Reduction of hexavalent chromium by *Serratia marcescens* immobilized on active carbon and their potencial use in bioremediation.

Reducción de cromo hexavalente, por *Serratia marcescens* inmovilizada en carbon activado y su potencial uso en biorremediación.

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RESUMEN

S. marcescens, cepa tolerante a cromato, presenta la capacidad de formar biopelículas y transformar el 95 % de cromato presente en el medio a Cr(III), compuesto menos tóxico, después de 48h de incubación, a una velocidad de transformación de 1.67 mg L⁻¹ h⁻¹. *S. marcescens* puede ser utilizado en biorremediación de residuos contaminados con cromato.

Chromium pollution due to wastewater discharges from manufacturing and domestic activities may pose a serious threat to human health and environmental quality (Campos *et al.* 2002). Indeed, Cr(VI) has been identified as a key contaminant due to its toxicity (Park *et al.* 2002). Biological reduction of Cr(VI) to Cr(III) is an attractive alternative to treat chromium contaminated effluents and soils, since the latter presents much lower toxicity (McLean & Beveridge 2001). A number of both aerobic and anaerobic bacteria presenting chromate reducing enzymatic ability have been identified (Shanker *et al.* 2005).

In particular, chromate-resistant *S. marcescens* bacteria, isolated from a tannery effluent has been shown to present chromate reducing activity (Campos *et al.* 2005) and could grow in the presence of a wide range of industrial contaminants. The use of such Cr(VI) reducing bacteria in chromium-contaminated soil and water bioremediation would be enhanced if *S. marcescens* was able to form stable biofilms. Biofilm formation appears to be a widespread attribute of bacteria and may allow increased survival ability under stressful conditions such as low nutrients or antimicrobial treatments (Mah and O'Toole 2001). Indeed, immobilised cells as a biofilm on inert supports enable high biomass retention in bioreactors and are frequently found in industrial applications (Nicoletta *et al.* 2000). Usually, cell immobilisation is achieved on inert support featuring large

specific surface area, where biofilms are formed (Friedman & Kolter 2004).

The aim of this work was to evaluate the efficiency of chromate reduction by *S. marcescens*, in Biofilm and free cells, under aerobic conditions and evaluated their biotechnological potential.

Biofilm formation was monitored by Scanning Electron Microscopy. Figure 1a to 1c, show biofilm development by *S. marcescens* cultured in the presence of granular activated carbon, where after 3 h incubation, small cells are randomly distributed on the surface of active carbon, with spaces non-colonized (Figure 1a). After 6h of incubation, bacteria were anchored to the carbon surface and to each other by means of fibrils, in order to bridge the space between bacteria. After 72 hours incubation, a mature biofilm was detected, with attached bacteria that excreted extracellular polysaccharides covering sessile cells and serving as a matrix for further biofilm formation.

In addition, Scanning Electron-microscopy revealed the presence of thin fibrillar pili at the cells surface (Figure 1a and 1b). These particular pili are similar to curli structures described by Olsen *et al.* (1989). The Curli (Pili or Microfibrillar) seem to be an envelope structure of major importance for surface colonization (Gualdi *et al.* 2008),

because provides stability to the biofilm, allowing strong hydrodynamic conditions support on prolonged culture or without interruption (Donlan & Costerton 2002).

As seen in Figure 1c, some cells were able to produce copious amounts of extracellular polysaccharides. The polymeric matrix observed appears similar to that previously described in other microorganisms (Kobayashi 2007; Lerner *et al.* 2009). These polysaccharides suffer different colloidal interactions, linking bacteria to the surface by a combination of weak chemical bonds, dipole interactions, and hydrophobic interactions (Hall-Stoodley *et al.* 2004). In

this respect, BATH tests showed that *S. marcescens* is highly hydrophobic (viz. hydrophobicity value around 67%) and, therefore, present a great affinity to solid surfaces.

Figure 2 shows the increase in the number of immobilised *S. marcescens* as the biofilm formation progresses. The results show counts of about 10^9 (CFU/g carbon) and 5×10^7 (bacteria/cm²), after 72 h of incubation. The rapid biofilm formation by *S. marcescens* (48-72 h) can be accounted by the multiplication of sessile cells, and the adherences of new planktonic cells to the carbon surface.

FIGURE 1. Biofilm formation of *S. marcescens* on activated carbon (a) 3h (b) 24 (c) 72 h. A= 7500x, P: Pilli; F: Microfibrils; EP: Exopolysaccharides.

FIGURA 1. Formación de biopelícula de *S. marcescens* sobre carbón activado. (a) 3h (b) 24 (c) 72 h. A= 7500x, P: Pilli; F: Microfibrils; EP: Exopolysaccharides.

FIGURE 2. Growth of *S. marcescens* on activated carbon. (•) Bacterial Growth, expressed as Log CFU/mL.

FIGURA 2. Crecimiento de *S. marcescens* sobre carbón activado. (•) Crecimiento bacteriano, expresado en Log UFC/mL.

The chromate tolerance study, demonstrated that *S. marcescens* could grow without any inhibition at a chromate concentration of 2 mM. It must be emphasized that resistance chromate is not a common property of *Serratia* spp. or others enterobacteriaceae.

The chromate bio-reduction analyses were performed using HPLC/HG/ASS. Chromate reduction by immobilised *S. marcescens* is illustrated in Figure 3a. These cells were able to reduce Cr(VI) under aerobic conditions, leading to about 95% Cr(VI) conversion to Cr(III) after 48 h culture. Mass balance shows that Cr(VI) was transformed to Cr(III) at a rate $1.67 \text{ mg L}^{-1} \text{ h}^{-1}$. As a comparison, *S. marcescens* (planktonic) were cultured in similar conditions yielding a 80% reduction in Cr(VI) to Cr(III), after 48 h and at cell concentration around 3×10^9 (CFU/mL) (Figure 3b), with a transformation rate of $1.40 \text{ mg L}^{-1} \text{ h}^{-1}$. No chromate reduction was observed in KSC media without bacteria.

Similar results were reported by Campos *et al.* (2005), with reduction percentages higher (86%), using non-immobilized *S. marcescens*. This difference is due to errors pre-treatment of the samples for chemical analyses. Also the ability to reduce chromate of this strain may be a secondary activity of a soluble reductase enzyme with a quite different physiological role. However, the immobilized cells were more efficient in transform chromate present in the medium.

It can be concluded that *S. marcescens* could be effectively used to remove chromate from highly contaminated soils and waters, given its ability to reduce Cr(VI) to Cr(III) and form a stable biofilm. Finally, it must be emphasised that since this bacteria was isolated from a natural environment, it should present resistance not only to chromium but also to other contaminants present in such conditions.

FIGURE 3. Chromium (VI) reduction by *S. marcescens*, with initial concentration $0.5 \text{ mmol L}^{-1} \text{ K}_2\text{CrO}_4$ at 30°C . A) Immobilized cells on activated carbon B) Planktonic cells.

FIGURA 3. Reducción de cromo (VI) por *S. marcescens*, con una concentración inicial de 0.5 mmol L^{-1} de K_2CrO_4 a 30°C . A) Células inmovilizadas en carbón activado B) Células planctónicas.

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