

MINI-REVIEW ARTICLE

Exploitation of Potential Extremophiles for Bioremediation of Xenobiotics Compounds: A Biotechnological Approach

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Abstract: Microorganisms that are capable of live and adapt in hostile habitats of different environmental factors such as extremes temperature, salinity, nutrient availability and pressure are known as extremophiles. Exposure to xenobiotic compounds is global concern influencing the world population as a health hazard. Hence their removal is warranted using biological means that is very sustainable, potentially cost-effective and eco-friendly. Due to adaptation in extreme environments and unique defense mechanisms, they are receiving more attention for the bioremediation of the xenobiotic compounds. They possess robust enzymatic and biocatalytic systems that make them suitable for the effective removal of pollutants from the contaminated environment. Additionally, the extremophiles act as microfactories having specific genetic and biotechnological potential for the production of biomolecules. This mini review will provide an overview of microbial degradation metabolic pathways for bioremediation along with the molecular and physiological properties of diverse extremophiles from variety of habitats. Furthermore, the factors affecting the bioremediation process is also summarized.

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1. INTRODUCTION

Microorganisms are cosmopolitan and ubiquitous living entities on the planet Earth. The diversity of microorganisms is variable in different habitat conditions. As per reported estimates, only about 1% of the total microorganisms present on the Earth have been cultured in selected culture media and cultivation conditions. Nevertheless, there are various unexplored habitats where enormous diversity of organisms may be present [1, 2]. From pertinent literature survey, it was observed that extremophilic microorganisms are currently less explored groups that have immense potential to thrive in extreme environmental conditions which were previously thought to be adverse for life. These microbes are found in hostile habitats of environmental variables such as extreme temperature, salinity, nutrient availability and pressure, *etc.* Extremophiles include psychrophiles that can grow at 20 °C while, thermophiles have potential optimization to grow at >50° C, acidophiles and alkalophiles can grow at pH 3 and pH 10, respectively and halophiles grow at high salt concentrations in 2.0-5.2 M sodium chloride [3]. In the years 1980 and 90s, the most important step was taken by the earlier researchers by analysing microbial community dynamics by using DNA sequencing for the exploration of uncultured microbes and their phylogenetic studies [4]. Culture based techniques limit the actual diversity of microbes from the

variety of habitats. Therefore, the metagenomic approach has been utilized by the researchers for comprehensive analysis of microbial dynamics in various habitats [5]. This technique can be used to isolate genomic DNA directly from the environmental samples and involve the PCR amplification 16S rRNA gene, cloning, DGGE, T-RFLP, *etc.* for the analyses of microbial diversity dynamics. In recent years the next generation sequencing (NGS) has been proven as a promising approach for analysing the diversity of microbes as well as elucidation of metabolic pathway. The NGS technologies provide high throughput, efficient and accurate studies of the whole genome of microbial community analysis. In the recent past, the NGS analysis of hot springs habitat in Central India revealed the presence of a variety of hydrocarbon degrading thermophiles and the pathways essential for bacterial survival under extreme environments [6]. Later, Rekadwad and Gonzalez [7] compiled the useful information on NGS and gene mining of extremophiles. Recently study uncovered the bacterial community structure analysis of hot spring soil using NGS of 16S rRNA gene. They investigated the 14 bacterial community phyla belonging to Aquificae, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Fusobacteria, Proteobacteria, Thermi, TM7, *etc.* [5]. Genomics and proteomics are linked together. Genomics approach depicts the structural information of microbial data from the various environmental components. Proteomics is the term used to describe the studies of the global protein complements of an individual, tissue or community and also deduce the expression of genes complements [8]. Few studies have been reported for the proteomics of extremophiles as well as the proteomics adaptation of extremophiles [8, 9].

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Xenobiotics are chemical substances of foreign origin. Chemical compounds such as polychlorinated biphenyls, polyaromatic hydrocarbon (PAH), *etc.* are toxic, mutagenic and carcinogenic in nature [10]. Their release and accumulation into the environment create various health hazards to human beings [11]. Due to the persistent nature and toxicity of various xenobiotic compounds in the environment, development of effective, eco-friendly and economical methods for its remediation has now become a prime concern for researchers. However, several physico-chemical methods such as pump and treat, air stripping, active carbon adsorption are utilized for the removal of aliphatic chlorinated compounds (trichloroethylene) and aromatic compounds (toluene, xylene, benzene, PAH, *etc.*). Yet, these processes are tedious, time-consuming, costly and generate secondary pollutants [12]. Hence, bioremediation is an attractive alternative for the removal of xenobiotics compounds using extremophiles because of low cost and eco-friendly in nature. They possess robust enzymatic and whole-cell biocatalytic systems that made them useful for adaptations in their genetic and metabolic machinery to thrive in extreme conditions. Therefore, it is interesting to review some potential new applications of extremophiles and their products, including enzymes, in bioremediation.

The biodegradation of xenobiotic compounds by extremophilic bacteria has received little attention. However, the literature survey suggests that extremophilic microorganisms possess robust enzymatic and catabolic versatility compared to other microorganisms hence their potential exploitation could be useful for the removal of xenobiotic compounds from contaminated environment. In this mini review article, an effort has been made to summarize and critically assess the recently published information on the biodegradation of xenobiotic compounds by extremophiles.

2. BIOTECHNOLOGICAL APPLICATIONS OF EXTREMOPHILES

2.1. Metabolic Pathway and Metabolic Engineering of Extremophiles

It is reported that available data on pollutant degradation by extremophiles thrive in extreme cold, high temperature and pressure is phenomenological, and scarce information is available in the literature on the metabolic mechanisms and enzymatic system involved in the pollutant degradation. However, Le Borgne *et al.*, [13] suggested the mechanisms of xenobiotic degradation by halophilic bacteria present in extreme saline environments in elaborated way. They discussed the biodegradation pathway of aliphatic and aromatic compounds by halophilic microbes and information regarding utilization of microbial enzymes and catabolic genes involvement of pollutant degradation. Earlier researchers have reported that the *Marinobacter* strain NCE312, isolated from a naphthalene-degrading culture inoculated from creosote-contaminated marine sediment. It has shown *Pseudomonas*-like naphthalene 1,2-dioxygenase [14]. This strain could grow on naphthalene as carbon and energy source produces yellow products from the *meta* ring cleavage of catechol *via* the action of a catechol 2,3-dioxygenase. Naphthalene 1,2-dioxygenase and the catechol 2,3-dioxygenase are the enzymes involved in the catabolism of naphthalene. The

phylogenetic analysis showed that this dioxygenase in the strain NCE312 was related to naphthalene 1,2-dioxygenase from *Pseudomonas*, *Burkholderia* and *Neptunomonas*. This may be due to the horizontal gene transfer. Peeples [15] describes the potential role of extremophiles for biodegradation of pollutants. They described the role of various exterozymes such as phosphotriesterase-like lactonase for degradation of organophosphate and phenol oxidase for degradation of phenolic compounds. Fig. (1) represents the isolation mechanism and application of omics tools in extremophiles for gaining desired products (enzymes, biofilms, siderophores, nanoparticles, *etc.*) for bioremediation purpose. For example, biogenic NP (Uraninite) synthesised by *Geobacter metallireducens* upon extracellular U(VI) reduction is worthy for uranium *in situ* remediation [16].

The mechanisms of degradation by extremophiles can be enhanced through the recombinant DNA technology with insertion of catabolic genes from the bacterium having high degradation potential [17]. Parrilli *et al.*, [18] investigated a psychrophilic bacterium for the bioremediation of aromatic compounds through recombinant DNA technology. They constructed a recombinant strain of bacterium *Pseudoalteromonas haloplanktis* TAC125,1 having the potential to convert a wide spectrum of aromatic compounds into catechols. Further, they constructed the recombinant enzymatic activity with the endogenous *P. haloplanktis* TAC125 laccase-like activity and enhance the degradation of aromatic compounds.

Earlier researchers have genetically engineered to enhance the potential of *P. haloplanktis* TAC125 for the production of the aromatic oxidative activity encoded by toluene-*o*-xylene monooxygenase isolated from bacterium *Pseudomonas sp.* OX1 [19, 20]. They found that toluene-*o*-xylene monooxygenase enzyme is able to convert a broad range of aromatic compounds such as phenol, cresols and dimethylphenols [18]. Biodegradation potential of *Geobacter sp.* has been enhanced by enrichment in acetate and demonstrated successful uranium removal and the biostimulation of activity by acetate enrichment had enhanced uranium reduction and *in situ*, bioremediation [21].

2.2. Bioremediation of Hydrocarbons

It is well proven that microorganisms act as better biocatalysts for the removal of xenobiotic compounds from contaminated environment [22]. However, biodegradation is affected by various factors such as hydrophobic nature of hydrocarbon, bioavailability and prevailing environmental conditions. Microorganisms possess an inherent potential for changing the outer membrane and enhance the hydrophobicity of the cell surface in order to facilitate the uptake of hydrocarbons. It is reported that under high saline conditions, biodegradation of PAH is low hence, biodegradation rates also become slow [23]. Extremophiles such as halophilic bacteria produce huge amount of biosurfactant that enhances the biodegradation of petroleum hydrocarbon [24]. Table 1 summarizes the details of extremophilic microbes for the biodegradation of pollutants from various environments.

In recent past study, microbial community analysis was performed based on 16S rRNA gene from geyser-discharge water effluent. The study revealed that bacterial genera such

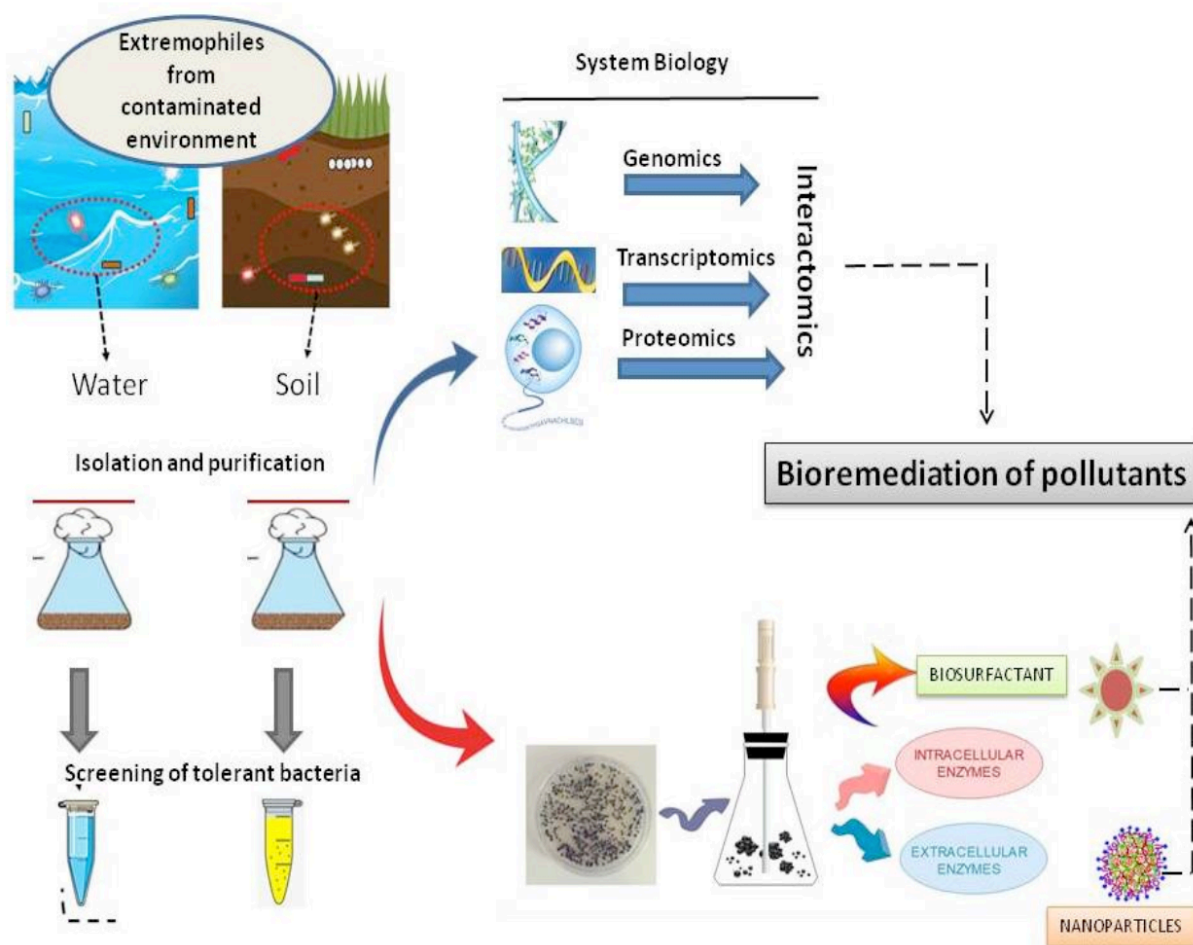


Fig. (1). Isolation of extremophiles and their application in bioremediation. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

Table 1. Utilisation of extremophiles for biodegradation of pollutants.

S. No.	Pollutants	Microbial Species/Strain	References
1.	Crude oil	<i>Streptomyces albiacialis</i> grow at 30% of crude oil, NaCl: 3–10%	[25]
2.	Benzoic acid, cinamic acid and 3-phenylpropionic acid	<i>Haloferax</i> sp. strain D1227	[26]
3.	Uranium	<i>Shewanella putrefaciens MR-1</i>	[27]
4.	Paraffin and other petroleum derivatives	<i>Dietzia maris</i> , NaCl: 10%	[28]
5.	Petroleum hydrocarbons	Bacterial consortia, <i>Marinobacter</i> spp., <i>Erwinia ananas</i> and <i>Bacillus</i> spp., NaCl: 0-22 % NaCl	[29, 30]
6.	4-hydroxybenzoic Acid	<i>Haloarcula</i> sp. D1	[31]
7.	Diesel	<i>Cellulomonas</i> spp., <i>Bacillus marisflavi</i> , <i>Dietzia maris</i> and <i>Halomonas eurihalina</i> , NaCl: 0 to 17.5%	[32]

(Table 1) contd....

S. No.	Pollutants	Microbial Species/Strain	References
8.	Phenol and catechol	<i>Halomonas</i> <i>Campisalis</i> , NaCl: 0-150 g/l	[33]
9.	Benzoic, <i>p</i> -hydroxybenzoic, cinnamic, salicylic, phenylacetic, phenylpropionic, <i>p</i> -coumaric, ferulic and <i>p</i> -aminosalicylic acids	<i>Halomonas organivorans</i>	[34]
10.	Hydrocarbon and organic pollutants	<i>Marinobacter</i> , <i>Chromohalobacter</i> , <i>Salinococcus</i> and <i>Halobacillus</i>	[35]
11.	Ferulic acid	<i>Halomonas elongate</i>	[36]
12.	Acetonitrile and propionitrile	<i>Natronocella acetinitrilica</i>	[37]
13.	Crude oil	<i>Ochrobactrum</i> sp.	[38]
14.	Fuel oil	<i>Acinetobacter</i> strain, Culture Collection (CC): JSC "Biocentras, Lithuania, NaCl: 35%	[39]
15.	Crude oil	<i>Fusarium</i> sp., NaCl: 35%	[40]
16.	Crude oil	<i>Phanerochaete chrysosporium</i> , Iranian Research Organization for Science and Technology (IROST)	[41]

Table 2. Exploitation of extremozymes for biodegradation.

S. No.	Microbes	Enzymes	Target Pollutants	References
1.	<i>Bacillus safensis</i> (CFA-06)	Oxidoreductase	Degradation of aromatic compounds	[47]
2.	<i>Thermus thermophilus</i> <i>HB27</i>	Laccase	Polyaromatic hydrocarbons	[15]
3.	<i>Geobacillus</i> <i>stearothermophilus</i>	Phosphotriesterase-like lactonase	Organophosphate	[15]
4.	<i>Nocardioides</i> sp. strain KP7	Dioxygenase	Polyaromatic compounds	[48]
5.	<i>Alcanivorax borkumensis</i> SK2T <i>Bacillus licheniformis</i> ATCC 14580T <i>Halomonas ventosae</i> AH12T <i>Idiomarina baltica</i> DSM 15154T	Alkane hydroxylases/Cytochrome P450	Alkanes	[49]
6.	<i>Pseudomonas stutzeri</i> DEH130 <i>Alcanivorax dieselolei</i> strain B-5	Haloalkane dehalogenases	Halogenated pollutants	[50]

as *Chloroflexi*, *Deinococcus-Thermus* and *Chlorobi*, etc. were the most prevalent microorganisms that reduced the arsenite concentration in the environment [42]. Other bacteria, such as *Geobacillus*, are also potential microbes capable of degrading organic pollutants [43]. Additional members of the same clade, such as *Geobacillus thermoleovorans* T80 degraded 70% of hexadecane at 60°C. Further, the study of Dennett and Blamey [44] isolated the hyperthermophilic archaea *Pyrococcus* sp. M24D13 and investigated as a novel thermostable cyanide-degrading nitrilase. This thermostable

enzyme may be useful to remediate cyanide contaminated waste streams [44].

2.3. Extremozymes

The exploitation of extremophilic microorganisms in industrial processes has grown rapidly over the last 2 decades. Various enzymes have been isolated and identified approximately more than 3,000 from the extremophiles. Out of them, majority have been used for biotechnological and

industrial applications. Extremozymes possess high stability and reduced risk of contamination of the organisms that produce them. These enzymes are categorized as polymer degrading enzymes such as amylases, proteases, cellulases, pullulanases, and xylanases [45]. Additionally, it is investigated that the extremozymes can act as good novel catalysts for industrial applications [46]. These enzymes have significant roles in chemical, food, pharmaceutical, paper, pulp, and waste-treatment industries [46]. Other important products of extremozymes are cyclodextrins, compatible solutes, and polyunsaturated fatty acids. Moreover, it is also reported that the extremozymes of marine origin have been proved to be useful tools for bioremediation applications. There are various sources of pollutants such as industrial wastes and contaminants, the mining activities for oil extraction or the accidental oil spills. All these activities are sources of several pollutants like hydrocarbons, polycyclic aromatic hydrocarbons, chlorinated hydrocarbons, pesticides, heavy metals, etc. The removal and detoxification of several pollutants can be achieved by means of extremozymes. Some extremozymes and their application in biodegradation process are listed in Table 2.

CONCLUSION AND FUTURE PROSPECTS

Though there have been plenty of reports on the bioremediation potential of fungi and bacteria from agroecosystem, in-depth assessment of the multifaceted role of extremophiles in bioremediation of xenobiotic compounds is lacking. Extremophilic microorganisms are largely unexplored groups that can thrive in extreme conditions than other mesophilic microbes. They are a source of extremozymes with a great variety of industrial applications due to their biodegradability and extreme stability. Therefore, application of extremozymes has made available a wide range of resistant biomolecules for industrial applications, such as cold-tolerant extremozymes, acid-tolerant extremozymes, alkali-tolerant extremozymes, and salt-tolerant extremozymes. These characteristic features of extremophiles offer new catalytic alternatives for the current scenario of climate change and the increasing level and diversity of pollutants. Therefore, innovative techniques are required to overcome bottlenecks related to uncultured extremophiles and obtaining the metabolites/enzymes from them at large scale.

Apart from Biotechnological potential, the ecological significance of extremophiles is of utmost importance in cracking black box of microbial adaptation under extreme conditions. Advancement in molecular tools and technologies, including NGS (Next Generation Sequencing) and NGP (Next Generation Proteomics) may play potential role in identifying and utilizing these amazing microbes in tackling issues such as restoration of polluted ecosystems, provide increased yields of degraded habitats, etc. Also, such studies and methods will reveal the mechanisms and strategies used by microbes to adapt to extreme conditions and are useful to understand the evolution of microorganisms subjected to extreme conditions. There is a huge diversity of microorganisms which are waiting to be explored, particularly from extreme habitats untouched by humans, hence many useful species and metabolites will need to be discovered and utilized for improving the quality of life. In a nutshell, extremophiles could be the incredible creatures that are going

to play a very crucial role in maintaining environmental sustainability.

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CONFLICT OF INTEREST

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