

# Extremophilic Microbes for Environmental Bioremediation: A Review

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**Abstract:** Bioremediation using extremophilic microbes has gained public attention due to their unique ability to thrive in various extreme environments through their natural biological process. Extremophilic microbes provide an effective, sustainable, and cost-efficient strategy to remediate toxic environmental pollutants under extreme conditions. Extremophilic microbes are categorized based on their capability to adapt and grow in diverse extreme environments, encompassing various microbes with distinct adaptive traits. Some of the extremophilic microbes include thermophiles, hyperthermophiles, psychrophiles, acidophiles, alkaliphiles, halophiles, piezophiles, metallotolerant, toxitolerant, radioresistant, and micro-aerophiles. Several bioremediation techniques include bioaugmentation, bioleaching, biosorption, bioprecipitation, bio-reduction, and many more. Bioaugmentation enhances natural biodegradation processes; bioleaching involves the oxidation of metal sulfides; biosorption focuses on metal adsorption onto biomass surfaces; bioprecipitation is the transformation of metal ions into solid precipitates; bio-reduction is the reduction of metal ions to less toxic or less soluble structures. Despite all the benefits of bioremediation using extremophilic microbes, it still has shortcomings and challenges, including complex maintenance, ethical concerns, and limited scalability, which require ongoing research to optimize their application in environmental pollution treatment. Further studies are needed to focus on understanding their ecology, gene expression, and metabolism to ensure sustainability and effectiveness on a global scale.

**Keywords:** extremophilic; microbes; environmental; bioremediation.

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## 1. Introduction

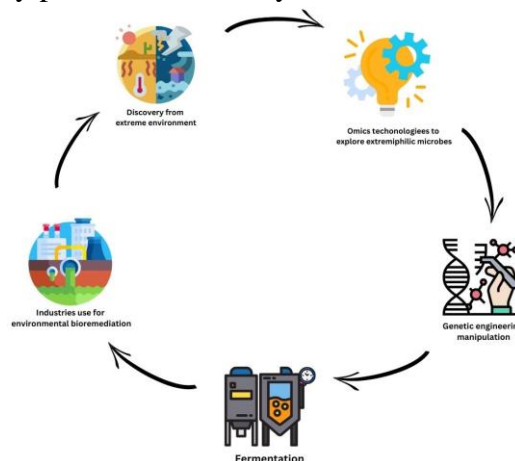
In recent decades, the rapid growth of industries has significantly threatened the environment due to the various toxic pollutants released and accumulated in the environment. These harmful substances could detrimentally affect human health and living organisms. Specifically, extremophilic microbes have gained significant attention due to their extraordinary ability to detoxify and restore polluted areas through their cellular metabolism under extreme conditions [1]. As a result, the incorporation of extremophilic microbes would significantly contribute to an effective and versatile environmental bioremediation solution. In addition, applying extremophilic microbes in environmental bioremediation offers lower-cost solutions. It decreases ecological impact as the naturally occurring extremophilic microbes can reduce the requirement for expensive physicochemical methods and potentially hazardous chemicals frequently used in conventional remediation processes [2]. With the unique

capability of extremophilic microbes to thrive and survive in extreme conditions, it promises to address environmental issues in many extreme environments on our planet.

The Earth is full of extreme environments, ranging from cold subzero arctic temperatures to the scorching heat at geothermal areas, the intense pressure under the deep ocean trenches, and even the highly acidic and alkaline environment. Despite the extreme conditions, scientists have found some microorganisms, known as extremophilic microbes, survive and thrive in this environment [3,4]. Extremophilic microbes can be found in various extreme environments, including hot springs, hydrothermal vents, saline lakes, acidic mine drainages, deep-sea floors, radiation-contaminated sites, and other extreme habitats [5]. These environments comprise extreme conditions of temperature, pressure, radiation level, pH, salinity, and toxic compounds present. In addition, low water availability, low nutrient concentration, low temperature, and pH are also part of the extremities [6,7]. These extreme environments are proven to be hostile and inhospitable for all life forms.

However, some microbial lives have evolved to withstand these harsh conditions throughout the decades [8]. These extremophilic microbes have modified their genetic and developed specialized metabolic systems to adapt to extreme environments. For example, acidophiles have developed unique proton-pumping mechanisms to regulate intracellular pH in acidic settings, while thermophiles have heat-stable enzymes that work best at high temperatures [9]. During the adaption process, extremophilic microbes have evolved to be immune to extreme conditions like toxic pollutants and could transform unstable toxic pollutants into stable and beneficial substances for their metabolic processes [1]. As a result, this unique ability of extremophilic microbes has attracted plenty of interest in different fields, especially in environmental bioremediation. Numerous studies have been undertaken to develop sustainable environmental bioremediation solutions employing the adaption mechanisms of extremophilic microbes [7].

Typically, extremophilic microbes are discovered within extreme environments and then cultivated in laboratory settings for thorough analysis using omics technologies. Researchers will explore the potential of enhancing their bioremediation efficiency by genetically modifying the microbes, intending to maximize their activities and other significant traits for eventual integration into industrial bioremediation applications [10]. This is because many common microbes cannot achieve the industrial requirements, such as enduring industrial requirements with high productivity in various temperature, pH, and aeration conditions [8]. Figure 1 shows a simple flow line of the application process of extremophilic microbes from the discovery process till industry uses [8].



**Figure 1.** Flow line sketch of extremophilic microbes' production for bioremediation.

## 2. Diversity and Distribution

The diversity of extremophilic microbes is an intriguing aspect of their potential application in environmental bioremediation, as they have unique adaptations to extreme environmental conditions. They cover all domains of life, including archaea, bacteria, and eukaryotes [11]. Extremophilic microbes can be categorized into various extremophiles based on their extreme condition's adaptations and distributions [12]. Some of the extremophilic microbes include thermophiles, hyperthermophiles, psychrophiles, acidophiles, alkaliphiles, halophiles, piezophiles, metallotolerant, toxitolerant, radioresistant, and micro-aerophiles [12] [13]. The summary of the diversity and distribution of different extremophilic microbes is shown in Table 1.

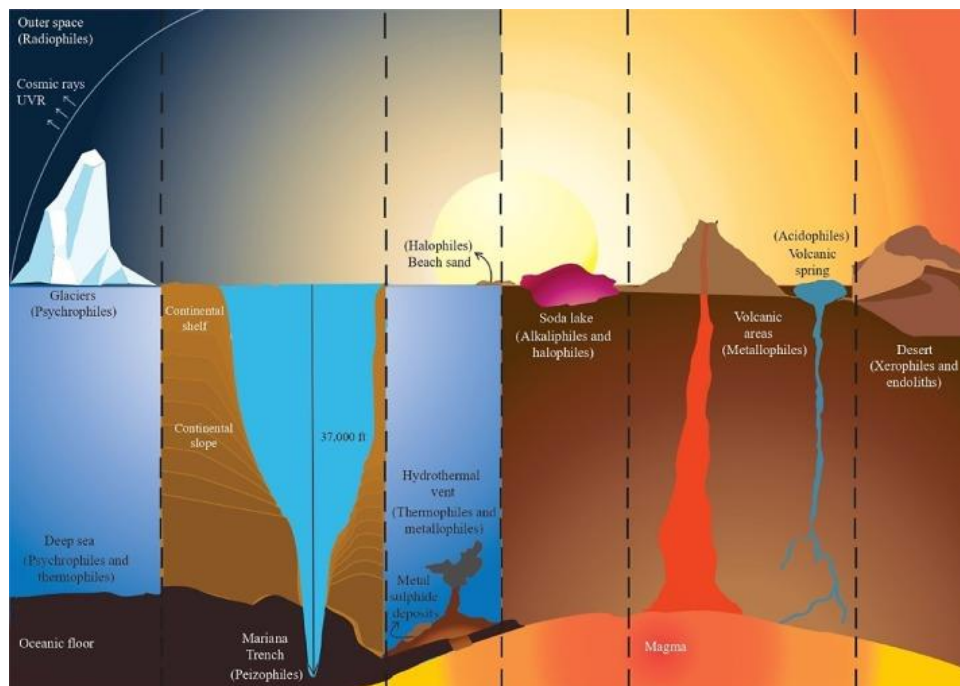
Thermophiles and hyperthermophiles are microbes that dominate high-temperature environments, and their optimum growth temperature is 60°C to 80°C and above 80°C, respectively [7, 14]. Usually, they are found in hot springs, mud pots, and volcanic environments [14]. Several studies on hot springs that have been found with thermophiles and hyperthermophiles are Ulu Slim hot spring (Malaysia) [15], Kirishima Natural Park (Japan) [16], and Domas and Cibuni (Indonesia) [17]. On the other hand, microorganisms that are capable of surviving and thriving at temperatures of -10°C to 20°C are known as psychrophiles [18]. Commonly, they are located in cold soils, glacial ice, cold ocean water, and alpine snowpacks such as the Arctic and Antarctic marine habitats [19].

Acidophiles are microorganisms that thrive in highly acidic conditions (pH 3 or below), whereas alkaliphiles are microorganisms that thrive in highly alkaline conditions (pH 9 or above) [20]. Acidophiles and alkaliphiles are typically found in acid mine drainage and soda lakes, respectively, primarily due to industrial discharges [20-22]. Halophiles are microbes that can withstand high salinity environments such as natural salt lakes, saltern pond brines, and hypersaline oceans [23]. The two most significant and well-researched hypersaline lakes are the Great Salt Lake in West America [24]. Piezophiles are microorganisms that can adapt to high hydrostatic pressure settings several times higher than at sea level [25]. They can be discovered at lithospheric sites, hydrothermal vents, and deep oceanic areas such as the deep waters of the Mediterranean and Sulu Seas [26, 27].

**Table 1.** Diversity and distribution of extremophilic microbes.

Category	Examples	Resistance	Distribution	Examples	References
Thermophile	<i>Geogemma barossii</i> <i>Pyrococcus sp.</i> <i>Pyrolobus fumarii</i>	High temperature, 60°C to 80°C	Hot springs, Mud pots, volcanic environments	– Ulu Slim Hot Spring (Malaysia). – Kirishima Natural Park (Japan). – Domas and Cibuni (Indonesia).	[7, 14-17]
Hyperthermophile	<i>Methanofollis tationis</i> <i>Thermotoga neapolitana</i>	High temperature, above 80°C			
Psychrophile	<i>Polaromonas vacuolate</i> <i>Psychrobacter cryopegellain</i> <i>Synechococcus lividus</i>	Low temperature, 20°C to -10°C	Cold soils, Glacial ice, Cold ocean water, Alpine snowpack	– Arctic and Antarctic marine habitat. – Deep, cold Pacific Ocean waters.	[7, 18-19]
Acidophile	<i>Acidithiobacillus ferrooxidans</i> <i>Ferroplasma sp.</i> <i>Sulfolobus sp.</i> <i>Thermoplasma sp.</i>	Acidic, pH 3 or below	Acid mine drainages	– Iron sulfide ore body.	[7, 20]

Category	Examples	Resistance	Distribution	Examples	References
Alkaliphile	<i>Arthrobacter sp.</i> <i>Halomonas alkaliphile</i> <i>Natronobacterium sp.</i> <i>Psychrobacter sp.</i>	Alkaline, pH 9 or above	Soda lakes	– Eutrophic soda (Na <sub>2</sub> CO <sub>3</sub> ) lakes harbor.	[7, 21, 22]
Halophile	<i>Dunaliella</i> <i>Halarsenatibacter silvermanii</i> <i>Halobacteriaceae</i> <i>Methanobrevibacter smithii</i> <i>Natrialba sp.</i>	High salinity, 2 to 5 M NaCl	Natural salt lakes, Saltern pond brines, Hypersaline ocean	– The Great Salt Lake, West America. – The Dead Sea, Middle East.	[7, 23, 24]
Piezophile	<i>Methanocaldococcus jannaschii</i> <i>Methanothermococcus sp.</i> <i>Pyrococcus yayanosil</i>	High hydrostatic pressure, up to 130 MPa	Lithospheric sites, Hydrothermal vents, Deep oceanic	– Deep waters of the Mediterranean and Sulu Seas.	[7, 25-27]
Metallotolerant	<i>Cupriavidus metallidurans</i> <i>Ferroplasma sp.</i> <i>Halbacterium sp.</i> <i>Ralstonia sp.</i> <i>Cyphellophora olivacea</i> <i>Rhinocladiella similis</i> <i>Exophiala mesophila</i>	High concentrations of heavy metals	Industrial sites, Mining sites	– Textile industries. – Heavy electrical plants. – Paper-manufacturing industries. – Leather-manufacturing industries.	[7, 18, 28-31]
Toxitolerant	<i>Pseudomonas putida</i>	High concentrations of toxic substances/organic solvents	Industrial sites, Oil spilt environments	– Textile industries. – Food industries.	[7, 18, 28-31]
Radioresistant	<i>Chroococcidiopsis sp.</i> <i>Halobacterium sp.</i> <i>Pyrococcus furiosus</i>	High level of radiation	High levels of ionizing radiation environments	– Radioactive waste storage facilities. – Nuclear power plants.	[7, 18, 32]



**Figure 2.** Different habitats of extremophiles.

Metallotolerant and toxitolerant are microbes that can withstand and live in environments with high concentrations of heavy metals such as arsenic, copper, cadmium, lead, mercury, zinc, and toxic substances such as benzene [18, 28]. Metallotolerants and toxitolerants

are frequently found in mining sites, oil-spilled environments, and industrial waste sites, which discharge plenty of heavy metals and toxic compounds such as textile industries, heavy electrical plants, paper-manufacturing industries, and food industries [29-31]. Besides, microorganisms capable of surviving high radiation levels are called radioresistant [18]. They can be found near radioactive waste storage facilities and nuclear power plants [32]. The radioresistant not only able to adapt to high ionizing radiation environments, but they also can accumulate and decontaminate radionuclides such as  $^{238}\text{U}$ ,  $^{137}\text{Cs}$ ,  $^{110}\text{Ag}$ ,  $^{65}\text{Zn}$ ,  $^{60}\text{Co}$ ,  $^{54}\text{Mn}$ , and  $^{14}\text{C}$  [32]. The different habitats of extremophiles are illustrated in Figure 2 [33].

### 3. Case studies

Petroleum contamination due to leaks and oil spills from exploitations, transportation, power plants, and industrial activities that use petroleum has been a severe environmental problem that negatively affected soil quality, aquatic ecosystems, and human health [34]. As petroleum serves as a significant raw material for many industries and the primary resource of energy, more than 9000 incidents with an average volume of 115,000 barrels of oil spill annually have been identified over the last decade, according to the National Oil Spill Detection and Response Agency (NOSDRA) [35]. These oil spills can cause adverse effects on human health and the environment as its toxicity from complex hydrocarbons encompassing short-chain n-alkanes and aromatics such as benzene, toluene, ethylbenzenes, and xylenes [36]. In addition, due to the hydrophobic nature of petroleum, the oil-water interface hinders the diffusion of oxygen and nutrients for normal microbes' growth [37]. This extreme condition makes extremophilic microbes the best candidate for sustainable and practical bioremediation for petroleum pollution due to their environmental and economic advantages compared to conventional remediation approaches when treating oil spills [38].

The contamination of the environment by oil spills is a pressing real-world concern as it will eventually affect all parties, including harming the health of humans, animals, and plants, degrading soil and water quality, damaging the coastal zones and seas, and risking the Earth's health sustainability [39]. One prominent worrying example is the oil contamination that often arises in the Antarctic region, one of the last pristine environments on our planet. The contamination generally resulted from extensive petroleum usage as a main source of heating, transportation, and electricity generation [40]. In Antarctica, in-situ bioremediation using extremophilic microbes is more beneficial compared to the physical extraction of oil-contaminated soil, which is impractical due to its possibility to disrupt the natural landscape, the substantial costs and logistically inefficiency associated with excavating and transporting soil for ex-situ treatment [41]. For instance, excavation could trigger the re-suspension of pollutants and initiate permafrost thawing, leading to alteration of groundwater flow, soil shrinkage, land subsidence, or salinization [42].

In contrast, in-situ bioremediation provides alternative low-cost and effective treatment solutions for petroleum contamination in the Antarctic regions, positively impacting the environment. Despite the frigid weather in Antarctica, extremophilic microbes, particularly psychrophile and psychrotolerant, not only can adapt to the Antarctic soils that are cold, dry, and low nutrients but also can break down petroleum compounds, metabolize hydrocarbons and reduce their presence in the oil-polluted land and water bodies [43]. Extremophilic bacteria such as *Pseudomonas*, *Rhodococcus*, and *Sphingomonas* are commonly found in Antarctica and used as they show a strong capacity for petroleum degradation, effectively breaking down alkanes and aromatic hydrocarbons through aerobic processes [44]. The soil and water



remediation using extremophilic microbes in Antarctic regions has directly contributed to restoring the polluted environment, reducing oil accumulation and toxic compounds in soil, water, and aquatic organisms, and mitigating the long-term environmental impacts.

The utilization of extremophilic microbes in remediating petroleum also has a favorable impact on humans, animals, plants, and their habitats, such as land and marine ecosystems. Commonly, human exposure to polycyclic aromatic hydrocarbons (PAHs) from petroleum contamination can cause nausea, skin irritation, inflammation, and even the formation of cancerous cells over the long term [45]. Besides that, soil contamination with petroleum could lead to severe damage and mutations in animals and plants, disrupting the productivity of agricultural activities [34]. In addition, the disruption of marine ecosystems by oil spills can result in a critical decline in marine life populations, causing biodiversity loss and ecological imbalance [46]. Due to its complex chemical structure, the complete degradation of PAHs is typically not easy [47].

Nevertheless, industries and researchers increasingly favor and commercialize bioremediation using extremophilic microbes for petroleum clean-up due to its ability to degrade a wide range of hydrocarbons comprising different chemical structures and molecular weight [48]. Adebuso *et al.* (2007) have discovered the rapid degradation of alkyl aromatic hydrocarbons by using thermophilic bacteria in marine sediment ecosystems [49]. In a tropical stream contaminated with petroleum in Lagos, Nigeria, some bacteria like *Alcanivorax*, *Cycloclasticus*, *Oleispira*, *Oleiphilus*, and *Thalassolituus* have demonstrated the capability to degrade petroleum hydrocarbons, which underscores the significance of microbial involvement in enhancing the degradation of petroleum pollutants [49, 50]. These extremophilic microbes can effectively bioremediate petroleum contamination, recovering marine and terrestrial ecosystems and reducing hazardous petroleum contamination in all living organisms.

Extremophilic microbes are also often employed in the treatment process of the petroleum industries. For example, biodesulfurisation (BDS) uses extremophilic microbes to remove sulfur from fuel and organic compounds, which can preserve the hydrocarbon structure and fuel value [51, 52]. In comparison to the commercial method, hydrodesulfurization (HDS), BDS demonstrates more significant benefits as it is more effective, less energy-intensive, low-cost, and safer as BDS is conducted under ambient temperature and pressure without producing toxic by-products like hydrogen sulfide [52]. Besides that, the application of extremophilic microbes is also promising in remediating petroleum industry wastewater that contains high concentrations of noxious pollutants such as toluene and phenols [53]. Scientists showcased the potential of halophilic microbes from the Great Salt Lake, West America, in remediating a simulated hypersaline oil field-produced water without the need for dilution [37]. Overall, utilizing extremophilic microbes in petroleum bioremediation highlights their possibilities for environmental problems. This approach positively influences the environment, organisms, and human health. Also, it demonstrates favorable outcomes concerning both environmental and economic advantages, comparing them to the conventional remediation approaches when treating oil spills.

#### **4. Pollutant treatment**

Nowadays, industrial activities produce and release various pollutants such as heavy metals, radioactive waste, and emerging contaminants into the environment. Therefore, bioremediation using extremophilic microbes has gained increasing interest due to its advantages over conventional remediation technologies in terms of environmental, economic,

and practicality. This is because extremophilic microbes' unique abilities to thrive under a wide range of extreme environments with their natural biological processes, offering a range of bioremediation techniques to remediate environmental contaminants. Some techniques include bioaugmentation, bioleaching, biosorption, bioprecipitation, and bio-reduction.

#### 4.1. Bioaugmentation.

Bioaugmentation is the introduction of extremophilic microbes to accelerate the degradation of target toxic contaminants, whether in situ or ex-situ [54]. The mixture of naturally occurring microbes and genetically modified variants is commonly used to enhance the treatment of contaminated soil or water in most commercial applications [55]. Due to its remarkable remediation enhancement capability, bioaugmentation is widely utilized in the wastewater treatment system to respond to unforeseen challenges in practical wastewater treatment operations [56]. Usually, in the complex process of treating wastewater, the toxicity of the contaminants can hinder the growth of normal functional microbes, thereby impeding the overall efficiency of bioremediation [57]. However, introducing specific degradation strains can effectively mitigate this toxic inhibition. Therefore, most bioaugmentation involves these introduced strains directly breaking down the targeted toxic contaminants, safeguarding other microorganisms within the system from toxic effects, and ultimately revitalizing the system's treatment process [58]. *Sphingomonas sp. NJUST37* strain with tricyclazole degrading ability was employed for treating wastewater with high toxicity and refractory tricyclazole [59]. This treatment improved tricyclazole removal efficiency, which increased from 5-30% to over 90.0% [59]. Furthermore, this process has substantially improved the microbial diversity within the treated environment.

Other than that, bioaugmentation can also be applied to remediate a wide range of pollutants, including BTEX, PAHs, methyl tert-butyl ether (MTBE), cyanides, nicotine, heavy metals, pesticides, and petroleum [60]. In addition, co-metabolism of bioaugmentation is noteworthy where methanol was added as a co-substrate in treating coal gasification wastewater, improving removal rates of refractory organic pollutants and increasing the abundance of bacteria capable of degrading phenol and polycyclic aromatic hydrocarbons (PAHs) [61]. Therefore, to achieve maximum efficiency for bioaugmentation, the strategic selection of strains with outstanding characteristics and the appropriate application of substrate addition to stimulating metabolic processes are effective and practical approaches [62].

#### 4.2. Bioleaching.

Bioleaching is a bioremediation method that uses extremophilic microbes to remove toxic heavy metals and other pollutants while simultaneously extracting metals from ores and minerals. This technique involves the microbial-catalyzed process of oxidation of insoluble metal sulfides into soluble forms so they can be leached out easily [63]. Bioleaching has drawn interest because of its ability to clean up metal-contaminated locations, low energy usage, and environmental friendliness. Direct contact and indirect leaching are the primary bioleaching methods [64]. The second method uses bacteria-produced acid to rove metals without direct inoculation. This method is very effective in some cases, such as with alkaline wastes where the conditions are unfavorable for the survival of acidophilic microbes. However, compared to employing mineral acids, both methods are considered to have fewer environmental and economic drawbacks when treating anthropogenic wastes [65].

Usually, bioleaching is applied commercially to recover metals from low-grade and waste ores, especially copper, as it would be uneconomic to use the conventional comminution and flotation method [66]. A study discovered that bioleaching of bottom ash and fly ash using a mixed culture of iron and sulfur-oxidizing microbes obtained from a natural system produced outstanding metal extraction yields, where approximately 90% of zinc (Zn), copper (Cu), and 10% of lead (Pb) of fly ash, and 100% of copper, 80% of zinc, and 20% of lead of bottom ash were successfully removed [67]. Another study explored bioleaching of bottom ashes using pure cultures of *Acidithiobacillus ferrooxidans* or *Leptospirillum ferrooxidans*, as well as a combination of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* [68]. According to the results, iron-oxidizing bacteria significantly improved the extraction of the metals, including aluminum (Al), chromium (Cr), copper (Cu), nickel (Ni), manganese (Mn), and rare earth elements like cerium (Ce), lanthanum (La), and erbium (Er) compared to abiotic controls. This is a critical discovery for potential industrial utilization because it can assist in recovering concentrated metals like Al and Cu, hence lowering the expenses involved with dumping the remaining leftovers. Bioleaching uses extremophilic microbes to generate minerals and organic acids and improve the solubility of metals by enzymatic reactions. In addition, copper metal has been successfully recovered by bioleaching techniques from various sources, such as smelting sludges, activated sludge generated from wastewater treatment plants, and shredded electronic waste [69-71]. Overall, this technique is less resource-consuming than chemical leaching and can access smaller particles than other methods [68].

#### 4.3. Biosorption.

Biosorption is a reversible physicochemical process using extremophilic microbes to enable specific biomaterials to passively bind harmful ions like toxic heavy metals and uranium to their cellular surfaces, removing the harmful ions from contaminated sites [72]. In other words, it can be described as the absorption of toxic pollutants from wastewater using extremophilic microbes. The process requires a solid phase, extremophilic microbes' surface, or the substance they produce, acting as an adsorbent to absorb the metal ions present in the liquid phase, contaminated wastewater. This method can remove inorganic and organic toxic pollutants, whether soluble or insoluble, in the wastewater [72, 73]. Biosorption is often referred to as a metabolic-independent bioremediation process as it does not rely on cellular metabolism to remove toxic contaminants from wastewater. The metabolism-independent biosorption mechanism involves complex interactions consisting of physical adsorption, ion exchange, electrostatic interaction, and precipitation [74]. Biosorption stands out among the bioremediation methods due to its profitability, high yield, and ecological nature [75].

Some examples of extremophilic microbes include *Geobacillus*, *Anoxybacillus*, *Thermus*, and *Thermococcus* play critical roles in the biosorption of various metals such as cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), and manganese (Mn) [76, 77]. For instance, studies found that *Geobacillus thermodenitrificans* (MTCC 8341) cells successfully remove 44% of  $\text{Fe}^{3+}$ , 39% of  $\text{Cr}^{3+}$ , 35% of  $\text{Cd}^{2+}$ , and 18% of  $\text{Pb}^{2+}$  when introduced to industrial effluent [77]. Besides that, a study on two obligate halophilic strains, namely *Aspergillus flavus* and *Sterigmatomyces halophilus*, isolated from a saline environment in Thailand exhibited a notable biosorption capacity of approximately 85% for iron and zinc after two weeks of incubation [78]. On the other hand, the biosorption process is not limited to the treatment of heavy metals from wastewater; it can also be applied to the remediation of various other contaminants such as dyes, antibiotics, antibiotic-resistant genes, antibiotic-resistant bacteria,



and polyaromatic hydrocarbons from waste effluents [72]. These findings suggest the potential application of biosorption using extremophilic microbes in treating industrial effluents.

### 3.4. Bioprecipitation.

Bioprecipitation is the application of extremophilic microbes to produce metabolic products that induce the precipitation of heavy metals, decreasing their mobility and toxicity [79]. One of the most significant extremophilic microbes in bioprecipitation is the sulfate-reducing microorganisms (SRM). SRM, mainly comprising bacteria, could derive energy for growth and cellular synthesis through the oxidation of low molecular weight organic substrates, such as acetate, ethanol, lactate, propionate, and others, under anaerobic conditions to reduce sulfate to sulfide [79]. Studies have shown that the metabolic activity of SRM can be effectively employed for the precipitation of metals and the generation of sulfide. The ions readily react with metal ions in contaminated water, precipitating insoluble metal sulfides. This finding presents the advantage of bioprecipitation over chemical treatment, which is the decrease of the sludge volume that is generated when carbonates are used for precipitation, and metal sulfides are more steady under anaerobic conditions compared to hydroxides or carbonates due to their low solubility product [80, 81].

The effectiveness of bioprecipitation has proven successful in removing copper contaminants from various sources, including swine wastewater, dredged sediment, and cheese production wastewater [82-84]. In another example of bioprecipitation, the bacterial strain *Bacillus thuringiensis MB497* was analyzed to produce intracellular and extracellular organophosphorus phosphatase (OPP) enzymes. The OPP successfully bio-precipitated 86 to 100% of heavy metals, including nickel, manganese, chromium, and cadmium. However, the activity of OPP could be inhibited by Ethylene diamine tetraacetic acid (EDTA) and sodium dodecyl sulfate (SDS) [85].

### 3.5. Bio-reduction.

Bio-reduction is a bioremediation method that uses extremophilic microbes to convert toxic contaminants into less hazardous or non-toxic forms, mostly in their oxidized forms. Extremophilic microbes, especially bacteria, use metal ions as electron acceptors in their metabolic pathways, lowering their oxidation states. Bio-reduction is usually applied for metal recovery, especially Chromium, Cr(IV), due to its efficiency, affordability, and environmental friendliness compared to conventional treatment methods such as activated carbon and electrochemical treatments, evaporation, ion exchange, and reverse osmosis [86].

There are mainly two main approaches for reducing Cr (IV): direct reduction and indirect reduction. In the direct reduction method, Cr(VI) is directly reduced to less toxic Cr(III) by Cr-reducing bacteria such as *Arthrobacter*, *Bacillus*, *Cellulomonas*, and *Pseudomonas*. These Cr-reducing bacteria could produce soluble chromate reductase enzymes that facilitate the conversion of Cr(VI) to Cr(III) under both aerobic and anaerobic conditions [87, 88]. On the other hand, the indirect reduction mechanism involves an enzymatic reaction where dissimilatory iron-reducing bacteria or sulfate-reducing bacteria produce iron, Fe<sup>2+</sup>, or Sulphur, S<sup>2-</sup> ions to reduce Cr(VI) into less hazardous forms [89]. Various exogenous nutrients like emulsified oils, lactates, alcohols, and saccharides are typically used as a source of carbon and energy for the microbes' activity that drives the reduction of Cr(VI) [90, 91]. For example, a sulfur-based mixotrophic bio-reduction process has effectively detoxified Cr(VI) with a

removal rate of  $95.5 \pm 0.74\%$  in 48 hours. Furthermore, volatile fatty acids (VFAs) generated through autotrophic sulfur oxidation serve as electron donors for the heterotrophic Cr(VI) reducers like *Desulfovibrio* and *Desulfuromonas*, which can enhance the bio-reduction process [92].

### 3.7. Advantages and disadvantages.

In recent years, bioremediation using extremophilic microbes has overtaken the traditional treatment method to remediate toxic pollutants in various industrial settings. Bioremediations using extremophilic microbes offer numerous advantages, including lower cost, lower environmental footprint, and high versatility, especially in extreme environments, compared to traditional remediation methods. However, it does have some shortcomings and challenges, which will be explained further in Section 4. Future research and prospect. One main disadvantage of bioremediation using extremophilic microbes is the uncertainty, as the success of the treatment depends on various aspects, including environmental conditions, microorganisms' activity, and types of contaminants to be treated. Extremophilic microbes are frequently site-specific, where certain microbes could adapt and remediate in certain extreme environments, so they may not be effective in treating all types of pollutants in all settings. An overview of the pros and cons of each bioremediation method is listed in Table 2 for a detailed comparison. Normally, selecting suitable bioremediation methods depends on the contaminants, contaminated site conditions, and intended outcomes. In addition, the most appropriate treatment method can be chosen by evaluating the pros and cons of each bioremediation.

**Table 2.** Pros and cons of different treatments.

Technique	Example	Pros	Cons	Reference
Bioaugmentation	<i>Sphingomonas sp. NJUST37</i>	<ul style="list-style-type: none"> <li>– Rapid restoration and enhancement of treatment performance, especially in response to sudden contamination or disturbances.</li> <li>– Effective treatment of complex and recalcitrant pollutants, leading to improved efficiency.</li> <li>– Low energy consumption.</li> <li>– Overcoming inhibitory effects of toxic pollutants on indigenous microorganisms.</li> </ul>	<ul style="list-style-type: none"> <li>– Introduced microbes need to establish and thrive within the existing microbial community.</li> <li>– Monitoring and controlling introduced strains' survival and activities in extreme environments can be complex.</li> <li>– Success depends on compatibility between introduced strains and target contaminants.</li> </ul>	[54-62]
Bioleaching	<i>Acidithiobacillus ferrooxidans</i> , <i>Leptospirillum ferrooxidans</i> , <i>Acidithiobacillus thiooxidans</i>	<ul style="list-style-type: none"> <li>– Oxidation of metal sulfides.</li> <li>– Lower energy usage.</li> <li>– It's not labor intensive.</li> <li>– Minimal investment costs compared to hydrometallurgy techniques.</li> <li>– Reduce strong mineral acids utilized in hydrometallurgy techniques.</li> </ul>	<ul style="list-style-type: none"> <li>– Not suitable for alkaline wastes.</li> <li>– A gradual process, mostly occurring in low pH.</li> <li>– It presents slow dissolution kinetics and low metal leaching yield, time-consuming.</li> <li>– Space-demanding.</li> </ul>	[63-71]
Biosorption	<i>Geobacillus</i> , <i>Anoxybacillus</i> , <i>Thermus</i> , <i>Thermococcus</i> , <i>Geobacillus thermodenitrificans</i> (MTCC 8341)	<ul style="list-style-type: none"> <li>– Metal adsorption onto biomass surfaces.</li> <li>– Applicable for all kinds of waste effluents.</li> <li>– No energy is required.</li> <li>– No secondary pollution.</li> </ul>	<ul style="list-style-type: none"> <li>– More efficient in alkaline pH.</li> <li>– Limited applicability. One biosorbent may not be suitable for all types of pollutants.</li> </ul>	[72-78]

Technique	Example	Pros	Cons	Reference
		<ul style="list-style-type: none"> <li>- Biosorbents can be regenerated and reused as the process is reversible.</li> <li>- Simple and rapid process.</li> </ul>		
Bio-precipitation	<i>Bacillus thuringiensis</i> <i>MB497</i> <i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Bacillus thuringiensis</i>	<ul style="list-style-type: none"> <li>- Conversion of metal ions into solid precipitates.</li> <li>- Metals could be easily recovered.</li> <li>- More stable under anaerobic conditions.</li> <li>- Reduction of sludge volume compared to the traditional precipitation method.</li> </ul>	<ul style="list-style-type: none"> <li>- High-cost remediation compared to the other bioremediation.</li> <li>- Inhibition at low pH values.</li> <li>- Large operating space is needed.</li> <li>- Nutrients are required to maintain microbial growth conditions.</li> <li>- Slow and incomplete bioremediation of uranium ions.</li> </ul>	[79-85]
Bio-reduction	<i>Arthrobacter</i> , <i>Bacillus</i> , <i>Cellulomonas</i> , <i>Pseudomonas</i> , <i>Desulfovibrio</i> , <i>Desulfuromonas</i>	<ul style="list-style-type: none"> <li>- Reduction of metal ions to less toxic or less soluble structures.</li> <li>- Long-term effectiveness.</li> <li>- Lower maintenance and lower energy consumption compared to the traditional reduction method.</li> </ul>	<ul style="list-style-type: none"> <li>- High-cost remediation compared to the other bioremediation.</li> <li>- Large operating space is needed.</li> <li>- Time-consuming process.</li> </ul>	[86-92]

#### 4. Future research and prospect

Despite the advantages and potentials of extremophilic microbes, there are also several shortcomings and challenges with utilizing extremophilic microbes in bioremediation. For example, it would be more expensive and complicated to maintain optimum conditions for growth and activity purposes compared to conventional bioremediation methods. Monitoring and controlling the extremophilic microbes' survival and activities in extreme environments can be very complex and difficult. Therefore, it is significant that research and development departments figure out how to use the least cost and limited resources to maximize the efficiency of the bioremediation process. Besides that, ethical concerns are raised by the intentional introduction of non-native extremophilic microbes into a new environment that might cause potential unintended ecological disruption [93]. There are also challenges in scaling up bioremediation due to inadequate knowledge of the microbes and the complexity of extreme environments [7].

The application of extremophilic microbes in environmental bioremediation is still under constant research and exploration for a deeper analysis of their capabilities and adaptations. Scientists are still studying the ideal conditions and strategies for introducing them to contaminated extreme environments to degrade and detoxify specific pollutants such as heavy metal, radioactive and organic pollutants [1, 7]. While extremophilic microbes could offer high potential for environmental pollution treatment in extreme locations, the bioremediation process's effectiveness must be improved and optimized. The ecology, gene expression, and metabolism of extremophilic microbes must be studied to have a thorough understanding of the application of extremophilic microbes in the treatment process. With this, large-scale extremophilic microbes in bioremediation can be developed and applied to worldwide industries, ensuring sustainability and effectiveness [94].

## 5. Conclusions

In conclusion, applying bioremediation using extremophilic microbes offers promising advantages such as low cost, low environmental footprint, and high versatility in treating pollution compared to conventional treatment methods. These extremophilic microbes could thrive and effectively degrade pollutants across various extreme conditions. Different microbes with different adaptive traits are classified into different categories, such as thermophiles, hyperthermophiles, psychrophiles, acidophiles, alkaliphiles, halophiles, piezophiles, metallotolerant, toxitolerant, radioresistant, and micro-aerophiles. There are also various bioremediation techniques, including bioaugmentation, enhancing the natural biodegradation process; bioleaching, oxidizing metal sulfides; biosorption, adsorbing metal onto biomass surface, bioprecipitation, precipitating metal ions into solid precipitates; and bio-reduction, reducing metal ions to less toxic or less soluble structures. One main disadvantage is the uncertainty, as the success of the treatment depends on various aspects, including environmental conditions, microorganisms' activity, and types of contaminants. There are several challenges in studying extremophilic microbes, including complex maintenance, ethical concerns, and large-scale application, so further studies are needed to understand their ecology, gene expression, and metabolism. As research progresses and technology advances in the future, these unique extremophilic microbes could lay the foundations for more effective and innovative strategies in bioremediation for the industries, contributing to a cleaner and healthier environment.

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## Conflicts of Interest

The author declares no conflict of interest.

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