4		Cellulases	Dicofol; cellulosic compounds	Hydrolysis of substrate to simple compounds.	[73]

Acidic residues are preferred in these conditions due to their great capacity for binding water molecules, but halophilic proteins also have repulsive electrostatic interactions at healthy pH levels. The carboxyl groups of glutamate and aspartate are used to maintain a hydrated protein surface. Polygalacturonase, a cold-active enzyme, was discovered to have low arginine concentrations, impairing thermo adaptation [83]. Psychrophilic enzymes have a reduced arginine/lysine proportion, more glycine residues for enhanced allosteric movement, fewer proline residues in loops but more in helices, and more non-polar residues on the protein surface, all of which make a contribution to a lower core hydrophobic nature but an increased surface chemistry [74]. The lack of surface lysine residues in halophilic proteins was a startling result. This observation corresponds to a decrease of hydrophobic surfaces, which results in an increase in hydration at the protein surface [84]. A small amount of bulky hydrophobic side chains has been identified on the surface of halophilic proteins [85]. *Thermovibrio ammonificans* makes an enzyme with a specific function. *Thermovibrio ammonificans* makes an enzyme with a distinctive core made up of two inter subunit disulfide connections and a single lysine residue from each monomer in the molecule's centre [85]. The stability and thermophilicity of extremozymes are greatly affected by modifying the N- and C-terminal residues. Thermophilic enzymes usually have high level of bisulfide bonds, shorter loops, which inhibit nonspecific interactions and improve structural rigidity and, as a result, resistance to unfolding at high temperatures [86].

Increased number of salt bridge formation/ increased ionic interaction

Ionic interactions have a big impact on protein stability. Extremophilic proteins interact to build salt bridges between oppositely charged neighboring residues, which aids protein folding, structure, and oligomerization while also improving enzyme stability at high salt concentrations [75]. Ion pairs have a vital role in the stabilization of hyperthermophilic proteins with a decreased hydrophobic impact, according to crystal structures of extremophilic proteins. Structure comparison, homology-based modelling, and site-directed mutagenesis are all aided by the availability of extremophilic protein crystal structures. The crystal structures of glutamate dehydrogenase, a hexameric protein isolated from hyperthermophilic organisms such as *Thermotoga maritima* (90°C), *Pyrococcus furiosus* (100°C), and *Thermococcus litoralis* (88°C), were studied [87, 88].

Modified protein structure

In nature, many proteins or protein families use the oligomerization mechanism in various ways, and they can form complexes with several polypeptide chains [89]. In oligomeric proteins, enzyme denaturation is frequently initiated by subunit dissociation, followed by irreversible monomeric denaturation in extreme cases. The oligomer structure of several extremozymes has been found to be more complex than that of mesophilic proteins, which are monomers or dimers [90]. In the other case, protein subunits undergo conformational changes that promote oligomer formation to compensate for the loss of individual component stability. The oligomerization method, on the other hand, can be utilized to promote protein flexibility and activity at low temperatures while decreasing protein stability [91]. Excellent lignocellulosic extremozyme production *in Pichia pastoris* has been demonstrated to have high enzyme properties and improved stability by a result of proper post-translational modifications [92]. A hyperthermophilic bacterium produced by iron hydrogenase, *Thermotoga maritime*, is a homotetramer, whereas dehydrogenases isolated from mesophilic species normally have one or two subunits [90].

Ionic charge changes at the surface

Extremozymes have unique properties in the solvent-exposed surface region, which contribute to their increased stability. Factors like extended nonpolar side chain exposure to solvent and an increase in total solvent accessible surface area aid in psychrophilic mannanase's thermal adaption at lower temperatures [93]. The very negative surface charge of halophilic enzymes like *Kocuria varians* and *Alicyclobacillus acidocaldarius* amylase improves their solubility and flexibility at high salt concentrations [94, 95]. The lack of surface lysine residues in halophilic proteins was an interesting finding. This finding is in line with the reduction in hydrophobic surfaces, which leads to an increase in hydration at the protein surface. Proteins that are halophilic have also been discovered and found to have a low content of bulky hydrophobic side chains on their surface [84].

Prominent catalytic mechanism

Extremozymes' catalytic processes are identical to those of their mesophilic counterparts, as evidenced by mechanistic research [96]. It's unclear

whether mesophiles and extremophiles share catalytic processes. Substrate specificities, pH dependences, kinetic isotope effects, and linear free energy connections were explored in recombinant glucosidase from *Pyrococcus furiosus*, a hyperthermophile, and *Agrobacterium faecalis*, for example [97]. As a result, enzymes with a diverse range of substrate specificities and pH sensitivity, similar Brønsted graphs, and a high correlation coefficient shared broad substrate specificities and pH sensitivity. Despite their somewhat differing optimal temperatures, both studies show that these enzymes have the same catalytic mechanism.

CONCLUSION

Due to urbanization and industrial expansion, the discharge of wastewater and the accumulation of contaminants in the environment has reached dangerous levels in recent years. These contaminants are poisonous and inflict significant harm to living beings. Extremophiles have consistently been investigated to be a great source of innovative extremozymes, which have a number of benefits over conventional chemical catalysts. Extremophile-derived enzymes, also known as extremozymes, have distinct molecular mechanisms for dealing with a variety of environmental extremes, including temperature extremes, acidic and increased metal concentrations, excessive salinity, and a basic pH. Extremozymes derived from microorganisms have been harnessed to biodegrade heavy metals, toxins, dyes, aromatic compounds, and plastics, among other contaminants. They disintegrate huge quantities of pollution quickly by using pollutants as feedstock. Extremozymes have biocatalytic activity in a specific substrate in challenging environments and have a long shelf life. Extremozymes are known for their consistency, repeatability, and higher yields under adverse conditions. Their ability to generate the least harmful and biodegradable industrial waste has pushed their use in a variety of products and processes. This review critically examines various aspects of extremozymes and their strategic protein structure adaptations for survival of extremophilic bacteria in various environmental conditions, as well as potential pollutant biodegradation during bioremediation for various industrial wastewater treatment.

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