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Cadmium Toxicology and it's Prevention through Lactic Acid Bacteria

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Abstract: Cadmium being one of the most toxic metals among heavy metals categories poses as a major environmental pollutant and potent toxicant to all living organisms. Food and water are the primary source of exposure of cadmium to humans and are accountable for numerous life- threatening diseases. Numerous conventional methods such as precipitation, membrane filtration, ion exchange, and flocculation have been applied so far for its removal, however, these techniques failed at very low metal concentrations and also are claimed to be uneconomic. Recently, the biosorption techniques for heavy metal removal are recommended as they are more effective, economical, and environmental friendly. Biosorption is a passive process, where heavy metal particles are adsorbed onto the surface of the bacterial cell walls. Lactic acid bacteria are a group of bacteria that is nonsporulating, fastidious, fermentative, and generally regarded as safe. Many of these species are probiotic and produce exopolysaccharides. Biosorption mechanisms and factors affecting the process make these strains competent in removing heavy metal cadmium. In the present article, the toxicology of cadmium and its biosorption through lactic acid bacteria is discussed thoroughly.

Keywords: Cadmium, Lactic acid bacteria, Exopolysaccharides, Biosorption, Heavy metals.

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1. Introduction

Heavy metal pollution is a serious environmental problem worldwide especially in developing countries (Duffus, 2001). Heavy metal contamination of the environment may be a result of anthropogenic activities as well as natural processes. Volcanic emissions, forest fires and leaching by acid rain are natural phenomena i.e. responsible for cadmium contamination in the environment (Robards *et al.* 1991), while, human-induced contaminants involve mining and industrial manufacturing, excessive burning of fuels, and waste yield (Gray, 1997). Leakage of sewage and sludge into soil and water may cause the transfer of cadmium, its accumulation and absorption by different plant parts and water bodies and from them to different strata of the food chain. Cadmium is one of the most toxic heavy metals that is widely used in industrial processes and has no significant biological function. Cigarette smoke is also a great source of cadmium exposure in the human population. A study showed that the blood samples of smokers had 4-5 times higher Cd (II) levels in the blood than the non-smoker's population. The chronic toxicity of cadmium to humans has been well documented (Satarug *et al.* 2003; Mc Gillis *et al.* 1990). The maximum intake by humans recommended by the WHO is 0.4–0.5 mg/ week, and the maximum admissible concentration in drinking water specified by the US EPA is 0.005 mg/L (Vilar *et al.* 2006). Various conventional techniques such as precipitation, coagulation, ion exchange, membrane processing, and solvent extraction have been employed for removal of cadmium. But these techniques are inefficient for complete the removal of metal. Conventional processes have numerous limitations in the elimination of heavy metals from water as these are not cost- effective, not viable for treatment of low concentration, renewable materials, and generation of toxic sludge and far away from their best possible performance (Popuri, 2007). However, in current days, the biosorption techniques for heavy metal removal are recommended as it is more effective, economical and environmental friendly (Xiao *et al.* 2010; Malik, 2004). The biosorption mechanism involves the use of microbes to detoxify or degrade the pollutants from an aqueous solution. Microorganisms are crucial to nutrient recycling in the ecosystem especially they can survive and grow under various extreme conditions of pH, temperature, and even at a toxic concentrations of metals (Congeeveram *et al.* 2007). Microbial remediation generally takes place either by bioaccumulation or biosorption. Biosorption is a passive process, where heavy metal particles are passively adsorbed onto the surface of biological materials (Volesky, 2003). The bacterial cell wall is the first effective eco-friendly way for adsorbing heavy metal particles because it contains many anionic functional groups capable of binding to heavy metals, such as peptidoglycan, teichoic acids, phospholipids, and lipopolysaccharides. Microorganisms therefore, have a high potential for use in bioaccumulation and biosorption processes for removal of heavy metals from polluted environments.

Lactobacillus is a low cost food grade, probiotic, and easy to obtain biological adsorbent. The xenobiotics, responsible for deterioration of food stuffs is easily inhibited and detoxify by probiotics (Srednicka *et al.* 2021). Several LAB strains utilized for the fermentation of milk have a good opportunity to survive through gastrointestinal conditions and it can be applied for probiotics. A study reported the mechanism between lactobacillus fermented rice and the removal rate of cadmium (Volesky, 2003). It is found that the interaction of lactic acid and enzymes in the fermentation contributed to the removal of cadmium and the main functional ingredient was lactic acid. The article aims to illuminate the adsorption mechanism between LAB and cadmium, the influential parameters on adsorption capacity, the concrete adsorption process, and the comparison between biosorption and accumulation in microbial cells.

2. Chemistry of Cadmium

Cadmium is a soft, bluish-white, or silver-white transition metal and in the periodic table, it is belonging to the second row of group 12 elements. The atomic number of cadmium is 48 and atomic mass is 112.41, low melting point, the oxidation state is almost +2 although few compounds have been reported with +1 and has a relatively high vapor pressure (1.0 mm Hg (133.3 Pa) at 392°C). There are eight naturally occurring isotopes of cadmium. It is slowly oxidized in moist air and forms brown-colored cadmium oxide fumes when heated in the air.

Cadmium is a less abundant, chalcophile element that is present generally as a substitute for Hg, Cu, Pb, and Zn. The principle source of cadmium is sphalerite (ZnS) and other Zn minerals such as smithsonite ($ZnCO_3$), sulfide, and sulphosalts may also carry a small amount of metal. The concentration of Cd in sphalerite ranges from 0.03 to 9.0 wt%, with a median value of around 5% has been reported. (Fergusson *et al.* 1990) Cadmium occasionally forms, most notably minerals greenockite (CdS) (a zinc ore deposits) but also the rarer octavite ($CdCO_3$) and monteponite (CdO) (Reimann *et al.* 2012). The cadmium compounds, such as cadmium sulfide (CdS), carbonate ($CdCO_3$) and oxide (CdO), are insoluble in water, but soluble in biological fluids, like the gastrointestinal tract and lung. Treatment through acids or light and oxygen, make the compound water-soluble (e.g. aqueous suspensions of cadmium sulfide are water-soluble which gradually photo oxidize to soluble cadmium, cadmium sulfate, nitrate, and halides) (WHO,1992a). The ratio of cadmium and zinc are present typically in 1:100 to 1:1000 ratios (Fairbridge, 2006; Fötstner, 1984).

3. Environmental Occurrence

Naturally, cadmium (0.1-0.5 mg/kg) occurs in the earth's crust and ocean. It is exposed to the environment occurs due to natural and anthropogenic activities. Natural sources of cadmium are weathering of rocks containing cadmium and sediments (15 mg/kg) (Bowen, 1966; WHO, 1992) volcanic activity, landfills, mobilization of cadmium from soils, sea spray, etc. Anthropogenic sources of cadmium involved the combustion of fossil fuels, the mining, and smelting of zinc-bearing ores, waste ignition, batteries, paint, medicinal industries, and municipal landfills. Fertilizers produced from phosphate ores constitute a major source of diffuse cadmium pollution. The solubility of cadmium depends upon its degree of acidity (Ros *et al.* 1987). In natural waters, cadmium is found mainly in bottom sediments and suspended particles.

3.1. Natural Occurrence

In the earth, crust cadmium is found in form of a complex of oxide, sulfide, and carbonate with ores containing zinc, lead, and copper. Higher concentrations of cadmium are found in ore deposits of these metals. The average 0.1-0.2 mg/kg of cadmium is present in the terrestrial region and about less than 5 to 110 ng/L in ocean water (National Resource Canada, 2007; ATSDR, 2008). Cadmium in its different forms like chloride, oxide and sulfide emitted to the atmospheric air from natural sources in which most important sources are weathering and erosion of cadmium bearing rocks. Cadmium does not break down in the environment but with minimal alteration, it is transported and deposited. Cadmium enters the aquatic environment through weathering and erosion of cadmium-containing rock that affect not only, aquatic but also the soil and the atmosphere (ATSDR, 2008). It is estimated that approximately 15000 tonnes of cadmium are produced by weathering and erosion to the global aquatic environment and about 900 and 3600 tonnes by both natural and industrial emission.

3.2. Anthropogenic Occurance

Minning and smelting, the combustion of fossil fuels, waste incineration, municipal landfills, and piles are anthropogenic sources of cadmium (ATSDR, 2008). Cadmium enters into the atmospheric air through nonferrous metal production and fossil fuel combustion, responsible for ferrous metal production, waste incineration, and cement production (ATSDR, 2008; WHO, 2000). According to the immediacy of population density and industrial source decided the cadmium concentration in air. Minning and smelting, drainage water of mine, rainy water overflow from mining areas, plating maneuver, phosphate-based fertilizers, sewage treatment plants, landfills, sludge, and waste disposal are various factors responsible for retention of cadmium in the aquatic environment (ATSDR, 2008; IARC, 1993). The release of these toxic metal contributes to the level of cadmium in water and soil, soil and water through plant and aquatic animals, from them to the food chain. Cadmium transfer rate depends upon different factors, soil type, categories of fertilizers, and the presence of some elements like zinc (WHO, 2000) lead, etc. Soil near smelters and other industrialized areas have been measured with levels >1 mg/kg.

4. World Wide Production of Cadmium

Total production of cadmium in Metric tons was estimated in the year 2020 as shown in Fig. 1. The world production of cadmium was estimated at 24,558 tons in 2017and 24400 tons in 2018. The production of cadmium in year 2018-19 was nil as compared to 47 tonnes in the previous year Most of the world's primary cadmium is produced mainly in China, Korea, Japan, Kazakhstan, Mexico, Canada, Russia, and Peru. As per the mineral commodity summary, January 2018 of USGS Report, the World refinery production of cadmium was estimated at 23,900 & 23,000 tons in 2016 & 2017, respectively. World's secondary cadmium production accounted for 20% of the total metal production. Most

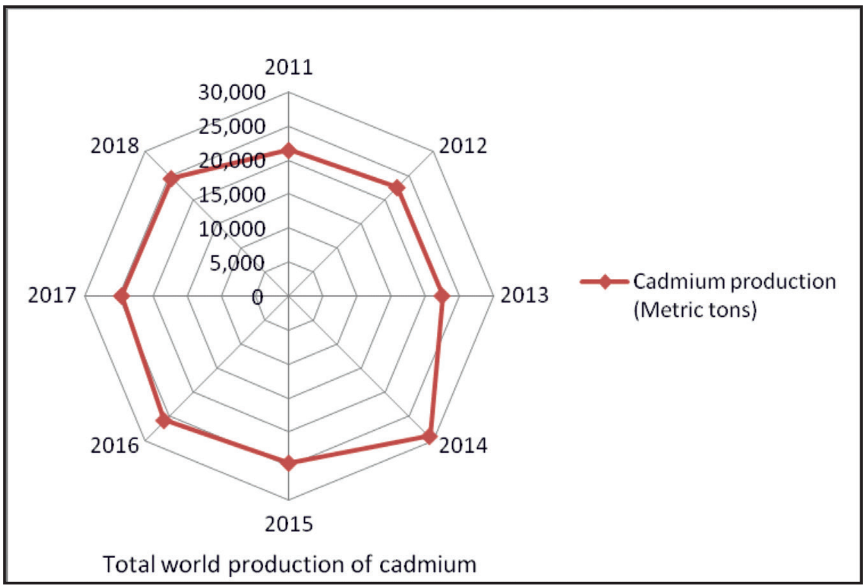

Figure 1: Total world production of cadmium (2011-2018)

secondary metal is produced at Ni-Cd battery recycling facilities in Asia, Europe, and the United States. China, Belgium, and Japan are by far the world's largest consumers of cadmium. The world production of cadmium from 2011 to 2017 by principal countries is furnished in fig 2 (Brown *et al.* 2017).

Cadmium is widely used in various industrial applications due to its outstanding corrosion resistance, lower melting point, and has higher thermal and electrical conductivity. Cadmium is widely used in making Ni-Cd batteries, coating of different materials, plating, stabilizers for plastics, semiconductors, etc. Impure cadmium is generally present in nonferrous metals, iron, and steel, fossil fuels, coal oil, cement, and phosphate group-fertilizers.

5. Recommended Level of Cadmium

Humans are exposed to cadmium mainly via air, water, food, or tobacco smoking. To prevent any further increase of cadmium in agricultural soils is likely to increase the dietary intake of future generations, a guideline of 5 ng/ m^3 (annual average) (Adriano, 2001) is established. According to the Bureau of Indian Standard, method 3025 (Part-41) cadmium in drinking water must be less than and equal to 0.003 mg/l beyond this the water becomes toxic. Food is the main source of cadmium intake for non-occupationally exposed people (Saha *et al.* 2010). It has been calculated that the average cadmium intake from food is about 8 to 25 mg/day (Egan *et al.* 2007; Lobet *et al.* 2003; Olsson *et al.* 2002) depending on food habit and country. Animal kidneys, and livers concentrate

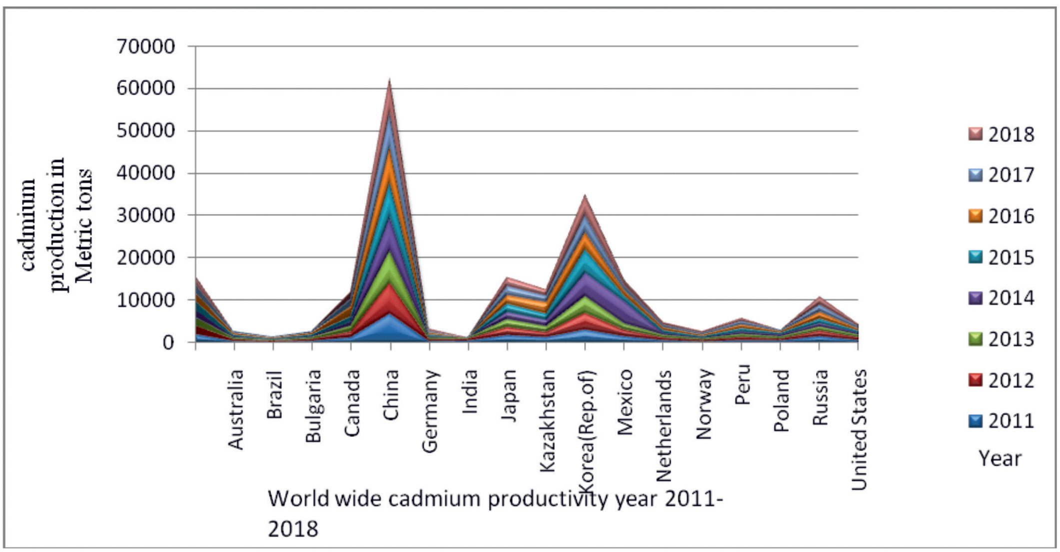

Figure 2: World Wide Cadmium Production (2011-2018)

cadmium. Levels in fruit, meat, and vegetables are usually below 10 μg/kg, in liver 10–100 μg/kg and in kidney 100–1000 μg/kg. In cereals, levels are about 25 μg/kg wet weights. In 1980–1988, average cadmium levels in fish were 20 μg/kg wet weights. High levels were found in shellfish (200–1000 μg/kg) (Galal-Gorchev 1991). The dietary daily intake of cadmium has also been estimated to be in the range 10–35 μg. In contaminated areas in Japan, daily intakes in 1980 were in the range 150–250 μg, based on measurements of cadmium in feces (Svartengren *et al.* 1986). Cadmium, whether absorbed by inhalation or via contaminated food or water, may give rise to various renal alterations. The lowest estimate of the cumulative exposure to airborne cadmium in industrial workers leading to an increased risk of renal dysfunction (low-molecular-weight proteinuria) or lung cancer is 100 μg/m^3-years for an 8-hour exposure.

6. Toxicity of Cadmium

Itai-Itai disease: Itai-Itai disease is one of the Four Big Pollution Diseases of Japan. It is chronic intoxication of cadmium causes softening of bone and kidney failure. The first detection occurred in the Jinzu river, Toyama Prefecture, Japan (Umemura *et al.* 2006). Cadmium is also one reason for the dismantling of bone, it directly interacts with bone cells and inhibits the production of collagen, procollagen C-proteinases (Staessen *et al.* 1999). Decreased bone density imparts increased risk for bone fractures leading to osteoporotic osteomalacia and severe skeletal decalcification (Nawrot *et al.* 2010).

Due to higher cadmium exposure serum parathyroid hormone levels decreased, which may provoke the release of calcium from bone tissue and is responsible for the weakening of the bone (Schutte *et al.* 2010). Cadmium is abundant for the metabolism of calcium, vitamin D3, and collagen synthesis. Decreased PTH level in serum leads to decrease calcium in the bone which causes osteomalacia and osteoporosis, observed in delayed manifestations of severe cadmium poisoning (Staessen *et al.* 1999). The direct effect of cadmium on bone caused osteoporosis and indirect effects cause loss of bone from cortex and trabecular bone, and a decrease in the number of osteocytes cell (Umemura *et al.* 2006).

Renal damage: Kidney and liver are the chief organs for accumulating Cd but it is also found in other tissues of the body. Exposure to cadmium causes renal damage, proteinuria, loss of calcium in its early stage. Biochemical analysis of urine explains signs of renal damage (Patrick 2003). Functioning of the glomerulus, proximal tubules (PCT), DCT, and overall nephron will be diminished, severe Cd toxicity may induce acute inflammation in nephrons with complications such as; glucosuria, polyuria, proteinuria, hyperphosphaturia, hypercalciuria, and uremia (Gonick, 2008).

Reproductive system: Studies reported that Cd shows an adverse effect on the reproductive system (Thompson *et al.*, 2008). Studies showed that cadmium lowers the volume and density although the number of immature sperm formation (Pizent *et al.* 2012). This is due to a defect in the functioning of accessory glands that are responsible for spermatogenesis. Likewise, the functioning of the ovary and oogenesis in females may affect. Hemorrhage and necrosis in the ovary can occur due to less production of steroids hormones (Thompson *et al.* 2008). Long-term exposure to Cd may increase the spontaneous abortion and the stillbirths rate (Pizent *et al.* 2012).

Carcinogenicity: International Agency for Research on Cancer (IARC) defined the carcinogenicity of Cd compounds (Kellen *et al.* 2007). It is defined carcinogen to the lung, renal, and prostate. Higher cadmium concentration affects testosterone production and hyperplasia of testicular interstitial cells (Gover *et al.* 2004). Studies reported that it alter DNA and lead to malignancies of liver, bladder, hematopoietic system, and stomach (Waalkes, 2003). Cadmium may be a potential risk for the development of breast cancer. Another study suggested that cadmium exposure may develop pancreatic cancer because it induces risk for neoplasia (Waalkes, 2003). Long-term exposure to Cd causes direct or indirect carcinogenesis which leads cell proliferation, cell differentiation, and apoptosis or cell death (Waalkes, 2003).

Cadmium and other systems: Exposure to cadmium can cause acute toxicity of the central and peripheral nervous system through lipid peroxidation, cell proliferation and deamination of neurotransmitters (Ismail *et al.* 2015). Cd provokes free radical generation in CNS (Lopez *et al.* 2003). Free radical formation in CNS leads to dysfunction of the olfactory region, and defects in memory, attention and psychomotor activity (Kim *et al.* 2005). Reactive oxygen species leads to degeneration of cells and degenerative diseases/disorders like Parkinson's, Alzheimer's, and Huntington's diseases.

Cadmium toxic exposure may increase cardiovascular mortality (Menke *et al.* 2007). Cadmium causes pulmonary diseases such as chronic obstructive disease and emphysema, Report by Agency for Toxic Substances and Disease Registry (ATSDR), denote that cadmium is one of the potent lung carcinogen (Lampe *et al.* 2008). It can decrease the vital capacity of lung and increase alveolar wall thickness due to pulmonary inflammation.

The absorption of cadmium depends upon the pH of the gastrointestinal tract. The acidity makes it absorbed readily and reaction of cadmium with HCl forms cadmium chloride. Cadmium chloride is an inflammatory compound that causes inflammation of GIT (Waisberg *et al.* 2005). Acute stage of cadmium toxicity leads to liver damage and chronic stage develop Itai-Itai disease (Baba *et al.* 2013).

Mechanism of Toxicity: Cadmium interacts with DNA, generates reactive oxygen species (ROS) and the induction of apoptosis occurs (Rani *et al.* 2014). Even at very low the concentration of cadmium can inhibit oxidative phosphorylation and respiration in mitochondria (Patrick, 2003). Its toxicity enhances reactive oxygen species like superoxide ion, hydrogen peroxide, and hydroxyl radicals due to reduced levels of glutathione and inhibits the activity of antioxidant enzymes and antioxidants (Filipic, 2012). Metallothionein consists of 33% protein in form of zinc concentrate scavenges free radical like hydroxyl and superoxide radicals, (Liu *et al.* 2009) and is able to resist the cell from cadmium toxicity.

7. Biosorption: The Process

Biosorption is a multidimensional rapid process. It can be defined as the passive uptake of toxicants, exclusion or binding of metal or metalloid species, from aqueous solution through metabolically independent or physiochemical pathway by low cost materials derived from biological sources (Patrick, 2003; Gadd, 2009). A large variety of active and inactive organisms have been employed as biosorbents to sorb heavy metal ions from aqueous solutions. Biosorption is made up of two words bio and sorption, bio means the association of biological material and sorption is a physicochemical process in which one substance is attached to another or it means absorption and adsorption both. Absorption involved both physical and chemical phenomena in which molecules or ions enter some bulk phase (liquid and solid). On the other hand, adsorption is a physical process that involves the binding of molecules or ions onto the surface of another molecule. It has been found that biosorbents are rich in the functional groups, which has a vital role in the removal of various heavy metal contaminants. The most important functional groups are carboxyl, sulfate, phosphate, and amine groups. It is the quick economical, eco-friendly method with less generation of sludge. It is equivalent to conventional ion exchange technology, where dead microorganisms may be used. Sorption through live biomass is known as bioaccumulation i.e. metabolism dependent active process. The number of bacterial species (Halttunen *et al.* 2007; Kinoshita *et al.* 2016), biofilms of microorganisms, and exopolysaccharide (Bai *et al.* 2016; Kumari *et al.* 2017) is used in the biosorption process. Fig.3 showed the

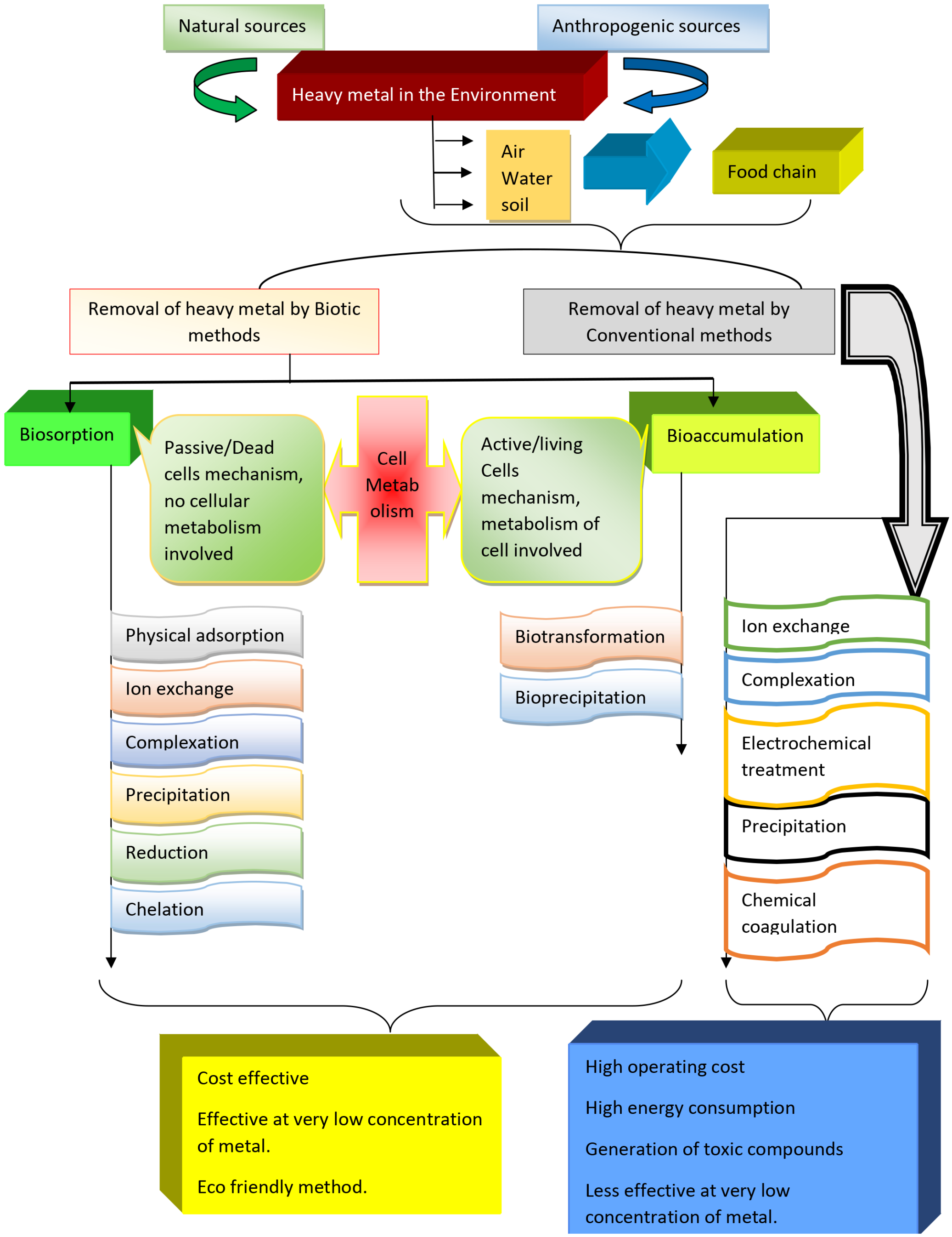

Figure 3: Sources of pollutant, biosorption process and their mechanism

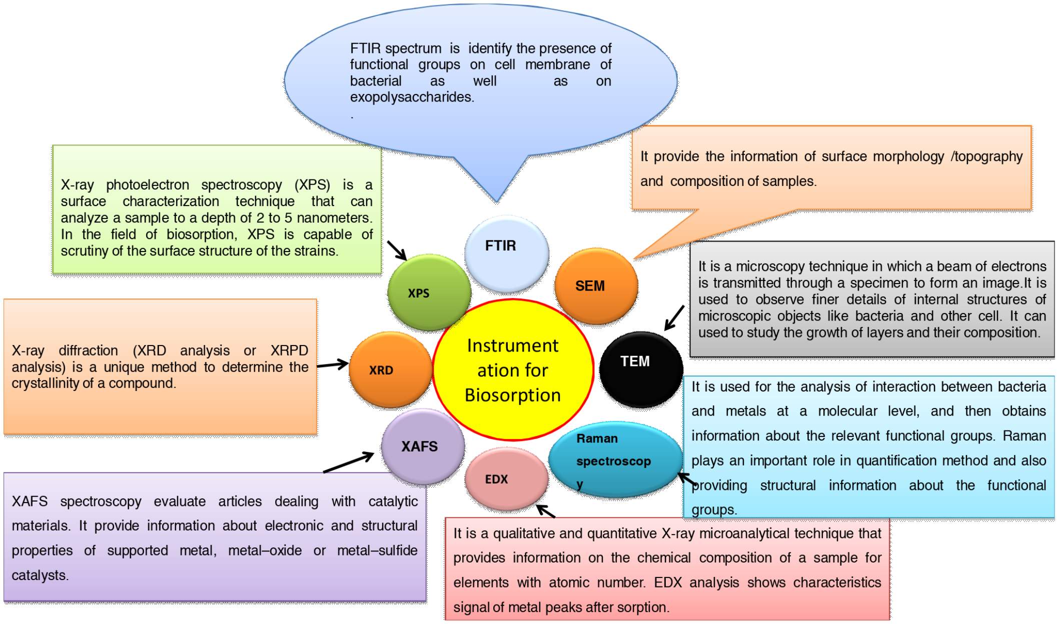

Figure 4: Instrumentation techniques applied in biosorption process

biosorption of metal through biological and conventional methods. Review studies have shown biosorption have following advantages over other conventional methods: chelating metal at very low concentration i.e. less than 100 mg/l, the raw material are easily available and fiscal due to utilizing the action of waste products and dead biomass, no requirement of costly growth media, rapid process, metal loading is high due to ion exchange process of nonliving materials, the process is reversible as sorption and desorption can take place, minimizes chemical and biological sludge. The only limitation of biosorption is that desorption of the metal from the biosrbent material is required for repeated use and the characteristic of biosorbents are difficult to be biologically controlled.

8. Biosorbent

Among various biosorbents as reported in literature various species of bacteria have shown a better biological approach. Bacteria have certain characteristics like smaller size, availability and flexibility. Numerous species of bacteria have such potential to remove pollutants that are not biodegradable like metal ions, and dyes. The cell structure, its biofilm capacity and bacterial biomass are usually used in the form of binding or supporting material to the adsorbent for the removal of heavy metals from the aqueous solution e.g. biofilm of *Escherichia* *coli* and *Bacillus sp.* for removal of Cr (VI) and also exopolysaccharide produced property has great importance in heavy metal removal (Khan *et al.* 2015; Gupta *et al.* 2017). Lactic acid bacteria (LAB) have great significance in the scientific community due to its valuable potential role in biological systems as immune booster and detoxification. Some species of LAB can remove mycotoxins (Pierides *et al.*, 2000), cyanotoxin, and heavy metals (Khan *et al.* 2015) as well. Some species are characterized by a metal-resistant LAB strain that has the potential to be a probiotic candidate for food (Bhakta *et al.* 2012). Proteins that are present on cell surface of LAB are also reported to be associated with heavy metal bioaccumulation (Kinoshita *et al.* 2013). LAB has developed a number of competent systems for detoxifying metals ions and resistance mechanisms for their survival (Mrvcic *et al.* 2012). The number of lactic acid bacteria (LAB) were screened for their potential probiotic capacity against Cd toxicity (Zhai *et al.* 2015). *Lactococcus lactis* subsp. *lactis*, isolated from pickles, a cadmium (Cd) tolerant bacteria along with Cd resistant properties of the lactis was evaluated under different Cd stresses and hydroxyl and amine functional groups play an essential role in biosorption process (Deng *et al.* 2016). Biosorbent used in biosorption process are usually dead cells. Maximum biosorption capacities calculated from Langmuir isotherm were 31.95 mg/g and 24.01 mg/g for dead cells and live cells of *Bacillus cereus* RC-1 (Huang *et al.* 2014). Heavy metal removal through *Lactobacillus acidophilus* showed maximum biosorption of lead (>69%) at pH 4 (Afraz *et al.* 2020).

9. Lactic Acid Bacteria

Lactic Acid Bacteria are gram-positive, nonsporulating, catalase-negative, devoid of cytochromes, of nonaerobic habit but aerotolerant, fastidious, acid-tolerant, and strictly fermentative with lactic acid as the major end product during sugar fermentation. The cell wall of lactobacillus is made up of peptidoglycan (present two sugar derivatives N- cetyl glucosamine (NAG) and N- acetylemuremic acid (NAM). Peptidoglycan also contains several different amino acids, with–D-glutamic acid, D-alanine, and meso- diaminopimelic acid to be the major ones. It rapidly provides amino, carboxylic, phosphate, and sulfate groups, which have great importance in binding heavy metals due to their small size, ubiquity, capability to grow under controlled conditions, and resistance against varying environmental conditions. The surface of LAB, like other Gram-positive bacteria, consists of a thick layer of peptidoglycan, (lipo) teichoic acids, protein, and polysaccharides (Delcour *et al.* 1999). A study has explored the mechanism of lactobacillus fermented rice and the removal rate of cadmium (Fu *et al.* 2016). They found that the interaction between lactic acid and enzymes in the fermentation contributed to the removal of cadmium biosorbent. Gerbino *et al.*, 2011 described the interaction between metal ions and S-layers of the *L. kefir* CIDCA 8348 and JCM 5818 with the help of TEM and FTIR (Gerbino *et al.* 2007). They explained that the precipitation in S-layers is caused by cadmium, the structure of S-layers changed, after absorption. The carboxyl group of Aspartic acid and Glutamate (Glu) along with a few N-H groups of peptide chain changed. The secondary structure of S layers changed because the number of a-helix decreased and the number of b- sheet increased. *Lactobacillus plantarum* CCFM8610 has shown good antioxidant property and cadmium binding ability. The functional groups such as OH, - C= O, -C- C, -C= C, SO_3 present on cell wall of *Bacillus cereus* are involved in the biosorption of cadmium (Arivalagan *et al.* 2014). *L. lactis subsp. lactis* a probiotic bacteria have a good antioxidant capacity against cadmium stress. The upregulated cadA was associated with the activated P-type ATPases that play an important role in cadmium resistance. A number of food sources of *Lactobacillus sps* used in daily diet in form of fruits, vegetables, milk, and milk products and fermented food products that have significant probiotic, antioxidant, and metal quenching properties inside the gut. Probiotic *L. plantarum* HD 48 was found to best cadmium binding and tolerance capacity i.e. 41.62% (Kumar *et al.* 2018). *Bacillus subtilis* reduces cadmium accumulation in rice and high adsorption has been seen in a study of Treesubsuntorn (Treesubsuntorn, 2018). *L. plantarum* MF042018 a novel marine lactic acid bacteria has, the efficient binding ability of Cd^{2+} and Pb^{2+}, from aqueous solutions was achieved at pH 2 and low incubation temperature (22 °C). Other species of lactobacillus shows different biosorption capacity in table:1. *L. plantarum* MF042018 is an inexpensive tool for detoxification of heavy metal-contaminated environments and/or foodstuffs that would be a reliable area for industrial-scale applications (Ameen *et al.* 2020). The LAB are known for maintaining homeostasis and suppression of pathogens in humans and animals. They also play a vital role in the bioremediation of certain heavy metals. Lactobacilli have been applied as a beneficial sorbent for heavy metals as well as removal of the food and microbial toxins (Zoghi *et al.* 2021). The study suggested that *Lactobacillus fermentum* L19 has cadmium removal effect in tomato and apple juice, and a way to biosorption of cadmium in the food industry(Shu *et al.* 2021). Kumar *et al.* 2018 reported that LAB-cadmium interaction plays a possible role in bioremediation of cadmium from foods and the environment to safeguard human as well as environmental health.

10. Exopolysaccharides

Exopolysaccharides (EPSs) are polymers that consist mainly of carbohydrates excreted by some bacteria, outside of their cell walls. They occur in two basic forms: as a capsule i.e. the polymer is closely associated with the cell surface, and slime i.e. loosely associated with the cell surface. Bacterial polysaccharides (alginate, dextran, gellan, pullulan, and xanthan gum) are commonly used as food additives for their gelling, stabilizing, or thickening properties (Acosta *et al.* 2005). EPS forms a protective layer against the harsh external environmental condition for cell, and also serve as carbon and energy reserves during starvation. EPS plays a wide variety of biological functions including prevention of desiccation, protection from environmental stresses like protection against toxins and antibiotics, adherence to surfaces, pathogenesis, and symbiosis (Bhaskar *et al.* 2006; Boels *et al.* 2001). Lactic acid bacteria are the well-known mesophilic group of EPS producer. Among mesophilic bacteria genera, *Bacillus* spp., *L. bulgaricus, L. helveticus, L. brevi, Lactococcus lactis, Leuconostoc mesenteroides, and Streptococcus* spp are the good EPS producer, lactic acid bacteria. As it is known that EPS contains different functional groups and these groups have a crucial role in the sequestration of heavy metals (Chmurny *et al.* 1998). Nowadays, sludge-associated EPS has been widely applied for treating metal contaminated sites. EPS are synthesized extracellular and intracellular, homopolysaccharides are generally synthesized extracellularly in this process the responsible enzymes involved in the transfer of activated precursor monosaccharides from the substrate to growing polysaccharide. Intracellular assembly of sugar nucleotide prepares the precursor of polysaccharides. Several metabolic pathways, co-enzymes, and cofactors participate in EPS biosynthesis. The process starts with the entry of the substrate (any form of sugar) in a bacterium, through active or passive process which undergoes catabolism through intracellular phosphorylation or extracellular oxidation (Frietas *et al.* 2005). EPS or intact microbial cells (live or dead), are negatively charged apart from this chitin having acetamido group, phosphodiester of teichoic acid, hydroxyl, and phosphate groups in polysaccharide imparts negative charge to the polymer, so it is possible and easy to interact with positively charged metal ions and these properties make it beneficial for biosorption (Wang *et al.* 2010). There are different strategies of EPS-mediated metal ion remediation. Homogenous associated EPS (biosorption of metals through pure bacterial cultures and their EPS (Kurita *et al.* 1979; Norberg *et al.* 1984; Norberg *et al.* 1982). Heterogenous Associated EPS (Use of mixed culture bacterial group has achieved profound success for heavy metal remediation).

EPS of *Ochrobactrum anthropi* bacterium removed cadmium ions and other toxic metals (30 mg/L cadmium ion) under the specific conditions of pH 2 and initial metal ion concentration 100.6 mg/L). Immobilized EPS (attachment of bacterial cells to solid surfaces grippingly. Polysaccharide gel and alginate beads have been widely applied for immobilizing various microorganisms as well as enzymes. The combination of calcium alginate and bacterial EPS enhances the absorption of cadmium and cobalt ion from aqueous solutions that is 64.10 mg/g and 55.25 mg/g (Ozdemir *et al.* 2005). Modification in EPS through chemical treatment as acetylation, carboxymethylation, methylation, phosphorylation, sulphonylation enhance the biological and absorptive activities of EPS which is able to make it potentially applicable and active.

Different instrumentation techniques are used to understand the structural and functional and compositional properties of cells and EPS of lactobacillus species. After biosorption process, the functional and structural change in cells and EPS have been done and this may change the physical and chemical characteristics of biosorbents. For achieving this goal fig.4 shows different instruments and their techniques are used such as Fourier Transform Infra-Red (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), X-ray Photo Electron Spectroscopy (XPS), Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD), Energy Dispersive X-ray (EDX) fluorescence spectrophotometry, Atomic Absorption Spectroscopy(AAS), etc.

11. Biosorption Mechanism

Mechanism of action of biosorption involves two main processes active absorption and passive adsorption. Living and nonliving biomass follows different mechanisms. Active absorption or metabolism dependent intracellular uptake takes place by living biomass and is called bioaccumulation wherein metal ions are transported across the cell membrane on the other hand non-metabolism dependent process can be rapid and reversible. Physical adsorption, reduction, ion exchange, precipitation, and chemical sorption are the key ingredients of biosorption (Ahalya *et al.* 2003). The cell wall of microbial biomass consists of polysaccharides, proteins, and lipids molecules. These molecules have different metal-binding functional groups such as carboxyl, sulfate, phosphate, and amino groups (Sardrood *et al.* 2013). The biosorption process required a solid phase absorbent, a liquid phase that is solvent, and dissolved species metal ions. The interaction among absorbent, solvent, and dissolve species make this process complete. The process continues till equilibrium is established between the absorbent and dissolved species present in the solution.

Biosorption is a physio-chemical process, that comprise mechanisms such as absorption, adsorption, ion exchange, surface complexation, and precipitation. The ability of bacteria biomass, EPS and biofilm to remove heavy metal ions or promote their transformation to less toxic forms has been identified by various environmental scientists (Chaney *et al.* 2007; Singh *et al.* 2010; Yu *et al.* 2013). The complex structure of biomass has ability to remove various pollutants, and several factors are responsible for influencing the mechanism of metal biosorption such as living or non-living, modified or natural type of biomass, types of origin, the chemistry of metal solution, and environmental conditions such as pH, temperature, etc. A living cell requires both processes for metal transport and deposition, but non-living cells in passive mode, i.e. metabolism independent, independent of energy, follow metal binding mechanisms such as complexation, ion exchange, physical adsorption, etc (Allard *et al.* 1987).

Physical adsorption: The process in which ions are transferred from a liquid phase to a solid phase usually involves boundary layer mass transfer across the liquid film surrounding the particles, internal diffusion/ mass transport within the pores/solid diffusion, adsorption within the particle and on the external surface. According to Allard *et al.* 1987, sorption may occur through two pathways one is physical adsorption, it is rapid and reversible and due to non-specific attraction force (van der Waals force); and electrostatic adsorption due to columbic attraction forces between charge solute species and adsorbent phase, which is usually rapid and largely reversible.

Complexation

This process involves metal removal through interaction between metal and the active group on the cell wall. It takes place by a complex formation on the cell surface. Complexation plays an important role in metal-ligand and sorbate-sorbent interactions. *Pseudomonas syringae* was found to be accumulating calcium, magnesium, cadmium, zinc, copper and mercury by complexation process.

Ion exchange

The exchange of ions takes place between an electrolyte solution and a complex. In most cases, the term is used to denote the processes of purification, separation, and decontamination of the aqueous solution via exchange of ions. The precise mechanism may range from physical binding followed by chemical binding. Biosorption of Pb (II) and Cd (II) by *A. rubescens* and *L. scrobiculatus* biomasses took place due to chemical ion exchange.

Membrane Filtration

It is a widely used chemical and biotechnology process, the process has valuable means of filtering and cleaning wastewater. There are a number of different methods of membrane technology. The most effective is pressure-driven membrane filtration. Other include reverse osmosis, nanofiltration, ultrafiltration, and microfiltration.

Microprecipitation

Refer to immobilization of metal species outside and inside the cells, for example, extra-cellular polymeric capsule or component.

12. Factor Affecting Biosorption

Effect of pH

It is the most significant factor in biosorption process as it regulates the chemistry of sorbate, activity of functional group present on biosorbents and chemical properties of metallic ions (Chaney *et al.* 2007), microbial metabolism, growth, and activity of functional groups present on the cell surface. Metal sorption is enhanced in a particular pH range. With low pH value, production of the large number of H_3O^+ which occupies the binding sites, enhance the electrostatic repulsion of functional groups in the cell surface and enhance sorption of anionic metals, while hydrogen ions inhibit the binding of metal cation through competing for binding sites due to strong repulsive force. As pH increases, there is a decrease in H_3O^+ and probability between the metal cation and cell functional groups increases. It is found that removal of lead was 55% at pH 2, highest removal (95%) was observed at pH 6, but as pH increased above 6, precipitation of metals occurred with the reduction in absorption (Halttunen *et al.* 2007). The maximum biosorption efficiency for cadmium was attained at pH 6 (Limcharoensuk *et al.* 1987). The study reported that L. acidophilus shows 87.68% removal of Pb2+ at the pH of 4 with the increased inoculum size inhance more available binding sites (Afraz *et al.* 2020).

Effect of Temperature

Usually, the biosorption process is temperature dependent, as temperature increase, it enhances the biosorptive removal of activity and kinetic energy of the adsorbate but sometimes very high temperatures may damage the physical structure of biosorbent. The sorption capacity of biosorbent for metal ions is dependent on the interaction between biosorbent and metal ions and exothermic or endothermic. Temperature alters the cell wall stability, configuration, and charges upon functional groups. These changes may have a positive and negative roles in biosorption of metals (Kao *et al.* 2009).

Influence of Contact Time

Biosorption consists of two main processes, extracellular adsorption, and intracellular uptake. The extracellular adsorption process is a rapid and metabolism-independent process. It involves surface conjugation such as surface complexation, ion exchange, and metal transport that occur within a few minutes as equilibrium is established. Initially, there are abundant available empty binding sites on the biosorbent and a high concentration of metal in solution so the rate of metal uptake is very fast due to the excited number of available sites present on biosorbent surface and decrease until equilibrium is reached (Abdolali *et al.* 2016). The latter stage is slow and metabolism-dependent, time taking. It involves active transport and transmembrane movement of metal uptake from the surface into the cell through metabolism and vectors. Rapid biosorption of metal ions was reported within 30 min and maximum removal was observed in 2 h (Akar *et al.* 2006). A study reported that cadmium uptake by *B. cereus* drastically increased after 14 h and was maximum at 20h (Wang *et al.* 2010).

Biomass Concentration and Binding Sites

The dosage of biosorbent strongly influences the extent of biosorption. Metal removal increases by increasing the biomass concentration generally along with increased surface area, binding sites and functional groups on the surface of the biosorbent. The quantity of biosorbed solute per unit weight of biosorbent decreases with increasing biosorbent dosages, which may be developed due to complex interaction of several factors. An important factor at high biosorbent dosages, overlapping and co-aggregation of biomass occurs due to electrostatic interaction, responsible to cut down the binding sites, and available exchangeable sites also lower the solute uptake (Brenner *et al.* 2005). Many factors can affect biosorption like the type and nature of the biomass, freely suspended cells or immobilized preparations and living biofilms, etc.

Initial Pollutant Concentration / Metal ion Concentration and particle size

It is also an important factor for biosorption process. If the initial concentration of metal is limited and within the range of biororbents or cell resistant capacity, then sorption capacity increases, but if the concentration is too high in the case of cell, it produces a toxic effects like inhibiting the growth and metabolism leading to cell rupture. Actually, initial metal ion concentration provides a necessary driving force to overcome the resistance of metal ions. So if the initial pollutant concentration is higher, it increases the quantity of biosorbed pollutant per unit weight of biosorbent but decreases its removal efficiency (Park *et al.* 2010) according to Arivalagan *et al.* (2014) rate of biosorption decreases with an increase in cadmium concentration. The smaller the particle size higher the biosorption effect because a small particle can expose more contact surface area. It is constructed only for batch processes and not for column processes due to its low mechanical strength and clogging of the column (Park *et al.* 2001).

Desorption

Desorption provides the possibility of regeneration, re-use, and recovery of biomass, decrease overall processing cost, and continuous supply of biomass. Successful desorption depends on the mode of removal of metal ions and maintained mechanical stability of biomass (Gadd *et al.* 1992). Generally, most biosorbents exhibit an ion-exchange method for cationic ions and mild acidic condition is required for the desorption process. Desorption with acid is most useful because acidic solutions are one of the common waste flushed out through industries and these waste have a tendency to regenerate biosorbent. But high acidity can cause aggregation and dose of the integrity of biomass that may lead to a decrease in performance. $CaCl_2$ and EDTA are effective desorbent when complexation, chelation, and microprecipitation mechanism are used, these require extensive screening for efficient desorption. A number of substances have been used as metal desorbents including acids, alkalis, and complex agents depending on the substance sorbed, process, requirements, and economic considerations.

13. Conclusion

Cadmium is a toxic heavy metal and one of the major causes of environmental pollution. So it is required to eradicate the toxicity of metal. For this number of physical and chemical methods was applied but the most promising biotechnological method was biosorption. Some strains of lactic acid bacteria have been used to remove toxins from food. As a biological adsorbent, it also has a wide range of applications. *Lactobacillus* or probiotic *Lactobacillus* species was found to be capable of removal capacity and remarkable cadmium and tolerance. Bacteria were selected as the research materials because of easy to obtain, inexpensive, and beneficial to humans and the environment. It is a biotherapeutic agent to discharge cadmium from the human body through excretion. In this article, the principle of biosorption is introduced in detail, and the interaction between lactic acid bacteria and cadmium is emphasized. Lactic acid bacteria its EPS and Biofilms have a better tendency to significantly eradicate heavy metals. The new biological-based technologies need not necessarily replace conventional treatment approaches but may complement them.

Biosorption is used to remove heavy metals from aqueous solution and recover rare and precious metals, is active, and can treat waste at particular, low concentrations where other conventional methods fail. Therefore, further

Table 1: Biosorption of cadmium through *Lactobacillus* strains

Lactic acid bacteria species and strains	*Heavy metal*	*Removal (%)*		*References*
L.plantarum 8PA3	Cd	8	%	(Kirillova *et al.*, 2017)
L.plantarum B-578	Cd	16	%	(Kirillova *et al.*, 2017)
L.plantarum S1	Cd	8	%	(Kirillova *et al.*, 2017)
L.plantarum Ga	Cd	8	%	(Kirillova *et al.*, 2017)
L.fermentum Na	Cd	4	%	(Kirillova *et al.*, 2017)
L.fermentum 3-2	Cd	8	%	(Kirillova *et al.*, 2017)
L.fermentum 3-3	Cd	12	%	(Kirillova *et al.*, 2017)
B. lactis Bb12 bioled biomass	Cd	32.1	%	(Halttunen *et al.*,2007)
B. lactis Bb12 freeze dried biomass	Cd	34.1	%	(Halttunen *et al.*, 2007
B. longum 2C boiled biomass	Cd	14.6	%	(Halttunen *et al.*, 2007
B. longum 2C freeze dried biomass	Cd	13.7	%	(Halttunen *et al.*, 2007
B. longum 46 boiled biomass	Cd	32.0	%	(Halttunen *et al.*, 2007
B. longum 46 freeze dried biomass	Cd	54.7	%	(Halttunen *et al.*, 2007
L. casei Shirota boiled biomass	Cd	19.0	%	(Halttunen *et al.*, 2007
L. casei Shirota freeze dried biomass	Cd	12.1	%	(Halttunen *et al.*, 2007
L. fermentum ME3 boiled biomass	Cd	26.7	%	(Halttunen *et al.*, 2007
L. fermentum ME3 freeze dried biomass	Cd	28.4	%	(Halttunen *et al.*, 2007
L. rhamnosus GG boiled biomass	Cd	12.5	%	(Halttunen *et al.*, 2007
L. rhamnosus GG freeze dried biomass	Cd	13.3	%	(Halttunen *et al.*, 2007
L. plantarum MF042018	Cd	18.8	%	(Ameen *et al.*, 2018)
Bacillus cereus RC-1	Cd	31.95	%	(Huang *et al.*, 2013)
L. sakei	Cd	48.1	%	(Kinoshita *et al.*, 2013)
L. plantarum/L. pentosus	Cd	84.8	%	(Kinoshita *et al.*, 2013)
L. rhamnosus	Cd	73.1	%	(Kinoshita *et al.*, 2013)
L. reuteri	Cd	81.7	%	(Kinoshita *et al.*, 2013)
L. gasseri	Cd	86.6	%	(Kinoshita *et al.*, 2013)
L. acidophilus	Cd	64.62	%	(Afraz *et al.*, 2020)
L. fermentum L19	Cd	51	%	(Shu *et al.*, 2021)

research should be carried out on the mechanisms and the regeneration of biosorption. Although it has different merits there aresome demerits of biosorption as it is not much applicable at broad level, developing a commercial biosorbent similar to an ion exchange resin is essential, which only need low growth conditions and is easy to carry out large-scale industrial cultivation and help to reduce the cost of biosorbent. Totally, the successful application of biosorption requires the joint efforts of workers from different backgrounds, so that biosorbents such as lactic acid bacteria can play an important role in the prevention and control of heavy metal pollution.

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