Review Paper:

Role of extremozymes in bioremediation

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Abstract

The extremozymes are the enzymes from extremophilic microorganisms. The extremophilic organisms can survive under extreme conditions. These groups of microbes have ability to degrade xenobiotics like pesticides and remove heavy metals from different water environment. The wastewater released from various industries is much polluted and needs to be treated before disposal to the landfills or any other water bodies. Also, it is necessary that the waste water meets the environmental standards. The enzyme-based technology is gaining wide interest and enzymes from the extremophilic microorganisms have many advantages.

The review mentions the extremophilic microorganisms along with their classification and diversity of extremophiles in different regions of India. The role of extremozymes viz. cellulases, laccases, dioxygenases, monooxygenases, peroxidases, lipases, esterases, nitrilase in bioremediation and degradation of various compounds are mainly focused in the review. The molecular mechanisms of extremophiles for adaptation to extreme conditions and role of extremozymes in biomining are also described in the review. The extremozymes in biomining (bioleaching) will explore the availability of precious metals which will have industrial applications.

Keywords: Enzymes, Wastewater, Pollution, Environment, Biocatalysts, Landfills.

Introduction

Various pollutants are present in the industrial wastewaters due to which industries makes the wastewater contaminated. The industries like coal conversion, petroleum refining, resins and plastics, textiles, oil milling, tanning, mining, pulp and paper contribute to the pollution of industrial wastewater. These pollutants must be removed before the wastewater is discharged to the landfills or any other water bodies and also it is necessary to achieve the required environmental standards. The various physical and chemical techniques used to remove the pollutants from wastewater have disadvantages viz. high cost, formation of toxic by-products, removal efficiency is not good etc. Therefore, there is need for alternative approach to remove the pollutants from wastewater and therefore, enzyme-based approach is gaining popularity in this aspect. Since pollution by organic compounds and heavy metals etc. is harmful to all the living beings and environment, research is therefore necessary in this aspect to study for minimization of pollution of water and also soil[91].

The bacteria and fungi along with their products like enzymes help in bioremediation[96]. The enzymes are able to degrade variety of recalcitrant compounds under *in-vivo* conditions which involve the sorption and complexing of enzymes in soil. The main objective of the review paper here is to focus on the role of extremozymes from extremophilic microorganisms and their role in the bioremediation and biodegradation of various toxic compounds and pesticides.

Extremophilic microorganisms and their classification

The extremophiles are the microorganisms which live in the extreme environmental conditions. Based on their ability to adapt various environmental conditions, the extremophilic microorganisms are classified as acidophiles, alkaliphiles, endoliths, thermophile, hyperthermophiles, hypolith, metalotolerant, oligotrophs, piezophiles, psychrophiles, radioresistant, toxitolerant and xerophiles[15]. The diversity of extremophiles in different regions is shown in table 1. The classification of extremophiles is shown in figure 1.

Acidophiles are the microorganisms at pH below 3.0. Alkaliphiles are the microbes which live in the alkaline conditions (pH 9-11)[26]. Endoliths are the organisms which live inside the rock, coral and animal shell. Hyper thermophiles are the organisms that can grow at temperatures between 80-122 °C. The organisms which live beneath the rocks in cold deserts are known as hypolith. The organisms which can tolerate high levels of toxic metals are known as metallotolerant. The organism which grows in nutritionally limited environment is known as oligotroph. The organisms which tolerate high hydrostatic pressure are known as piezophiles.

The organisms which grow at temperatures of about -10 to 20°C are known as psychrophiles[26]. They are the cold environment loving microorganisms and found mostly in the Arctic and Antarctic oceans. The characteristic feature of the enzymes from psychrophiles is correlation of high catalytic activity and low thermal stability at moderate temperatures. The microorganisms which can tolerate high levels of ionizing radiation are known as radio resistant. The organisms which can tolerate high levels of toxic compounds are toxitolerant. The organisms which live in extreme dry environment are called as xerophiles.

Table 1
Diversity of extremophiles in different regions of India

Isolation Site	Diversity	Category
Bakreshwar hot water spring, West Bengal	Temperature= 54 °C Firmicutes (65%) Temperature= 65 °C Synergistetes (27.24%), Firmicutes (96.10%), Proteobacteria (3.36%).	Thermophile[11]
Taptapani hot water spring, Orissa	*Bacillus* sp., *Exiguobacterium* sp., *Alcaligenes faecalis*, *Aeromonas veronii* and *Stenotrophomonas maltophilia*, *Aspergillus tubingensis*, *Coriolopsis polyzona* and *Trichoderma* sp.	Thermo- alkalophilic, amylolytic[84]
Taptapani hot water spring, Orissa	Proteobacteria (45.17%), Bacteriodetes (23.43%), Cyanobacteria (10.48%)	Thermotolerant[75]
Manikaran hot water spring, Himachal Pradesh	*Bacillus arsenicus* NBM47, *Bacillus mycoides* NBM19, *Bacillus pumilus* NBM31, *Bacillus subtilis* NBM48, *Bacillus thermoamylovorans* NBM38, *Geobacillus* sp. NBM49, *Paenibacillus glycanilyticus* NBM30, *Paenibacillus thiaminolyticus* NBM71, *Planococcus* sp. NBM37, *Thermonema lapsum* NBM28	Thermophilic protease, amylase, xylanase and cellulose producers.[74]
Manikaran hot water spring soil, Himachal Pradesh	Proteobacteria (45%) Actinobacteria (36%), Cyanaobacteria (5%), *Verrucomicrobia* (2%), *Bacteriodetes* (3%), *Chloroflexi* (2%), Acidobacteria (2%), *Chlorobi* (1%), *Deinococcus* (1%), *Rhodothermeota* (1%)	Thermo- alkalophilic[39]
Unkeshwar hot springs	1. Proteobacteria a.α-Proteobacteria *Rhizobiales* (17.62%) *Rhodospirillales* (5.7%) *Caulobacterales* (25.5%) *Rhodobacterales* (0.66%) *Sphingomonadales* (1.32%) b. β-Proteobacteria *Burkholderiales* (8.14%) *Rhodocyclales* (0.22%) c. γ-Proteobacteria Pseudomonadales (3.52%) *Enterobacterales* (1.54%) *2.* Actinobacteria *Actinomycetales* (1.98%) *Micrococcales* (4.40%) *Corynebacteriales* (1.32%) *Micromonosporales* (1.10%) *Propionibacteriales* (0.88%) *Streptomycetales* (0.20%) *3.* Firmicutes (42.95%) 4. *Deinococcus–Thermus* (0.66%)	Thermophilic, cellulase, xylanase, amylase and protease producers and heavy metal tolerance (chromium, arsenic) potential[53]
Lasundra, Gujarat	Abundant Prokaryotic phyla: Firmicutes (95.50%) Proteobacteria (2 %) Actinobacteria (0.80%) Bacteroidetes (0.1%) Cyanobacteria (0.10%) Euryarchaeota (0.09%) Dominant bacterial families: Bacillaceae (90.1%) Paenibacillacea (1.3%), Clostridiaceae (0.8%), Listeriaceae (0.5%) Staphylococcaceae (0.5%)	Thermophilic lipase[48]

Tuwa hot spring, Gujarat	Firmicutes (97.0%), Proteobacteria (1.3%) and Actinobacteria (0.4%). Euryarchaeota (0.05) was abundant archea	Thermophilic microorganisms carrying genes for stress response and aromatic compound degradation potential.[49]

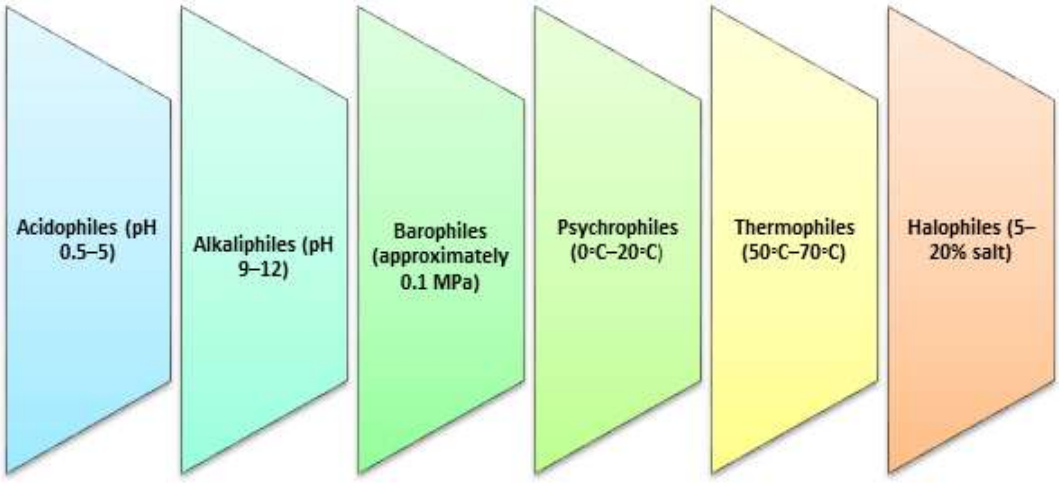

Figure 1: Classification of the extremophiles.

Halophiles are the organisms which can grow in very high salt concentration. Halophiles from the archaeal domain are the main source of extremely halophilic enzymes. Halophiles encapsulated in reverse-micelles can be used in bioremediation to eliminate toxic chemicals and other hazardous wastes[83]. The extremophilic sites and extreme sites in India are represented in figure 2.

Extremophilic microorganisms have many biotechnological applications and therefore, the research for extremophilic microorganisms has increased. Enzymes isolated from extremophilic microbes play important role as metabolic catalysts[1]. Due to growth of biotechnology, the enzymes have gained interest to reach biobased economy[70,81]. The importance of enzymes from extremophiles in many processes and industries has been investigated long before[35,61,86].

Molecular mechanisms of extremophiles for adaptation to extreme conditions: The molecular mechanisms of extremophiles for their adaptation to extreme conditions are as follows:

Acidophiles - (i) Potassium antiporter releases protons towards the extracellular medium (ii) ATP synthase (iii) Chaperones.

Thermophiles - (i) Upregulated glycolysis proteins (ii) Lipids with iso-branched chain fatty acids and long chain dicarboxylic fatty acids.

Halophiles - (I) High salt-in strategy: (i) chloride transporters (ii) potassium uptake into the cells by the action of bacteriorhodopsin and ATP synthase. (II) Low-salt strategy: (i) *de novo* synthesis.

Psychrophiles - (i) high degree of unsaturated, cyclopropane containing fatty acids and short chain fatty acids (ii) Cold shock proteins (CSP) (iii) Chaperones (iv) Anti-freeze proteins (AFP) which stops the ice growth on protein surfaces

Alkaliphiles - (i) Electrochemical gradient of Na^+ and H^+ by antiporters for proton accumulation (ii) Na^+- solute uptake system (iii) Cytochrome c-552 enhances terminal oxidation function by electron and H^+ accumulation.

Industrial enzyme production and market of enzymes: The large scale production of enzymes is possible with extremophilic microbes. These microbes have the ability to survive up to a high temperature during fermentation. The market of industrial enzymes is nearly US$ 7,100 million with a yearly progression rate of 8%[18]. The extremophilic microorganisms produce enzymes with industrial applications showing good activity at low temperatures[51]. The extremozymes act as biocatalysts and are active under extreme environmental conditions.

The microorganism-derived enzymes will dominate the market by 2024. The microbial enzymes have immense industrial demand because they are easily available and the production cost is also less.

Advantages of enzyme-based technology in bioremediation

The enzymes from extremophilic microorganisms have gained wide application due to their stability and biodegradation ability. One of the most important biotechnological applications is in the bioremediation of various pollutants and toxic compounds from water and sediments[9,33,59] and extremozymes from halophilic

microorganisms have found application in bioremediation[20]. These microbes are advantageous to remove pollution from adverse environmental conditions. The cell wall, capsule, S-layer proteins, extracellular polymer substances (EPS) and siderophores structural and functional properties present make extremophilic microorganisms potent for metal biosorption under extreme environmental stress.

Enzymes have main advantages as high degree of specificity, highly stable under high temperatures, pH, salt and metal concentrations etc. and also high reaction velocity which reduces the cost of processing. The enzymes have capacity to act under different environmental conditions, have catalytic power and also the enzymes can reach substrates in pores with small dimensions[69]. The enzymes speed the chemical reactions by lowering the activation energy. The advantages of extremophilic enzymes are viz. carry reactions at high temperatures, strong to many organic solvents and can be over-expressed in suitable host-vector.

Due to selectivity and versatility properties, the extremozymes have diverse applications in the treatment of wastewater i.e. bioremediation[60]. The extremozymes include enzymes viz. amylases, pullulanases, xylanases, proteases, cellulases, proteases, pectinases, keratinases, lipases, esterases, catalases, peroxidases and phytases[82]. The study of extremozymes and their application in bioremediation has emerged in the recent years. The extremozymes as green chemistry approach are shown in figure 3.

The efficiency of extremozymes is improved through genetic and chemical modification, immobilization strategies which increase their activity and stability for use in different industries. The extremozymes and their purification, K_m and V_{max} are shown in table 2.

Various extremozymes useful in the bioremediation

The extremozymes used to degrade various compounds are represented in table 3.

Pectinases: Fruits are rich in the pectin polysaccharide due to which waste water originated from fruit juice industry contains high concentration of pectin as a by-product which affects treatment due to production of methane.

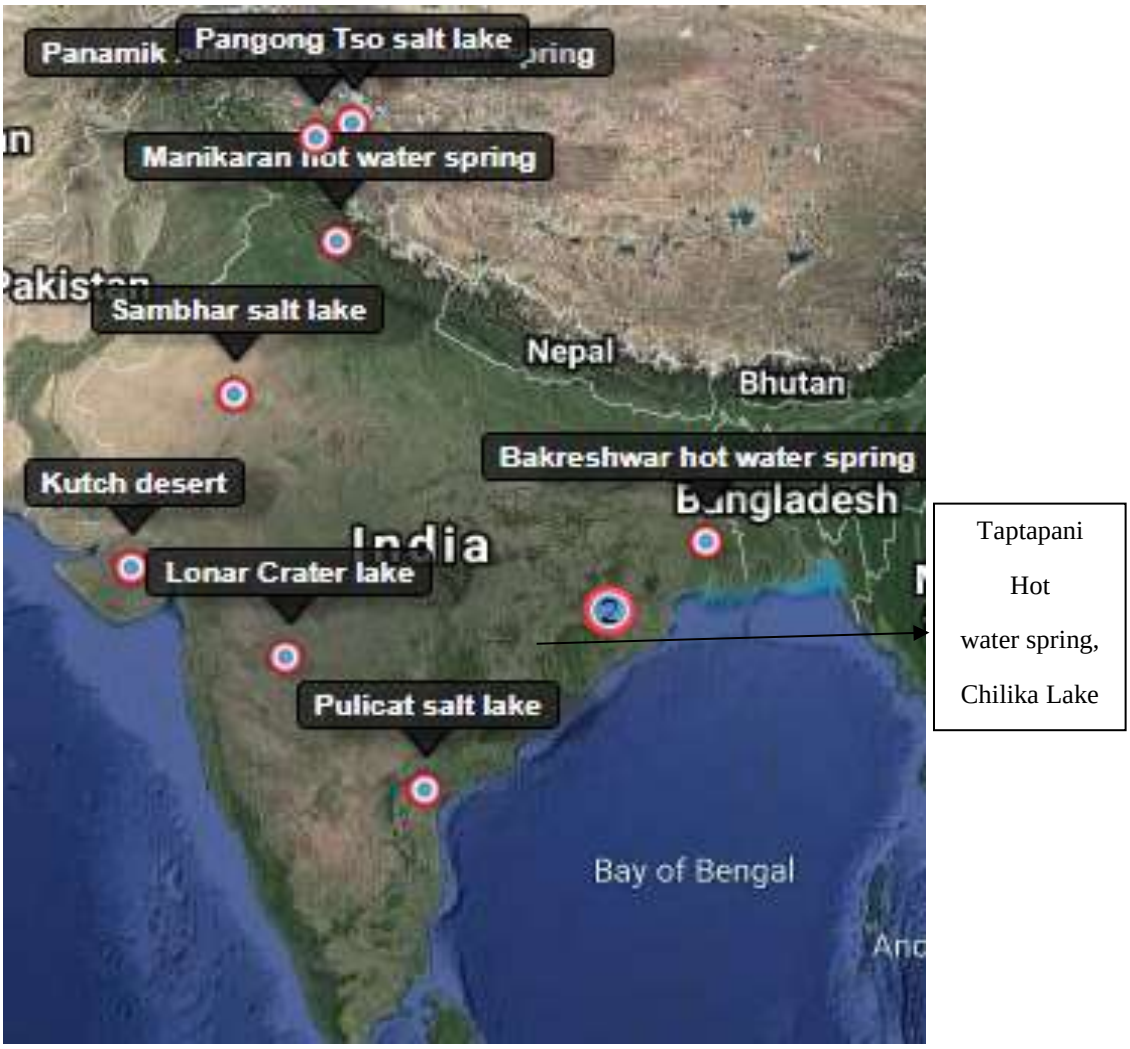

Figure 2: Extreme sites in India.

Table 2
Extremozymes, their purification, K_M and V_{max}

Microorganism	Gene	Molecular weight	Purification	Km	Vmax
Archaeon					
Nitrilase					
Pyrococcus sp. M24D13	NitM24D13	37-38.5 KD	Step I: Preparation of Cell-Free Extract Step II: Ammonium Sulfate (55%) Fractionation Step III: Hydrophobic Interaction Chromatography Using Octyl Sepharose column Step IV: Size Exclusion Chromatography Using Superdex-200 Step V: Ion Exchange Chromatography Using Q-HiTrap HP anion exchange column[17]	0.3 mM for benzonitrile substrate	333.3 µM min-1 for benzonitrile substrate
Chitinase					
Thermococcus chitonophagus	Chi70	70KD	Step I: Pretreatment of cells Step II: Ammonium Sulfate (40-80%) Fractionation Step III: Hydrophobic Interaction Chromatography Using butyl-TSK-NPR, HR 5/5 column (TosoHaas) column Step IV: Anion Exchange Chromatography Using Mono Q, HR 5/5 (Pharmacia) column[3]	1.3 (mg/ml) for colloidal chitin substrate	0.37 (mg product/ min-mg protein) for Pure colloidal chitin
Amylase					
Haloferax mediterranei	-	58 kDa	Step I: Medium supernatant Step II: Hydroxylapatite Chromatography Step III: Affinity chromatography using Sepharose-4B Step IV: Ion exchange chromatography using DEAE Cellulose Step V: Gel filtration chromatography using Sephadex-G50[65]	-	-
Laccase					
Haloferax volcani	LccA	63 kDa	Step I: Cell free extract Step II: Ethanol precipitation Step III: Anion exchange chromatography using MonoQ 5/5 (Pharmacia) column Step IV: Size exclusion chromatography using Superdex 200 HR 10/30 column (Pharmacia)[90]	670 µM for 2,2,-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)	-

Table 3
Extremozymes used to degrade various compounds

Enzymes	Compounds
Laccase	Anthracene and benopyrene[19]
	2,4,6 trinitrotoluene[93]
	4-amino-2, 6-dinitrotoluene[93]
	2,4, dicholorphenol[89]
	Pentachlorophenol[89]
	2,6 dimethoxyphenol[31]
	4-hydroxybiphenyl[40]
	Diketonitrile[56]
	Delor 106 polychlorinated biphenyls[62]
Manganese peroxidase	2,4,6 trinitrotoluene[10]
	Delor 106 polychlorinated biphenyls[62]
Lipase peroxidase	Amino dinitrotoluenes[10]
	Delor 106 polychlorinated biphenyls[62]
Arylsulfotransferase	Phenols[57]
l-Haloacid Dehalogenase	2-halo-carboxylic acids[46]

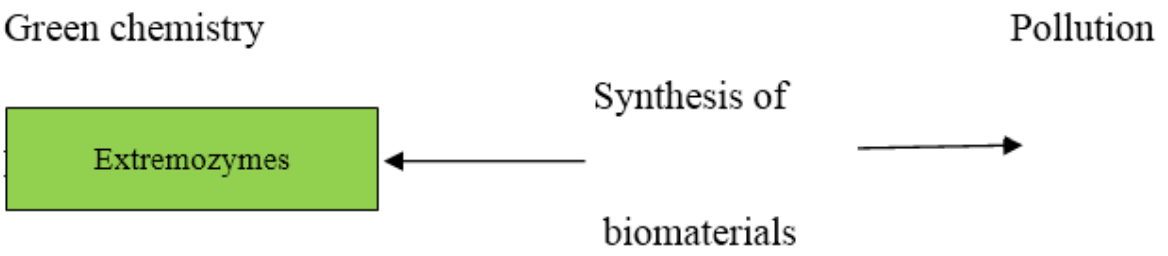

Figure 3: Representation showing extremozymes as green chemistry approach

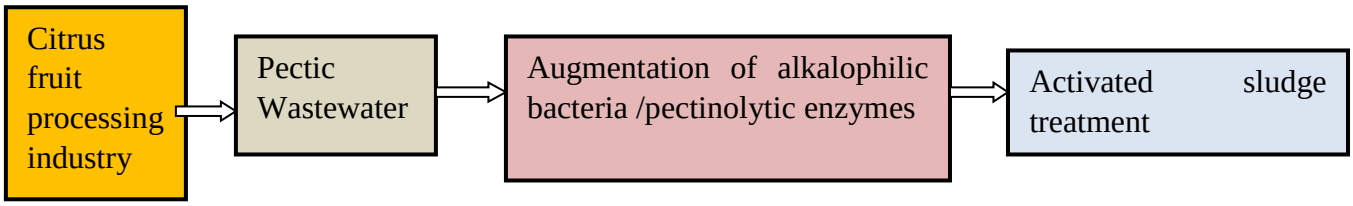

Figure 4: Schematic representation of application of alkalophilic pectinolytic microorganisms or pectinase in wastewater treatment

Hence, pectinolytic enzymes treated waste water make feasible activated sludge treatment[28]. Alkalophilic *Bacillus* spp. has been reported for the pretreatment of pectinacious wastewater before discharge into activated sludge tank[88]. The application of alkalophilic pectinolytic microorganisms or pectinase in wastewater treatment is shown in figure 4.

Cellulases: The three groups of cellulases are viz. endoglucanase which attacks low crystallinity in the cellulose fiber and creates free chain ends; exoglucanase or cellobiohydrolase which degrades the cellulose by removing cellobiose units from the free chain ends and β-glucosidase which hydrolyzes cellobiose to glucose units[37]. The enzyme cellulase Puradax HA produced from alkaliphilic organism *Bacillus* sp. has been reported for the removal of stains and color preservatives in textiles. Indigo dye has application in textile industry which releases in the water bodies from textile effluent. The cellulase enzyme from alkaliphilic bacteria plays a role in the reduction and decolorization of indigo dye.

Laccases: Laccase is a benzenediol:oxygen oxidoreductase, multi copper enzyme. The extremozyme laccases are reported in the degradation of PAHs[54,72]. The high redox potential laccases occur in white rot fungi[13,29] and low redox potential laccases occur in molds. Laccases require oxygen as a co-substrate. During the process of oxidizing of substrate, laccases reduce oxygen to water[40,34]. The enzyme laccases produced by microorganisms catalyze the oxidation of phenol and phenolic compounds, polyamines, lignins and aryl diamines[47,89].

Laccases decarboxylate these compounds and attack their methoxyl groups. These enzymes also depolymerize lignin. Laccases have great applications in bioremediation due to their high stability in extracellular fluids. Polycyclic aromatic hydrocarbons (PAHs) are mutagenic and carcinogenic chemicals. PAHs are distributed in the environment due to incomplete combustion of organic matter, emission sources and automobile exhaust[78]. There are reports on degradation of PAHs by laccases[78,96]. The enzyme dioxygenases oxidizes the aromatic ring which leads to degradation of PAHs and a complete biotransformation into CO_2 and water[96].

There is a report on degradation of PAHs by MnP *Irpex lacteus*[5] and *Pleurotus ostreatus*[68] which are white-rot fungi. Laccases can also oxidize xenobiotic and non-phenolic compounds. There is a report on biodegradation of a mixture of pentachlorophenol (PCP), 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP) and 2,4,6-trichlorophenol (2,4,6-TCP) using laccases from *Trametes pubescens*[24]. Azo dyes are the major group of dyes used in textile industries[87]. About 7 x 10^5 tons of dyestuff is produced annually and nearly 1.5 tons is released in wastewaters[71]. This dye is toxic to aquatic life and carcinogenic to human beings. Nearly 10-15% of azo dye is lost during dyeing process[63].

The laccases from extremophiles have application in bioremediation of azo dyes[23]. There is a report on degradation of azo dyes -Bezactiv Blue-S matrix 150 and Tubantin Brown GGL by laccase enzyme from *Pseudomonas resinovorans*, alkali-halotolerant bacteria. The laccases are also used in the degradation of dyes viz. remazol black-5, remazol blue-19 and remazol orange-16[92].

Microbial dioxygenases: Dioxygenases are enzymes which add molecular oxygen into their substrate. Dioxygenases enzyme has applications in bioremediation because they oxidize aromatic compounds. The dixoygenases enzyme degrades aromatic compounds and transforms into aliphatic products. The intradiol and extradiol cleaving enzymes utilize Fe (III) and Fe (II) respectively[37]. *Nocardioides* sp. strain KP7 was isolated from Kuwait beach. The dioxygenase causes degradation of phenanthrene and helps in detoxification action after an oil spill accident[76].

Monooxygenases: Monooxygenases act as biocatalysts in bioremediation because of stereo selectivity on various substrates. These enzymes use substrates as the reducing agent[4,14] and are used in degradation of aromatic and aliphatic compounds. Methane monooxygenase enzyme is mainly involved in the degradation of hydrocarbons.

Peroxidases: The peroxidases enzymes also have important application in bioremediation. The peroxidases enzymes are used in bioremediation of solids and waste waters polluted by hydrocarbons and oil[50]. Many phenolic compounds are oxidized by peroxidases enzymes. Lignin peroxidases (LiPs) and manganese peroxidases (MnPs) are important enzymes under the category peroxidases. The reactions include C_α-C_β cleavage, hydroxylation of benzylic methylene groups, oxidation of phenolic groups and benzyl alcohols[30]. LiPs catalyze the reactions in the presence of hydrogen peroxide[21]. Lignin peroxidase and manganese peroxidase degrade polychlorinated biphenyls (PCBs) compounds. MnP oxidizes Mn^{2+} to Mn^{3+}. These enzymes are also used in degradation of dyes viz. orange G, orange I, remazol brilliant blue R, amaranth, crystal violet and malachite green[58].

Lipases: Lipases are used in the biodegradation of waste water containing oil[85]. There is a report on lipase enzyme from *Moraxella* sp. isolated from Antartic sea water and also yeast strains for the degradation of oil[94]. The enzyme lipases are used in the treatment of saline wastewater and oilfield waste treatment. The lipases are reported to be used for the treatment of effluents from dairy and tannery industries which are rich in oil and proteins respectively. Lipases are used in the degradation of polyester waste and removal of biofilm which accumulates in cooling water units[85].

Esterases: The esterases belong to the hydrolase group and hydrolases ester containing compounds viz. carbamates, pyrethroids, organophosphates etc. The genes responsible for degrading cypermethrin, sulfosulfuron and fipronil are *estP*, *pytH*, *pye3* and *pytZ*[73]. The esterases extremozyme isolated from the thermophilic microorganism has been reported to be useful in the removal of xenobiotic and various other toxic compounds[95].

Nitrilase: Commercial application of nitrilases includes surface modification, waste treatment and biofactories for carboxylic acids synthesis[25]. Nitrilase acts on carbon-nitrogen triple bond converting nitrile compounds to carboxylic acid with the liberation of ammonia. Nitrilases act on plant nitrile compounds [organic compounds with cyano group (-CN) like cyanolipids, phenylacetonitrile, ricinine and cyanoglycosides][6]. The enzymatic pathway for hydrolysis of nitriles is represented in figure 5.

Catabolism of nitrile compounds occurs by two distinct pathways:

Nitrilases (EC3.5.5.1)
Nitriles……> Carboxylic acids+ ammonia (i)

Nitrile hydratases (NHases; EC 4.2.1.84) (ii)

Nitrilases hydrolyze carbon-nitrogen triple bonds and produce ammonia and carboxylic acid or amide product[42,98]. Nitrilases have been identified in several bacteria, fungi, yeast and plants[25]. Nitrilases are mainly used for amides and carboxylic acid production due to their efficiency and environmental friendliness.

As compared to the action mesophilic nitrilase, the activity of thermophilic nitrilase will be useful as the synthesis at elevated temperature has advantages like enhanced transfer rate, substrate solubility, viscosity reduction and less chance of contamination[22]. The thermophlic nitrilase producers are *Acidovorax facilis* 72W[12], *Bacillus pallidus* Dac 521, *Geobacillus pallidus* RAPc8 (NRRL: B-59396)[97] and hyperthermophilic anaerobic archaeon *Pyrococcus* sp. M24D13. The cyanide-degrading nitrilase enzyme isolated from hyperthermophilic archaea *Pyrococcus* sp. M24D13 has been reported in bioremediation of cyanide[17].

Tyrosinases: Industries dealing with coal conversion, plastic and resin manufacturing, petroleum refining discharge wastewater which contains phenolic compounds[44]. Toxicity of phenol affects biological treatment process i.e. activated sludge process. Enzymatic based method has been found to be suitable since 1980s where researchers employed horseradish peroxidase to oxidize phenol[43]. β-Tyrosinase extracted from thermophile *Symbiobacterium* sp. SMH-1 showed the potential of enzyme in bioconversion of phenol found in phenolic resin manufacturing industry to value-added product L-tyrosine[44].

Enzyme based technology for the purification of phenolic wastewater has been shown to be valuable technique as it leads to generation of valuable product with less cost of purification. The thermophilic tyrosinase or thermophilic tyrosinase producers for phenolic industrial wastewater is shown in figure 6.

Catalase: Strong oxidant, hydrogen peroxide is one of the commonly used chemical in textile and semiconductor industries. Hence, these industrial effluents contain higher concentration of H_2O_2 which needs to be removed from water to save flora and fauna. Chemicals are used to remove hydrogen peroxide before activated sludge treatment. Augmentation of catalase or catalase producers can be the effective method for textile industrial wastewater[38].

Production of extremophilic enzymes by extremophiles: The production of extremophilic enzymes by extremophiles is shown in table 4.

Role of extremozymes in biomining: The acidophiles viz. *Acidithiobacillus*, *Ferroplasma*, *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* are used to mine metals gold, silver, copper, zinc, nickel and uranium. The enzymes of extremophiles have applications in the mining process[66].

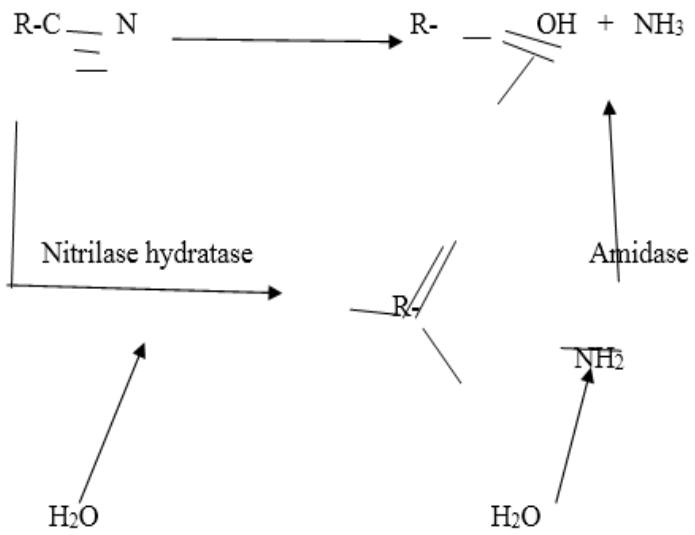

Figure 5: Enzymatic pathway for hydrolysis of nitriles

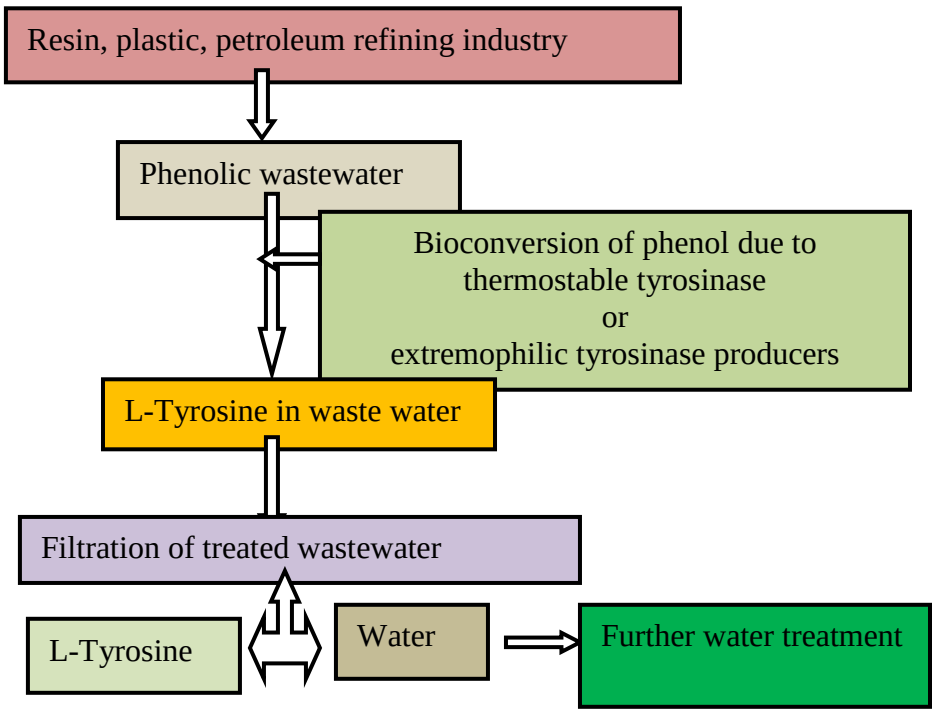

Figure 6: Schematic representation of treatment of thermophilic tyrosinase or thermophilic tyrosinase producers for phenolic industrial wastewater.

The process is called bioleaching which is eco-friendly to extract the metals. The extremophiles in biomining are shown in figure 7. Bioleaching is preferably used for the extraction of base metals while biooxidation is for the pretreatment of minerals which contain gold and silver[27]. Researchers are looking for thermophilic microorganisms for the extraction of metal sulphides from chalcopyrites which are copper iron sulfide mineral.[32]

Pesticide degrading microbes as extremophiles: Pesticides are used widely in agricultural sectors to reduce the pest with crops. But due to their harmful effects on living beings, it is necessary to remove the toxic pesticides from environment. To reduce the biomagnification, effects of these toxic pesticides and microbial based approaches are popular. Individual microbial cells or mixed cultures are used for degradation of pesticides from agricultural fields or other environments. Microorganisms produce the enzymes that play important role in the biodegradation of pesticides. There is broad category of pesticides used globally.

Carbamates, organophosphates and pyrethroids are the pesticides which belong to the ester bond containing pesticides. Microbial esterases have the potential to degrade these ester containing pesticides. Carbamates and pyrethroids are apolar compounds (lipophilic xenobiotics) and may accumulate in fatty tissues.

Table 4
Production of extremophilic enzymes by extremophiles

Enzymes	Enzymes produced by		
	Thermoalkaphiles/ alkaphiles	**Halophiles**	**Psychrophiles**
Amylase	*Alkalimonas amylolytica*	*Halothermothrix orenii*	*Bacillus*
	Bacillus subtilis	*Bacillus dipsosauri*	*Clostridium*
	Bacillus sp. KSM K-38	*Halobacillus* sp. strain MA-2	*Alteromonas haloplanctis*[2]
		Haloferax mediterranei	
Lipase	*Pseudomonas* sp. LBA34	*Marinobacter lipolyticus* SM19[64]	*Psychrobacter okhotskensis* sp. nov
	Halomonas sp. LBB1	*Salicola* sp. IC10[55]	
Protease	*Bacillus pumilus*	*Pseudoalteromonas ruthenica* CP76[79]	*Pseudomonas* strain DY-A
	Arthrobacter ramosus	*Halobacillus karajensis*[36]	*Sporosarcina aquimarina*[80]
	Bacillus alcalophilus	*Halorubrum ezzemoulense* ETR14[16]	*Algoriphagus antarcticus*[80]
	Nocardiopsis sp.	*Salicola* sp. IC10[55]	*Janthinobacterium lividum*[41]
Xylanase	*Bacillus firmus*	*Halorhabdus utahensis*	*Pseudoalteromonas haloplanktis*
Cellulase	*Geobacillus* sp. HTA426[67]	*Haloarcula* sp. LLSG7[45]	*Pseudoalteromonas haloplanktis*
Pectinase	*Thermoascus aurantiacus*[52]	*Streptomyces coelicoflavus* GIAL86[77]	*Cryptococcus cylindricus*
	Anoxybacillus sp.		*Mrakia frigida*
			Cystofilobasidium capitatum

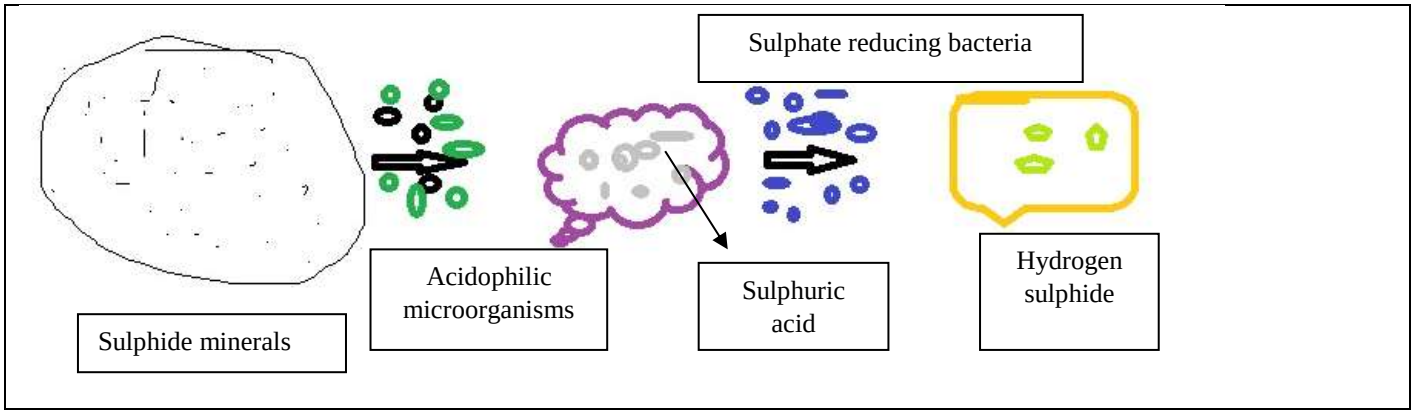

Figure 7: Extremophiles in biomining

Hydrolysis increases the water solubility of these compounds by converting them into alcohol and carboxylic acid. Extremophilic enzymes from the microbes can be used for bioremediation of these pesticides from environment. It was reported previously that various groups of microbes have the ability to degrade the pesticides into non-toxic and environmentally accepted form[7]. The pesticide induced gene expression pattern was also reported with microorganisms from agricultural fields[8]. The enzymes from the cold regions microbes are used as bioremediation and the whole microbial cell can be used for psychrophilic biofertilizer.

Conclusion

The extremozymes have immense applications in bioremediation and biodegradation of many toxic compounds, pesticides and other wastes. The enzyme-based technology can be used for the treatment of soil, wastewater and other wastes coming from various industrial processes.

The use of extremozymes for bioremediation and biodegradation of various toxic compounds and pesticides will be eco-friendly and fast and this will help to achieve environmental standards before the wastewater is discharged to the landfills or other waterbodies and also will ultimately help to reduce the pollution.

Also, the role of extremozymes in biomining process (bioleaching) will explore the availability of precious which will have many interesting industrial applications. The extremophilic microorganisms are sustainable sources which can be used for biotechnological aspects, thus leading to a bio-based economy.

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