



## Biosorption of hexavalent chromium by microorganisms



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### ABSTRACT

Microorganisms perform an important function in the bioremediation of contaminated soils, water and effluents. Bacteria, fungi, yeasts and microalgae and cyanobacteria, are low-cost biotechnological tools for the treatment of large volumes of complex effluents containing Cr(VI). As from the selection and identification of new microorganisms and the evolution of Modern Biotechnology, molecular techniques and the characteristics of microorganisms such as their versatility and capacity to adapt to different environments, permit that the biosorption of Cr(VI) be an alternative for the removal of contaminants. Variations in the cell composition, morphology and way of growth, as also a study of the operational conditions, allow for the application of aerobic or anaerobic microorganisms, live or dead, in the removal of Cr(VI). Thus the characteristics of the biosorbents applied in the removal of Cr(VI), the biosorption processes, reactors and bioreactors, and the research developed with a view to the biosorption will be discussed in this review.

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### 1. Introduction

The population growth and potential increase in industrial activity in recent decades have contributed to a worsening of environmental problems, such as, for example, water pollution. Industrial effluents are generated by the incorporation of organic and inorganic contaminants, in addition to disposing of metals such as chromium, lead, mercury, cadmium and selenium. They are frequently launched into the soil and water as from various polluting sources, such as foundries, tanneries, textile, microelectronic, fertilizer and pesticide industries, mining activity and other industrial activities.

Studies have provided evidence that in addition to water and soil contamination, there has been contamination of rice and soybean (Silva et al., 2014), of mango orchards (Silva et al., 2012a,b), of birds and swine (Alkmim Filho et al., 2014), of cattle (Souza et al., 2009), amphibians (Fernando et al., 2016), and of wild animals (Curi et al., 2012). The toxicity of heavy metals is reproduced by individual action mechanisms of specific natures: i-exerting immunosuppressive activity, reducing the activity or efficiency of the immune system; ii-competing at fixation sites of enzyme

activity co-factors; iii-inhibiting vital enzymes such as oxidative phosphorylation and iv-altering cell structures, principally in the lipoprotein zone of membranes (Strandberg et al., 1981).

The maximum permitted discharge level of total Cr into surface and potable waters has been set to below 0.05 mg l<sup>-1</sup> by the Environmental Protection Agency (USA) (Baral and Engelken, 2002) and the European Union (EC, 1998). Industrial effluents containing Cr(VI) are often released into natural water resources without proper treatment, resulting in anthropogenic contamination (Viti et al., 2003; Cefalu and Hu, 2004; Cheung and Gu, 2007). Chromium stands out due to its wide range of use in industrial processes. It is used in the production of steel and metal alloys, cement, galvanized plastic, tanneries, paints, fertilizers and fungicides. It can be present in waters and liquid effluents in the hexavalent form as chromate (CrO<sub>4</sub>)<sup>-2</sup> or dichromate (Cr<sub>2</sub>O<sub>7</sub>)<sup>-2</sup> salts or as chromic oxide, in addition to being highly water soluble and all forms of the metal can be toxic, depending on the concentration.

It has been estimated that Cr(VI) is about a hundred times more toxic than Cr(III). Cr(III) is essential to human metabolism, being involved in the maintenance of glucose, cholesterol and triglyceride levels, and playing an essential role as a nutrient for live organisms (Frois et al., 2011). It should be remembered that Cr(III) can oxidize in nature, transforming into its more toxic form, Cr(VI).

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Cr(VI) shows elevated toxicity, being related to nasal irritation and ulceration, hypersensitivity reactions and contact dermatitis, chronic bronchitis and emphysema, cause liver and kidney damage, internal hemorrhage, lung and skin cancer, in addition to damaging DNA by its interface with the enzyme DNA-polymerase (Harte et al., 1993; Chhikara et al., 2010). Inhaling and/or ingesting Cr(VI) compounds has been shown to be cancerous due to its easy permeation of the cell membrane and elevated oxidizing potential, causing lung and stomach cancer (Frois et al., 2011; Miranda Filho et al., 2011; Mutongo et al., 2014). The lethal dose ( $DL_{50}$ ) of Cr(VI) by oral ingestion in rats, varies from 50 to 100 mg kg<sup>-1</sup>, a very small value when compared to the  $DL_{50}$  of Cr(III) of between 1900 and 3300 mg kg<sup>-1</sup>.

Heavy metals such as Cr(VI) have been removed from industrial effluents by the application of conventional metal removal techniques such as reverse osmosis, solvent extraction, lime coagulation, ion exchange and chemical precipitation are encountered with certain major disadvantages such as high energy requirements, incomplete metal removal and generation of a large quantity of toxic waste sludge due to various reagents used in a series of treatment such as reduction of Cr(VI), neutralization of acidic solution and precipitation. (Ahalya et al., 2003; Mane et al., 2011; Alpatova et al., 2004). Consequently the search for alternative solutions has become of eminent importance, searching for techniques that aid in the removal of these contaminants, such as, for example, the application of biosorbents, either alone or coupled with another conventional method already used to remove Cr(VI).

The potential for growth and adaptation of microorganisms when faced with adverse conditions and their versatility in biotechnological applications linked to modern biotechnology, has qualified microorganism as biosorption agents known as biosorbents. The use of biosorbents in the removal of Cr(VI) is a promising technique when compared to the conventional techniques mentioned above.

To better understand the importance of the theme, a survey was carried out in the Web of Science database. The terms *biosorption* and *hexavalent chromium*, and *biosorption* and *Cr(VI)*, were used as the title words, resulting in 49 and 138 documents, respectively, with a total of 187 studies carried out between 1991 up to the date of the survey, carried out on March 15th, 2016. It can be seen that few studies have been presented in this research area with the objective of finding new solutions to preserve the environment. Considering the importance of the socio-environmental impact created by the presence of Cr(VI) in industrial effluents and by the deleterious potential for human and animal health, the objective of the present review was to present and discuss the potentialities of removing Cr(VI) by applying microbial biosorbents, and discuss the main characteristics and factors involved in the biosorption processes.

## 2. Biosorbents

Biosorption is defined as a passive, rapid, reversible and independent metabolic energy process carried out by active or inactive microorganisms. The biosorption process carried out by way of biosorbents has been shown to have great potential in the removal of heavy metals from industrial effluents. This process is represented by disequilibrium of the surface forces by the contact of a solid surface with a liquid phase, forming a surface layer of solutes on the adsorbent and resulting in the accumulation of metals by physicochemical interactions of metal ions with cell components of biological species (Buratto et al., 2012).

The search for alternative processes to the conventional treatments for the removal of Cr(VI) makes it possible to use microalgae, fungi, bacteria and yeasts in biosorption processes. As compared to

conventional methods, these biosorbents show characteristics such as low cost, high capacity and removal efficiency, reduction in the generation of chemical and biological residues, low nutritional requirements of the biosorbents and regeneration of the biosorbent by recovering the metal. The high specific growth rate of many microorganisms stands out, which makes cell multiplication possible or even reuse of the microbial biomass in industrial applications, such as in the case of brewery yeast.

The mechanisms underlying the biosorption process depend on factors intrinsic and extrinsic to the biosorbents. The nature of the microbial biomass is one of the most important factors in the choice of a biosorbent for heavy metals, although other factors, such as cell viability, specific growth velocity, the nutritional requirements (substrates and nutrients), the metabolic products and the culture conditions such as temperature, pH and dissolved oxygen, are important characteristics in the selection of a biosorbent. In addition the metallic species and its respective concentration and the type and composition of the effluent, should also be taken into consideration when choosing an adequate biosorbent.

The majority of microorganisms exhibit a biphasic response, represented by the stimulus to cell growth when submitted to low concentrations and growth inhibition, perceived as from the minimal inhibitory concentration (MIC). This represents the lowest concentration of the contaminant that causes inhibition of microbial growth, and can therefore directly affect the population size, biological activity and microbial biodiversity (Sadler and Trudinger, 1967; Kavamura and Espósito, 2010).

Various types of biosorbents have been employed in the removal of heavy metals and other contaminants from effluents, aiming at biosorption by algae, microalgae and cyanobacteria (Saravanan et al., 2009; Mane et al., 2011; Monteiro et al., 2011; Khoubestani et al., 2015; Kwak et al., 2015; Nemr et al., 2015), fungi (Akar and Tunali, 2006; Khani et al., 2012), yeasts (Ferreira et al., 2007; Canuto et al., 2007; Martorell et al., 2012; Mahmoud, 2015), bacteria (White et al., 1995; Wierzbza, 2010; Chen et al., 2009; Cabral et al., 2014; Wu et al., 2015; Huang et al., 2016), sludge (Chen et al., 2015; Michailides et al., 2015), aquatic macrophytes (Módenes et al., 2009; Lima et al., 2011); plant, fruit and vegetable residues (Mutongo et al., 2014; Reddy et al., 2014; Huang et al., 2015; Sultana et al., 2015; Wang et al., 2016); and inorganic substances (Khelaifia et al., 2016).

Microbial biomass has the capacity to adsorb inorganic contaminants due to its cell composition (Fig. 1). The anionic ligands phosphoryl, carbonyl, sulfhydryl and hydroxyl groups contribute greatly to the biosorption processes (Volesky, 1987). Depending on the species, microalgae present a diversified biochemical composition of carbohydrates, proteins, lipids and fatty acids (Cardoso et al., 2011). In contrast, the cell walls of fungi, especially the filamentous ones, are composed of polysaccharides such as  $\beta$ -glucan, chitin and chitosan, glycoproteins, lipids, melanins, D-galactosamine polymers and polyuronides, being considered a location with a prevalence of metal binding sites such as the chemical groups acetamido, amide, phosphate, amino, amine, sulfhydryl, carboxyl and hydroxyl (Vimala and Das, 2011).

The fungal cell wall is composed mainly of the polysaccharides  $\beta$ -1,3 glucan,  $\beta$ -1,6 glucan, mannan-binding protein and a low concentration of chitin (Fleuri and Sato, 2010), whereas that of Gram-positive bacteria is constituted of a thick layer composed of peptidoglycan, responsible for its rigidity. On the other hand the cell wall of Gram-negative bacteria has a thin layer of peptidoglycan, conferring greater fragility (Rosa, 2008). Aquatic macrophyte biomass is characterized by its chemical composition, presenting concentrations of proteins, lipids, cell wall fractions and soluble carbohydrates, together contributing an important nutritional source of vegetable biomass (Henry-Silva and Camargo,

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