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Research progress of biosorption between LAB and Pb

The research progress in mechanism and influence of biosorption between lactic acid bacteria and Pb(II): a review

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Abstract

Currently, due to high surface to volume ratio; large availability, rapid kinetics of adsorption and desorption and low cost, the exploitation of microbial biosorption of heavy metals is regarded as reliable alternative compared to the conventional bioremediation approaches. In parallel with the increasing attractiveness of biosorption research, its pace of advance is also boosted. The barrier that prevent biosorption as an effective method from being applied into wastewater purification listed, (1) There is not enough data on multi-component biosorption, (2) It remains to be seen physical-chemical characteristics of different biomasses. (3) Studies on surface modification of strains for enhancement of heavy metals removal efficiency is lack. And extensive literatures involving the mechanism and model of biosorption for particular metal and microbial strains are not available. The present literatures lack systematization, the theory on interaction between acid bacteria and Pb is far from complete. Therefore, the review tries to give a comprehensive explanation about the mechanism of Pb removal from Lactic acid bacteria and provide a brief overview of distinction between biosorption and bioaccumulation, biosorption technology, highlight the underlying features of biosorption and the various affecting factors such as pH, required, initial concentration, temperature, and treatment performance as a reference. mechanisms can be briefly generalized into several pathways, which are ion exchange, complexation, precipitation, reduction and chelation. Many views holds that complexation is the major absorption mechanisms of Pb. Biosorption mechanisms can be roughly classified as

biosorption and bioaccumulation, which have great differences between each other. Biosorption metabolism-independent but fast, while bioaccumulation is metabolism-dependent but slow. The slight advantages of the bioaccumulation are the metabolite (lactic acid), lactobacillus surface-layers, enzymes and so on. Many factors can greatly affect adsorption process, different factors have different influence and the effects of pretreatment, pH and temperature are relatively greater. Desorption is not a fully reversible process of biosorption, but could not only achieve the goal of the recycle of microorganism, but also contribute to release of trace metal elements. Also the technologies for observation of biosorbents characterics and effect on the metal binding process are reviewed.

Keywords

lead, lactic acid bacteria, biosorption, bioaccumulation, technologies, desorption, affecting factors

Nomenclature

EDTA ethylene diaminetetra-acetate

EDX energy-dispersive X-Ray spectroscopy

EPS exopolysaccharides

FTIR fourier transform infra-red spectroscopy

LAB lactic acid bacteria

MPW mango peel waste

Pb lead

SEM scanning electron microscopy

SLP S-layer proteins

TEM transmission electron microscopy

XAFS X-Ray absorption fine structure spectroscopy

XPS X-ray photoelectron spectroscopy

XRD X-Ray diffraction

Introduction

Lead, a kind of amphoteric heavy metal, is widely applied to modern industry including metallurgy, printing, military, medicine, electronics, ceramics, pigment industry and transportation industry as an important raw materials. That's why the pollution of lead through a variety of ways causes serious environmental problems. It has been found as ubiquitous environmental pollutant in the form of cations and damages the nervous system, bone marrow hematopoietic function, and growth.

The great harm caused by lead pollution to human and environment has aroused people's attention. The traditional methods for removing lead are precipitation, coagulation, adsorption ion exchange. The precipitation agents used in precipitation are lime, caustic soda and soda ash. Alum, ferrous sulfate and ferric sulfate can also be used for lead treatment, in which using alum and anionic polypropylene as composite flocculant to settle complex colloid has obtained better results (Lee et al., 2014). In addition to biosorption, there are also adsorption of ceramsite, adsorption of ferric oxide and so on (Xu et al., 2009). Iron oxide has been widely used in the treatment of heavy metals in waste water, but it is not suitable to use the iron oxide to fill in the pipe column directly, which is poor permeability and low efficiency. Ion exchange is to lead in the waste water to the fiber so as to remove the lead. But these methods of removing lead involve narrow areas and are traditional than biosorption. Some of these methods are not good enough to meet the more and more stringent standards of waste water discharge. On the other

hand, some of them are not economically feasible and greatly limit their practically applicable value.

In recent years, biosorption process, a new method to eliminate heavy metal pollution, has been researched as a popular topic due to the advantages of low investment, high efficiency and safety with no side effects. The general ideas that extract different kind of microorganisms with a high tolerance of lead from soil or animal intestinal fecal to solve lead contamination in food and natural environment are approximately similar. And many studies involve the isolation and identification of strains, the adsorption mechanism of heavy metals, and the influential factors for metal adsorption in order to perfect related theories. Unfortunately, the researches of lactic acid bacteria (LAB) adsorption lead are relatively few.

LAB has been acknowledged as the safety level microorganisms which are widely used to produce fermented food, such as yogurt (*streptococci and lactobacilli*), cheeses (*lactococci*), sauerkraut (*Leuconostoc*), wine (*oenococci*), or cured sausages like Salami (*pediococci, lactococci*)(Solioz et al.,2011). Not only that, several strains of lactic bacteria have been studied the removal of various toxins originated from food, such as aflatoxins, zearalenone, and fusarium (Elnezami et al., 2000; Elnezami et al., 2002; Rawal et al., 2014; Pierides et al., 2000). dietary mutagens, and mutagenic pyrolyzates (Zhang and Ohta. 1991; Zhang and Ohta. 1992; Turbic et 2002). For it has good tolerance of hydrochloric acid in gastric juice, bile and enzymes. The oral agents of it can also reach the intestines smoothly and produce a large amount of cytokines

can improve the body's physiological function by adjusting the micro environment. Besides, the resource of LAB is extensive such as pickle water, soil and animal intestinal fecal. According to the above reasons, LAB draws wide attention on application for a biosorbent. Halttunen et al. found that it's more effective to remove heavy metal by the mixture of different kind of LABs (Halttunen et al., 2008). Zhai et.al screened the lactic acid bacteria of the highest affinity and tolerance to lead from 33 kinds of strains (Zhai et al., 2015).

The aim of this article is to elucidate the adsorption mechanism of between LAB and lead, the influential parameters on adsorption capacity, the concrete adsorption process and the comparison between biosorption and accumulation in microbial cells. The comprehensive induction and analysis are expected to be used to provide the theoretical basis and the best experimental conditions for LAB adsorption experiments.

Biosorption Mechanisms

The biosorption process consists of solid and liquid phases. The solid phase is called biosorbent, such as LAB. And correspondingly the liquid phase is water solution with Pb. Pb is combined with biosorbent, the LAB, with different mechanisms leading to distinction of biosorbent affinity for the sorbate. The process finishes when the equilibrium between absorption and desorption is established (Lin et al., 2014; Lin et al., 2015).

Although several speculations have been proposed about biosorption mechanism, the actual one remains mysterious due to the interaction between the specific biosorbents and heavy metals and the selectivity or affinity towards different metals. Moreover, extensive literatures involving the mechanism and model of interaction between lead and microbial strain are not available (Figure 1). However, the main factors which control and characterize these mechanisms are similar which is shown in Figure 1, from that we can find there are some mechanisms occurring simultaneously. mechanisms referred biosorption can be classified into metabolism dependent and metabolism independent, on the basis of the location of the sorbate species, they are assorted into extra cellular accumulation/ precipitation, cell surface sorption and intra cellular accumulation. Other mechanisms for biosorption are transport across cell membrane, ion exchange and complexation. Therefore, we can only analogize general characteristic of metals to specific metal, and then try to extract speciality through dispersive research results to perfect the theory of biosorption.

According to cell metabolism, biosorption mechanisms can be roughly classified as metabolism dependent and metabolism independent, while on account of the metal binding sites, they are divided into bioaccumulation and biosorption (Ahemad and Kibret, 2013).

The former is so complicated that it involves transmembrane transport, intracellular accumulation, metabolic regulation and so on. This means that this may happen only to living

cells. It often related to an active defense system of the microorganism, reacting in the existence of toxic metal (Ahalya et al., 2003).

Bioaccumulation is a metabolism-dependent process which needs the energy supply to carry out the metabolic activities. There is the general understanding that the biosorption of living cells consists of two steps. First, the interaction between the metal and the functional sites displayed on the cell surface reaches the adsorption equilibrium within 30--40 minutes, passively binding to the cell wall. And second, metal ions penetrate the cell membrane and enter into the cells which are called metabolism dependent intracellular uptake. The cell wall contains a variety of polysaccharides and proteins and hence provides massive active sites capable of separating metal ions. Due to the difference in the cell wall binding sites, the different groups of microorganisms cause significant differences in the type and amount of metal ions (Vimala and Karthika, 2008). There exist two possible models: One is that the soluble metal ions are transported to cytoplasm through the cell membrane and isolated in the specific regions in order not to damage important organelles or cellular components (Wang et al., 2014). Another can be that the metal-binding protein plays an important role in elimination of metal ion, thus, metal exist in the form of innocuous and inactivating metal chelates (Sun et al., 2007). Kim et al.(2007) demonstrated the view that the Pb uptake of the cells decreased sharply by pretreatment with alkaline solution due to the decrease in crude protein content (Kim et al., 2007). The further researches suggested that the amount of –SH from cysteine in MTs helps a lot in metal binding (Xu et al., 2004). What's

more, carboxyl can also improve the affinity between polypeptide and metals and stabilize the metal-protein complex (Xu et al., 2004). The metal-binding protein combined with metals through covalent bonds, therefore, it can be speculated that combination is irreversible. And there is a possibility that the growth of biosorbent may be inhibited because the protein is likely to execute special functions such as essential housekeeping enzymes (Kinoshita et al., 2014). While other researches proved that the growth is not affected to some extent, because metal-binding protein functions as chaperone transferring metal to special sites apart from cell functional domain, protecting cell from metal damage. Beyond the range, cell metabolism should suffer irreversible damage. Kinoshita, Hideki, et al. studied the affinity of metallothionein (MT) to the heavy metals. The order that Hg (II) S Ag (I) > Cu (I) > Cd (II) > Pb (II) > Zn (II) is parallel to their data on the amount of biosorption of LAB in order of Hg (II) > Cd (II) > Pb (II) and As (III) (Kagi and Kojima, 1987; Ryvolova et al., 2011). Hg (II) intended to influence the growth of the bacteria while there are few signs that Cd (II), Pb (II), and As (III) had the effect. This suggests the toxicity of heavy metals on the strains is related to its high affinity to LAB (Kinoshita et al., 2014).

Correspondingly, the latter includes surface complexation and extracellular precipitation, mainly owing to the functional groups in the cell wall and the metabolites exported to the external. Cell walls of microorganism, mainly consisting of polysaccharides, proteins and lipids possess massive metal binding sites such as carboxyl, sulphate, phosphate and amino groups.

This kind of biosorption, non-metabolism dependent, is relatively rapid and can be reversible (Kuyucak and Volesky, 1988).

Biosorption mechanisms can be briefly generalized into several pathways, which are ion exchange, complexation, precipitation, reduction and chelation (Table 1).

There are negative-charge molecules such as carboxyl and phosphoryl groups in the cell membrane able to bind with positive-charge molecules. Proton binding with functional sites was replaced by heavy metal ions, confirming the existence of ion exchange. Halttunen et al found that ion exchange was probably at least partly responsible for the observed metal binding based on the effect of pH in their work (Halttunen et al., 2007). Later, Halttunen et al conducted a further research on comparison of the removal of cadmium, lead, aflatoxin B1 and microcystin-LR by Lactobacillus rhamnosus GG, L. rhamnosus LC705, Propionibacterium freudenreichii shermanii JS and Bifidobacterium breve Bbi99/E8, separately and in combination. They had found that the net surface charge of Lactobacillus rhamnosus GG was evidently higher than other strains with a range of PH and inferred that the negative charges on strains' surface were optimal for binding cations, such as Pb^{2+} (Halttunen et al., 2008). What's more, Teemu et al. used transmission electron micrographs which showed the cell membrane binding with Pb to prove the existence of ion exchange (Teemu et al., 2008). It's worth noting that Shen et al found the concentration of K^+ dropped from 1.76% to 0, while Pb^{2+} went from 0 to 11.72%. Based on

this, they thought ion exchange happens between K^+ and Pb^{2+}, but the absorption peak of Pb^{2+} outclassed that of K^+ which meant ion exchange is not the main pathway (Shen et al., 2006).

Complexation is that metal ions interact with organic molecules by means of covalent or electrostatic force. Masses of researches hold the same view that complexation is the major absorption mechanisms of Pb. Tobin et al.(1984) found alkali metal ions were not bound, obviously, showing that they could not match the ligand in the cell wall(Tobin et al., 2002). From another angle, the complexation is related to the functional groups on cell surface. The carboxyl groups of peptidoglycan polymers and the carboxyl groups and phosphate groups of phosphoric acid play an important role in metal complexation, which has been demonstrated by several researchers using chemical modifications (Beveridge et al., 1980; Sonnenfeld et al., 1985; Graham et al., 1994; Vieira wt al., 2000).

Metal precipitation results from the interaction between heavy metals and extracellular polymers or anions produced by microbes. Metal cations may not only bind on cell surfaces but also within the cell wall and in turn, and metal uptake is increased through micro-precipitation (Ahemad and Kibret, 2013). Allan et al (2002) separated a kind of Citrobacter, having acid phosphatase of Cd tolerances. This kind of bacteria is able to decompose 2-glycero-phosphate, generate HPO_4^{2-} which is combined with Cd^{2+}, therefore forming precipitation (Allan et al., 2012). Strandberg et al (1981) viewed uranium, radium and palladium were accumulated in the aeruginosa by the dense precipitation observed under electron microscopy (Strandberg et al.,

1981). Volesky et al (1993) found that cadmium adopting the form of phosphate precipitation deposited in the cytosol not in the cell wall when using beer yeast to absorb cadmium. Therefore, a proper explanation for enzymatic action is that living cells is likely to absorb metals by carrier protein (Volesky et al., 1993).

Arief et al (2008) found that biosorbents have low BET surface area due to its low porosity and smoothness (Arief et al., 2008). Compared with almost previous studies, they inferred the physical sorption which is spontaneous and exothermic might not the major mechanism in the biosorption. But in several rare biosorption cases, physical adsorption can be dominant. Halttunen et al. (2007) thought that physical adsorption to the cell surface may be the main mechanism for lead (Halttunen et al., 2007). Cultured LAB was more efficient in the removal than the lyophilized cells, which indicated that these biosorption mechanisms can take place simultaneously (Halttunen et al., 2008).

The interaction between LAB and Pb

As a biological adsorbent, LAB have low cost and easy to obtain excellent performance. They have the ability to selectively eliminate heavy metals, adsorption and desorption kinetics of pH and temperature range.   Compared with other microorganisms studied, LAB binds lead to moderate conditions. And according to literature, several LAB strains utilized for fermentation of milk have a good opportunity to survive through the gastrointestinal conditions and it can be

applied for probiotics (Akhmetsadykova et al., 2013). On the basis of massive researches, it can be included that mechanisms of absorption of Pb by LAB are almost the same as that of gram positive bacteria. 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). The slight difference is the metabolite (lactic acid), lactobacillus surface layers and so on. Fu et al (2016) has researched on the mechanism between lactobacillus fermented rice and removal rate of cadmium. They 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. What's more, the removal of cadmium is not only because of the low PH, but also the functional groups like α-hydroxy group and carboxyl group. Lactic acid can promote the protein dissolution. They also included a decrease in protein in rice, an increase in cadmium removal rate (Fu et al., 2016). Gerbino et al (2011) researched the interaction between metal ions and S-layers of the L. kefir CIDCA 8348 and JCM 5818 with the aid of TEM and FTIR. Their findings were as follows: (1) Cadmium generated precipitation in S-layers. (2) After absorption, structure of S-layers changed. The carboxyl of Aspartic acid (Asp) and Glutamate (Glu) along with a few N-H groups of peptide chain changed. (3) The secondary structure of S-layers changed because the number ofα-helix decreased and the number of β- sheet increased. This can reduce energy consumption of combination of metals by changing its appearance (Gerbin et al., 2011).

The bacterial cell walls play an important role in chelating metal. LAB belongs to gram positive bacteria, compared with gram negative bacteria, whose surface is a thicker and sealed reticular formation mainly composed of multilayer peptidoglycan (Nasir et al., 2007). Beveridge et al found peptidoglycan layer in the cell wall from B. subtilts demonstrated that it could combine with a lot of transition metals (Beveridge et al., 1995). The binding process in the cell wall is metabolism-independent. When the binding sites provided by macromolecule substance are saturated, complexation begins to work in the accumulation of Pb (Sun et al., 2007). Teichoic acids appear to extend to the surface of the peptidoglycan, and, because they are negatively charged, lead to the cell wall carrying negative charge (Wang et al., 2009). The teichuronic acids are free of phosphate and made up of hexuronic acid linear chains. There is a proof that the teichoic acids and teichuronic acids are involved in metal tripping, for both the phosphoryl groups of the secondary polymers and the carboxyl groups of the peptide chains possess negatively charged sites in the Gram-positive cell wall (Remacle et al., 1990); (Urrutia et al., 1997). Gourdon et al. compared the Cd^{2+} biosorption difference between Gram positive and Gram negative bacteria. The reticular formation is able to enhance adherence and absorption of lead. It was suggested that glycoproteins existed in the outer side of Gram positive bacterial cell walls have more possible functional sites for Cd^{2+} than the phospholipids and LPS from Gram negative bacteria and thereby accounts for the observed difference in capacity (Gourdon et al., 1990). Special components with many charged groups, such as polysaccharide, murein and

associated protein, exist in the cell wall (Liu et al., 2016). Clark et al used chemical ways to neutralize some special groups in the cell surfaces, the absorption capacity was dramatically decreased which indicated that these groups function in the process of binding Pb (Clark et al., 2008). LAB is able to produce slime layer above the bacterial cell wall, which contain much hydrate and has a loose distribution of carbohydrates and proteins. S-layers are composed of polysaccharides and a few consist of proteins or polypeptides. It is accepted that S-layers are associated with peptidoglycan of a gram-positive cell, which could be an analogy of LAB. Recent studies have focused on the capacity of lactobacilli bearing SLP for metal removal (Mrvcˇicˊ et al., 2009); (Mrvcˇicˊ et al., 2012); (Dohm et al., 2011); (Schut et al., 2011);(Gerbino et al., 2012). In some of them, the part of SLP regarded as functional structures to remove metals was suggested but not directly proved (Mrvcˇicˊ et al. 2009); (Gerbino et al. 2012). Gerbino et al. researched the interaction between metal ions and S-layers of the L. kefir CIDCA 8348 and JCM 5818 with the aid of TEM and FTIR. Their findings were as follows: (1) Cadmium deposited in S-layers. (2) After absorption, structure of S-layers changed. The carboxyl of Asp and Glu along with a few N-H groups of peptide chain changed. (3) The secondary structure of S-layers changed because the number of α-helix decreased and the number of β-sheet increased. This can reduce energy consumption of combination of metals and show a greater thermal stability by changing its appearance (Gerbino et al., 2011). While the new research conducted by Gerbino et al. shows that the phosphate groups from teichoic acid and the carboxylates from superficial

structures including SLP are involved in the interaction of both L. kefir CIDCA 8348 and JCM 5818 with Pb^{2+} with the help of Raman spectroscopy. Another finding is that microorganisms without SLP removed more Pb^{2+} than those bearing SLP and isolated SLP removed less Pb^{2+} than those combined with whole strains. It can be deduced that when carrying SLP, microorganisms have enough charged groups on their surfaces leading to low efficiency in Pb^{2+} biosorption. That is to say, SLP play an inessential role in Pb^{2+} removal. Meanwhile, the research found that Lactobacillus kefir without SLP were more likely to suffer the detrimental effect of Pb^{2+}, which suggested that SLP probably acts as a protective instead of a sequeatrant layer (Gerbino et al., 2015).

Macromolecule metabolites produced by LAB, like polysaccharide and nucleic acid, contain different kinds of charged groups like carboxyl, hydroxyl and phosphate groups, which have electrostatic interaction with Pb. LAB secreted exopolysaccharides (EPS) into the extracellular environment during growth, which is regarded as biosorbents for heavy metal removal. The interaction between metal cations and negative charges with acidic functional groups of EPS, such as–OH, COO–, C = O, and –NH, can account for the mechanism of removing heavy metals (Gerbino et al., 2011). On the one hand, H^+ ions in the solution finish Pb(II) ions for combination with the active binding sites on surface of 70810 EPS results in the decrease of the number of negatively charged adsorption sites with positively charged heavy metal ions (Feng et al., 2012). On the other hand, the components of EPS with various charges have different absorption

performance with metal ions. Taking some common bivalent metal cations, Pb^{2+}, Cd^{2+} and Cu^{2+} as an example, Pb^{2+} has the smallest radius (450 pm), followed by Cd^{2+} (500 pm) and Cu^{2+} (600 pm). So their charge density order is Pb > Cd > Cu. However, their actual adsorption affinity orders with EPS were surprisingly different: Pb > Cu > Cd at pH 6 and Cu > Cd at pH 7–8 (Comte et al., 2008). It is concluded that the ion exchange mechanism is not the only reason for the metal adsorption of EPS (Li et al., 2014). In Figure 2, we can find not noly the ion exchange, complexation as well as surface precipitation are all involved in the biosorption but also due to a high diversity and complexity of EPS composition, in practical niches multiple adsorption mechanisms usually occur simultaneously.

It's worth noting that while there are other co-exist ions in the solution, these ions should compete with Pb(II) ions for limited negatively charged groups so that the biosorption of Pb is inhibited. Actually, biosorption is used to handle multi-metal systems because there is more than one type of metallic ions in the medium. The elimination of one metal ion is inevitably influenced by other metal species (Deng and Wang, 2012). For example, cobalt absorption by different microorganisms is nearly inhibited accompanied by uranium, lead, mercury and copper (Ahalya et al., 2003). It can be concluded that the biosorption of microorganisms has a selection for metals.

Comparison between Biosorption and Bioaccumulation

The mechanism of the effect of heavy metals on biology

As an important common pollutant, the toxic action of heavy metals to biology mainly manifests in two aspects. On the one hand, heavy metals are likely to combine with some biological micromolecules such as the active center of enzyme and electron-donating group such as the sulfonyl and carboxyl in protein, basic group and phosphate in nucleic acid resulting in activation of biological micromolecules. Ultimately, the biont is disease and death. On the other hand, since most heavy metals can't be biodergradable, they will combine with a large number of metallothionein, metallothionein-like and small molecular ligands such as glycine, taurine. They are extremely easy accumulated in organisms and the accumulation function will transfer with food chain which is harmful to the whole food chain especially the human health and life safety.

Comparison between biosorption and bioaccumulation

The absorption aims to isolate the heavy metal from contaminated water with the aid of functional components and characteristic structures. The biosorbents are abundant in nature, such as algae, lichen, fungi, bacteria and so on. The complicacy of microbial structures and difference in affinity between one microrganism and different metals determine the biosorption mechanism is very complex, which has not yet been recognized.

According to the distribution of heavy metal ions in cells, it can be classified into two parts, which are biosorption and bioaccumulation (Table 2, Figure 3). In this figure, we can clearly see the significant differences between biosorption and bioaccumulation. These differences will be described in detail below.

Biosorption is a passive and metabolism-independent process by means of the microbial cell wall, including ion exchange, complexation, precipitation and chelation. Brady and Duncan (1994) drew a conclusion that coordination bonds play an important role in metal ions binding for Cu^{2+} binds to a greater degree than Na^+. At the same time, they considered that some ionic bonding is also involved because copper was partly displaced by sodium ions (Brady and Duncan, 1994). Under favorable conditions, metal affinity is considerably high. The rate of metal uptake is generally rapid. The equilibrium only needs a few seconds for outer cell wall accumulation to be reached. One study shows that the biosorption of dead cells is faster than that of living cells for the physiology of cell plays an important role in metal uptake. The reason may be that lack of metabolism and proton gradient lead to negative polarization which improves the affinity between metal ions and biosorbents (Brady and Duncan, 1994). The process of biosorption is poor selectivity due to a variety of ligands with various affinities for different metals. The complexity of microbial cell wall compositions implies that there are multiple classes of metal cation binding sites in the surface (Wang et al., 2014). Three most important chemical ligands may be carboxyl, amino, and hydroxyl groups. Hydroxyl groups, which are

originated from the carbohydrate polymers, are far more than the other two groups. However, they have low affinity for metal. Thanks to their collective advantage, hydroxyl groups are considered to be crucial in metal binding. The carboxyl groups exist in the side chains of acidic amino acids, while the amino groups also constitute proteins (Brady and Duncan, 1994). Obviously, the cell wall components play a role in biosorption. Within a modest range of temperature, the biosorption is efficient. Matal uptake is likely affected by anions or other molecules. Teemu et al.(2008) found that certain cations had an influence on binding Cd^{2+} and Pb^{2+}, such as Mg^{2+}, Ca^{2+}, Fe^{2+} and Zn^{2+} (Teemu et al., 2008). Extracellular polymeric substances are secreted by microorganism. The main compositions are polysaccharide and polypeptide. Protein, nucleic acid and other else are relatively few. These compositions with carboxyl, phosphate groups and hydroxyl groups have interaction with metal ions (Wang et al., 2014). And massive experiments prove that extent of metal uptake is usually pH dependent. In the process of biosorption, the hydrogen ions have been displaced during cation absorption. The extent of proton displacement may affect significantly by the ambient pH, which would even determine the protonation status of the cell wall ligands (Brady and Duncan, 1994). Bioaccumulation is quite different from the biosorption. For living cells, many metal ions are premature on the cell surface, and then several will pass through the transmembrane into the cell. The latter is an active and metabolism-dependent process of metal removal by living cells referred as bioaccumulation. The mechanism of bioaccumulation and detoxification at least includes two sides. One is that

ligands inherent or induced in some conditions bind immediately with heavy metals for accumulation. At the same time, these metals can not react with intracellular bioactive substances like enzyme and nucleic acid so that their toxicity decrease. In the cells, microorganisms utilize conjugation to restrict metal ions in inactive areas or combine metals with heat-stable protein which turn it into low toxicity. For example, the absorbed Sr, Co ions are accumulated in the vacuole, while the Cd, Cu ions lie in the soluble areas of the living yeast. Another is that the metals converge in subcellular granule by cell metabolism and are packed by biofilm so that they are separated from other cells (Kinoshita et al., 2013). Metal toxicity wound affect living cells binding metal uptake on inhibiting enzyme activities or even leading to death. And the metabolism is affected by various factors, which conduces its limitation. But the recent researches have a different finding that some shellfishes in the ocean have an accumulation on metals. Although the accumulation of metal in vivo is rather high, this kind of organism shows no signs of intoxication. It can be speculated that in spite of limited metal-binding proteins and others executing similar function, the conjugation is reversible. On the basis of the specificity of functional components, the selectivity of bioaccumulation is better than biosorption, but less than chemical technologies. Besides, the rate of metal absorption is usually slower than that of biological adsorption. The reason may be that the bioaccumulation requires an energy source and depends on plasma ATPase activity.

According to the comparison between biosorption and bioaccumulation, it is obvious that two methods have their own superiority in different aspects. It also lays the foundation for the mechanism of metal uptake.

Influential Factors for Metal Adsorption

Effect of pretreatment

According to the biosorption mechanism of LAB, the adsorption capacity mainly depends on the amount, accessibility and chemical state of the sites and metals (Ahemad and Kibret, 2013). Therefore, preprocessing is an effective way to increase the biosorption of lead by enhancing the interaction between cell binding sites and lead, because of exposure of binding sites and increase of good affinity. A large number of studies have shown that the pretreatment methods such as heat treatment, acid and alkali treatment, crosslinking with organic solvent have significant effects on the adsorption capacity of lead. Paul et al. got a conclusion the autoclaving of bacterial biomass enhanced the adsorption capacity of heavy metal (Paul et al., 2012). The reason why the treatment works is that the cell wall ruptures and exposes more number of potential binding sites for heavy metal (Aryal and Liakopoulou-Kyriakides, 2015). The speculation could also account for the chemical pretreatment with detergent, acid and alkali treatment. Among these methods, unlike heat treatment and washing with detergent/acids/alkalies which increase absorption efficiency by exposing additional metal binding sites on the surface, enzymatic treatments

destroy undesired components to facilitate biosorption. Yan et al. reported that in these chemical ways, the cellular internal autolytic enzymes causing putrefaction of biomass may be destroyed and the cell wall will release certain biopolymers which have high affinity to the heavy metal ions by removing the lipid and proteins which cover binding sites primitively (Yan and Viraraghavan, 2000). Generally, all the pretreatments aim to increase the number of binding sites and enhance the affinity and accessibility of it, but the same pretreatment has a different effect on different heavy metals.

Effect of pH

pH is one of the most significant parameters by influencing microbial metabolism and growth, the chemical properties of metal ions, the activity of cell functional groups and the competition between coexisting ions in variable extent. The relationship between adsorption capacity and pH value is not a linear function but most metal sorption is enhanced with pH in a particular pH range until a certain value, then, a reduction follows with the continuing increase pH (Arief et al., 2008). The impact mechanism is that a large number of H_3O^+ occupies the binding sites enhancing the electrostatic repulsion of functional groups in the cell surface when at a low pH value, while the hydrogen ions will hinder the approach of metal cations by the competition with metal cations about the binding sites and the metal cations are in a disadvantage due to the strong repulsive forces. On the contrary, there will be more groups with

negative charge with the decrease of H_3O^+ with the gradually increasing pH. The increasing combined probability between metal cations and cell functional groups bring about increasing biosorption capacity naturally but only limited a certain value. Halttunen et al. have found that with the help of lactic acid bacteria, the removal of lead was high (55%) already at pH 2 and the highest removal (95%) was achieved at pH 6, but pH values higher than 6, the precipitation of metals will reduce the absorbing capacity on the contrary (Halttunen et al., 2007).

Effect of temperature

Temperature affects the adsorption capacity to a certain extent within the normal growth temperature range while beyond the range the too high temperature will lead to harmful results because some available binding sites were destroyed. The absorption capacity of bacterial for heavy metal ions mostly depends on whether the interaction between metal ions and binding sites is exothermic or endothermic. The exothermic or endothermic nature of a process is determined by the temperature changes which alter the adsorption equilibrium. The surface activity and kinetic energy of the solute increase with higher temperature at an appropriate range are another reason. Many researchers have found this trend (Guzel et al., 2008; Uslu and Tanyol, 2006; Shen and Duvnjak, 2004). And they have proved the phenomenon by exploring the impact of temperature on the adsorption isotherm of cupric and cadmium ions. However, the optimum adsorption temperature of different microorganisms is various and most of the literatures

determined the optimum temperature for heavy metal sorption between 20 and 35 °C (Aryal and Liakopoulou-Kyriakides, 2013).

Effect of contact time

Biosorption is divided into two main processes: extracellular adsorption and intracellular bioaccumulation. The former is a rapid and metabolism-independent process. Metal ions differ from solution to adsorbent surface in a short time which involves surface conjugations such as ion exchange and surface complexation. The passive transport stage can only last a few minutes when the equilibrium between the adsorbed metal ions and free metal ions in solutions is reached. It can be speculated that the abundant availability of empty binding sites on the biosorbent and the high concentration of heavy metal ions in solution lead to a fast phase. And it may involve these mechanism such as the adsorption of the groups in cell wall surface, the exchange and complexation of ion, microprecipitation and so on. As contact time prolongs, the adsorption becomes slower because the gradual occupation of these sites and repulsive forces from metal ions adsorbed on adsorbents surface. But there was another speculation that the latter stage is a slow metabolism-dependent time-consuming and slow effect process which connects with active transport and transmembrane transport. In this process, the metastasis of adsorbed metal ions from surface into the cells may be limited by cell metabolism and vector. Akar. et al (2006) have indicated that rapid biosorption of ion was observed in 30 min and the maximum

removal occurred in 2h(Akar and Tunali, 2006). There was no significant change beyond that interval. Many studies have explained that beyond that there is less vacant surface sites remained and it's more difficult for metal ions to occupy.

Effect of initial metal ion concentration

The initial metal ion concentrations are also an important parameter for adsorption capacity. Two main reasons may account for the influence. On the one hand, it's related to saturation of binding sites. If the metal ion concentration is limited within the range of cell resistant capacity, microbes can combine with heavy metal ions normally and the adsorption capacity increases with the higher ion concentrations, but if the metal concentration is too high for cell to resist which arrived the maximum tolerance dose, the superfluous ions will compete the limited binding sites resulting in lower biosorption efficiency and even show toxic effects in cells like inhibiting the growth of its metabolism and leading to rupture. On the other hand, the initial concentration serves as a driving force to overcome mass transfer resistance between metal ion and the surface of the biomass. At present, many studies have summarized the regularity about the effect of initial heavy metal concentration on metal ion (Khambhaty et al., 2008; Baral et al., 2007). Aryal et al. also reported the reason why the adsorption capacity enhances with the increase of metal ion concentrations is that the metal ion concentration provides the necessary driving force to overcome the resistance to the mass transfer of metal ions between aqueous and

solid phases, but when the ion reaches the upper-limit the biosorption efficiency still decreases (Aryal et al., 2010).

Effect of biomass concentration

The biomass concentration is also one of the most important parameters for biosorption, but it usually shows composite effects with metal ion concentration together on biosorption efficiency. Generally speaking, adsorption capacity will improve with the increased biomass concentration due to the available adsorption sites or functional groups were also increased. However, the biosorption efficiency begins to decline beyond the equilibrium point due to the electrostatic interactions produced by thallus. That means when the biomass concentration is too high, the overlapping and coaggregation of bacteria to form particles of more than one cell would cut down the binding sites on the surface. Yu et al (2016) have indicated that the removal rate of lead ions increased at first and then decreased. When the biomass concentration is 1~4 g/L, the removal rate increase gradually but at 4~7g/L the removal rate begins to decline to 84. 10%. The highest removal rate is 97.85% at 4g/L.

Effect of interfering ions

Most microorganisms are usually capable of adsorbing various heavy metals, but are selective for metals. There are two different competitions impacting the absorption. When

multiple heavy metal cations co-exist in solution, there will be an inter-constraint between each other. If the affinity between coexisting ions and binding sites is higher than target ions, all the ions will compete for a fixed number of negatively charged groups causing decrease combining target ions. Interestingly, co-exist metal cations have a synergistic effect on improving the absorption efficiency in few cases. The reason can be that certain metal cations destroy cell membrane and make it easy to transmembrane transport. Incharoensakdi et al. have proved that Ca(II), Hg(II), and Pb(II) showed an inhibitory effect on each other. But in some special cases, the coexisting ions may increase biosorption efficiency by a synergy between coexisting ions and target ions (Incharoensakdi and Kitjaharn, 2002). Tu et al.(1995) found that Cu^{2+} can increase the adsorption of rhizopus nigricans to Cr (VI) anion can be inferred that Cu^{2+} neutralize the negative charges in cell wall improving the opportunity of the combination between anions and active sites. While anions in solution will have a competition with binding sites on cell surface for free metal ions (Tu et al., 1995). The stability of binding forces between anions and free metal cations has a great impact on absorption capacity.

Effect of other factors

There are some other factors can also affect biosorption capacity such as microbial cell structure, the affinity between binding sites and metal ions, metabolites, thallus age, composition of culture medium and so on, but all of these are not the main factors.

Halttunen et al. concluded that the removal efficiency of a combination of LAB is less than a simple sum of their individual capacities. Hence, pure single strains should be used when it is aimed at removal of single compounds. The combinations of strains may work well when several compounds are mixed (Halttunen et al., 2008).

Chen et al. have found that inoculating times also influence the biosorption capacity due to the activity change of microorganisms. There used to be an experiment showing that the adsorption rate of a sort of bacterial strain declined from 84.2% to 32.2% at the third inoculating time and even declined to 6.0% at fifth time. But the degradation effects vary from different species of bacterial strain (Chen and Zhao, 2001).

Instrumentation for Biosorption Research

The metal binding mechanism on the cell surface can be inferred on the basis of the change of the physical and chemical characteristics of biosorbents. In order to make a full sense of the structure, morphology and composition of the strains, various techniques are utilized for the purpose, such as Fourier Transform Infra-Red (FTIR) spectroscopy, X-ray Photo Electron Spectroscopy (XPS), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Energy Dispersive X-ray (EDX) fluorescence spectrophotometry, nitrogen sorption, etc (Arief et al., 2008). Srivastava et al. proposes a framework on instrumental analysis for characterization of the adsorbent and their outcomes (Figure 4). In this figure, except the mentioned methods above,

there are other methods to characterize the adsorbents and their applications, and the content of this diagram is very detailed, and various features are also explained. When it is used properly, the proper technology can be chosen according to the specific conditions.

FTIR Spectroscopy

FTIR Spectroscopy method works when infrared ray with specific wavelengths is absorbed by the substance under test, which is only able to provide a qualitative description of the structure and composition of it. The method adopted in the researches is aim to determine the certain functional groups in the surface, making it possible to further probe the effect of these groups in the metal binding process. Based on the FTIR conclusions, there are some assumptions emerging about how the functional groups work in metal binding.

Pavasant et al. focus on the link between functional groups and the heavy metal adsorption potential of the dried marine green macro-alga Caulerpa lentillifera with the help of FTIR (Pavasant et al., 2006). Combining with the transmission spectra shift at certain wave-numbers indicated that some functional groups are related to the binding of Pb(II). For example, as for the amine groups, N-H stretching attributed to Pb(II) binding. It can be speculated that carboxylic acid, amine, amide, and $S = O$ within sulfonyl and sulfonate groups play an important role in metal binding.

Yu et al. observed a remarkable shift in the N-H stretching vibration peaks after the combination through FTIR, which convincingly proved the interaction between the Pb(II) and amide groups (Yu et al., 2007). Raize et al. also studied the biosorption mechanism of Cd(II), Ni(II) and Pb(II) by brown marine macroalga (Raize et al., 2004). The result is the same as the Pavasant's. And Gupta and Rastogi conducted that the amino, carboxyl, hydroxyl and carbonyl groups of the green algae Spirogyra should be responsible for binding lead (Gupta et al., 2008). Feng et al. used FTIR spectrum to identify the role of functional groups of EPS in Pb(II) biosorption. They found that there were several absorption peaks at 3392 cm^{-1} (indicative of –OH groupand –NH stretching), 2934 cm^{-1} (indicative of –CH_2 asymmetric stretching vibration), 1652 cm^{-1} (indicative of $C = O$ stretching vibration, amide I), 1554 cm^{-1} (indicative of N–H bending and C–N stretching, amide II), 1422 cm^{-1} (indicative of COO– anions), 1242 cm^{-1} (indicative of $C = O$ deformation vibration and–OH stretching vibration), and 1048 cm^{-1} (indicative of –OH stretching) (Bl´azquez and others 2010; Cao and others 2011). However, a shift of the peaks at 3369, 2927, 1650, 1548, 1409, 1241, 1054 cm^{-1} was observed after Pb(II) biosorption onto 70810 EPS surface, respectively, which showed that these functional groups is relevant to the mechanism (Feng et al., 2012).

Scanning Electron Microscopy

The Scanning Electron Microscopy (SEM) is on the basis of the interaction between electron and the substance. It has a large depth of field and high resolution which makes the high magnification possible to produce a clear image (Gupta and Rastogi, 2008; Das and Guha, 2007; Raize et al., 2004; Han et al., 2006; Yavuz et al., 2006; Srivastava and Thakur, 2007). The device produces the high-energy electron beam scanning the specimen to excite the topographical and elemental information. That is to say, when the electron beam hit a point in the specimen, there is a corresponding light on the fluorescent screen. What's more, the luminance is in proportion to the electron energy. Through the procedure of acceptances, magnifications, images, the topographical aspects of material can be obtained.

SEM pictures and EDX spectra adopted, before and after the heat inactivated Botrytis cinerea binding Pb(II), evidently proved the deposition of Pb which seemed "billiard balls" on the cell's surface (Akar et al., 2005). Feng et al. carried out a research about the surface morphology of the 70810 EPS before and after the binding of Pb(II) (Figure 5). In Figure 5, we can observe that the surface of EPS was rough with many visible particles depositing on the surface after the Pb(II) biosorption compared to the smooth surface before which indicated that Pb(II) ions are adsorbed onto the 70810 EPS surface. And the EPS is a high-molecular weight sorbent and with many binding-sites which can effectively adsorb the Pb(II) ions from the aqueous solution (Feng et al., 2012).

Transmission Electron Microscopy (TEM)

The principle of Transmission Electron Microscopy (TEM) aim to throw the electron beam accelerated and gathered into the very thin specimen, and then induce the change of direction of motion of electron beam, thus producing a solid angle scattering. The thickness and density of the specimen are responsible for the scattering angle, forming images with light and dark distribution. In general, TEM is capable of providing information on internal crystalline structures, topography and the density of substance. Although the TEM has a high resolution and magnification, the limitation of it is the costly price.

Kim et al.(2007) found that CPB4 cells have the different mechanism with different heavy metals. What's more, the TEM scanning results are consistent with the data that approximately 70.4, 35.5 and 6.1% of Pb deposited on the cell wall, cell membrane and cytoplasm, respectively (Figure 6). In this figure, electron-dense granules are mainly gathered on the cell wall and cell membrane in Pb-uptake cells, on the cell membrane in Cd-uptake cells and on the outside but located very close to cells in Cu-uptake cells. The results not only indicated the CPB4 cell has a mutative types of biosorption with different heavy metals, but also showed the cell wall and cell membrane are likely to uptake the heavy metals (Kim et al., 2007). Halttunen et al. (2008) employed the TEM to observe the results of lyophilized B.longum 46 and L. fermentum ME3 before and after lead sorption (Halttunen et al., 2008). A large deposits of Pb on the surface after

binding is a strong proof to speculate that LAB has a potential in lead biosorption (Teemu et al., 2008).

X-ray Photoelectron Spectroscopy (XPS) Analysis

X-ray photoelectron spectroscopy (XPS) is a special chemical analysis technique which utilizes the X-ray to irradiate the surface of specimen producing the photoelectric effect. According to the energy of photoelectron released to the vacuum, the information about the elemental composition, the chemical and electronic states of the elements can be obtained.

In the field of biosorption, XPS is capable of scrutiny of the surface structure of the strains (Vijayaraghavan and Yun, 2008; Yu et al., 2007a; Yu et al., 2007c; Raize et al., 2004; Zhou et al., 2005; Taty-Costodes et al., 2003; Sheng et al., 2004; Park et al., 2005; Deng et al., 2006; Yu et al., 2007b; Tong et al., 2010). In a research XPS was employed to scrutinize the surface structure change of backer's yeast which was modified by grafting polyamic acid. And the results showed that the interaction of Pb(II) with amide and hydroxyl groups results from ion exchange or electrostatic interaction (Yu et al., 2007c).

Energy-Dispersive X-Ray Spectroscopy

The application of Energy-Dispersive X-Ray Spectroscopy (EDX) is similar to XPS, which can also be used to analyze elements or chemical characterization of a specimen. And the method

is employed in concert with SEM to obtain more comprehensive information (Bueno et al., 2008; Davis et al., 2003; Panda et al., 2008; Han et al., 2006; Srivastava and Thakur, 2007; Taty-Costodes et al., 2003; Akar et al., 2007; Liu and Xu, 2007; Majumdar et al., 2008; Basha et al., 2008; Chen and Wang, 2008). The sample emitted X-ray when it suffers electron beam bombardment. The X-ray which carries a special energy is relevant to their molecular weight (Arief et al., 2008).

Further determination of the involvement of ion exchange in the adsorption of heavy metals through MPW was detected by Energy-Dispersive X-Ray Spectroscopy (EDX). Iqbal et al. shows a phenomenon that a release of cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and proton H^+ from MPW with the corresponding absorption of Cd^{2+} and Pb^{2+}, which confirmed that the main mechanism of sorption was likely to be ion exchange (Figure 7). The Figure 7 displays the representative EDX type for MPW before and after the sorption of Pb^{2+}. The Figure 7a did not show the characteristic signal of Pb^{2+}, but that was found in Figure 7b. These finding indicate the involvement of ion exchange mechanism for the removal of metal ions by MPW.

X-Ray Absorption Fine Structure Spectroscopy (XAFS)

X-ray absorption fine structure spectroscopy (XAFS) is working to utilize X-rays to explore the physical and chemical structure of substance at an atomic scale. An adjustment of the X-ray energy is made to be paralleled with the inner electronic shell and then utilize X-rays to detect

sample. The correction between the amount of absorbed X-rays and its energy can indicate what has a strong connection with attached metals in the study of biosorption mechanism.

The research carried out by Guiné et al. concluded that for the two bacterial samples (*C. metallidurans* and *E. coli*), Zn is bound to O and S neighbors. While in the study of C (Guiné et al., 2006). Chen et al.(2008) found that the nearest neighboring atom bound to Zn is oxygen atom (Chen and Wang, 2008).

X-Ray Diffraction (XRD) Spectroscopy

As the sample is unaltered by X-rays, X-ray diffraction (XRD) is regarded as a non-destructive method to acquire detailed information on the crystallographic structure of substance. The main principle of XRD analysis is the interference of monochromatic X-rays and a crystalline sample: The X-rays are generated from a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the rays with the specimen produces constructive interference while conditions fulfill Bragg's Law ($n\lambda = 2d \sin \theta$). The characteristic x-ray diffraction pattern produced provides a unique "key" of the crystals present in the sample. When properly interpreted, compared with standard reference patterns and measurements, this key ascertains the crystalline pattern. As an assist in the characterization of biosorbents and biosorption mechanism, XRD helps a lot (Volesky, 2001; Zhou et al., 2005; Liu and Xu, 2007; Lin et al., 2005).

Based on the XRD image of the Pb-loaded cellulose/chitin beads displayed distinct and complex peaks, indicating the deposition of crystallized lead, Zhou et al.(2005) deduced it likely to be lead hydroxides ($Pb(OH)_2$) (Huang and Lo,2004) and lead carbonate ($Pb_3(CO_3)_2(OH)_2$, $PbCO_3$) (Lee et al., 1997)(Zhou et al., 2005).

Raman spectroscopy

Raman spectroscopy is used for the analysis of interaction between bacteria and metals at a molecular level, and then obtains information about the relevant functional groups. The utilization of Raman spectroscopy on the metal biosorption plays an important role in not only acting as a quantification method but also providing structural information about the functional groups.

The great differences were observed in the comparison of the bacteria/metal samples and the controls from the Raman spectra results on the research carried out by Gerbino et al. It supports that the S-layers participate in the interaction of metal and bacteria, which is related to carboxylate and phosphate groups and polysaccharides exposed to the surfaces (Gerbino et al., 2012).

Desorption and Metal Recovery

Desorption occurred after the biosorption processing. There are many methods used in desorption processing such as common chemicals washing. Huang et al.(2010) have showed that the desorption effect of sodium carbonate and EDTA is best. After the first washing of sodium carbonate, desorption rate is 92.5% and it could even reach up to 97% after the third washing. In addition, adding surfactant to bacterium is another method. Surfactant can take up the adsorption site of heavy metals to finish the desorption (Ma et al., 2010).

Desorption could not only achieve the goal of the recycle of microorganism, meanwhile, the rare heavy metals could be extracted from the desorption and be recycled after special processes in order to save cost and reduce environmental pollution.

Conclusions and Outlook

In recent years, some achievements have been made in the field of lead pollution at home and abroad. There are many traditional methods for treating lead, such as precipitation, biosorption, condensation, ion exchange and so on. Nowadays, biosorption have attracted more and more attention. Lactic acid bacteria were selected as the research materials, which are easy to obtain, inexpensive, and beneficial to humans and the environment. 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. In this paper, the principle of biosorption is introduced in detail, and the interaction

between lactic acid bacteria and lead is emphasized. Many factors can greatly affect adsorption process, such as pretreatment, pH, temperature, contact time, initial metal ion concentration and so on. On the basis of many researches, different factors have different influence and the effects of pretreatment, pH and temperature are relatively greater.

Biosorption is uesd to remove toxic heavy metals from waste water and recover rare and precious metals, which has the advantages of high efficiency, low cost, simplicity and selectivity. In particular, low concentration heavy metal in waste water which is not treated with conventional methods but can be treated by biosorption. Therefore, biosorption is a promising method for the treatment of heavy metal in waste water.

Although biosorption is an effective way to remove heavy metals from waste water, its industrialization has been slow, and the study of the mechanism is not thorough. Therefore, further research should be carried out on the mechanisms and the regeneration of biosorption. At present, there are many problems to be solved for biosorbent, and the practical application of biosorption is also facing a severe test. On the one hand, because the biosorption of dead cells is difficult to be applied, and the removal of some metals is not good, people are turning to the biosorption of living cells. On the other hand, developing a commercial biosorbent similar to an ion exchange resin is essential,which merely need low growth conditions and is easy to carry out large-scale industrial cultivation and help reduce the cost of biosorbent. To sum up, 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.

Author Contributions

Dering Lin, Ran Ji, Mengshi Xiao designed the structure of this review. Dering Lin, Ran Ji, Mengshi Xiao, Dan Wang and Tao Qin drafted the manuscrift and completed the presentation of periodical. Peng Liu collected and sorted out the references. Dering Lin, Baoshan Xing gave some valuable advices about the structure of the manuscript and made contribution to the revision of the manuscript. Yuan Chen made certain contribution to the language and form modification of the review.

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Table 1. Biosorption data of different biosorbent with lead

strains	Efficiency of the process (as uptake (Q, mg/g) or removal efficiency (Re, %))	references
E. faecium Pb12	Q = 0.0460 mg/h/g of cells	(Bhakta et al., 2012)
Lact. reuteri strains Pb71-1	Re = 59%	(Bhakta et al., 2012)
Lactobacillus pentosus ITA23	Re = 90.78%	(Jahromi et al., 2016)
Lactobacillus acidipiscis ITA44	Re = 90.00%	(Jahromi et al., 2016)
Lactobacillus fermentum ME3	Re = Over 90.00%	(Teemu et al., 2008)
Lactobacillus fermentum ME3	Re = 66.6%	(Rayes, 2012)
Lactobacillus acidophilus X37	Re = 92.8%	(Rayes, 2012)
Najas indica	Re = 92%	(Singh et al., 2010)
	Q = 3.554	
Bifidobacterium longum 46	Re = Close to 80.00%	(Teemu et al., 2008)
Propionibacterium freudenreichii shermanii JS	Re = 69.6%	(Halttunen et al., 2008)
G.thermodenitrificans	Re = 18.22%	(Chatterjee et al., 2010)
Bacillus firmus	Q = 467 (mg/g^{-1})	(Salehizadeh and Shojaosadati, 2003)
Bacillus sp.	Q = 92.3(mg/g^{-1})	(Tunali et al., 2006)
Corynebacterium glutamicum	Q = 567.7(mg/g^{-1})	(Choi and Yun, 2004)
Enterobacter sp.	Q = 50.9(mg/g^{-1})	(Lu et al., 2006)
Pseudomonas aeruginosa	Q = 79.5(mg/g^{-1})	(Chang et al., 1997)

Pseudomonas aeruginosa	$Q = 0.7 (mg/g^{-1})$	(Lin and Lai, 2006)
Pseudomonas putida	$Q = 270.4 (mg/g^{-1})$	(Uslu and Tanyol, 2006)
Pseudomonas putida	$Q = 56.2 (mg/g^{-1})$	(Pardo et al., 2003)
Streptomyces rimosus	$Q = 135 (mg/g^{-1})$	(Selatnia et al., 2004c)

Table 2. Comparisons of characteristic features of heavy metal biosorption and bioaccumulation processes

Feature	Biosorption process	Bioaccumulation process
Definition	The biosorption is a passive and metabolism-independent process by means of the microbial cell wall.	The bioaccumulation is an active and metabolism-dependent process of metal removal by living cells.
Process	Single stage passive process	Double stage active process.
Metal affinity	Under favorable conditions, the metal affinity is considerably high.	Toxicity affects metal uptake by living cells, but in some instances, there is high metal accumulation.
Rate of metal uptake	The rate of metal uptake is Extremely rapid. The equilibrium only needs a few seconds.	The rate of metal uptake is normally slower, for the high toxicant concentration with the detriment to living cells.
Metal binding regions	Metals are bound to the cell surface	Metals are bound to cellular surface and interior structures.
Selectivity	The process of biosorption is poor selectivity due to a variety of ligands with various affinities for different metals. However, it can be improved by modification/processing of biomass.	On the basis of the specificity of functional components, the selectivity of bioaccumulation is better than biosorption.
Temperature	Within a modest range of temperature, the biosorption is efficient.	The bioaccumulation is inhibited by low temperatures.
Versatility	Metal uptake may be affected by anions or other molecules. Extent of metal uptake usually pH	The process require an energy source and is dependent on plasma membrane ATPase activity.

	dependent.	
Toxicant recovery	With proper selection of elutant, toxicant recovery is possible. Several examples proved that acidic or alkaline solutions are effective to recover toxicants.	The biosorbents are non-renewable.

Reference"**Dutton and Fisher, 2011**","**Vijayaraghavan and Yun, 2008**"suggested the following comparison of characteristic features of heavy metal biosorption and bioaccumulation processes.
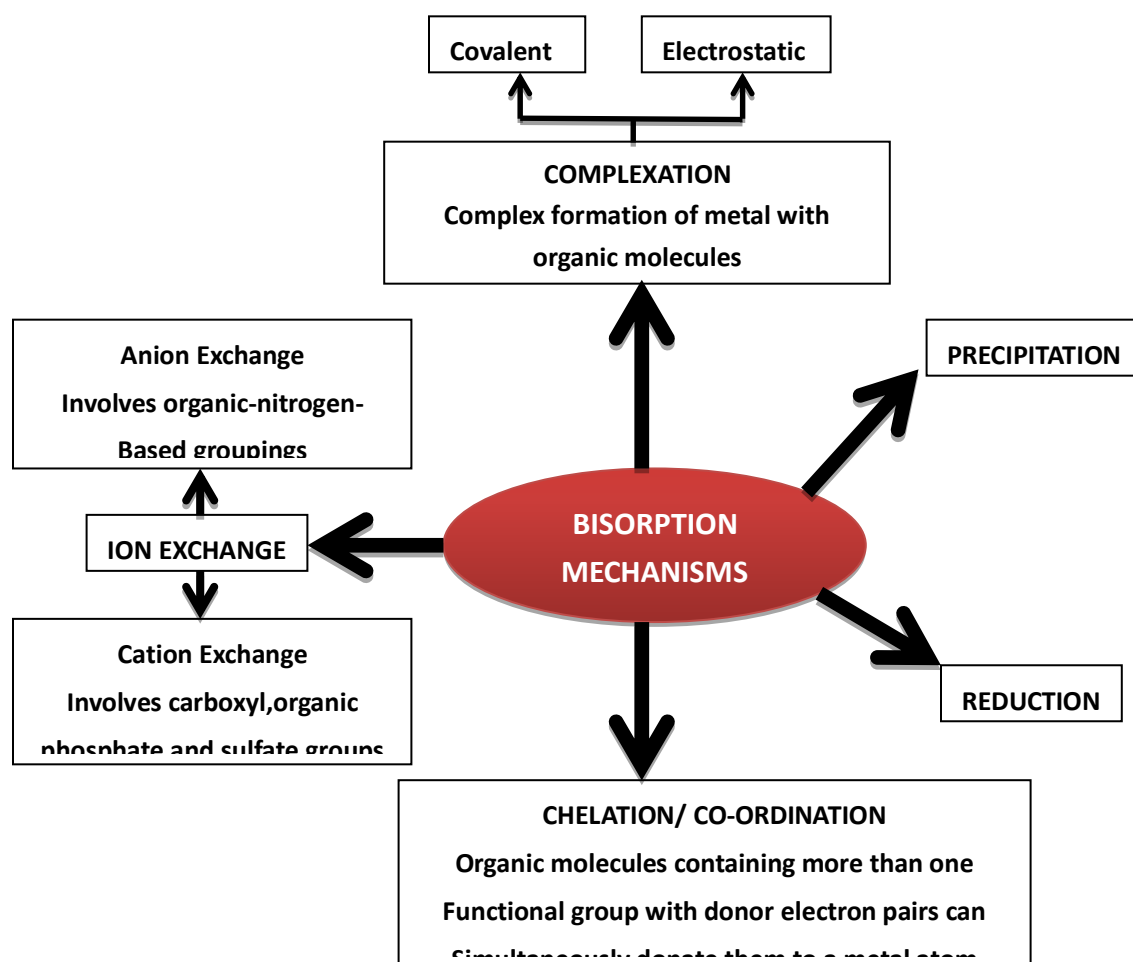
Figure 1 The mechanism of adsorption and it incorporates numerous passive (non-metabolic) mechanisms(Taken from (Ahemad and Kibret, 2013)).
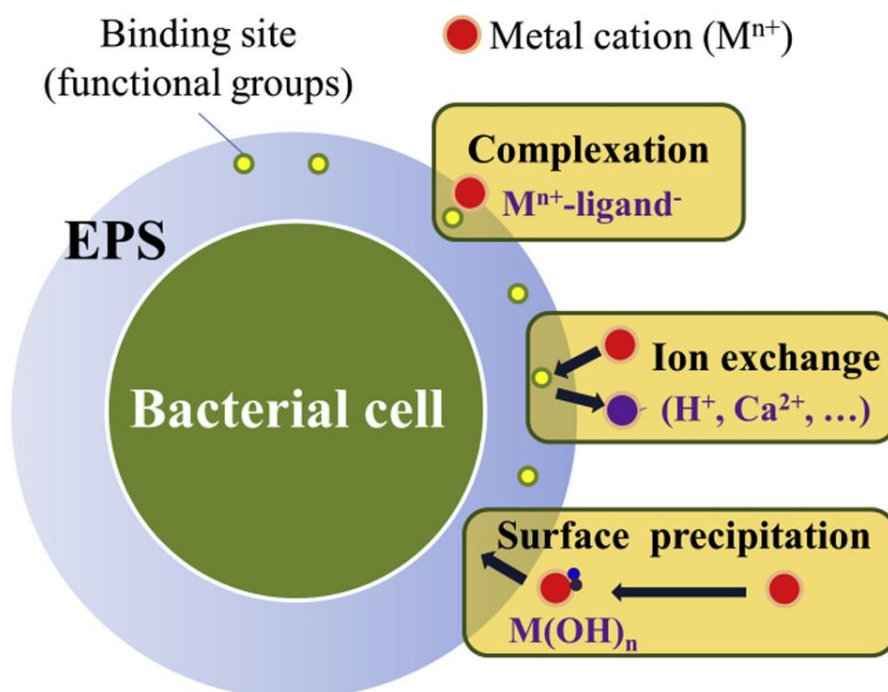
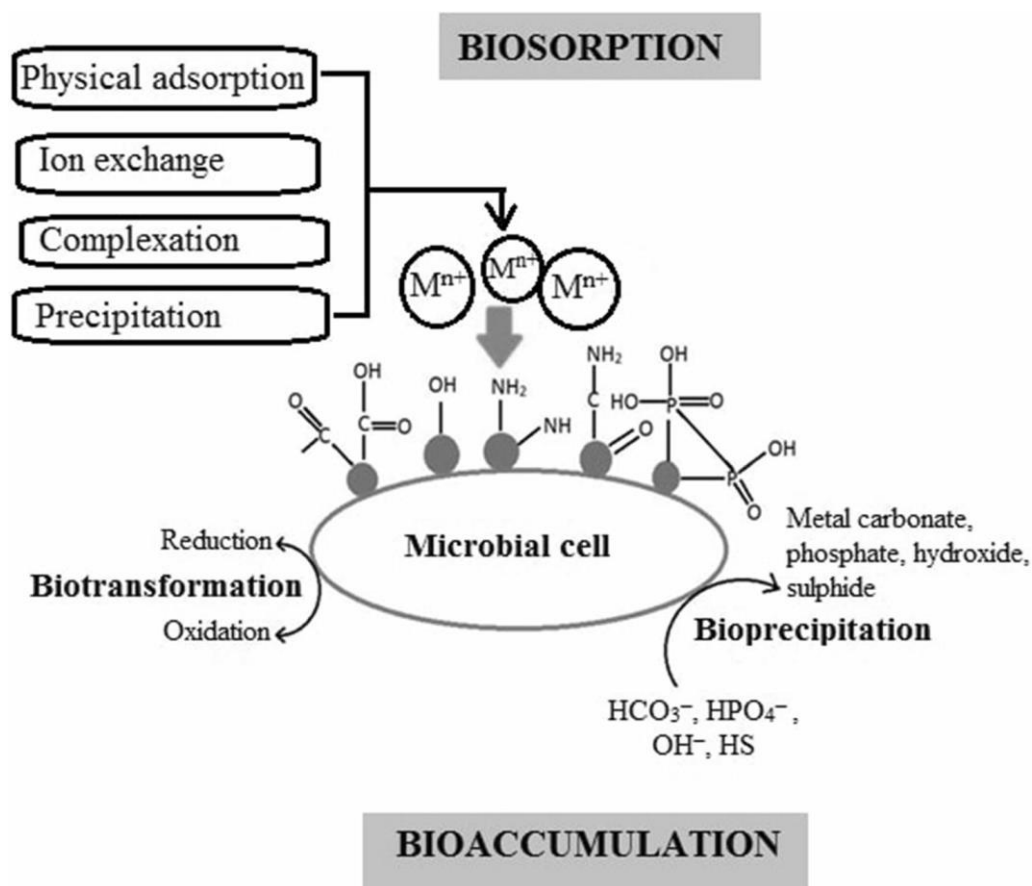Figure 2 Schematic diagram of mechanism of metal–EPS interactions(Taken from (Li et al., 2014)).

Figure 3 The mechanism of biosorption and bioaccumulation of a microbial cell (Taken from (Veglio and Beolchini, 1997)).
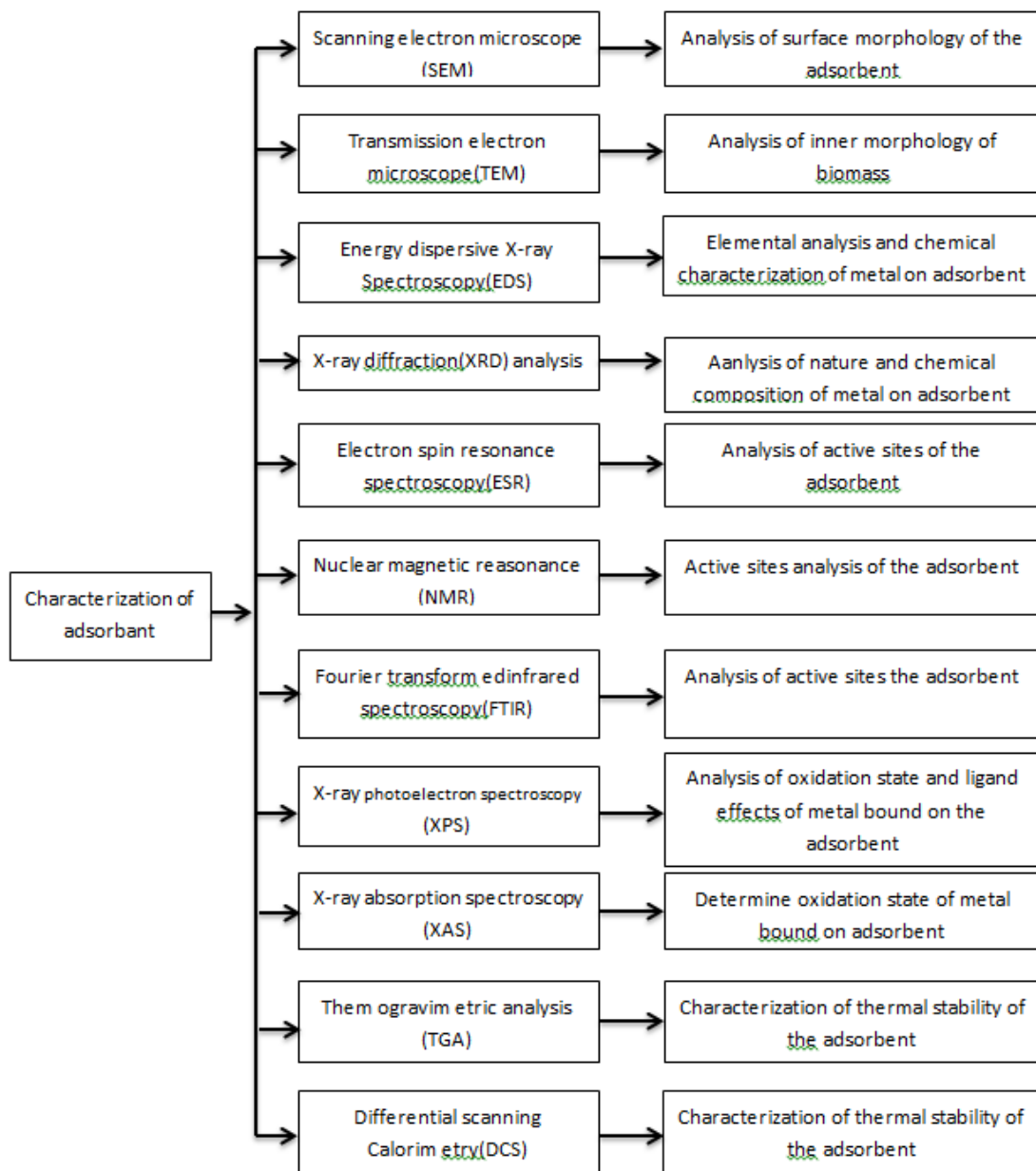
Scanning electron microscope (SEM)	→	Analysis of surface morphology of the adsorbent
Transmission electron microscope(TEM)	→	Analysis of inner morphology of biomass
Energy dispersive X-ray Spectroscopy(EDS)	→	Elemental analysis and chemical characterization of metal on adsorbent
X-ray diffraction(XRD) analysis	→	Aanlysis of nature and chemical composition of metal on adsorbent
Electron spin resonance spectroscopy(ESR)	→	Analysis of active sites of the adsorbent
Nuclear magnetic reasonance (NMR)	→	Active sites analysis of the adsorbent
Fourier transform edinfrared spectroscopy(FTIR)	→	Analysis of active sites the adsorbent
X-ray photoelectron spectroscopy (XPS)	→	Analysis of oxidation state and ligand effects of metal bound on the adsorbent
X-ray absorption spectroscopy (XAS)	→	Determine oxidation state of metal bound on adsorbent
Them ogravim etric analysis (TGA)	→	Characterization of thermal stability of the adsorbent
Differential scanning Calorim etry(DCS)	→	Characterization of thermal stability of the adsorbent

Characterization of adsorbant

Figure 4 Instrumental analysis for characterization of the adsorbent and their outcomes(Taken from (Srivastava et al., 2015)).
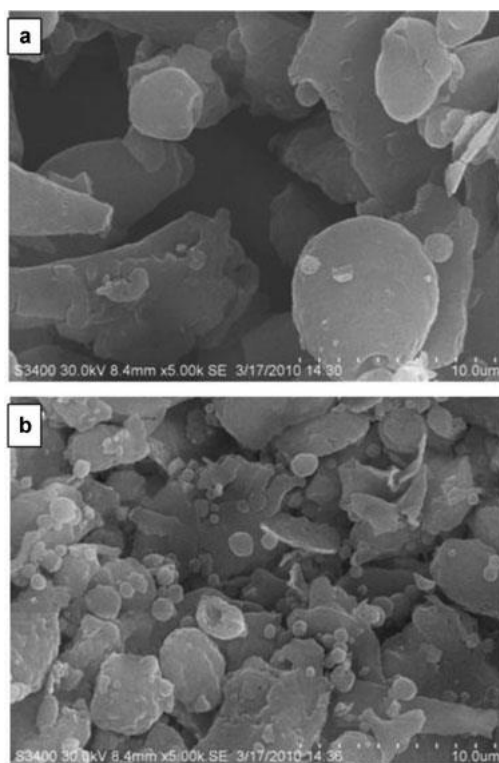
68

Figure 5 Scanning electron micrograph (SEM) of 70810 EPS (a, before biosorption; b, after lead
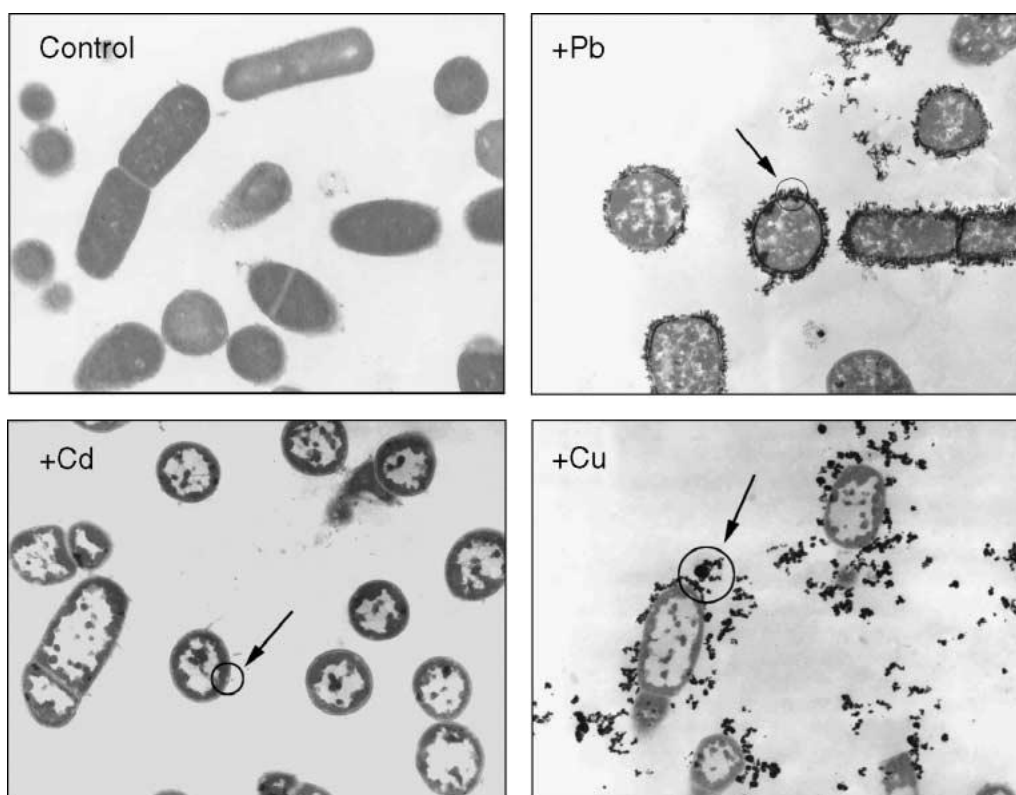
biosorption)(Taken from (Feng et al., 2012)).

Figure 6 TEM observation of uptake of heavy metals on the cell surfaces of the bacterium CPB4 strain, with magnification (12,000X). Arrows indicate the area of the EDS analysis (Taken from (Kim et al., 2007)).
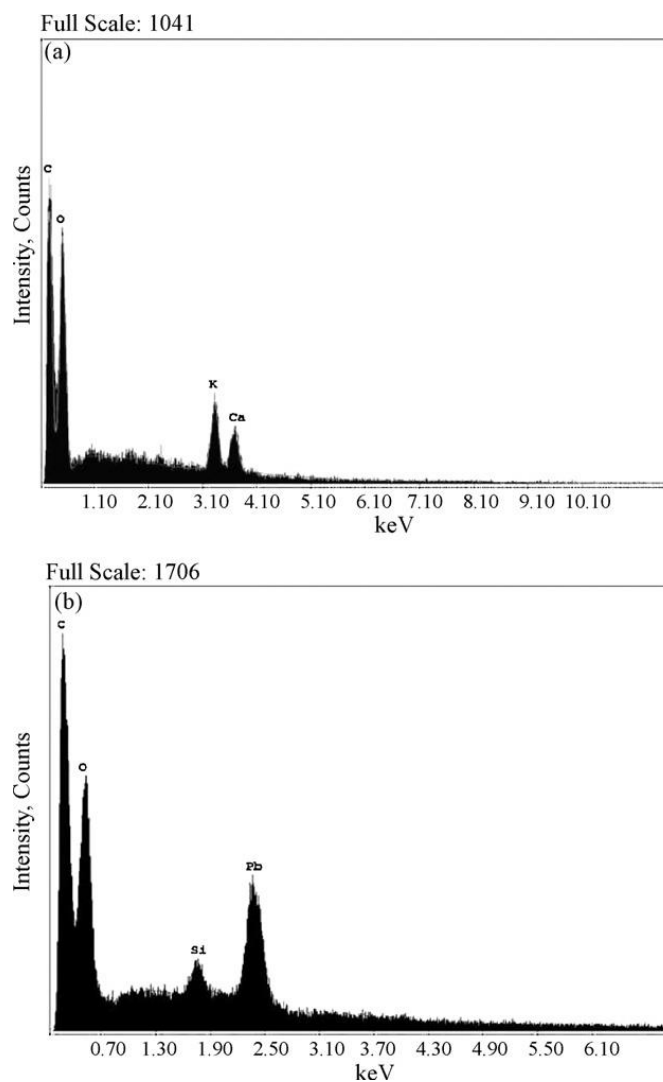
Figure 7 Energy dispersive X-ray (EDX) analysis of mango peel waste (a) before and (b) after the sorption of Pb^{2+} (Taken from (Iqbal et al., 2009)).