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POTENTIAL OF THERMOPHILIC BACTERIA IN BIOREMEDIATION OF HEAVY METALS

Shailesh Kumar Vishwakarma, Mamta Arya and Sachin Singh
Department of Zoology and Biotechnology
HNB Garhwal Unviersity, Uttarakhand (India) 246174
Email-mamtaarya.biotech@gmail.com

ABSTRACT:

Pollution is like cancer to the mother earth. Anthropogenic activities like industries, agriculture, mining, and daily lifestyle produces pollution in an exaggerated manner. Among the various types of pollutions heavy metals pollution is a serious issue because unlike organic waste materials, heavy metals are not biodegradable. Heavy metals are known for their persistence in nature and bioaccumulation properties. Even some heavy metals are essential for living organism but in excess quantity, they are toxic and carcinogenic. Microorganism mediated bioremediation process is very significant and economical. Among the microorganism (bacteria, algae, fungi, protozoan) thermophilic bacteria are gaining attention in the field of bioremediation because of their wide application in biotechnology and commercial use. The adaptation to survive in higher temperature and harsh condition make thermophilic bacteria instrumental in the bioremediation process. This manuscript review outlines the hazardous of heavy metals, bacterial strategies to eliminate heavy metal contamination and the recent research on thermophilic bacteria mediated heavy metal bioremediation.

Keywords: *heavy metals, bioremediation, thermophilic bacteria, biosorption, bioaccumulation*

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Introduction

Pollution is a global problem. Pollution is continuously gripping all the types of ecosystems terrestrial, aquatic and aerial because pollution exists in physical, chemical and biological forms. This hideous condition agitated to the whole world. Even some natural phenomenon is also contributed but the major responsible factor for pollutions are industrialization and urbanization. According to the composition of pollutants, they can be simply categorized as biodegradable and non-biodegradable. Natural organic substances Food waste, paper waste, and manure are fall in degradable pollutants category while plastics, pesticides, heavy metals belong to non-biodegradable (Ramírez-García et. al., 2019). Heavy metals and their metalloids forms are ubiquitous in nature but at higher concentration, they are very harmful to human life and to the environment. Toxic effects and caused diseases of heavy metals are well-known. Various methods and process have been developed to eliminate heavy metals contamination from the waste and environment. Physical and chemical methods can be used to remove contamination from the environment and industrial effluent, but these are not commercially viable because of high operating cost or difficulty in treating the solid wastes generated (Thakur et. al., 2015; & Vijayaraghavan et. al., 2008) and only effective when the concentration of metals above 1 to 100mg/l. (Green-Ruiz et. al., 2008) and the disposal of hazardous residual material is an also problem. Microorganism mediated treatment of pollutants is called bioremediation. Bioremediation processes are getting attention because they are cost effective in environmental pollution control and waste treatment and eco-friendly (Umrania, 2006). Microorganisms are a good biological indicator and many of them have the potential of bioremediation to remove heavy metals from the contaminated site. Among the other microorganisms, thremophile bacteria are more versatile because they are adopted to survive in a harsh environment. Thermophile bacteria are adapted to grow at 50-80^{0}C temperatures while hyper-thermophilic Achaea can survive up to 80-120^{0}C (Sharma et. al., 2013). Many thermophile bacteria and archaea have been reported from various naturally occurring site hot springs, sulfatara and geo-thermally heated soils (Adiguzel, 2009; Sen et. al., 2010) as well as from man-made site e.g., effluent site, waste water treatment site, waste dumping site and biological waste (Mehta et al., **2016**; Oshima & Moriya **2008)**. The early studies on diversity and characterization of thermophile came from Yellowstone National Park (Marsh & Larsen,1953). The thermophilic bacteria have been isolated and identified from the different geographical sites throughout the globe e.g., Iceland (Kristjansson & Alfredsson, 1983; Marteinsson et. al., 2001), Kamchatka Peninsula, Russia (Belkova et. al., 2007), Tunisia (Sayeh et. al, 2010), New Zealand (Niederberger et. al., 2008), India (Ghosh et. al., 2003), Jordan (Mohammad et. al., 2017), China (Xue et. al., 2001), Japan (Takai & Horikoshi 2000), Indonesia (Mantiri et. al., 2019). This review provides an insight into the heavy metals, their toxicity, and impact on human life, and recent updates on the potential application and role of thermophilic bacteria in the bioremediation of heavy metals.

Heavy metals and their impact on human being and environment: Heavy metals are also known as trace metals because of their presence in the environment is less than 10ppm. In general, the metals with 5 times the specific gravity of water are considered as heavy metals. They are total 53 and can be categories as low toxic (e.g. Fe, Mo, Mn), average toxic (e.g. Zn, Ni, Cu, V, Co, W, Cr) and high toxic (e.g. As, Ag, Sb, Cd, Hg, Pb) on the basis of their physiological side effects (Tchounwou et. al., 2012). Some heavy metals (Cu, Co, Ni, Mn, Fe, and Zn) are essential to maintaining body metabolism but toxic when present at a certain higher level (Pandey & Madhuri, 2014, a). Heavy metals cannot be degraded or destroyed. Heavy metals in the environment may be present in mainly three forms, first Ionic forms, this the most toxic form for the living organism, second is ion bound with different ligands forms complex compounds and third forms are precipitated molecules. At high concentrations, metals form unspecific complex compounds in the cell, which lead to various toxic effects depending on the metal and the micro-organism considered. Small size and flexible metabolic pathways make microorganism efficient and versatile in bioremediation processes. Removal of toxic heavy metals from industrial waste waters is essential from the standpoint of environmental pollution control (Guangyu & Thiruvenkatachari, 2003). Table 1 represents the toxic effect of some metals on human health and their WHO prescribed permissible limits.

TABLE:1. SOME HEAVY METALS AND EFFECTS OF HUMAN HEALTH

Heavy metal	**Used in**	**Toxic Effect**	**Permissible limits (WHO,** 2011**)**	**Reference**
Tin (Sn)	Alloying, coatings and powder, Coating steel cans, Solder for joining pipes or electric circuits.	gastrointestinal tract irritation, abdominal pain and anemia	No guideline	Chang et. al.,1996; Cima, F. (2018)
Nickel (Ni)	alloys and coatings chemical compounds and in stainless steel	Dermatitis, induces carcinogenesis, immunological disorders	0.02mg/l	Flint, (1998); Zambelli et. al., 2018
Chromium (Cr)	industry, paints and metal platings as a corrosion inhibitor,	Hexavalent chromium Cr(VI) is mutagenic, reactive oxygen	0.05mg/l	Pandey & Madhuri (2014)b; Costa & Klein

	photographic and pharmaceutical industries	species such as superoxide ion, hydrogen peroxide, and hydroxyl radical, ultimately leading to oxidative stress in the cell causing damage to DNA and proteins		(2006)
Selenium (Se)	Industrial and agricultural, glass manufacture, photocells	the propensity to bioaccumulation, neurodegenerative effect, cardiovascular disease, and amyotrophic lateral sclerosis	0.01mg/l	Sharma et. al., 2019; He et. al., 2018;
Lead (Pb)	lead-acid batteries, ammunitions, metal products (solder and pipes), and devices to shield X-rays, oxides for paint, glass, pigments, and chemicals	the nervous system, Headache, poor attention spam, irritability, loss of memory and dullness, neuro-developmental abnormalities in offspring	0.01mg/l	Harvey (2002); Huel et. al., 1992;
Mercury (Hg)	electrical industry, dentistry (dental amalgams), nuclear reactors, antifungal agents for wood processing, preservative of pharmaceutical products	Accumulates in the kidneys, neurological tissue, and the liver. All forms of mercury are toxic and their effects include gastrointestinal toxicity, neurotoxicity, and nephrotoxicity	0.001mg/l	Tchounwou et. al., 2003; Shenker et. al., 2000;
Cadmium (Cd)	industrial activities, manufacturing of batteries, pigments, stabilizers, and alloys	severe pulmonary and gastrointestinal irritant, nausea, vomiting, salivation, muscle cramps, vertigo, shock, loss of consciousness, a hormonal disorder	0.003mg/l	O' Brien et. al., (2003); Singhal et. al., (1976)

Arsenic (As)	insecticides, herbicides, fungicides, algicides, sheep dips, wood preservatives	inhibition of various mitochondrial enzymes, Fetal exposure,	0.01mg/l	Wang et. al., (1998); Vahter et. al., (2001)
Copper (Cu)	Pesticide production, chemical industry, pharmacy, metal piping	affects the liver, damage to renal tubules, the brain, and other organs	2mg/l	Singh et. al.,(2011); Gaetke et. al., (2003)

Table-2. Bacterial strategies for Bioremediation with reference to thermophilic bacteria

Process	**Description**	**Example**
Biotransformation	enzyme-catalyzed stoichiometrically conversion of one chemical into another that may be more or less toxic	A thermophilic Bacillus spp mediated transformation of nitrile into corresponding carboxylic acids Graham et. al., (2000)
Biomineralization	Biomineralization is defined as the synthesis of inorganic crystalline or amorphous mineral-like materials by living organisms.	thermophilic Geobacillus bacterium catalyzed the formation of 100-µm hexagonal crystals at 60°C in a hydrogel containing sodium acetate, calcium chloride, and magnesium sulfate. Yoshida et. al., (2010)
Bioconversion	The enzyme or whole cell assisted conversion of material into a different	bioconversion of lignocellulose to hydrogen using novel moderately thermophilic bacteria T.

	valuable product	thermosaccharolyticum M18 Cao et. al., (2014)
Bioaugmentation	If appropriate biodegrading microorganisms are not present in soil or if microbial populations have been reduced because of contaminant toxicity, specific microorganisms can be added as "introduced organisms" to enhance the existing populations. This process is known as bioaugmentation.	bioaugmentation with a small amount of the strain T. thermosaccharolyticum W16 (5% of total microbes) increased the hydrogen yield Maier et. al., (2009); Zhang et. al., (2019)
Biostimulation	Addition of amendments to contaminated water/soil to encourage the growth and activity of bacteria already existing in the contaminated sites.	the oil-consumption potential of 15 thermophilic bacterial isolates was enhanced by calcium (II) - and dipicolinic acid (DPA)-supplement. Isolates also revealed high heavy metal resistance. Radwan et. al., (2017)
Bioaccumulation	Bioaccumulation refers to the accumulation of toxic material in the cell or tissue of a particular organism	The thermophilic bacterium, Thermus scotoductus SA-01 was found to survive at higher conc. of europium (Eu). TEM-EDX analysis showed that the bacterium can accumulate Eu both intracellularly and extracellularly. Maleke et. al.,

		(2019)
Biodeterioration	Breakdown or corrosion of materials by microbial action	thermophilic bacteria *Desulfotomaculum nigrificans* was capable of corrosive stainless steel. Lata et. al., (2012)
Biovolatilization	The microorganism can methylate different elements, these methylated compounds are gaseous and highly volatile	Bacillus subtilis 168 was genetically engineered to express the arsenite S-adenosylmethionine methyltransferase gene (CmarsM) from the thermophilic alga Cyanidioschyzon merolae to remove arsenic from organic waste. The modified strain significantly enhanced As volatilization. Huang et. al., (2015)
Bioleaching	Microorganism are used to industrially for recovery of various metals from low-grade mineral resources	moderately thermophilic consortia (Ferroplasma thermophilum, Leptospirillum ferriphilum and Acidithiobacillus caldus) were investigated and it was found that these bacteria can intensify chalcopyrite bioleaching. Marafi et. al., (2010); Liu et. al., (2019)

Bioflocculant	bioflocculants include extracellular polymeric substances such as polysaccharides, proteins, glycoproteins or nucleic acids. biopolymers induce particle to particle flocculation through the formation of bridges resulting in the agglomeration and settling of suspended fine particles	exopolysaccharides (EPSs) producing two thermophilic bacterial strains *Rhodothermus marinus* were reported. Sardari et. al. (2017). EPSs can be utilized in the area of wastewater treatment, drinking water processing, and industrial fields. Shahadat et. al., (2017); Natarajan, (2015)

BACTERIAL STRATEGIES FOR THE BIOREMEDIATION OF HEAVY METALS:

Bioremediation techniques are diverse and on the basis of their implementation they are basically two types, in-situ (bioslurping, bioventing, biosparging and phytoremediation) and ex-situ (biopile, window, bioreactor and land farming) Azubuike et. al., (2016). Table 2 contains the description of some bacterial mechanisms and thermophilic stains that are found to be effective in bioremediation processes.

THERMOPHILIC BACTERIA IN BIOREMEDIATION:

Bioremediation can be defined as a pollution treatment technology that exploits microorganisms to reduce, eliminate, contain, and transform environmental contaminants to benign products (Tabak et. al., 2005). Bioremediation is an innovative and promising technology available for removal of heavy metals and recovery of the heavy metals in polluted water and lands. Since microorganisms have developed various strategies for their survival in heavy metal-polluted habitats, these organisms are known to develop and adopt different detoxifying mechanisms such as biosorption, bioaccumulation, biotransformation, and bio-mineralization. The cost-effective and ecofriendly newer biotechnological processes viz. bioremediation and biobenefication through microbial metal reabsorption have been widely accepted.

During the last two decades, extensive attention has been paid on the management of environmental pollution and its control due to hazardous materials such as heavy metals. Bacterial strategies (sequester, precipitate, or change the oxidation state) to eliminate the deleterious effect of heavy metals to survive and grow are exploited to the assessment of metal contamination. These strategies lead to the biosorption or bioaccumulation, biosorption is a metabolically-independent process whereas bioaccumulation is a metabolically active process. In the case of biosorption techniques, the major advantage is that there is no need to maintain a suitable environment for life-supporting conditions and biosorbent can recover. Hg(II) and Pb(II) resistances of thermophilic *E. profundum* were investigated and its was found that the thermophilic E. profundum can be applied for removal and recovery of toxic metals from industrial wastewater (Akkoyun et. al., 2019). It was suggested that the *E. profundum* can be also used as a bioindicator for the detection of toxic metal pollution in natural water samples because the presence of antioxidant enyzme activities.

Two strain, *Geobacillus thermocatenulatus, and Geobacillus thermodenitrificans* were studied for biosorption capacities of metals copper, lead and zinc (Babak et. al., 2013). Affinity of metals to bacteria was determined in the order Pb2+ > Cu2+ > Zn2+. The results show, that *Geobacillus thermocatenulatus* has better biosorption capabilities than *Geobacillus thermodenitrificans*. The strain *G. thermocatenulatus was* also studied for the cadmium ion biosorption and its surface complexation models (SCMs) was analyzed (Hetzer et. al., 2006). This strain was recommended for the monitoring of potable water and to improve the waste treatment of polluted-water and soil. The bioaccumulation of Cd2+, Cu2+, Co2+, and Mn2+ and their effect on the growth of thermophilic bacteria *Geobacillus thermantarcticus* and *Anoxybacillus amylolyticus* were studied and it was found that both bacterial show maximum efficiency during the exponential stage of growth (Özdemir et. al., 2013). It was concluded that G. thermantarcticus and A. amylolyticus could be used for the removal of Mn2+ ions. Another two strain *Geobacillus toebii* sub.sp. *decanicus* (G1) and *Geobacillus thermoleovorans* sub.sp. *stromboliensis* (G2) were tested for biosorption and bioaccumulation of heavy metals Cd, Cu, Ni, Mn and Zn in two different studies (Özdemir et. al; 2012 & 2009). The highest metal bioaccumulation was performed by Geobacillus toebii subsp. decanicus for Zn (36,496 lg/g dry weight cell). Biosorption experiments were carried out in a stirred batch culture system. Different parameters, Scatchard plot, Langmuir, Freundlich, Dubinin–Radushkevich (D–R) isotherms were analyzed. According to the parameters of the Langmuir isotherms, the maximum biosorption capacities of Cd2+, Cu2+, Ni2+, Zn2+ and Mn2+ for G2 were 38.8, 41.5, 42, 29 and 23.2 mg/g, respectively, while 29.2, 48.5, 21, 21.1 and 13.9 mg/g for G1 was found. Alkan et. al., (2015) reported a newly isolated thermophilic haloalkalitolerant bacterial strain (KG9) and used its dead mass as biosorbent. in this experiment dead biomass of KG9 was immobilized with Amberlite XAD-4 as used it as biosorbent of Cd, Cu and Ni ions. It was effective and reusable at least 25 times without any loss of biosorbent property. After 16S rRNA gene sequencing this isolate was found a close member of *Bacillus licheniformis.* A novel thermophilic bacterium was patented (*Patent No. 8,828,238.*) by Yin-Lung, et al., (2014) that was capable of excreting extracellular proteins having the excellent metal-ion binding ability. This thermophilic bacterium is being useful in the treatment of boiler equipment, pipelines, geothermal wells or industrial wastewater or hard water. In order to understand the removal of such types of toxins, Chalaal and Islam (**2001**) used two strains of thermophilic bacteria belonging to the *Bacillus* family isolated from the hot water stream, to remove strontium from aqueous stream systems. These bacteria were able to concentrate strontium in one side of a two-compartment bioreactor. Ilyas et. al., (**2007**) found that metals can be recovered from electronic scrap by microbial leaching using moderately thermophilic bacteria such as *S. thermosulfidooxidans.* Chaterjee et. al., (**2010**) studied that the metal binding capacity of the thermophilic bacteria *Geobacillus thermodenitrificans* isolated from Damodar river, India was assessed using synthetic metal solutions and industrial waste water. Biosorption preference of dead biomass of G. thermodenitrificans for the synthetic metal solutions was in the following order Fe+3 > Cr+3 > Co+2 > Cu+2 > Zn+2 > Cd+2 > Ag+ > Pb+2. It reduced the concentration of Fe+3 (91.31%), Cr+3 (80.80%), Co+2 (79.71%), Cu+2 (57.14%), Zn+2 (55.14%), Cd+2 (49.02%), Ag+ (43.25%) and Pb+2 (36.86%) at different optimum pH within 720 min. A possibility of using simultaneous sewage sludge digestion and metal leaching (SSDML) process at the thermophilic temperature to remove heavy metals and suspended solids from sewage sludge is explored in a study (Mehrotra et. al., **2015**). The results indicated that SSDML at thermophilic temperature can be effectively used for the reduction of heavy metals and suspended solids from sewage sludge.

CONCLUSION:

In view of the eco-friendly, easy and approachable procedure, the bioremediation has been an alternative to eliminating heavy metals contaminations from the contaminated site. In the past few years, increasing numbers of the study on bioremediation properties of thermophilic bacteria indicate that thermophilic bacteria can provide a better bioremediation strategy in contamination sites with elevated temperature and harsh condition. The various findings suggest that the living bacterial cell and dead biomass can the used to remove heavy metals but the industrial application would need further investigation and optimization of various parameters for maximum uplifting of process.

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