



Two combined mechanisms responsible to hexavalent chromium removal on active anaerobic granular consortium

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HIGHLIGHTS

- Anaerobic granular consortium proves a potential application on metal bioremediation.
- Reduction and bioadsorption were combined to remove Cr VI in anaerobic consortium.
- Bioadsorption is the major removal pathway of Cr VI at concentrations >50 mg/L.
- Cr VI enhances the CH₄ production of up to 30% at concentrations <100 mg/L.
- The suitability of the removal capacity of the anaerobic consortium is the high IC₅₀.

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ABSTRACT

Hexavalent chromium (Cr VI) from industrial wastewaters represents a highly toxic source at low concentrations. Biological treatments with anaerobic granular biomass are a promising alternative for the Cr VI bioremediation. This study evaluated the Cr VI removal in a range of 5–500 mg/L, using an active anaerobic granular consortium. Two removal mechanisms were differentiated from the assays: 1) biological reduction of 70 mg/L to Cr III at a concentration of 250 mg Cr VI/L and 2) physical bioadsorption of 297 mg of Cr VI/L or 31.39 mg of Cr VI/g_{biomass} at concentration of 500 mg Cr VI /L. The half-maximal inhibitory concentration (IC₅₀) values for the rate and production of methane were 1.4 and 253 mg/L, respectively. In addition, Cr VI is a biostimulant that increase the methane production, in a range from 5 to 100 mg/L, of the anaerobic consortium. This work demonstrates the potential application of the anaerobic granular consortium in metal bioremediation.

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1. Introduction

Chromium is a heavy metal that can exist in the environment in two main forms: Hexavalent Chromium (Cr VI) and Trivalent Chromium (Cr III), the first being the most toxic and dangerous. Cr VI is mainly found in the form of strong oxides and dichromates and is the second most common heavy metal in polluted waters, soils and sediments (Kang et al., 2014). Cr VI is highly soluble and potentially dangerous for all forms of life because of its capacity to

alter or denature proteins (Singh et al., 2011). Different studies have corroborated the toxic effect due to the presence of Cr VI in different matrices. Velma and Tchounwou (2013) report damages in goldfish DNA due to Cr VI bioaccumulation at a concentration above 4.0 mg/L. The Cr VI mutagenic activity has been demonstrated in numerous studies using bacterial cells of *Salmonella typhimurium*, *Escherichia coli* or *Bacillus subtilis* due to the chromosomal damage that it causes (Grevatt, 2010). On the other hand, some authors argue that at concentrations above 10.0 mg/L, Cr VI causes a decrease in the productivity of agricultural soils (Singh et al., 2011) and that there is a risk of bioaccumulation in crops (Chen et al., 2014a, 2014b).

The main sources of Cr VI pollution can be both natural and

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derived from anthropogenic activities, such as the tanning, mining and textile industries. Cr VI has been detected in different industrial effluents in a range of 50 to 4000 mg/L (Leyva et al., 2008; Owlid et al., 2009). As a result of this problem, different methods are used for the treatment of these effluents, such as chemical reduction followed by precipitation (Rashmi et al., 2013), ion exchange (Alvarado et al., 2013), bioremediation (Acosta-Rodríguez and Cárdenas-González, 2010) and membrane technologies (Doke and Yadav, 2014). Since some of these treatments generate dangerous byproducts, they have higher costs and special requirements; therefore, in recent years, the adsorption processes have become the most attractive technologies for the removal of heavy metals (Kumar and Jena, 2017).

Different adsorbents have been applied for the removal of heavy metals, among them activated carbon (Jung et al., 2013), metal oxides (Jiang et al., 2013), immobilized nanomaterials (Rajput et al., 2014), bioadsorbents such as microorganisms (Fadel et al., 2017) and biological sludge generated during the treatment of wastewater (Chen et al., 2015). However, the current application of bioadsorbents allows the use of wastes and low-cost materials for the removal of pollutants, in addition to being an environmentally friendly and efficient option (Fu and Wang, 2011). Some research have used inactive biological sludge for the adsorption of heavy metals, demonstrating the capacity to capture ions and form metal complexes due to the interactions present in the cell wall (Ahlwalia and Goyal, 2007). In this sense, Wang and Chen (2009) established that Cr VI has an affinity toward bioadsorbents, such as bacteria, fungi and microalgae, due to the presence of functional groups (hydroxyl, carboxyl, amino, phenol) in the cell wall.

Currently, the most efficient Cr VI biological removal processes are those that apply axenic cultures, with the highest reported adsorption capacities for *Pseudomonas* sp. (95 mg/g) and *Aeromonas caviae* (284 mg/g). However, a disadvantage of using axenic cultures is the need to maintain strictly controlled conditions that avoid the contamination of such cultures by other bacteria and/or compounds, which is reflected in a portion of the cost of these processes. Therefore, the application of mixed cultures or microbial consortium in granular or floccular form becomes a viable option for the biological removal process of metals due to the synergy that can occur between the different groups of microorganisms (Michailides et al., 2013; Nancharaiah et al., 2010). The application of microorganisms as bioadsorbents in the form of flocs, despite being the most commonly used, presents various disadvantages, such as the difficulty of separating the flocs from the treated effluent. This disadvantage requires immobilization processes, which increases the complexity and cost of the process (Liu et al., 2012). Moreover, other authors have established that for the removal of phenols and heavy metals, the granular consortia have a greater advantage over flocs because of their morphological characteristics, which provide the granules high permeability and porosity, in addition to suitable separation from the effluent due to their high sedimentability (Ma et al., 2014; Sun et al., 2010). Evidence suggests that the extracellular polymeric substances (EPS), which allow the formation and stability of the granules, contain organic molecules such as proteins, humic acids, lipids, nucleic acids and polysaccharides, which increase the interaction between the adsorbate and bioadsorbent due to the presence of functional groups: amino, hydroxyl, carboxyl, etc. (d'Abzac et al., 2013).

Some species of microorganisms thriving in the Cr VI contaminated environments have evolved novel strategies to cope with its toxicity. For example, the decrease in the uptake or exclusion of Cr VI through membranes, biosorption and the upregulation of genes associated with the oxidative stress response are some of the resistance mechanisms in microbial cells. The most important mechanism is the microbial reduction to Cr III, since it transforms

toxic and mobile chromium derivatives into a harmless reduced species and is considered an essential trace element that is involved in glycolysis and lipid metabolism (Ahemad, 2014). In order to carry out this reduction, Cr VI must cross the cell membrane through an anion carrier (Ding and Shi, 2002), and once inside the cell it can be reduced by an enzyme or non-enzymatic action to Cr V, Cr IV and Cr III (Borthiry et al., 2007; Myers, 2012). During the reduction process, Reactive Oxygen Species (ROS) are generated. Many studies suggest that the toxic and carcinogenic effects of Cr VI is partially associated with the overproduction of ROS and associated oxidative stress, since it is an important step in the mechanism of induced DNA damage (Fu et al., 2008; O'Brien et al., 2003; Valko et al., 2005). In the respiratory chain and energy metabolism, there is evidence that Cr VI affects the electrons transfer, leading to apoptosis by alteration of the mitochondria-related genes (Yang et al., 2012; Yuan et al., 2012). Previous research has focused on exploring the influence of Cr VI on the performance and reducing capacities of this metal by some microorganisms (Jin et al., 2017). In an aerobic membrane biofilm reactor, Cr VI could be reduced to Cr III and removed from wastewater in form of solid (Dogan et al., 2011). Cr VI reduced by *Pseudomonas putida* was partially associated with extracellular polymeric substances (Priester et al., 2006), because EPS could protect biomass and reduce the toxic effect on Cr VI reduction, which is carried out by membrane-associated chromate reductase enzymes on the anaerobic activated sludge's. However, although these investigations show up evidence of how the Cr VI is reduced, consider only the removal efficiency is inaccurate, since its reduction was not taken into account with respect to total Cr.

Furthermore, there are signs that the application of microorganisms as bioadsorbents for the treatment of effluents polluted with Cr VI can occur through the following phenomena: (i) the binding of the cation to the cell wall, which is known as biosorption, followed by the translocation of this cation inside the cell, and (ii) the reduction of Cr VI to Cr III, either enzymatically or as a consequence of the interactions of the microorganisms with the medium that contains the metal (Gokhale et al., 2008). Several authors establish three biological pathways by which the reduction of Cr VI can be performed under anaerobic conditions, where Cr VI acts as electron acceptor: 1) due to the presence of chromate reductase enzymes in the membrane, 2) during the electron transport in the anaerobic respiration and 3) through different components present in the cell protoplasm (amino acids, nucleotides, carbohydrates, vitamins, organic acids, flavonoids and proteins) that serve as electron donors (Ahemad, 2014; Cheng et al., 2012). Therefore, the main goal of this work was conducted to explore, on the one hand, the mechanism that govern the removal capacity of Cr VI either by adsorption or biological reduction on the active anaerobic granular consortium and, on the other hand, the effect of this metal ion in the specific methanogenic activity of this consortium.

2. Materials and methods

2.1. Origin of the anaerobic granular consortium

The anaerobic consortium was obtained from an upflow anaerobic sludge blanket (UASB) reactor used inside the wastewater treatment plant of a brewery located along the road between the cities of Obregón and Navojoa, Mexico. The anaerobic sludge was first washed multiple times with a 0.9% saline solution until a clear supernatant was obtained. Subsequently, three more washes were performed with distilled water, and the Total Solids (TS), Total Volatile Solids (TVS), Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were determined according to the established in APHA (2012).

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