Heavy Metal Removal Processes by Sulfate-Reducing Bacteria

13

María Isabel Neria-González and Ricardo Aguilar-López

Abstract

Actually, natural phenomena and anthropogenic activities have led to large environmental pollution in all kinds of ecosystems. From the above exist the needed to implement technological actions in order to diminish the pollution problems. Among these technological actions, the biotechnology processes have the great advantage of high specificity for the removal of chemical compounds as heavy metals with low energy consumption. The microorganisms are the biological agents most used in the treatments of polluting compounds, due to their degradation capacities of organic and inorganic pollutants, but the metals are not degraded but only can be modified in their redox state, converting them to less toxic forms. Precipitation and biosorption are the most employed process for metal ion removal from water. Microalgae, fungi and bacteria have been used successfully in the removal of metals, but in the last decade, sulfate-reducing bacteria (SRB) have taken great relevance in the processes of metal removal from wastewater. The removal of heavy metals is promoted when hydrogen sulfide is produced; it reacts with metal ion and forms metal sulfides, which are insoluble and tend to precipitate; it is not the only mechanism by which metal ions are removed, biosorption mechanisms can also be carried out (with biomass and production of exo-polysaccharides), immobilization and enzymatic reduction of the metal ion to less toxic and insoluble forms, but this will depend on the type of bacteria and their tolerance to metals.

M. I. Neria-González (✉)
División de Ingeniería Química y Bioquímica, Tecnológico de Estudios Superiores de Ecatepec, Ecatepec de Morelos, Edo. México, Mexico
e-mail: mineriag@tese.edu.mx

R. Aguilar-López
Departamento de Biotecnología y Bioingeniería, CINVESTAV-IPN, Ciudad de México, Mexico

R. Prasad (ed.), *Environmental Pollution and Remediation*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-5499-5_13

Specifically, the species of the genus *Desulfovibrio* have been the most studied for metal removal with a high efficiency, where the main mechanism is the precipitation of metal sulfides among others. *Desulfovibrio alaskensis* 6SR exhibits a high metallic resistance with respect to other sulfate-reducing bacteria, including other microorganisms; since it shows strong resistance to Cr(VI), Cd (II), Pb(II) and Zn(II), some of these are considered as extremely toxic to biota. *Desulfovibrio alaskensis* strain 6SR is capable to remove more of the 98% Cr (VI) Cd(II), Pb(II) and Zn(II) in solution. The main mechanism of removal is the precipitation of the corresponding metallic sulfides, followed by adsorption of these by the produced EPS, and transmission electron micrographs show a slight metal accumulation at the intracellular level and periplasmic space. Also, the chromium reducing for the hydrogen sulfide has been analyzed by the sulfate reduction in independent reactors, as well as in culture per batch of *D. alaskensis*. The results indicate that the bacterium is able to grow up to a concentration of 18 mg/L of Cr(VI), and contrary to *D. vulgaris*, the reduction of sulfate does not interrupt at any time of the chromium reducing. Finally, a molecular analysis with respect to cadmium and chromium resistance mechanisms demonstrated the presence of *cad*A and *chr*A genes. Both genes are induced by Cd, Zn, Pb and Cr; the codified proteins by these genes are involucre to abate the oxidative stress provoked by heavy metal non-essentials.

Keywords

Heavy metals · Sulfate-reducing bacteria · Wastewater · Microbial reduction · *Desulfovibrio alaskensis*

13.1 Introduction

Ever since that the human is done sedentary, the usage of metals was incrementing; now they are used for everything, from construction to improve our health. Some metals are also necessary for the vital processes of any organisms. But where do we obtain the metals? In answer to this question, the Earth's crust is the main source of metals. So, gold, silver, platinum and others are found as the uncombined elements or native or free state, known as non-reactive metals too. In general, most metals are found combined with other elements to form compounds; on both cases, these are in rocks named ores. Most metals are extracted from ores by different extraction methods that depend upon the metal's position in the reactivity series. In principle, any metal could be extracted from its compound using electrolysis, but the using of large amounts of electrical energy results expensive, and other types of extraction methods are required, but this is another story. The fact is that the high demand of metals for various anthropogenic activities as the manufacture of steel, foundries, electroplating, auto parts, fuel production, manufacture of electronic devices, manufacture of agrochemicals and manufacture of batteries, among others, is the main source of metallic contamination (Haferburg and Kothe 2010). The metals are

released to the environment as solutes or particles that can reach high concentrations, especially near the discharge site (Krishna and Govil 2007). In general, heavy metals accumulate in aquatic environments, mainly in sediments, debris and organic matter, where they may be consumed by fish, which tend to accumulate metals in the gills and intestines magnifying their concentration to a toxic level. This generates serious problems in health and the trophic chain of organisms affecting all kinds of ecosystems.

In actuality exist the needed to implement technological actions in order to diminish the pollution problems. Among these technological actions, the biotechnology processes have the great advantage of high specificity for the removal of chemical compounds as heavy metals with low energy consumption. The microorganisms are the biological agents most used on the treatments of polluting compounds, due to their degradation capacities of organic and inorganic pollutants, but the metals are not degraded but only can be modified in their redox state, converting them to less toxic forms (Wood and Wang 1983). Precipitation and biosorption are the most employed process for metal ion removal from water. Microalgae, fungi and bacteria have been used successfully in the removal of metals, but in the last decade, sulfate-reducing bacteria (SRB) have taken great relevance in the processes of metal removal from wastewater (López-Pérez et al. 2016). The removal of heavy metals is promoted when hydrogen sulfide is produced; it reacts with metal ions and forms metal sulfides, which are insoluble and tend to precipitate; however, it is not the only mechanism by which metal ions are removed, but biosorption mechanisms can also be carried out (with biomass and production of exo-polysaccharides), immobilization and enzymatic reduction of the metal ion to less toxic and insoluble forms, but this will depend on the type of bacteria and their tolerance to metals (Li et al. 2018). Specifically, the species of the genus *Desulfovibrio* have been the most studied for metal removal with a high efficiency, where the main mechanism is the precipitation of metal sulfides between others. In particular, *D. alaskensis* 6SR exhibits a high metallic resistance with respect to other sulfate-reducing bacteria, including other microorganisms. Therefore, this strain is considered as a model for the removing of metals under anaerobic conditions.

13.2 Heavy Metal Removal

Heavy metals are chemical elements; in principle, these are the simpler substances of the matter. The chemical elements are found ordered and classified in the periodic table. Currently, this contains 118 chemical elements; the most were discovered and some synthesized. Metals are the most abundant chemical elements, and these are classified in the periodic table as alkali metals, alkaline earth metals, transition metals, lanthanides and actinides.

However, the term “heavy metals” has been widely used to refer to any metallic or semimetallic chemical element. In the scientific literature, an authoritative definition is not found. But the authors have considered the density to refer to the term “heavy”; some of them have proposed densities among 3.5–7.5 g/cm. Therefore, a

heavy metal is a metal or metalloid with a relatively high density and associated with pollution and toxicity (Duffus 2002). Other criteria as the atomic weight or mass were used to give consistency to the term "heavy", but not prospered. With respect to pollution and toxicity, heavy metals are highly toxic to very low concentrations. The heavy metals tend to accumulate with respect to time, exceeding their permissible concentration in the environment; the metallic accumulation unchains damages on the biological systems (human, animals, microorganisms and plants), provoking important problems of toxicity for environmental health and safety (Velea et al. 2009). Also, the term "heavy metal" in the legal aspects implies that the pure metal and all its compounds have the same physicochemical, biological and toxicological properties, which is false, as will mention below.

Heavy metals can be classified as (1) toxic metals, (2) essential metals for living organisms and (3) radionuclides such as uranium (Gadd 2010; Wood and Wang 1983). (1) Toxic metals – the toxicity of the heavy metals can define as the ability of a metal to cause negative effects on living organisms and depends on the bioavailability of the metals, and it is aggravated by their long-term persistence in the environment. Arsenic, fluorine, cadmium, mercury, chromium and lead are some examples of extremely toxic elements to biota, even at very low concentrations. Lead is one metal with the most retention time in soil (150–5000 years). (2) Essential metals, of all elements in the periodic table, 30 are required for microbial life, although not all are necessary for the growth and cell division of every microbial species. Among them, the carbon, nitrogen, hydrogen and oxygen are the bulk elements, and 26 of them are required in intermediate to trace amounts. Twenty-two of the 26 elements are found to be essential for life in higher organisms; see Fig. 13.1. An overabundance of any of these elements can cause build-up to an intracellular toxic level, which can result in death (Wood and Wang 1983). (3) Radionuclides, the radioactive elements, are formed by chemical elements whose atomic nuclei are unstable. As a consequence of this instability, its atoms emit subatomic particles intermittently and randomly. The imbalance is corrected by the release of excess neutrons or protons, in the form of α particles that are really helium nuclei and β particles that can be electrons or positrons. Among the radioactive elements are polonium, astatus, radon, francium, radio, actinium, thorium, protoactinium, uranium, neptunium, plutonium, americium, lawrencio, curio, berkelio, california, einsteinium, fermium, mendelevian, and nobel.

The contamination for heavy metals is caused by natural phenomena and human activities. The human activities demand the development of new chemicals, materials and enormous quantities of energy and exploit natural resources and discharge of wastewater from metal-related industry, which result in environmental pollution, mainly of water-body (Haferburg and Kothe 2010; Thakare et al. 2021). Generally, the heavy metals are present in form of soluble salts in water, that these cannot be separated by ordinary physical separation systems. Physicochemical processes such as chemical precipitation, chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, ion exchange and membrane technologies have been widely used to remove metallic ions from industrial wastewater (Das et al. 2008; Li et al. 2018). But these processes may be ineffective or

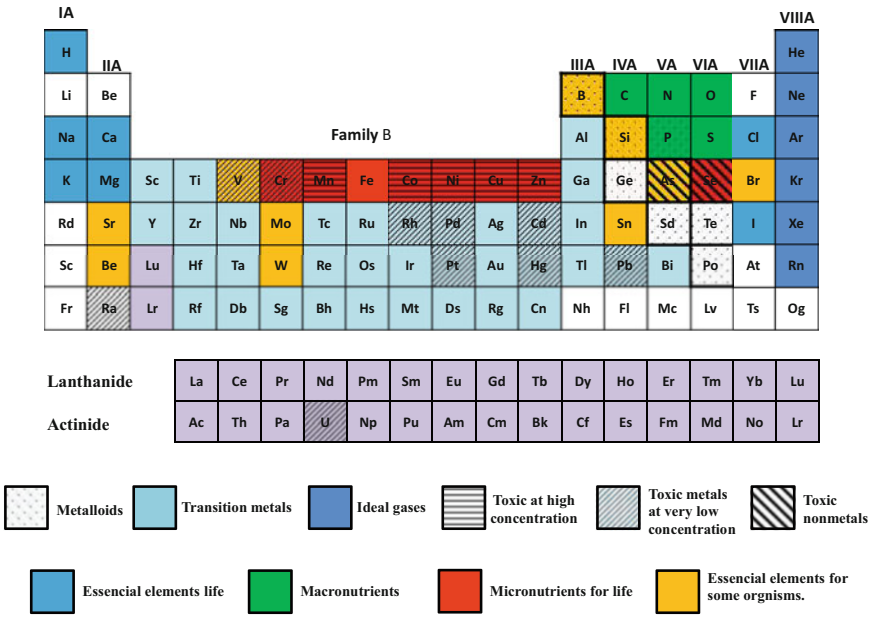

Fig. 13.1 Periodic table of the elements. This table illustrates the essential elements of life. Most of the transition metals are considered as toxic and cannot be processed by living organisms, but some are essential for life

expensive by the high energy requirement, especially when the solutions contain of 1–100 mg metal per litre (Das et al. 2008). Therefore, the technical applicability, cost-effectiveness and plant simplicity are the key factors in selecting the most suitable treatment method to remove heavy metals. However, the biotechnology offers alternative biological methods to the removal of heavy metals (Joo et al. 2015). Although the inorganic elements cannot be destroyed, microorganisms can alter their redox state. Oxidation-reduction reactions mediated by microorganisms help in the conversion of a highly toxic, soluble, and mobile species into a species less soluble and toxic, e.g. the biological reduction of Cr(VI) to Cr(III). When some metals as Cu, Zn, Ni, Cd, As, U, Np and Tc combines with the sulfide, hydroxide or carbonate anion form salts whose solubility is low and precipitate in aqueous solution.

13.3 Main Mechanisms of Biological Removal

13.3.1 Biosorption and Bioaccumulation

Biosorption is the most employed technique for the removing of metals in solution, it is a form of passive uptake of metallic ions by a sorption material, and it can be an alternative to the conventional technologies. Microbial biomass and agricultural waste are biomaterials most used (Ahluwalia and Goyal 2007; Singh and Goyal

2007). Bacteria of the genera *Bacillus* and *Streptomyces* and yeasts possess the capacity of adsorbing high amounts of metals from solution, due to the adsorption of the metallic ions by ionizable groups of the cell surface constituents, such as peptidoglycan, cellular membrane and capsule (carboxyl, amino, phosphate and hydroxyl groups) (Das et al. 2008; Ahluwalia and Goyal 2007). Macroalgae and alginate derivatives exhibit high affinity towards many metal ions. The major advantages of biosorption over conventional methods include low cost, high efficiency, minimization of chemical or biological sludge, the regeneration of biosorbents and possibility of metal recovery chemical or biological sludge (Gavrilescu 2004).

Heavy metal ions are also adsorbed by extracellular biopolymers or exopolysaccharide (EPS) produced by bacteria, which form the bacterial capsules and other bacterial covers. The carboxyl groups of polysaccharides hold back and accumulate metal ions. Extracellular biopolymers of *Enterobacter cloacae*, *Marinobacter* sp., *Klebsiella aerogenes* and *Acinetobacter* sp. have shown metallic accumulation. However, a considerable accumulation of ions of copper, lead and zinc was demonstrated on *Pseudomonas aeruginosa* cell biofilms. EPS acts as a barrier of protecting bacterial cells inside of biofilm of the toxicity of metal ions (Ianieva 2009).

The accumulation of intracellular metal ions responds to changes in cell membrane permeability. Thus, some metallic ions can enter the cell via the systems responsible for the uptake of essential elements, as in the case of *Ralstonia metallidurans*, the ions of cadmium, zinc, cobalt, nickel and manganese enter at the cell using systems of magnesium transport. Another example is chromate using sulfate transport system (Ianieva 2009; Gadd 2010). The passage of metal ions into the cell causes response detoxification of toxic metals, which is based on the expression of proteins capable of being complex with metallic ion. These metallic complexes are out from the cell, and these accumulate in cell membrane surface or in the periplasmic space or are deposited in internal vacuoles called inclusion bodies. These are called resistance mechanisms and are associated with metallothioneins and metallohistins, molecules of intracellular storage and detoxification able to bind heavy metals (Haferburg and Kothe 2010; Thakare et al. 2021). This may provide an excellent source of applicable mechanisms in environment biotechnological decontamination.

13.3.2 Precipitation

In the precipitation produced chemically stable forms of metal and only use to reducible metals. Metabolic activity of the microorganisms contributes to the removing indirect or direct of metals, e.g. metal precipitation by secreted phosphate generated from polyphosphate hydrolysis. In this context, the precipitation by phosphates and sulfides has been investigated due to the low solubility of their metal compounds. This mechanism is suggested to remove metals and actinides from wastewater. Therefore, the selective precipitation of metals with the hydrogen sulfide produced by sulfate-reducing bacteria (SRB) is the most studded (Joo et al.

2015; Li et al. 2018). The main process of removal of heavy metals followed by SRB is definitely the precipitation of metal sulfides. The generation of sulfide diminishes the acidity promoting the precipitation of metals as insoluble metal sulfides that can be easily separated. The process consists of two stages: (1) the production of H_2S by SRB and (2) the precipitation of metals by the biologically produced H_2S; it reacts with metal ions and produces insoluble metal sulfides that can easily separate from a solution.

$$\underset{(e^- \text{ donor})}{\text{Lactate}} + SO_4{}^{2-} \rightarrow \underset{(e^- \text{ acceptor})}{2CH_3COO^-} + HS^- + HCO_3{}^- \tag{13.1}$$

$$Me^{2+} + HS^- \rightarrow MeS \downarrow + H^+ \tag{13.2}$$

13.3.3 Microbial Reduction of Metallic Ions

Microbial reduction of some metals and metalloids as Cr(VI), Mn (IV), Tc(VII), U (VI) and Se(VI) has been proposed like a bioremediation strategy; particularly, their reduced forms [Cr(III), Mn (II), Tc(IV), Se(0) and U(IV) Se(0)] are insoluble and less toxic precipitates. Uranium reduction can carry out under aerobic and anaerobic conditions. The reduction of U(VI) under anaerobic conditions forms uraninite (U (IV)), which is an insoluble mineral. It oxidized to U(VI) with nitrate acting as the electron acceptor; this could provide a strategy for solubilizing and extracting microbial U(IV) precipitates from the subsurface (Finneran et al. 2002; Silver and Phung 2005). Generally, the microbial metallic reduction utilizes electron donors such as ethanol and acetate and the metallic ions as electron acceptors under anaerobic conditions, sulfate-reducing bacteria being an excellent alternative (Cabrera et al. 2006).

Finally, the using of microorganisms in the removal of metals is due to the metallic resistance capacity of each strain, which are enveloped as an evaluative characteristic to survive in a hostile habitat. But it is clear that such resistance is due to resistance mechanisms that microorganisms induce in the presence of toxic heavy metals. The main mechanisms of metal resistance studied in bacteria are related to (1) cellular components that capture metal ions for neutralizing their toxicity, (2) enzymes that modify the redox state of metals or metalloids to less toxic forms and (c) transporters of the membrane that eject harmful species outside cellular cytoplasm (Ramírez et al. 2008). Resistance mechanisms of transporters of the membrane are efflux systems, which contain proteins belonging to three families: resistance, nodulation, and cellular division (RND), cation diffusion facilitator (CDF) and P-type ATPases. In Gram-negative bacteria, both P-type ATPases and CDF proteins are predominant; proteins transport specific substrates through the plasma membrane into the periplasm. P-type ATPases predominantly transfer metal ions with high affinity for sulfhydryl groups [Cu(I)/Ag(I), Zn(II)/Cd(II)/Pb(II)], while CDF proteins specifically interact with ions of divalent metals [Zn(II), Co

(II), Ni(II), Cd(II) and Fe(II)]. The most relevant resistance mechanism in bacteria is related to the P1B-type ATPases and chemiosmotic systems (Naghma et al. 2005; Silver and Phung 2005). Genomic studies of the interrelationships to metal-induced proteome and metabolome changes allow in silico searches for genes encoding metal-responsive proteins (Haferburg and Kothe 2010; Chance et al. 2004). The proteins encoded could be either involved in metal homeostasis, thus being of interest for improving metal resistance of strains for bioremediation.

13.4 Sulfate-Reducing Bacteria

Reducing sulfates constitute a group of obligate anaerobic prokaryotes (bacteria and archaea); they present a morphological and physiological diversity. These microorganisms live in anoxic habitats and have great ecological importance in the carbon and sulfur cycles, because they mineralize the organic matter of the anaerobic environments. In marine sediments, 50% of organic matter is oxidized by sulfate reduction with an equivalent or higher yield than in an aerobic process (Barton 1995). Within this group of prokaryotes, the sulfate-reducing bacteria (SRB) are the most abundant and are widespread in natural habitats such as marine sediments, lakes and saltwater lagoons and oil fields, as well as in the gastrointestinal tracts of many animals, including humans. In environments with low sulfate levels, such as bodies of freshwater, they have relevance in the mineralization of organic matter (Muyzer and Stams 2008). Some SRB are able to survive in the presence of oxygen, but no growth has been observed (Fournier et al. 2004). Also, SRB have industrial (biocorrosion), environmental (bioremediation) and health (inflammation of the intestine) implications (Bartosch et al. 2004); consequently, the SRB have been studied extensively.

Sulfate-reducing bacteria were discovered by Martinus Willem Beijerinck in 1895 and described as the use of enrichment cultures for “sulfur fermentation”. With his cultivation technique, he was able to isolate colonies surrounded by a black precipitate, ferrous sulfide. Beijerinck had isolated the first sulfate-reducing bacteria from the Dutch city canal in Delft. The morphological description of the bacterium corresponded to curved bacilli with movement, which is the reason why it was named *Spirillum desulfuricans*. Beijerinck also suggested studying other terminal electrons acceptors, besides sulfate, and studying the distribution of these bacteria in marine environments and soil (Voordouw 1995). SRB are chemolithotrophic microorganisms, capable of using sulfate as the final electron acceptor in the degradation of organic matter, a process called sulfate reduction, where hydrogen sulfide is generated. The dissimilatory reduction of sulfate is a large-scale process limited to SRB; however, they can also reduce other oxidized forms of sulfur such as sulfite and thiosulfate and other inorganic compounds as nitrite or nitrate. Some SRB are able to integrate elemental sulfur as a substrate in the respiration, and other SRB can even respire with oxygen. SRB can grow in a sulfate-dependent manner using hydrogen and a wide range of organic compounds, but polymeric compounds as

polysaccharides and proteins are not typically used by them. Therefore, SRB are very versatile with respect to the electron donors and acceptors for their growth.

Sulfate-reducing bacteria most studied are *Desulfovibrio* spp., which are curved bacilli, are spiroidal bacilli and occasionally are straight bacilli, and present mobility by polar flagella. Under stress conditions, some bacteria are polymorphic, such as *D. africanus*, *D. salexigens* and *D. gigas*. Nutritionally, the cultures are easily enriched with lactate, a reducing agent for their growth and in some cases of vitamins. Lactate is partially oxidized to acetate for most of the species. Some strains are capable of growing in the presence of H_2 as a source of energy and acetate plus CO_2 or yeast extract as a source of carbon, while carbohydrates are a source unusual of carbon. Very few members carry out a fermentative metabolism. Others are moderate halophiles by their requirement of NaCl (20–30 g/L). Not only is sulfate the electron acceptor, but also the sulfite and thiosulfate and in some cases nitrate are final electron acceptors (Devereux et al. 1990; Voordouw 1995). Some bacteria have the capacity to reduce iron (III), uranium (VI), chromium (VI), pertechnetate (VII), selenite (VI) and arsenate (VI), but these reduction processes are not coupled to growth (Cabrera et al. 2006; Muyzer and Stams 2008).

13.4.1 Classification and Phylogeny

During the first six decades of the last century, the knowledge of new SRB with similar cellular characteristics but different morphologies generated serious problems in the classification of this bacterial group. In 1936, Kluyver and Niel gave the prospects for a natural system of classification of bacteria, as an attempt to devise a bacterial natural classification of their own, based primarily on morphological criteria, but attaching great weight to physiological characters. Based on the morphological and physiological criteria, the genus *Desulfovibrio* was established, and "*Spirillum desulfuricans*", discovered by Beijerinck, was the first species, but with a novel name *Desulfovibrio desulfuricans*. Later, Campbelly and Postgate proposed the genus *Desulfotomaculum* (1965). Both continued studding the Gram-negative SRB and discovered the presence of cytochrome c_3, indicated by an absorption band at 552–554 mμ, forming a "pyridine hemochromogen" of the hematoheme class, observed by other investigators too. This generated a greater understanding of Gram-negative SRB that the genus *Desulfovibrio* was improved (Postgate and Campbell 1966). For 25 years, other SRB were isolated, and other genera that appeared such as *Desufobacter*, *Desulfobulbus*, *Desulfococcus*, *Desulfonema*, *Desulfobotulus*, *Desulfobacterium*, *Desulfomonile*, and *Desulfoarculus* were described (Widdel and Pfennig 1984). Thermophilic Gram-negative bacteria able to reduce sulfate were classified inner of the genera *Thermodesulfomicrobium* and *Thermodesulfobacterium*, and thermophilic Gram-positive sulfate reducers were placed in *Desulfotomaculum*. On the other hand, two isolate sulfate reducers from anaerobic submarine hydrothermal areas were denominated as archaeal sulfate reducers, which were classified as *Archaeoglobus fulgidus* and *A. profundus*. Then, three basic cell groups of SRB were proposed:

gram-negative eubacteria, gram-positive eubacteria and archaebacteria (Barton 1995).

In the 1990s of the last century, advances in molecular biology and DNA technology, as well as the classification of organisms proposed by Woese, based on ribosomal gen analysis, had a great impact on the traditional classification, particularly of microorganisms (Woese et al. 1990). The phylogenetic analysis based on the comparative analysis of the sequences of the 16S rRNA gene establishes phylogenetic relationships among microorganisms up to a genus and species approach. Therefore, the importance generated around SRB and with the development of rRNA phylogenetic analysis gave a great step for the taxonomy and phylogeny of the sulfate-reducing prokaryotes. The phylogenetic analysis of the 16S rRNA gene showed that the sulfate- reducing prokaryotes are divided into four phylogenetic lineages: mesophilic Gram-negative SRB, Gram-positive spore-forming SRB, thermophilic sulfate-reducing archaea (SRA) and SRB (Castro et al. 2000). Gram-negative SRB are located in the Deltaproteobacteria subdivision, where there are other non-sulfate-reducing bacteria, but within this division, there are two relevant families of SRB: Desulfovibrionaceae and Desulfobacteriaceae. The family Desulfovibrionaceae is basically constituted by species of the genus *Desulfovibrio* (Voordouw 1995). The main genera of the family Desulfobacteriaceae are *Desulfobacter*, *Desulfococcus*, *Desulfobulbus*, *Desulfobacterium*, *Desulfosarcina*, *Desulfoneum* and *Desulfotulus*, among others. The genus *Desulfotomaculum* is the most representative of Gram-positive SRB, and their species form spores. The thermophilic reducing sulfate prokaryotes are divided into bacterial and archaea species (Castro et al. 2000).

In the first decade of this century, Muyzer and Stams reported that due to the discovery and isolation of new species (Muyzer and Stams 2008) and the increasing of the number of 16S rRNA gene sequences reported in the Genebank, this group of microorganisms is divided in seven phylogenetic lineages: five within the Bacteria and two within the Archaea. Most SRB belong to the class Deltaproteobacteria with approximately 23 genera, and the Gram-positive bacteria within the *Clostridia*, followed by lineages *Nitrospirae* (genus *Thermodesulfovibrio), Thermodesulfobacteria* and *Thermodesulfobiaceae* (only thermophilic sulfate reducers). The lineages to SRA belong to genus *Archaeoglobus* in the Euryarchaeota and the genera *Thermocladium* and *Caldivirga* in the Crenarchaeota.

In the next decades up to today, using of molecular technique and functional markers as *dsr* or *asr*, functional genes that codify dissimilatory sulfite reductase and anaerobic sulfite reductase, respectively, has increased the knowledge of novel sulfate/sulfite-reducing microorganisms (Jiang et al. 2009; Meyer and Kuever 2007; Wagner et al. 2005). In parallel, different studies revealed the capacity sulfate or sulfite-reducing in at least 13 additional bacterial and archaeal lineages, which at the moment were not associated with the metabolism dissimilatory of sulfate or sulfite. These include the phyla Acidobacteria, Planctomycetes, Verrucomicrobia and Armatimonadetes, among others. Besides, 8 of these 13 are candidate phyla without isolated representatives, which only represent uncultured microorganisms (Müller et al. 2015; Wörner and Pester 2019).

Currently, the application of next-generation sequencing methods of DNA in studies of metagenomic and metatranscriptomic has revealed the existence of novel sulfate reducers not yet cultivated in different environments, mainly marine habitats, and they are classified in others lineages to those already known (Anantharaman et al. 2018). Therefore, the knowledge of phylogenetic relationships of this particular microbial group allows a better understanding of their energy and nutritional demands, as well as a perception of their way of life in a given environment, and their possible biotechnological application.

13.4.2 Biotechnological Implications

Biotechnology offers an alternative to develop and to innovate methods using as tools the knowledge biochemistry and the organism's manipulation, with the goal of obtaining valuable products or improving an industrial process while maintaining the natural environment. In this sense, environmental pollution and waste treatment are mainly treated by microorganisms (Mani and Kumar 2014). In this way, SRB have great implications in the context of environmental biotechnology.

Sulfate-reducing bacteria can cause serious problems for industries, the production of sulfide, a highly reactive and corrosive compound, provoking corrosion of iron and steel, which affects mainly to oil, hydroelectric and metal-mechanic industry (Gadd 2010; Hernández-Gayoso et al. 2004). Metallic corrosion is one of the main damages causing great economic losses in pipeline systems of the petroleum industry. In particular, *D. desulfuricans*, *D. vulgaris*, *D. vietnamensis* and *D. alaskensis* play an important role in the biocorrosion of metallic surfaces due to H_2S production, which modifies the environmental pH and leads to the formation of the corrosion product FeS. SRB also promote the development of biofilms in aquatic environments, which are associated with located corrosion of the metal surfaces of oil pipelines. The production of hydrogen sulfide by SRB sours oil and gas; this reduces the quality and the cost of them (Dinh et al. 2004; Muyzer and Stams 2008: Neria-González et al. 2006). Also, SRB have health implications since some species of *Desulfovibrio* can act as opportunistic pathogens; they are associated with primary bacteremia and abdominal infections, such as abscesses and cholecystitis (Urata et al. 2008).

Due to the demand of sulfuric acid on the manufacture of fertilizer and its use in applications in oil refining, pigment production, steel treatment and non-ferrous metal extraction and manufacture of explosives, detergents, plastics and fibres, it results in the occurrence of sulfate in wastewater. The presence of sulfate has an impact on the biological treatment in the wastewaters. In nature, sulfate reducers coexist with other microorganisms, which drifts in metabolic interactions, i.e. the sulfate reducers compete with the methanogens and acetogens for common substrates, such as hydrogen and acetate, but in the absence of sulfate, sulfate reducers grow acetogenically in syntrophic with the methanogens. These microbial interactions are important in the treatment of wastewater. The heavy metals are other pollutants very common of the wastewater; its presence is a very serious threat to the

environment owing to its toxicity, even at a low concentration, and bioaccumulation potential (Velea et al. 2009). Thus, SRB are an alternative method for sulfate and heavy metal removal from wastewater, as well as oxidized sulfur compounds from gas and water (Klonowska et al. 2008). Comparing the biological sulfate reduction with conventional physical and chemical methods, it is an effective technique to treat and reduce both heavy metals and sulfate from contaminated systems.

On the other hand, the toxicity of heavy metals in the microorganisms, including SRB, deactivates enzymes because these react with the functional groups, provoking the denaturation of proteins; also, there is a competition with essential cations. In both cases, they can reduce bacterial metabolic activity or cause death. The concentration of the metal in solution is an important variable, since this depends on the bacterial ability to immobilize the heavy metals. Several studies have determined the capacity of tolerance towards the heavy metals on different cultures of SRB, since their high metal tolerance and removal capabilities are particularly attractive in heavy metal removal processes. The toxic concentration of heavy metals for SRB can reach up to 100 mg/L but in a mixed culture of SRB for some metals is as follows: Zn (25–40 mg/L), Pb (75–80 mg/L), Cu (4–20 mg/L), Cd ([4–20 mg/L), Ni (10–20 mg/L) and Cr (60 mg/L) (Cabrera et al. 2006; Utgikar et al. 2002). In marine SRB, a high tolerance level (500 μM) towards the most toxic metals, Hg(II) and Cd (II), has been reported (El-Naggar 2009; Joo et al. 2015). The application of SRB as an alternate technology on removal metal offers advantages such as high metal removal at low pH, stable sludge, very low operation costs, and minimal energy consumption (Ayangbenro et al. 2018).

13.5 Removing of Heavy Metal by *Desulfovibrio*

13.5.1 *Desulfovibrio alaskensis* as a Model of Removing of Metals

The species of the genus *Desulfovibrio* are the most studied in this field (Joo et al. 2015; Qian et al. 2016). The bacterial response to heavy metals depends on the concentration and availability of metals, and on the response depends the mechanisms of action towards the metals, which include precipitation, reduced uptake, formation and sequestration of heavy metals in insoluble complexes, enzymatic oxidation or reduction to less toxic species, efflux from the cell, metabolic bypass and repair (Gadd 2010; Haferburg and Kothe 2010). Such mechanisms can be evaluated, particularly, in *D. alaskensis* strain 6SR showing high efficiency in the removal of different metallic ions as Cd, Zn, Pb and Cr (López-Pérez et al. (2015); Neria-González et al. 2011; Peña-Caballero et al. 2016).

Desulfovibrio alaskensis was isolated from a soured oil well in Prudhoe Bay, Alaska, and its morphological description corresponds to gram-negative, mobile curved bacilli with a single polar flagellum. The optimal growth conditions of the bacterium in Postgate medium C correspond at a pH 7, 2.5% (w/v) NaCl and temperature 37 °C, using lactate as the main source of carbon and energy (Postgate 1984). Therefore, it is considered as a mesophilic and moderated halophilic

Table 13.1 Minimum inhibitory concentrations of metallic ions for different species of *Desulfovibrio*

Species	Cr (VI)	Cd (II)	Zn (II)	Pb (II)	References
Desulfovibrio alaskensis strain 6SR	18	200	130	200	López-Pérez et al. (2015), Peña-Caballero et al. (2016), and Morón-Vázquez (2015)
Desulfovibrio vulgaris	5	20	20	80	Klonowska et al. (2008) and Cabrera et al. (2006)
Desulfovibrio magneticus	–	1.3	–	–	Arakaki et al. (2002)
Desulfovibrio desulfuricans	–	50	50	1.2	Li et al. (2018) and Muyzer and Stams (2008)
Desulfovibrio sp.[a]	15		15	–	Cabrera et al. (2006)

[a]The strain was evaluated with Cr(III)

bacterium (Feio et al. 2004). Otherwise, the 6SR strain was isolated from a biofilm formed within an oil pipeline from the southeast of Mexico; the phylogenetic characterization showed strong homology with *D. alaskensis* (Neria-González et al. 2006). Subsequent researches indicate that *D. alaskensis* is an SRB frequently found in biofilms developed in the oil pipelines, and these are associated to metallic biocorrosion of pipelines of the oil industry (Hernández-Gayoso et al. 2004; Neria-González et al. 2006).

Desulfovibrio alaskensis strain 6SR grows in Postgate medium C with a concentration of 3.0% NaCl, pH 7 at 45 °C. The growth of the bacterium and the generation of -SH favour the production of EPS. In a natural environment, the EPS induces the development of biofilms that allows microorganisms to survive under stress conditions; also, it offers protection to environmental changes and toxic substances. But, this situation is not very different in pure cultures because when hydrogen sulfide accumulates, the EPS is produced as a protection mechanism of the toxicity of hydrogen sulfide, since despite the sulfate-reducing nature of *D. alaskensis* strain 6SR, its growth is inhibited by the accumulation of this (Barton 1995; Neria-González et al. 2011). On the other hand, the EPS have an important role in the adsorption of heavy metals for their chelating properties, an important factor in the removal of metals in solution. The production of EPS by *D. alaskensis* strain 6SR under optimal growth conditions in Postgate medium C is estimated in 780 mg/L, highest compared with the ones in cultures of *Desulfovibrio* H0407 (239 mg/L) and *Desulfovibrio* LM1 (169 mg/L), obtaining a yield of 6.14 mg EPS/mg cellular protein (Neria-González et al. 2011).

Desulfovibrio alaskensis strain 6SR is able to grow in the presence of Cr, Cd, Pb and Zn; some of these are considered as extremely toxic to biota. The minimal inhibitory concentration (MIC) shows that the strain 6SR has a strong resistance to these metals, and in comparison with other SRB, *D. alaskensis* strain 6SR is the most resistant; see Table 13.1.

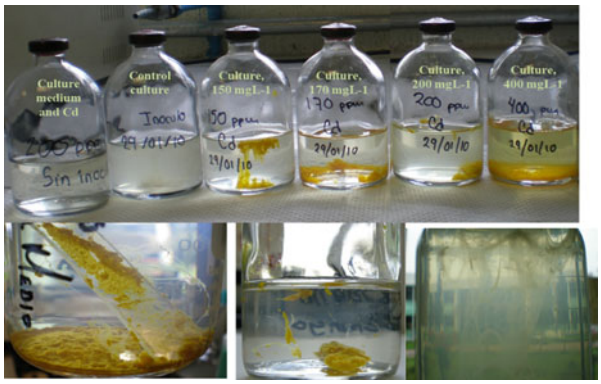

Fig. 13.2 Growth of *Desulfovibrio alaskensis* strain 6SR in Postgate medium C with a high cadmium concentration (0–400 mg/L). Images show biofilm with the precipitated cadmium sulfide and the control culture. *D. alaskensis* strain 6SR was up to 200 mg/L Cd(II)

Based on to their metallic resistance, the removal of Cr(VI), Cd(II), Pb(II) and Zn (II) has been studied in cultures of *D. alaskensis* strain 6SR. Such studies showed a removal rate of 78.3% for 18 mg/L Cr(VI), and for 150 mg/L Cd(II), Pb(II) and Zn (II), removal rates were greater than 98%. The main mechanism of removal is the precipitation of the corresponding metallic sulfides, followed by adsorption of these by the produced EPS and a slight metal accumulation at the level of the periplasmic space, as shown in the image obtained by transmission electron microscopy (Avilés Trejo and Salazar López 2014; López-Pérez et al. 2013b, 2015; Peña-Caballero 2016). The removal mechanisms for zinc, lead and cadmium is related to the production of H_2S, generated from the anaerobic respiration of the bacterium, which reacts with zinc, lead and cadmium, forming the respective metallic sulfide, allowing simple recovery methods. Specifically, for cadmium, *D. alaskensis* 6SR is able to grow under high cadmium concentrations, of the order of 200 mg/L Cd (II) without affecting the sulfate-reducing metabolism, and the production EPS is considerable (López-Pérez et al. 2013a, b); see Fig. 13.2.

Precipitation is the main mechanism of cadmium removal, cadmium sulfide being a yellow precipitate, followed by its adsorption in EPS. The highest amount of cadmium was contained in the EPS or biofilm (99.4%); see Fig. 13.3. The accumulated cadmium in the biofilm could be associated with a quick production of EPS. In the free biomass, only 0.47% Cd (0.8 mg/L) was detected. Electronic micrographs show a very low intracellular and periplasmic accumulation of cadmium in the cells (López-Pérez et al. 2015). The capacity to remove cadmium by cultures of *D. alaskensis* strain 6SR overtake the capacity of other sulfate-reducing systems, including other bacterial species; see Fig. 13.4 and Table 13.2. This confirms that the capacity of removing toxic metals by *D. alaskensis* strain 6SR is higher than some physicochemical methods and other microorganisms, which require special conditions, i.e. the microalgae require illumination conditions and large areas, and the fungi generate a large amount of biomass that demands an aeration special system and mixing. Also, species as *D. vulgaris* (11 mg/L), *D. magneticus* (1.3 mg/L), *D. desulfuricans* (56 mg/L) and *Desulfovibrio* sp. (20 mg/L) have a lower cadmium removal capacity (Fig. 13.3). Further, we

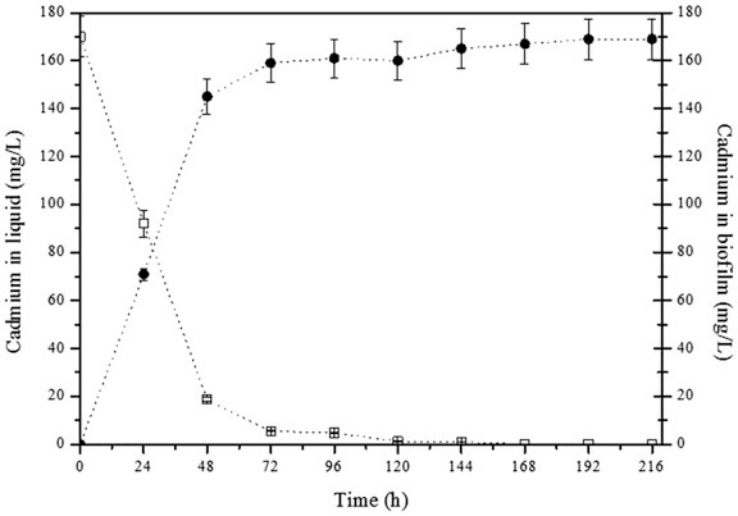

Fig. 13.3 Cadmium removal by *Desulfovibrio alaskensis* strain 6SR. Symbols: (□) concentration of cadmium in the liquid phase and (●) concentration of cadmium adsorbed in the biofilm

think that the anaerobic of culture and handling conditions of SRB could be a disadvantage in the removal metals but it is quite the opposite. Bacterial growth under anaerobic conditions produces less biomass, and its simple removal metal mechanism allows easy recovery of the metallic sulfide, and some of them have an added value in the manufacture of solar cells (López-Pérez et al. 2015; Rangel-Chávez et al. 2015).

In the case of hexavalent chromium, *D. alaskensis* strain 6SR is able to grow up to a concentration of 18 mg/L Cr(VI); see Figs. 13.5 and 13.6. However, some species as *D. vulgaris* Hildenborough have the capacity to reduce Cr(VI) to Cr(III) by the action of dehydrogenase and the cytochrome c_3, but cells are unable to use Cr (VI) like a terminal electron acceptor linked to growth (Klonowska et al. 2008; Franco et al. 2018). This has been demonstrated using a fresh culture medium with sulfate and chromium, which was inoculated with cells harvested in the exponential phase and washed. The results indicated that chromium decoupled lactate consumption from sulfate reduction, while chromium reduction is carried out. This explains that electron flow from cytochrome c_3 is a non-specific process that can be diverted from sulfate by other redox partners including Cr(VI). Then, the reduction of chromium in culture for the batch of *D. alaskensis* strain 6SR was studied under the same methodology and conditions followed by Klonowska et al. (2008). The results revealed that the reduction of Cr(VI) is due to the production of H_2S, since the sulfate-reducing process was not inhibited by chromium hexavalent, such as demonstrated to *D. vulgaris* Hildenborough; see Fig. 13.7 (Avilés-Trejo and Salazar-López 2014; Peña-Caballero et al. 2016). Therefore, *Desulfovibrio alaskensis* strain 6SR is not able to reduce Cr(VI) via enzymatic reduction.

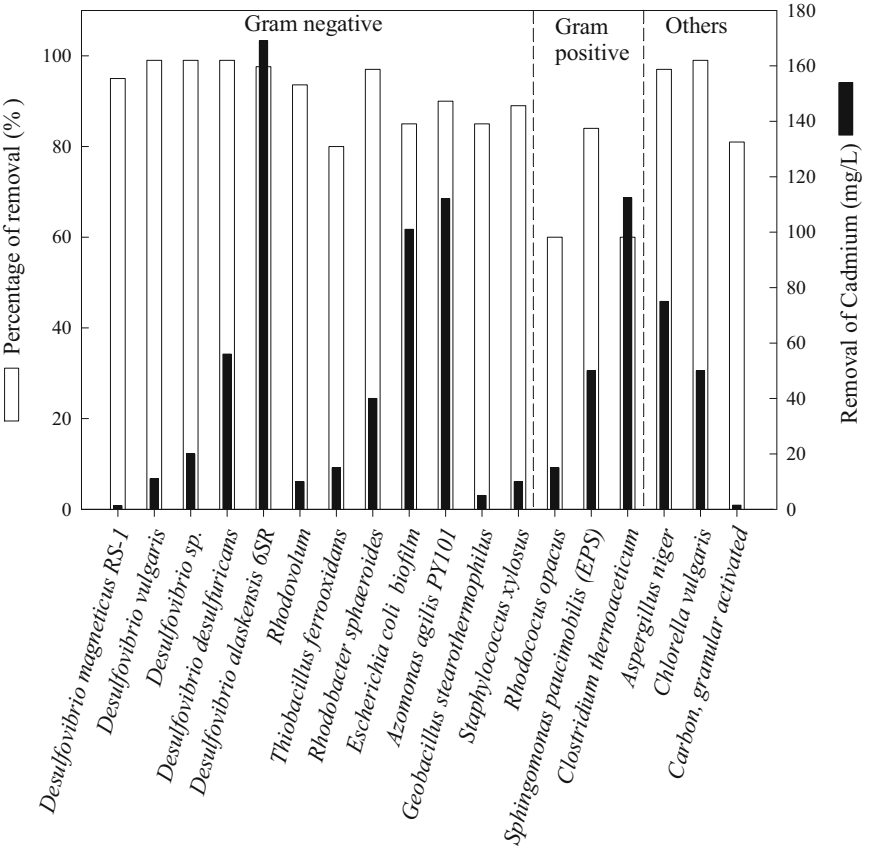

Fig. 13.4 Removal of cadmium obtained among *Desulfovibrio alaskensis* 6SR and other microorganisms. The graphic was made using data previously reported (Arakaki et al. 2002; Cunningham and Lundie 1993; Yun-guo et al. 2006; Quintelas et al. 2009; Selatnia et al. 2004; Sinha and Mukherjee 2009; Vásquez et al. 2007; Watanabe et al. 2003; White and Gadd 1998; Ziagova et al. 2007)

The chromium reduction carried out between 16 and 24 hours under the sulfate-reducing process. The measuring of chromium in liquid phase and biofilm indicated a deficit of chromium added to culture medium; see Fig. 13.8. The remaining chromium was localized in the free biomass, this was reveled by a TEM analysis when evaluate the accumulation of chromium in the cells. The micrographs obtained from *D. alaskensis* strain 6SR were not stained when chromium was present, whereas cells not exposed to the metal were stained with lead citrate. Comparison of both micrographs showed an accumulation of chromium on extern and cellular membrane and periplasmic space in the cell; see Fig. 13.9. For this reason, the chromium removal follows a precipitation by chromium sulfide and bioaccumulation in the biofilm and cellular surface.

Table 13.2 Cadmium removal by different microorganisms

Kind of microorganisms	Cd (mg/L)	Adsorbent (g)	Uptake capacity (mg/g)	Method	References
E. coli biofilm	85	6.54	13	Batch experiments – Biosorption performance	Quintelas et al. (2009)
Pseudomonas aeruginosa	11	0.26	43	Immobilized on granular activated charcoal	Sinha (2009)
Pseudomonas sp.	10	0.03	278	Biosorption	Ziagova et al. (2007)
Rhodobacter sphaeroides (S)	20	0.87	23	Batch experiments – Biosorption performance	Watanabe et al. (2003)
Rhodovulum sp. (PS88)	20	0.54	37	Batch experiments – Biosorption performance	Watanabe et al. (2003)
Rhodococcus opacus	9	2.31	4	Batch-scale basis	Vásquez et al. (2007)
Staphylococcus xylosus	9	0.04	250	Biosorption	Ziagova (2007)
Streptomyces rimosus	210	3.32	63	Batch experiments – Biosorption performance	Selatnia et al. (2004)
Clostridium thernoaceticum	110	–	–	Precipitation of cadmium	Cunningham and Lundie (1993)
Mixed-culture SRB (*Desulfotomaculum*)	7	–	–	Batch experiments	White and Gadd (1998)
Desulfovibrio magneticus RS-1	1.3	–	–	Batch experiments	Arakaki et al. (2002)
Desulfovibrio desulfuricans	100	–		Batch experiments	Joo et al. (2015)
Desulfovibrio alaskensis 6SR	170	2.03[a]	83	Batch and continue experiments	López-Pérez et al. (2013b, 2015)
Aspergillus niger	75	4.84	16	Agitation rate on the biosorption	Yun-guo et al. (2006)
Chlorella vulgaris	150	1.73	87	Batch stirred system	Aksu and Donmez (2006)
Spirulina platensis	2	0.03	48	Batch experiments	Murugesan et al. (2008)
Dead algae, marine	252	3.15	80	Batch experiments	Herrero et al. (2006)

[a]Based on the dry mass of biofilm

Fig. 13.5 Growth of *Desulfovibrio alaskensis* strain 6SR. Postgate medium C supplemented with 2.5% NaCl, pH 7, and 18 mg/L Cr(VI). A control with culture medium and Cr (VI) indicates that the culture medium does not affect chromium speciation or precipitate it. Postgate medium C is the negative control

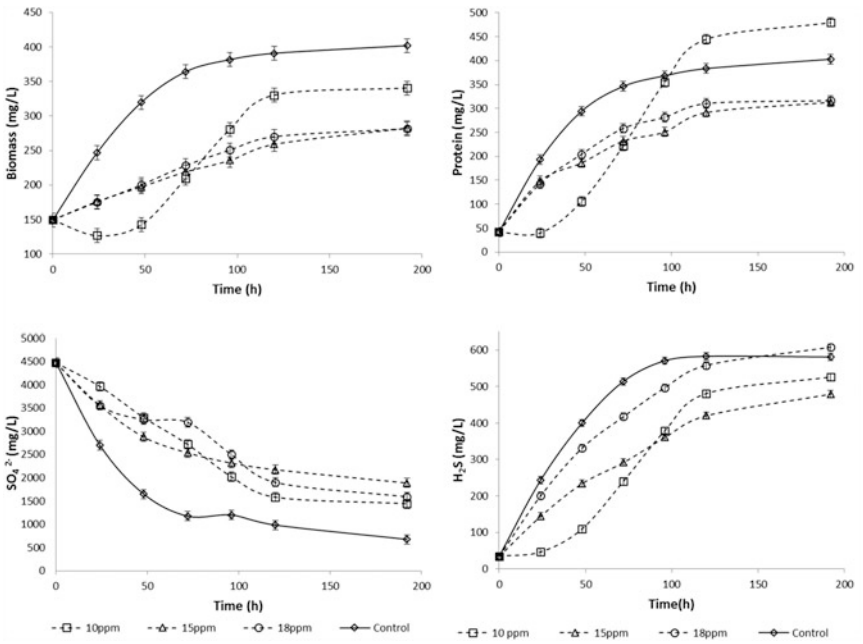

Fig. 13.6 Growth of *Desulfovibrio alaskensis* strain 6SR in Postgate medium C with 0, 10, 15 and 18 mg/L Cr(VI)

Due to the industrial interest of chromium, *D. alaskensis* is employed for hexavalent chromium and sulfate reduction. Chromium is reduced by hydrogen sulfide produced by the sulfate reduction in independent reactors; this alternative process was studied in the electrochemical treatment of Cr(VI) from wastewater (Peña-Caballero et al. 2016), as shown in Fig. 13.10.

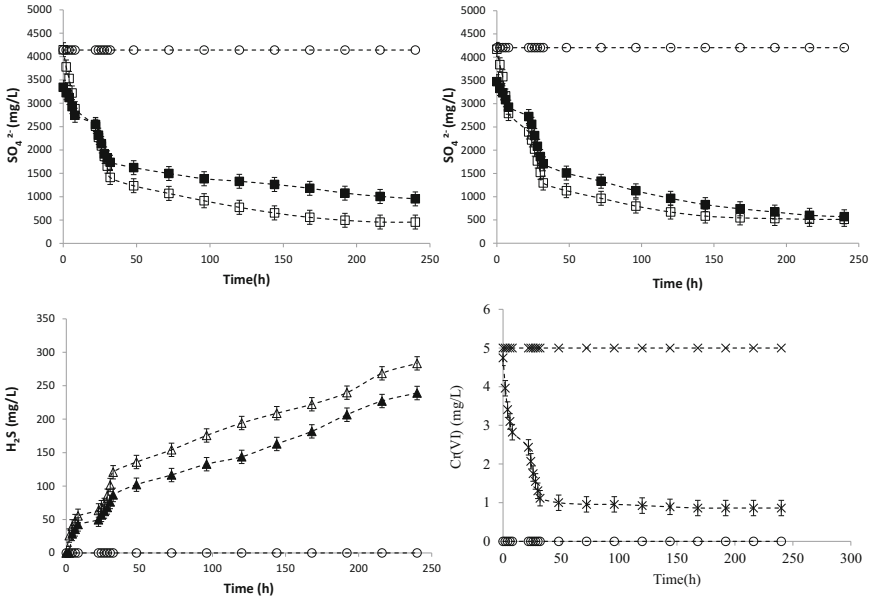

Fig. 13.7 Growth of *Desulfovibrio alaskensis* strain 6SR in Postgate medium C and 5 mg/L Cr (VI), under the same conditions of growth followed by Klonowska et al. 2008. The sulfate reduction process was not inhibited. Full markers represent the culture with Cr(VI). Empty markers represent control culture

Metallic removal studies in *D. alaskensis* strain 6SR showed that the bacterium is a strong candidate for the development of metallic removal processes of contaminated wastewater. *Desulfovibrio alaskensis* strain 6SR showed that there is no inhibition of biomass growth or sulfate reduction in the presence of high concentrations of Cd(II), Pb (II) and Zn(II) (150 mg/L), contrary to other SRB capable of growing in concentrations below 100 mg/L of metal ions. But even more, the metallic removal observed in *D. alaskensis* strain 6SR cultures was almost 100%, in the particular case of cadmium; the removal was very similar to concentrations higher than 150 mg/L Cd(II), (López-Pérez et al. 2015), the removal efficiency of 5 and 18 mg/L Cr(VI) was around 80% to both concentrations (Fig. 13.8b). Studies on the harmful effects of these heavy metals have revealed that cadmium and divalent metals are able to replace the essential ions to cell, provoking a build-up on cellular structure and block functional groups of macromolecules, promoting damage to the integrity of the cellular membrane and inactivation of cellular enzymes (Hossain et al. 2012); likewise, zinc at 40 mg/L initial concentration and above inhibits the cellular growth (Sani et al. 2001). However, the molecular studies showed that *D. alaskensis* 6SR has a resistance mechanism relationship to ATPase type P1B system, which responds to cadmium, lead and zinc (Morón-Vázquez 2015). The main mechanisms of metal resistance studied in Gram-negative bacteria are related to the transporters of the membrane

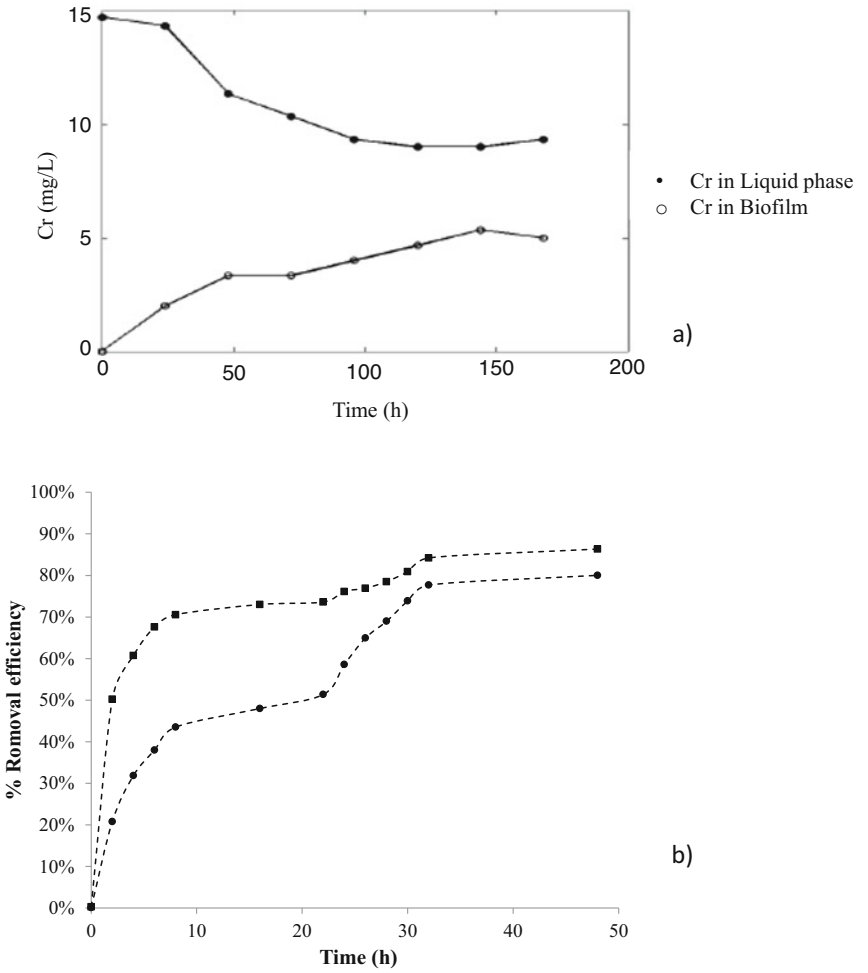

Fig. 13.8 Chromium removal. (**a**) Balance for chromium in liquid phase and biofilm (EPS). Chromium was assayed by atomic absorption spectrophotometry (AAS) (Atomic Absorption Spectrometer Specter AA-20 plus, Varian). (**b**) Efficiency of chromium removal: • 18 mg/Cr and ◆ 5 mg/L

that throw out harmful species of cellular cytoplasm such as ATPases type P1B (Nies 1999; Ramírez et al. 2008). Besides, the most relevant resistance mechanism in bacteria is related to the P1B-type ATPases and chemiosmotic systems. Metallomic specific researches have showed the interrelationships of metal-induced proteome and metabolome changes (Lara-Chavero et al. 2018; Metallomics 2012). The increase in genomic sequencing opens the knowledge of the genes that are related to the mechanisms of metallic resistance. Furthermore, the influence of metal ions on gene expression is of great interest in understanding metal resistance in bacteria. Some genes as *cad*A encode for such resistance mechanisms, which are

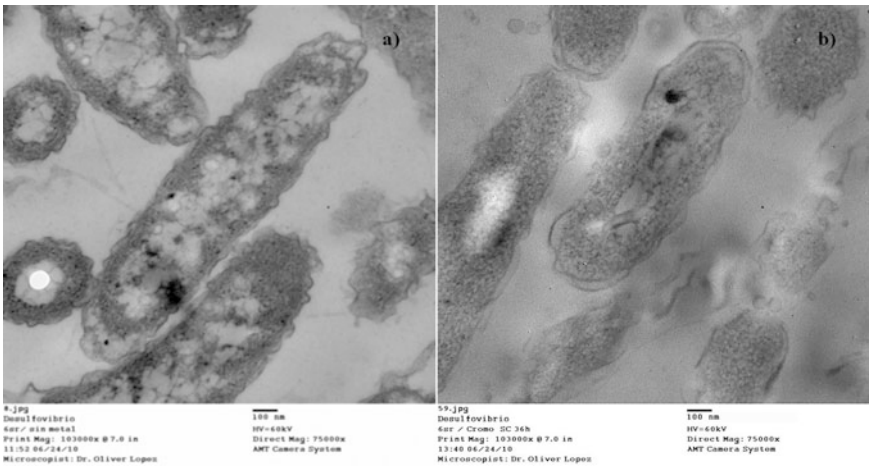

Fig. 13.9 Transmission electron micrographs of *Desulfovibrio alaskensis* strain 6SR. (**a**) Cells of *D. alaskensis* strain 6SR stained with lead acetate. (**b**) *D. alaskensis* strain 6SR grown in the presence of 18 mg/L Cr(VI) (72 h incubation)

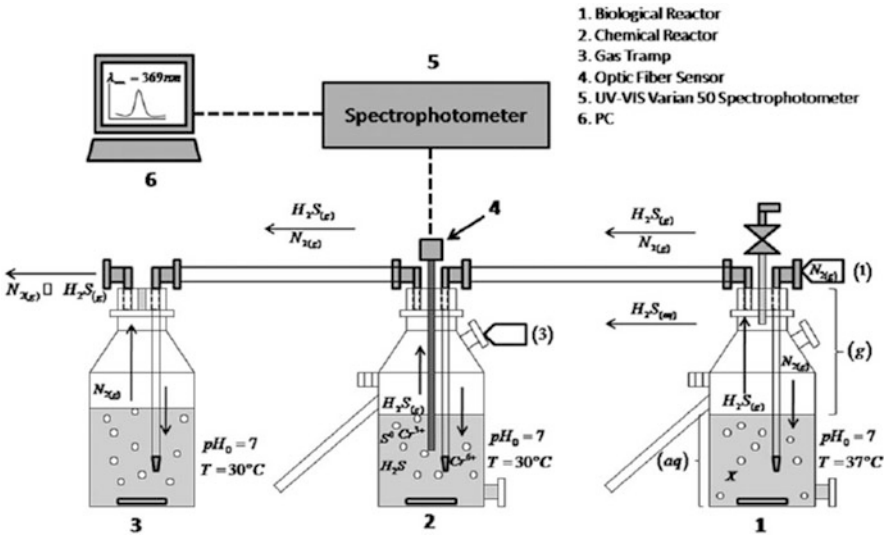

Fig. 13.10 Schematic diagram of the chromium removal from wastewater by biological sulfide. (Reported by Peña-Caballero et al. 2016)

induced by cadmium and zinc, and *chr*A gene is induced by hexavalent chromium (Naghma et al. 2005; Ding et al. 2005). The molecular analysis with respect to cadmium and chromium resistance mechanisms demonstrated the presence of *cad*A and *chr*A genes, when the partial sequences were analysed phylogenetically; see Figs. 13.11 and 13.12. Both genes are induced by Cd, Zn, Pb and Cr; the codified

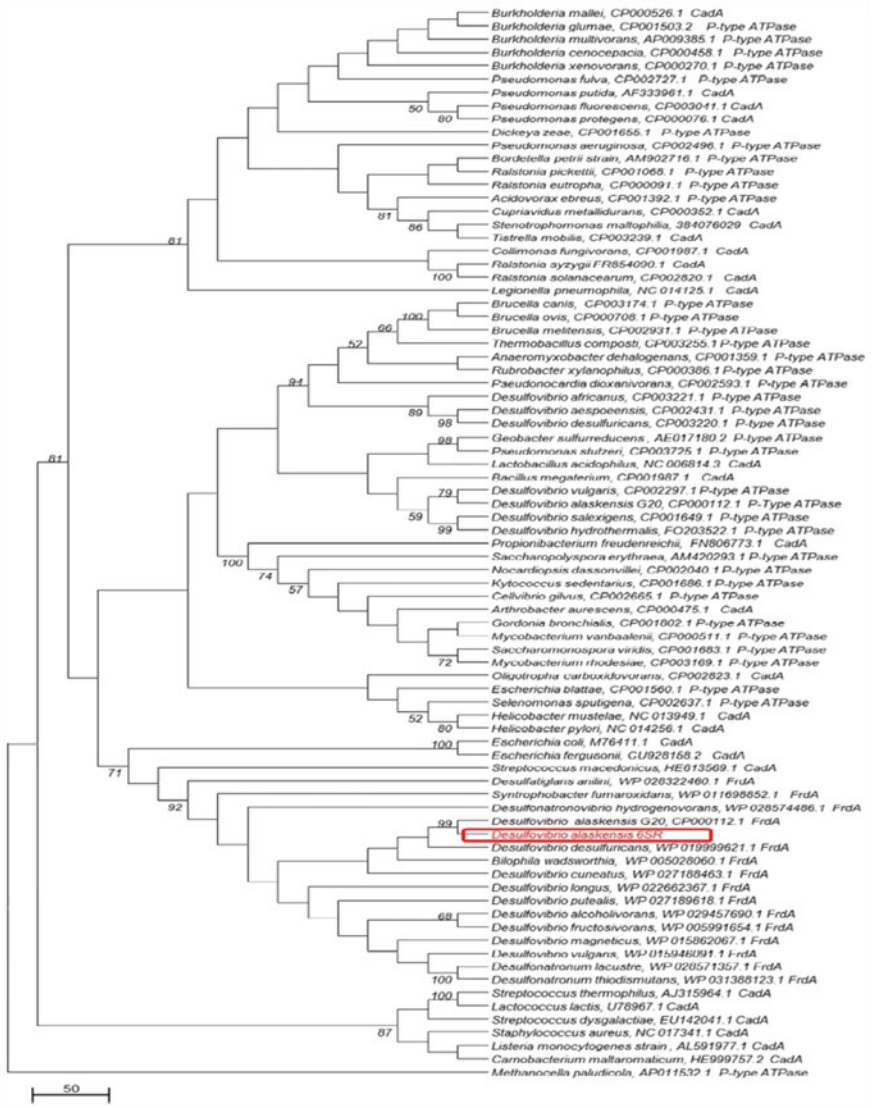

Fig. 13.11 Phylogenetic tree for the sequence obtained of *cad*A gene. The analysis was carried out with 550 aligned amino acids with related sequence to species that exhibit resistance to cadmium. The tree was built using a maximum likelihood algorithm. The scale bar represents 50 nucleotide substitutions per 100 amino acid. Bootstrap values, expressed as a percentage of 1000 repetitions, are shown at branching points. Only values greater than 50% are displayed

protein by these genes is involucre to abate the oxidative stress provoked by heavy metal nonessentials. The presence of thioredoxin codified by *chr*A gene has been reported in enzymatic chromium reduction processes. The capacity to reduce U

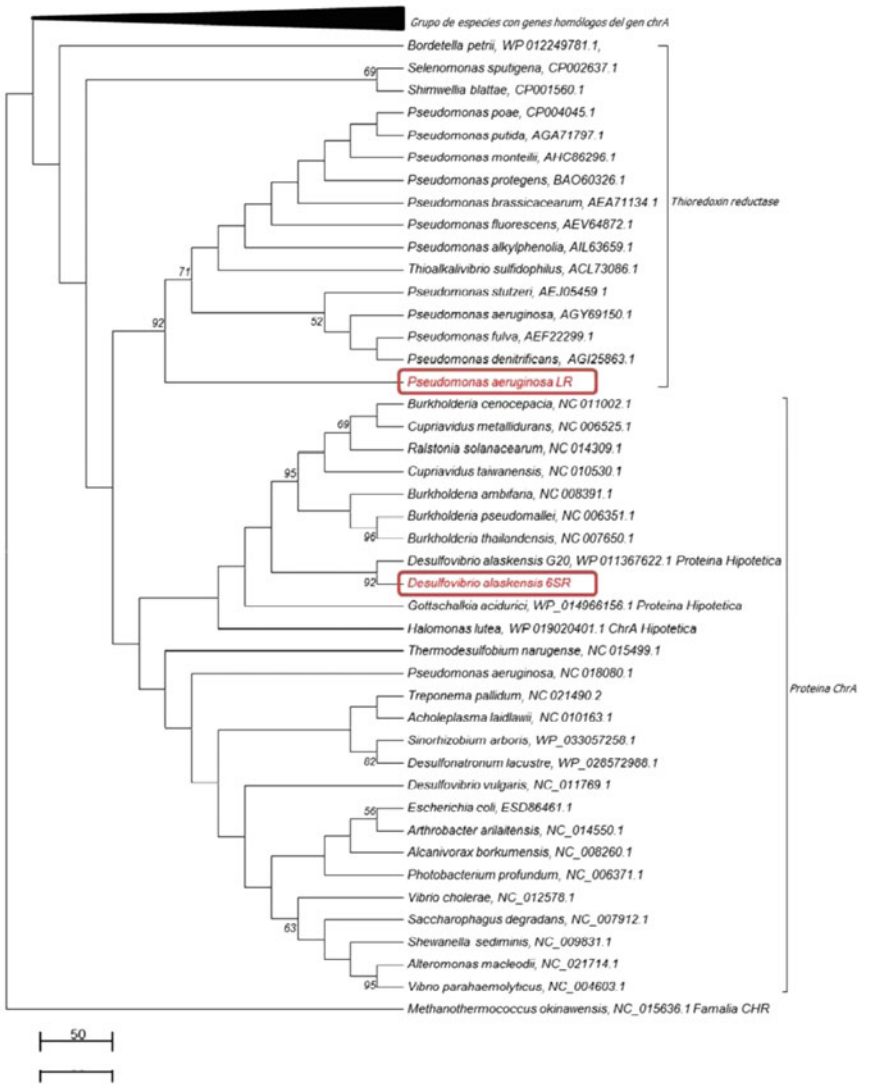

Fig. 13.12 Phylogenetic tree for the sequence obtained of *chr*A gene. The analysis was carried out with 550 aligned amino acids with related sequence to species that exhibit resistance to cadmium. The tree was built using a maximum likelihood algorithm. The scale bar represents 50 nucleotide substitutions per 100 amino acid. Bootstrap values, expressed as a percentage of 1000 repetitions, are shown at branching points. Only values greater than 50% are displayed

(VI) and Cr(VI) of *D. desulfuricans* G20 is related to the action of the thioredoxin reductase and NADPH (Li and Lee 2009). This suggests that the strain 6SR could have a resistance mechanism involving enzymatic reduction of Cr(VI), like a response to the oxidative stress generated by metallic ion.

References

Ahluwalia SS, Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol 98:2243–2257. https://doi.org/10.1016/j.biortech.2005.12.006

Aksu Z, Donmez G (2006) Binary biosorption of cadmium (II) and nickel (II) onto dried *Chlorella vulgaris*: co-ion effect on mono-component isotherm parameters. Process Biochem 41:860–868. https://doi.org/10.1016/j.procbio.2005.10.025

Anantharaman K, Hausmann B, Jungbluth SP et al (2018) Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. ISME J 12:1715–1728. https://doi.org/10.1038/s41396-018-0078-0

Arakaki A, Takeyama H, Tanaka T, Matsunaga T (2002) Cadmium recovery by a sulfate-reducing magnetotactic bacterium, *Desulfovibrio magneticus* RS-1, using magnetic separation. Appl Biochem Biotechnol 98–100:833–840. https://doi.org/10.1385/abab:98-100:1-9:833

Avilés Trejo M, Salazar López M d C (2014) Reduction of Cr (VI) to Cr (III) via cellular, using Desulfovibrio alaskensis 6SR as a biological model. Tesis Ingeniería Bioquímica, Tecnológico de Estudios Superiores de Ecatepec. (in Spanish)

Ayangbenro AS, Olanrewaju OS, Babalola OO (2018) Sulfate-reducing bacteria as an effective tool for sustainable acid mine bioremediation. Front Microbiol 9:1986. https://doi.org/10.3389/fmicb.2018.01986

Barton L (1995) Biotechnology handbooks. Sulfate-reducing bacteria. Springer

Bartosch S, Fite A, Macfarlene GT, McMurdo MET (2004) Characterization of bacterial community in faces in healthy elderly volunteers and hospitalized elderly patients by using Real-Time PCR and effects of antibiotic treatment on the fecal microbiota. Appl Environ Microbiol 70:3575–3581. https://doi.org/10.1128/AEM.70.6.3575-3581.2004

Cabrera G, Perez R, Gomez JM, Abalos A, Cantero D (2006) Toxic effects of dissolved heavy metals on *Desulfovibrio vulgaris* and *Desulfovibrio* sp. strains. J Hazard Mater 135:40–46. https://doi.org/10.1016/j.jhazmat.2005.11.058

Campbell LL, Postgate JR (1965) Classification of the spore-forming sulfate reducing bacteria. Bacteriol Rev 29:359–363

Castro HF, Williams NH, Ogram A (2000) Phylogeny of sulfate-reducing bacteria. FEMS Microbiol Ecol 31:1–9. https://doi.org/10.1111/j.1574-6941.2000.tb00665.x

Chance MR, Fiser A, Sali A, Pieper U, Eswar N, Xu G, Fajardo JE, Radhakannan T, Marinkovic N (2004) High-throughput computational and experimental techniques in structural genomics. Genome Res 14:2145–2154. https://doi.org/10.1101/gr.2537904

Cunningham DP, Lundie LL Jr (1993) Precipitation of cadmium by *Clostridium thermoaceticum*. Appl Environ Microbiol 59:7–14

Das N, Vimala R, Kartika P (2008) Biosorption of heavy metals – an overview. Indian J Biotechnol 7:159–169

Devereux R, He S-H, Doyle C et al (1990) Diversity and origin of Desulfovibrio species: phylogenetic definition of a family. J Bacteriol 172:3609–3619. https://doi.org/10.1128/jb.172.7.3609-3619.1990

Ding H, Harrison K, Lu J (2005) Thioredoxin reductase system mediates iron binding in IscA and iron delivery for the iron-sulfur cluster assembly in IscU. J Biol Chem 280:30432–30437. https://doi.org/10.1074/jbc.M504638200

Dinh HY, Kuever J, MuBmann M, Hassel AW, Stratmann M, Widdel F (2004) Iron corrosion by novel anaerobic microorganisms. Nature 427:829–832. https://doi.org/10.1038/nature02321

Duffus JH (2002) "Heavy metals" – a meaningless term? (IUPAC technical report). Pure Appl Chem 74:793–807. https://doi.org/10.1351/pac200274050793

El-Naggar MM (2009) Heavy metals removal by marine sulfate reducing bacteria. Egypt J Exp Biol (Bot) 5:215–219. http://www.egyptseb.org

Feio MJ, Zinkevich V, Beech IB, Llobet-Brossa E, Eaton P et al (2004) *Desulfovibrio alaskensis* sp. nov., a sulphate-reducing bacterium from a soured oil reservoir. Int J Syst Evol Microbiol 54:1747–1752. https://doi.org/10.1099/ijs.0.63118-0

Finneran KT, Anderson RT, Nevin KP, Lovley DR (2002) Bioremediation of uranium-contaminated aquifers with microbial U(VI) reduction. Soil Sediment Contam 11:339–357. https://doi.org/10.1080/20025891106781

Fournier M, Dermoun ZM-C, Dolla A (2004) A new function of the *Desulfovibrio vulgaris* Hildenborough [Fe] hydrogenase in the protection against oxidative stress. J Biol Chem 279:1787–1793. https://doi.org/10.1074/jbc.M307965200

Franco LC, Steinbeisser S, Zane GM, Wall JD, Fields MW (2018) Cr(VI) reduction and physiological toxicity are impacted by resource ratio in *Desulfovibrio vulgaris*. Appl Microbiol Biotechnol 102:2839–2850. https://doi.org/10.1007/s00253-017-8724-4

Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156:609–643. https://doi.org/10.1099/mic.0.037143-0

Gavrilescu M (2004) Removal of heavy metals from the environment by biosorption. Eng Life Sci 4:219–232. https://doi.org/10.1002/elsc.200420026

Haferburg G, Kothe E (2010) Metallomics: lessons for metalliferous soil remediation. Appl Microbiol Biotechnol 87:1271–1280. https://doi.org/10.1007/s00253-010-2695-z

Hernández-Gayoso MJ, Zavala-Olivares G, Ruiz-Ordaz N et al (2004) Microbial consortium influence upon steel corrosion rate, using polarization resistance and electrochemical noise techniques. Electrochim Acta 49:4295–4302. https://doi.org/10.1016/j.electacta.2004.03.038

Herrero R, Cordero B, Lodeiro P, Rey-Castro C, Sastre de Vicente ME (2006) Interactions of cadmium(II) and protons with dead biomass of marine algae *Fucus* sp. Mar Chem 99:106–116. https://doi.org/10.1016/j.marchem.2005.05.009

Hossain MA, Piyatida P, Teixeira da Silva JA, Fujita M (2012) Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J Bot 872875:1–37. https://doi.org/10.1155/2012/87287

Ianieva OD (2009) Mechanisms of bacteria resistance to heavy metals. Mikrobiol Z 71:54–65. (Article in Russian)

Jiang L, Zheng Y, Peng X et al (2009) Vertical distribution and diversity of sulfate-reducing prokaryotes in the Pearl River estuarine sediments, Southern China. FEMS Microbiol Ecol 70:249–262. https://doi.org/10.1111/j.1574-6941.2009.00758.x

Joo JO, Choi J-H, Kim IH, Kim YK, Oh BK (2015) Effective bioremediation of Cadmium (II), Nickel (II), and Chromium (VI) in a marine environment by using *Desulfovibrio desulfuricans*. Biotechnol Bioprocess Eng 20:937–941. https://doi.org/10.1007/s12257-015-0287-6

Klonowska A, Clark ME, Thieman SB, Giles BJ, Wall JD, Fields MW (2008) Hexavalent chromium reduction in *Desulfovibrio vulgaris* Hildenborough causes transitory inhibition of sulfate reduction and cell growth. Appl Microbiol Biotechnol 78(6):1007–1016. https://doi.org/10.1007/s00253-008-1381-x

Kluyver AJ, van Niel CB (1936) Prospects for a natural system of classification of bacteria. Zentr Bakteriol Parasitenk Abt II 94:369–403

Krishna AK, Govil PK (2007) Soil contamination due to heavy metals from an industrial area of Surat, Gujarat, Western India. Environ Monit Assess 124:263–275. https://doi.org/10.1007/s10661-006-9224-7

Lara-Chavero ZZ, Neria-González MI, Aguilar-López R (2018) Chromium-resistant *Pseudomonas stutzeri* LRO and its possible resistance mechanism. In: X biochemical engineering international congress. J Bioeng Biomed Res, pp 55–57. XVI biomedicine and molecular biotechnology scientific meetings proceedings, Merida, Yucatán, México

Li X, Lee KR (2009) Thioredoxin is involved in U (VI) and Cr (VI) reduction in *Desulfovibrio desulfuricans* G20. J Bacteriol 191:4924–4933. https://doi.org/10.1128/JB.00197-09

Li X, Lan SM, Zhu ZP, Zhang C et al (2018) The bioenergetics mechanisms and applications of sulfate-reducing bacteria in remediation of pollutants in drainage: a review. Ecotoxicol Environ Saf 30:162–170. https://doi.org/10.1016/j.ecoenv.2018.04.025

López-Pérez PA, Neria-González MI, Aguilar López R (2013a) Cadmium concentration stabilization in a continuous sulfate reducing bioreactor via sulfide concentration control. Chem Pap 67:326. https://doi.org/10.2478/s11696-012-0274-8

López-Pérez PA, Neria-González MI, Flores-Cotera LB, Aguilar-López R (2013b) A mathematical model for cadmium removal using a sulfate reducing bacterium: *Desulfovibrio alaskensis* 6SR. Int J Environ Res 7:501–512. https://doi.org/10.22059/IJER.2013.630

López-Pérez PA, Aguilar-López R, Neria-González MI (2015) Cadmium removal at high concentration in aqueous medium: mediated by *Desulfovibrio alaskensis*. Int J Environ Sci Technol 12:1975–1986. https://doi.org/10.1007/s13762-014-0601-4

López-Pérez PA, Neria González MI, Pérez García MR, Aguilar López R (2016) Concentrations monitoring via software sensor for bioreactors under model parametric uncertainty: application to cadmium removal in an anaerobic process. Alex Eng J 55:1893–1902. https://doi.org/10.1016/j.aej.2016.03.013

Mani D, Kumar K (2014) Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. Int. J. Environ Sci Technol 11:843–872. https://doi.org/10.1007/s13762-013-0299-8

Metallomics: whence and whither (2012) Whence: the inorganic side of life. (Editorial) Metallomics 4: 1017–1019. https://doi.org/10.1039/C2MT90041F

Meyer B, Kuever J (2007) Molecular analysis of the diversity of sulfate-reducing and sulfur-oxidizing prokaryotes in the environment, using the *apr*A as functional marker gene. Appl Environ Microbiol 73:7664–7679. https://doi.org/10.1128/AEM.01272-07

Morón-Vázquez MA (2015) Identification of metal resistance genes in the sulfate reducing bacteria *Desulfovibrio alaskensis* 6SR. Tesis Maestría en Ciencias, Tecnológico de Estudios Superiores de Ecatepec. (in Spanish)

Müller AL, Kjeldsen KU, Rattei T, Pester M, Loy A (2015) Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi)sulfite reductases. ISME J 9:1152–1165. https://doi.org/10.1038/ismej.2014.208

Murugesan AG, Maheswari S, Bagirath G (2008) Biosorption of cadmium by live and immobilized cells of *Spirulina platensis*. Int J Environ Res 2:307–312. https://doi.org/10.22059/IJER.2010.209

Muyzer G, Stams AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. Review. Nat Rev Microbiol 6:441–454. https://doi.org/10.1038/nrmicro1892

Naghma N, Hilary K, Nuzhat A, Geoffrey M (2005) Cadmium accumulation and DNA homology with metal resistance genes in sulfate-reducing bacteria. Appl Environ Microbiol 71:4610–4618. https://doi.org/10.1128/AEM.71-4618.2005

Neria-González I, Wang ET, Ramírez F, Romero JM, Hernández-Rodríguez C (2006) Characterization of bacterial community associated to biofilms of corroded oil pipelines from the Southeast of Mexico. Anaerobe 12:122–133. https://doi.org/10.1016/j.anaerobe.2006.02.001

Neria-González MI, Figueroa-Estrada JC, Cruz-Diaz MR, Aguilar-López R (2011) Adaptive smooth observer design for state estimation 4 in *Desulfovibrio alaskensis* 6SR cultures. Rev Mex Ing Quím 10:137–146

Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51:730–750. https://doi.org/10.1007/s002530051457

Peña-Caballero V, Aguilar-López R, López-Pérez PA, Neria-González MI (2016) Reduction of Cr (VI) utilizing biogenic sulfide: an experimental and mathematical modeling approach. Desalin Water Treat 57:13056–13065. https://doi.org/10.1080/19443994.2015.1055811

Postgate JR (1984) The sulphate reducing bacteria, 2nd edn. Cambridge University Press, New York

Postgate JR, Campbell IL (1966) Classification of *Desulfovibrio* species, the nonsporulating sulfate-reducing bacteria. Bacteriol Rev 30:732–738

Qian J, Wei L, Liu R et al (2016) An exploratory study on the pathways of Cr (VI) reduction in sulfate-reducing up-flow anaerobic sludge bed (UASB) reactor. Sci Rep 6:23694. https://doi.org/10.1038/srep23694

Quintelas C, Rocha Z, Silva B et al (2009) Biosorptive performance of an *Escherichia coli* biofilm supported on zeolite NaY for the removal of Cr(VI), Cd(II), Fe(III) and Ni(II). Chem Eng J 152:110–115. https://doi.org/10.1016/j.cej.2009.03.039

Ramírez M, Díaz C, Vargas E, Riveros H, Campos J, Cervantes C (2008) Mechanisms of bacterial resistance to chromium compounds. Biometals 21:321–332. https://doi.org/10.1007/s10534-007-9121-8

Rangel-Chávez RG, Neria-González MI, Márquez-Herrera A et al (2015) Synthesis of CdS nanocrystals by employing the by-products of the anaerobic respiratory process of *Desulfovibrio alaskensis* 6SR bacteria. J Nanomater 2015:Article ID 60397. https://doi.org/10.1155/2015/260397

Sani RK, Peyton BM, Brown LT (2001) Copper-induced inhibition of growth of *Desulfovibrio desulfuricans* G20: assessment of its toxicity and correlation with those of zinc and lead. Appl Environ Microbiol 67:4765–4772. https://doi.org/10.1128/AEM.67.10.4765-4772.2001

Selatnia MZ, Bakhti A, Madani L, Kertous Y, Mansouri K (2004) Biosorption of Cd^{2+} from aqueous solution by a NaOH-treated bacterial dead *Streptomyces rimosus* biomass. Hydrometallurgy 75:11–24. https://doi.org/10.1016/j.hydromet.2004.06.005

Silver S, Phung LT (2005) A bacterial view of the periodic table: genes and proteins for toxic inorganic ions. J Ind Microbiol Biotechnol 32:587. https://doi.org/10.1007/s10295-005-0019-6

Singh SA, Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol 98:2243–2257. https://doi.org/10.1016/j.biortech.2005.12.006

Sinha S, Mukherjee SK (2009) *Pseudomonas aeruginosa* KUCD1, a possible candidate for cadmium bioremediation. Braz J Microbiol 40:655–662. https://doi.org/10.1590/S1517-838220090003000030

Thakare M, Sarma H, Datar S, Roy A, Pawar P, Gupta K, Pandit S, Prasad R (2021) Understanding the holistic approach to plant-microbe remediation technologies for removing heavy metals and radionuclides from soil. Curr Res Biotechnol. https://doi.org/10.1016/j.crbiot.2021.02.004

Urata T, Kikuchi M, Yusuke TH, Kiyoko Y et al (2008) Bacteremia caused by *Desulfovibrio fairfieldensis*. J Infect Chemother 14:368–370. https://doi.org/10.1007/s10156-008-0629-9

Utgikar VP, Harmon SM, Chaudhary N et al (2002) Inhibition of sulfate-reducing bacteria by metal sulfide formation in bioremediation of acid mine drainage. Environ Toxicol 17:40–48. https://doi.org/10.1002/tox.10031

Vásquez TGP, Botero AEC, de Mesquita LMS, Torem ML (2007) Biosorptive removal of Cd and Zn from liquid streams with a *Rhodococcus opacus* strain. Miner Eng 20:939–944. https://doi.org/10.1016/j.mineng.2007.03.014

Velea T, Gherghe L, Predica V, Krebs R (2009) Heavy metal contamination in the vicinity of an industrial area near Bucharest. Environ Sci Pollut Res 16:S27–S32. https://doi.org/10.1007/s11356-008-0073-5

Voordouw G (1995) The genus *Desulfovibrio*: the centennial. Appl Environ Microbiol 61:2813–2819

Wagner M, Loy A, Klein M et al (2005) Genes for identification of sulfate-reducing prokaryotes. Methods Enzymol 397:469–489. https://doi.org/10.1016/S0076-6879(05)97029-8

Watanabe M, Kawahara K, Sasaki KN (2003) Noparatnaraporn, biosorption of cadmium ions using a photosynthetic bacterium, *Rhodobacter sphaeroides S* and a marine photosynthetic bacterium, *Rhodovulum* sp. and their biosorption kinetics. J Biosci Bioeng 95:374–378. https://doi.org/10.1016/81389-1723(03)80070-1

White C, Gadd GM (1998) Accumulation and effects of cadmium on sulphate-reducing bacterial biofilms. Microbiology 144:1407–1415. https://doi.org/10.1099/00221287-144-5-1407

Widdel F, Pfennig N (1984) Section 7. Dissimilatory sulfate- or sulfur reducing bacteria. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 1. Williams & Wilkins, Baltimore, pp 663–679

Woese C, Kandlert O, Wheelis M (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci 87:4576–4579. https://doi.org/10.1073/pnas.87.12.4576

Wood JM, Wang H-K (1983) Microbial resistance to heavy metals some microorganisms have developed "strategies" for combating effects of toxic inorganics, and several may prove useful for their removal from wastewater. Environ Sci Technol 17:12. https://doi.org/10.1021/es00118a002

Wörner S, Pester M (2019) The active sulfate-reducing microbial community in littoral sediment of oligotrophic lake constance. Front Microbiol 10:1–12. https://doi.org/10.3389/fmicb.2019.00247

Yun-guo L, Ting F, Guang-ming Z, Xin L, Qing T, Fe Y, Ming Z, Wei-hua X, Yu-e H (2006) Removal of cadmium and zinc ions fi-om aqueous solution by living *Aspergillus niger*. Trans Nonferrous Met Soc China 16:681–686. https://doi.org/10.1016/S1003-6326(06)60121-0

Ziagova Z, Dimitriadis G, Aslanidou D et al (2007) Comparative study of Cd(II) and Cr (VI) biosorption on *Staphylococcus xylosus* and *Pseudomonas* sp. in single and binary mixtures. Bioresour Technol 98:2859–2865. https://doi.org/10.1016/j.biortech.2006.09.043