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Optimization of production of extracellular polymeric substances by *Arthrobacter viscosus* and their interaction with a 13X zeolite for the biosorption of Cr(VI)

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In this work we aimed to optimize the production of extracellular polymeric substances (EPS) by an *Arthrobacter viscosus* biofilm supported on 13X zeolite to be used in the biosorption of Cr(VI). The optimization parameters were agitation rate, work volume, pH and glucose concentration. Following the optimization of EPS production, the biofilm was used in the biosorption of hexavalent Cr from liquid solutions. Differences between the use of dead or active biomass and between the performance of zeolite in powder or in pellet form were also studied. The optimized EPS production allowed values of metal uptake between 2.72 mg/$g_{biosorbent}$ and 7.88 mg/$g_{biosorbent}$ for initial Cr(VI) concentrations of 20–60 mg/L. For an initial concentration of 20 mg/L, the optimal conditions of EPS production allowed an increase of 10% on the removal percentage of total Cr, and the use of zeolite as a powder rather than the pelleted form produced an increase of 46.5% in the removal percentage. For the initial concentration of 60 mg/L, the use of active biomass compared to dried biomass allowed a reduction of the time required for the total removal of Cr(VI) from 20 to 13 days.

Keywords: *Arthrobacter viscosus*; biofilm; biosorption; chromium (VI); 13X zeolite

1. Introduction

The pollution caused by heavy metals, in particular by chromium (VI), deserves special attention because their deposition in aquatic systems is very detrimental for animals and for humans. The search for new technologies for the treatment of chromium liquid solutions has led to the development of methods using sorbents of biological origin.

The advantages and disadvantages of biosorption are known, as is its potential relative to the traditional methods of treatment of heavy metal-contaminated effluents. In this context, many studies have been performed using different biosorbent materials such as yeasts [1], algae [2,3], agricultural waste [4–6], bacteria [7, 8] and biofilms [9–13]. It is generally accepted that a biosorption process involves several mechanisms, mainly ion exchange, chelation, adsorption and diffusion through cell walls and membranes; depending on the species used, the origin and the processing of the biomass and the solution chemistry of some will be more relevant than others.

Lameiras *et al.* [12] revealed that the major groups present on the *Arthrobacter viscosus* biomass are hydroxyl, carbonyl, carboxyl, amino and sulfonate groups. These groups were also found in studies undertaken with different biomasses, with good results on the biosorption of hazardous materials [1]. Vijayaraghavan and Yun [14] confirmed that the major binding groups in biosorption are the hydroxyl, carbonyl, carboxyl, sulfonate, amide, imidazole, phosphonate and phosphodiester groups, many of them present on the *A. viscosus* biomass.

A biofilm is originated when a mass of microbial cells immobilized in the heterogeneous mass of extracellular polymeric substances (EPS) becomes attached to a solid surface [15]. A major player in microbial aggregation or biofilm formation is the natural ability of cells to produce these biopolymers during their growth. According to Eboigbodin and Biggs [16], the term EPS refers to polysaccharides, proteins, nucleic acids and other biopolymers located outside the cell. EPS are complex heterogeneous substances, and their composition and location depend on several metabolic processes such as changes in growth phase, cell breakage due to cell death, active secretion, release of cell surface macromolecules (outer membrane proteins and lipopolysaccharides) and interaction with the environment. A number of studies have proved that biofilm EPS play an important role in the sorption of inorganic substances: Joshi and Juwarkar [17] studied the role of EPS from

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Azotobacter in the immobilization of heavy metals; Pal and Paul [15] described the EPS as central elements in heavy metal biosorption; Morillo-Pérez *et al.* [18] applied EPS produced by *Paenibacillus jamilae* to the biosorption of heavy metals; and Scott and Palmer [19], while studying the biosorption of cadmium, found that exopolysaccharides from *A. viscosus* have an accumulation capacity 2.3-fold greater than the equivalent weight of intact cells, and that they are 13.7-fold more effective than the cells of *Arthrobacter globiformis*, an organism that does not produce exopolysaccharides.

Several factors influence the bacterial production of EPS and consequently the biosorption process, and these include culture medium composition, solution pH, temperature, ionic strength, biosorbent dosage, biosorbent particle size, initial solute concentration, work volume and agitation rate.

Zeolites are aluminosilicates formed by tetrahedral units of SiO_4 and AlO_4, which are connected by shared oxygen atoms, allowing an almost infinite number of possible structures [20]. Zeolites are commonly used as ion exchangers, where a cation in the fluid phase replaces the balancing cation in the exchange sites. The behaviour of the 13X zeolite during the capture of heavy metals was described by Covarrubias *et al.* [21], who studied the retention of Cr(III), and by Chandra Rao *et al.* [22], who investigated the capture of cadmium and zinc by 13X zeolite as well as 4A zeolite and bentonite.

The hexavalent form of chromium is usually present in liquid solutions in the form of chromate (CrO_4^-) and dichromate ($Cr_2O_7^-$), both anions. Although it is generally thought that zeolites have little affinity for anionic metals, this behaviour may be changed by surface pretreatment or surface coverage by a specific biofilm capable of reducing the anionic hexavalent chromium to the cationic trivalent form [23]. Several bacterial species are capable of this reduction, and *Arthrobacter sp*. is one of them [13].

The aim of this work was the optimization of EPS production by an *A. viscosus* biofilm supported on 13X zeolite, in view of their application to the biosorption of Cr(VI). The optimized parameters were agitation rate, work volume, pH and glucose concentration. Following the optimization of EPS production, the biofilm was used in the biosorption of hexavalent Cr from liquid solutions. Differences between the performance of dead and active biomass and between zeolites in powder or in pellet form were also studied.

2. Materials and methods

2.1. *Materials*

The bacterium *A. viscosus* was obtained from the Spanish Type Culture Collection of the University of Valencia. The selection of this bacterium was based on previous studies developed by this group that proved its active behaviour on the biosorption of hazardous materials [9–13,24]. Aqueous chromium solutions were prepared by dissolving $K_2Cr_2O_7$ (p.a., Riedel) in distilled water. Glassware used for experimental purposes was washed with 60% nitric acid and subsequently rinsed with deionized water to remove any possible interference by other metals. The 13X zeolite (13X MS Bead 5.0–8.0 mm) was obtained from Xiamen Zhongzhao Imp. & Exp. Co., Ltd. It was dried at 200 °C for 48 h under a dry air stream prior to use. All the experimental work was performed in duplicate.

2.2. *Methods*

2.2.1. *Optimization of EPS production*

Parameters such as agitation rate, work volume, solution pH and glucose concentration of the culture medium were optimized to obtain the maximum EPS production. The sequence of experiments is presented in Table 1.

For the first assay, a medium with 10 g/L of glucose, 5 g/L of peptone, 3 g/L of malt extract and 3 g/L of yeast extract was used for microorganism growth [25]. The medium was placed in four different Erlenmeyer flasks (500 mL) and was sterilized at 121 °C for 20 min, cooled to room temperature, inoculated with bacteria (≈ 0.3 g/L) and kept in an incubator at 28 °C for 24 h with agitation rates of 125, 150, 175 and 200 rpm. The 24-h period of incubation was chosen according to previous studies [13]. For the second assay the same media were prepared but the work volume varied from 20% to 80% and the selected agitation rate was the best one obtained during the first assay. For the third assay, the pH varied (with agitation rate and work volume fixed accordingly to assays 1 and 2). For the final assay the glucose concentration varied and the other parameters were kept fixed according to previous assays (Table 1). All experiments were conducted in triplicate. The data presented are an average of the results of the different assays. The relative standard deviation and relative error of the experimental measurements were less than 2% and 5%, respectively.

2.2.2. *Determination of free and bound EPS*

In this work, free and bound EPS were estimated as the quantity of polysaccharides and proteins. Quantification of polysaccharides and proteins was made for all the assays, using colorimetric methods. The polysaccharides were determined by the difference between the total sugars and the reducing sugars present in the samples. For the determination of total sugars, the phenol-sulfuric acid method was used, measuring the

Table 1. Operational parameters evaluated aiming the optimization of the EPS production, optimal and poor conditions for EPS production and characteristics of the biosorption assays.

Optimization assays				
	Agitation rate	Work volume		Glucose concentration
Assay	rpm	%	pH	g/L
1	125, 150, 175, 200	40	7	10
2	175	20, 40, 60, 80	7	10
3	175	20	6, 7, 8, 9	10
4	175	20	7	5, 10, 15, 20
	Agitation rate	Work volume		Glucose concentration
Conditions	rpm	%	pH	g/L
Optimal	175	20	7	20
Poor	200	40	9	10

Biosorption assays			
Assay	Characteristics	Zeolite	Initial Cr (VI) concentration mg/L
A	Active biomass; optimal conditions	powder	20, 40, 60
B	Dry biomass; optimal conditions	powder	20, 40, 60
C	Autoclaved biomass; optimal conditions	powder	20, 40, 60
D	Active biomass; poor conditions	powder	20
E	Active biomass; optimal conditions	pellets	20

absorbance at 490 nm of the yellow-orange complexes [26]. The reducing sugars were determined by the dinitrosalicylic acid (DNS) method [27], by absorbance readings at 595 nm. The proteins present in the samples were determined by the Bradford method [28], by evaluation of the absorbance at 595 nm. In order to normalize the results obtained for free and bound EPS the determination of biomass concentration in each assay was essential, and was performed by spectrometry at 620 nm.

After the end of each assay, samples of the culture medium were collected to determine the free and bound EPS. The samples were centrifuged in order to separate the supernatant and the biomass. The free EPS were directly determined in the supernatant phase by the spectrophotometric methods described above. In order to evaluate the bound EPS, it was necessary to first solubilize the polysaccharide and polymeric net attached to the biomass with a solution of glutaraldehyde. These suspensions were placed in Erlenmeyer flasks and kept in an orbital incubator for 3 days, with moderate agitation, at room temperature. The suspension was then centrifuged and the supernatant was dialysed for the elimination of the glutaraldehyde molecules, salts and other small molecules. Finally, the solution obtained after the dialysis process was analysed for the determination of bound EPS, using the analytical methods described.

2.2.3. *Batch biosorption studies*

A medium with 20 g/L of glucose (or 10 g/L), 5 g/L of peptone, 3 g/L of malt extract and 3 g/L of yeast extract was used for the growth of the microorganism. The medium was sterilized at 121°C for 20 min, cooled to room temperature, inoculated with bacteria and kept at 28°C for 24 h with moderate stirring in a incubator. The cells were then harvested by centrifugation at 7000 rpm for 15 min and re-suspended in a smaller volume of residual culture medium to obtain a suspension with a biomass concentration of 5 g/L. Batch experiments were conducted in 250 mL Erlenmeyer flasks using 1 g of 13X zeolite, 15 mL of *A. viscosus* suspension and 150 mL of a potassium dichromate solution (20, 40 and 60 mg/L). These concentrations were selected based on typical values of Cr emission in wastewater. For comparison purposes the microorganism was cultivated at optimal and at poor conditions for EPS production, for an initial metal concentration of 20 mg/L.

The performances of powder/pellets zeolite and active/dead biomass were also compared. For the biosorption assays with powder zeolite, the beads were previously macerated using a mortar and pestle. Two types of dead biomass were used in this study: autoclaved and dried biomass. To obtain the autoclaved biomass, a certain volume of the culture medium was centrifuged after the incubation at the optimal conditions for EPS production. The biomass pellets then were

re-suspended in the residual culture medium and this suspension was autoclaved at 121°C for 20 min. The dried biomass was also obtained at the same optimal conditions, but in this case the cells were inactivated by drying at 105 °C for 3 days. The dried biomass was then powdered using a mill and re-suspended in the residual culture medium. The autoclaved and dead biomass were used in the biosorption assays at the same concentration used in the other experiments. The pH of the chromium solutions was adjusted at pH 4 in all the assays. The Erlenmeyer flasks were kept at 28 °C with moderate stirring until equilibrium was reached. The time required to reach thermodynamic equilibrium depended on the conditions of the assay. Samples of 1 mL were collected, centrifuged and analysed for chromium quantification. Hexavalent chromium was determined by measuring the absorbance at 540 nm of the purple complex of Cr(VI) with 1,5-diphenylcarbazide, in acidic solution [29]. For total Cr determination, the Cr(III) was first oxidized to Cr(VI) at approximately 100 °C, by the addition of an excess of potassium permanganate previous to the reaction with 1,5-diphenylcarbazide [29]. The Cr(III) concentration was calculated by the difference between total Cr and Cr(VI) concentration. The uptake and removal percentage were calculated as the mass of chromium removed per mass of biosorbent used and the ratio between the amount of metal removed (g/L) and its initial concentration, respectively.

2.2.4. *Scanning electron microscopy*

Samples of the supported biofilm were taken and analysed (after dehydration with different concentrations of ethanol) by scanning electron microscopy (SEM) (Leica Cambridge S360). Samples were gold coated prior to SEM observation. The images presented are intended to demonstrate that the biofilm uniformly covered the zeolite surface, and each is an example of many pictures taken of various zoomed areas.

3. Results and discussion

3.1. Optimization of EPS production

According to Eboigbodin and Biggs [16], EPS can be classified by their relative proximity to the cell surface. EPS tightly linked via a covalent or non-covalent association are known as capsular EPS (or cell-bound EPS), while EPS which are not directly attached to the cell surface are known as free EPS. in the present work both free and bound EPS were quantified, and the total EPS as well as the percentage of free and bound EPS are presented in Table 2.

3.1.1. *Effect of agitation rate and work volume*

According to López *et al.* [30] the agitation rate affects the EPS production because it interferes with the rheological complexity of the culture growth media and the rate of oxygen transfer.

In order to determined the influence of the agitation rate on EPS production, batch experiments were carried out over a range of stirrer speeds from 125–200 rpm (Table 2). The biomass concentration was also evaluated to allow the normalization of the results of EPS production obtained in each experiment (Table 2).

Table 2. Total EPS (g_{EPS}/L), free EPS (%, $g_{EPS}/g_{biomass}$) and bound EPS (%, $g_{EPS}/g_{biomass}$).

Conditions	$C_{biomass}$ g/L	Bound EPS $g_{EPS}/g_{biomass}$	Free EPS $g_{EPS}/g_{biomass}$	Total EPS g_{EPS}/L	Free EPS %	Bound EPS %	Ratio Free EPS/ Bound EPS –
125 rpm; 40%; pH 7; 10 g/L	2.6	0.21	0.47	1.77	69.4	30.6	2.27
150 rpm; 40%; pH 7; 10 g/L	2.9	0.21	0.40	1.76	65.4	34.6	1.89
175 rpm; 40%; pH 7; 10 g/L	3.1	0.23	0.45	2.12	65.8	34.2	1.92
200 rpm; 40%; pH 7; 10 g/L	3.5	0.07	0.41	1.68	84.8	15.2	5.57
175 rpm; 20%; pH 7; 10 g/L	4.3	0.71	0.23	4.06	24.3	75.7	0.32
175 rpm; 40%; pH 7; 10 g/L	3.2	0.82	0.09	2.91	10.2	89.8	0.11
175 rpm; 60%; pH 7; 10 g/L	3.1	0.71	0.05	2.36	6.6	93.4	0.07
175 rpm; 80%; pH 7; 10 g/L	2.9	0.91	0.09	2.92	9.4	90.6	0.10
175 rpm; 20%; pH 6; 10 g/L	4.5	0.52	0.20	3.24	27.9	72.1	0.39
175 rpm; 20%; pH 7; 10 g/L	4.4	0.732	0.21	4.16	22.5	77.5	0.29
175 rpm; 20%; pH 8; 10 g/L	4.8	0.67	0.15	3.97	18.4	81.6	0.23
175 rpm; 20%; pH 9; 10 g/L	4.2	0.52	0.08	2.51	13.8	86.2	0.16
175 rpm; 20%; pH 7; 5 g/L	3.7	0.85	0.24	4.06	22.2	77.8	0.29
175 rpm; 20%; pH 7; 10 g/L	4.6	0.644	0.20	3.88	23.6	76.4	0.31
175 rpm; 20%; pH 7; 15 g/L	4.6	0.67	0.21	4.01	23.6	76.4	0.31
175 rpm; 20%; pH 7; 20 g/L	5.6	0.73	0.17	**5.01**	18.7	81.3	0.23

Enhancements in batch cell growth were detected due to the increase in dissolved oxygen levels and to better nutrient distribution on the cell growth media. In terms of EPS production, the best results were obtained for an agitation rate of 175 rpm (2.12 g_{EPS}/L). The worst results in terms of EPS production were obtained for the agitation rate of 200 rpm (1.68 g_{EPS}/L), but they corresponded to the best results in terms of free EPS (84.8%) (Table 2). This evidence may be explained by the fact that at this speed an important amount of bound EPS was released from the bacterial surface. It is important to point out that for the other agitation rates tested, the ratio between the free EPS and the bound EPS was constant.

The effect of working volume on EPS production was also studied over a range of working volumes from 20–80% (Erlenmeyer flasks of 500 mL) (Table 2). The results for the biomass concentration are also present in Table 2. With an increase in working volume a decrease in the cell growth was observed. A possible explanation is that for the same agitation rate, an increase in the working volume negatively affects the dissolved oxygen levels and does not favour a suitable nutrient distribution in the cell growth media. For the same agitation rate, less volume allows better mass and oxygen transfer. In terms of total EPS production, the best results were obtained for a working volume of 20%. For this working volume, the total EPS obtained was 4.06 g_{EPS}/L, being 75.7% of bound EPS and 24.3% of free EPS (Table 2).

3.1.2. Effect of pH and glucose concentration

The effect of variations in pH values on the EPS production and biomass concentration was analysed and results are presented in Table 2. The biomass concentration produced a higher value for pH 8 (4.8 g/L), but the differences between the values obtained for other pH values were not significant (Table 2). Studies developed by Novak *et al.* [31] using *A. viscosus* showed higher EPS production for a pH value of 8 when xylose was used as carbon source. On the other hand, López *et al.* [30] obtained a maximum production of EPS at pH 7.5 when glucose was the carbon source and at pH 8 using xylose, in accordance with the results obtained by Novak *et al.* [31]. In the present report, pH 7 was found to produce an optimum EPS value of 4.16 g_{EPS}/L (Table 2), 22.5% being free EPS and 77.5% being bound EPS. The worst results were obtained for pH 9 for both EPS production and biomass concentration. The optimal pH recommended by the bacteria supplier (CECT) is 7, so pH 9 is probably detrimental to the bacteria and this explained the decrease in the biomass concentration and EPS production.

Glucose concentration was another of the parameters studied, and the results in terms of EPS production and biomass concentration are presented on Table 2. There was an increase in both biomass concentration and EPS production with increasing glucose concentration. Biomass concentration increased 51% when the glucose concentration increased from 5 g/L to 20 g/L. EPS production was enhanced to approximately 23% for the same increase on the glucose concentration. Pal and Paul [15] confirm that, for some biofilms, the presence of excess carbon and limitations of nitrogen, potassium or phosphate promoted the synthesis of EPS. The optimal value of EPS, 5.01 g_{EPS}/L, was obtained with an agitation rate of 175 rpm, 20% of work volume, pH 7 and 20 g/L of glucose concentration. For these conditions, 81.3% of bound EPS and 18.7 % of free EPS was obtained (Table 2).

It is important to explain the difference in the ratio of free EPS/bound EPS between assay 1 (when the agitation rate was the parameter to be optimized), and the following assays. This difference is a consequence of a slight change in the inoculum conditions. In assay 1 the bacteria were directly inoculated in the Erlenmeyer flasks from slant cultures. For the other assays the bacteria were transferred to the Erlenmeyer flasks (10% of the culture medium) during the exponential phase of their growth.

For the optimal growth conditions (175 rpm; 20%; pH 7; 20 g/L) a total EPS value of 5.01 g_{EPS}/L was obtained, which is higher than that obtained by López et al. [30]. These authors obtained a maximum value of polysaccharides excreted by *A. viscosus* of 2 g/L using glucose as carbon source (800 rpm, pH 7.5, 20% work volume, 25 g/L glucose) and a value of 3.35 g/L using xylose as carbon source (800 rpm, pH 8, 20% work volume 25 g/L xylose).

3.2. Biosorption studies

As is generally known, a sorption process is significantly affected by the initial pH. Yin *et al.* [32] confirm that in acid solutions, a large number of H^+ occupy active sites on cell surfaces and this leads to reduced adsorption capacity. Conversely, hydroxide precipitation of metal ions occurs easily in alkaline surroundings, and this will decrease their opportunity of contacting with biosorbents. Previous studies [33] about the effect of pH on Cr(VI) reduction and removal by *A. viscosus* fixed the optimal pH for the biosorption by these bacteria at pH 4. In all the experimental assays reported here the pH was adjusted to this value.

Studies developed by Park *et al.* [34] suggested that Cr(VI) can be reduced to Cr(III) by the biomass through two different mechanisms: in the first, Cr(VI) is directly reduced to Cr(III) in the aqueous phase by contact with

the electron-donor groups of the biomass, and the second consists of three steps: 1) binding of anionic Cr(VI) ion species to the positively charged groups present on the biomass surface; 2) reduction of Cr(VI) to Cr(III) by adjacent electron-donor groups; and 3) release of the Cr(III) ions into the aqueous phase due to electronic repulsion between the positively charged groups on the cell surface and the Cr(III) ions, or the complexation of the Cr(III) with adjacent groups capable of Cr binding.

The results of the biosorption assays indicated that the percentage of total Cr removal decreased as the initial concentration of Cr(VI) was increased. The uptake values were enhanced with the increase on the initial chromium concentrations (Table 3). This evidence has been thoroughly discussed in previous studies [24,35–37]. Total Cr removal ranged from 96.4–89.2% at initial Cr(VI) concentrations of 20–60 mg/L, for assay 1, from 95.8–86.2% for assay B, and from 100–80.2% for assay C. The maximum uptake obtained, 7.88 mg/$g_{biosorbent}$, was achieved with active bacteria and optimal conditions of EPS production, with an initial Cr concentration of 60 mg/L. The values of uptake and total Cr removal efficiency obtained at the optimal conditions of EPS production and initial Cr concentration of 20 mg/L were 2.72 mg/$g_{biosorbent}$ and 96.4%, respectively. In poor conditions, for EPS production and for the same initial ion concentration, an uptake of 2.46 mg/$g_{biosorbent}$ and a removal efficiency of 88% were obtained. These results show an increase in the uptake and removal percentage when the optimal conditions of EPS production were used, in comparison with the poor conditions. Therefore, the uptake of total Cr was enhanced by 10.6% and the removal efficiency increased 9.5%.

Assays A and E allow the comparison between the behaviour of powder and pelleted zeolite. The values of uptake and removal efficiency obtained for assay E were 1.77 mg/$g_{biosorbent}$ and 65.8%, respectively. Thus, the use of zeolite in pellets represented a decrease of 32% in the removal percentage of total Cr and a decrease of 35% in the uptake value. These results may be explained by the decrease of the surface area for the pelleted zeolite in comparison with the powder zeolite, which means there are fewer active sites available for the metal entrapment and an increase of diffusional and sterical limitations imposed by the pelleted form.

At the end of the experimental assays, samples of the solutions inoculated with active bacteria were seeded onto Petri plates with nutrient agar to assess and confirm the metabolic activity of the microorganism.

Figure 1 presents the residual Cr(VI) concentration in solution as a function of contact time. From the analysis of Figure 1a, it can be concluded that for the assay with active bacteria the biosorption process occurs in two stages. The first is very fast and reversible, and is mainly based on physical adsorption, ion exchange and chemisorption. The second is a much slower and irreversible metal binding process that may include covalent bonding, surface precipitation, redox reactions or crystallization on the cell surface. It is important to note that, despite the fact that the uptake values were similar for the three assays for each initial concentration (Table 3), the biosorption for assay A (active biomass, optimal conditions of EPS production) was faster: the total elimination of Cr(VI) took 13 days against 20 days for assay B (Figure 1a). For assay C, the total removal of Cr(VI) was not reached before 25 days. The time needed for a biosorption process to reach equilibrium is one of the most important parameters to be taken into account for an industrial implementation of such a system. Faster kinetics implies less energetic and operational requirements.

Figure 1b shows the variation in Cr(VI) and total Cr concentration for an initial concentration of 40 mg/L. It can be seen that the total elimination of Cr(VI) was achieved, and this results from the bacterial reduction of Cr(VI) to Cr(III). It is possible to observe an increase of total Cr after 2 days of experimentation, which means a release of chromium to the solution. This is probably related to the electronic repulsion between the positively charged groups of the cell wall and the cationic Cr(III) species resultant from the reduction of hexavalent chromium on the bacterium surface.

A comparison between the results obtained with the optimal conditions of EPS production for the biosorption of Cr(VI) and others found in the literature are presented in Table 4. The biosorption of Cr(VI) was well studied by several authors but, in general, the biomass tested in this study exhibited higher adsorption capacity. It is important to highlight that this optimization

Table 3. Uptake values (mg/g) and removal percentages (%), of total Cr, for all the biosorption assays.

	Cr $(VI)_{initial}$	Uptake	Removal
Assay	mg/L	mg/g	%
A	20	2.72	96.4
	40	5.38	92.3
	60	7.88	89.2
B	20	2.70	95.8
	40	5.42	92.9
	60	7.59	86.2
C	20	2.83	100
	40	5.19	89.4
	60	7.00	80.2
D	20	2.46	88.0
E	20	1.77	65.8

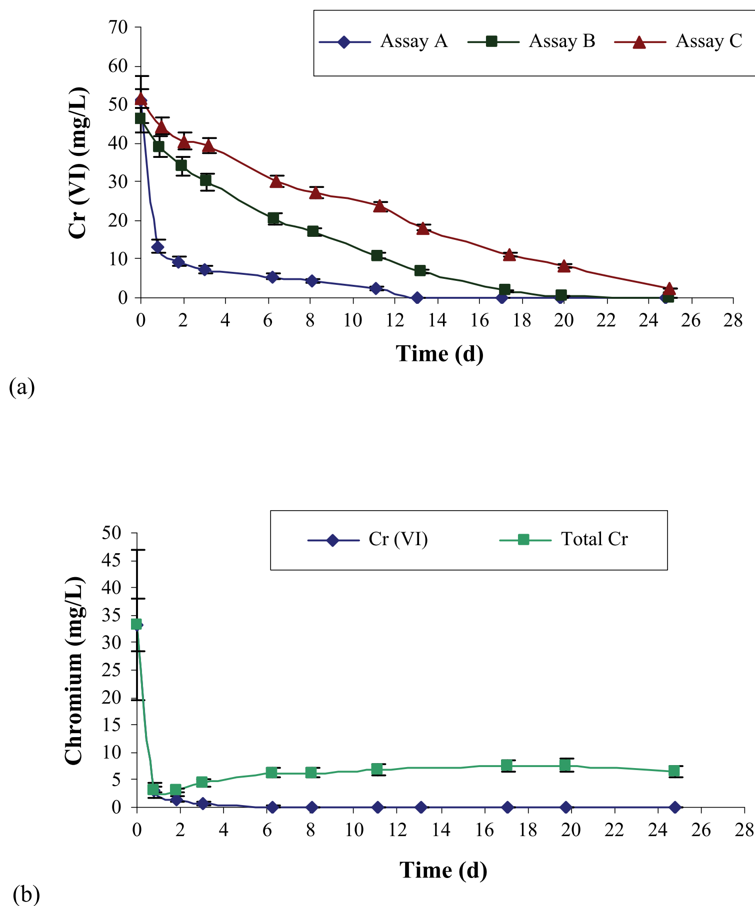

Figure 1. Residual chromium concentration in solution as a function of contact time. (a) Comparison between the removal of Cr(VI) for assays A, B and C, for an initial Cr concentration of 60 mg/L, (b) Cr(VI) and total Cr variation, for an initial Cr concentration of 40 mg/L.

Table 4. Uptake and removal percentages of Cr by different biosorbents.

Initial Cr (VI) Conc.		Uptake	Removal	
mg/L	Biosorbent	mg/g	%	Reference
200	*Saccharomyces cerevisiae*	6.60	–	[1]
20	EPS of *Nostoc punctiforme*	–	90.1	[38]
20	EPS of *Gloeocapsa calcarea*	–	90.0	[38]
70	*A. viscosus* + natural zeolite	0.57	18	[12]
70	*A. viscosus* + GAC	1.55	19	[12]
70	*A. viscosus* + natural zeolite +GAC	3.58	42	[12]
100	*A. viscosus* + NaY zeolite	3.00	–	[13]
75	*Bacillus coagulans* + GAC	38.87	46.9	[35]
75	*Escherichia coli* + GAC	–	36.6	[36]
75	*Streptococcus equisimilis* + GAC	–	72.0	[36]
30	*Escherichia coli* + kaolin	1.1	23.6	[37]
20	*Optimimazed EPS A. viscosus+13X*	2.72	96.4	This report
40	*Optimimazed EPS A. viscosus+13X*	5.38	92.3	This report
60	*Optimimazed EPS A. viscosus+13X*	7.88	89.2	This report

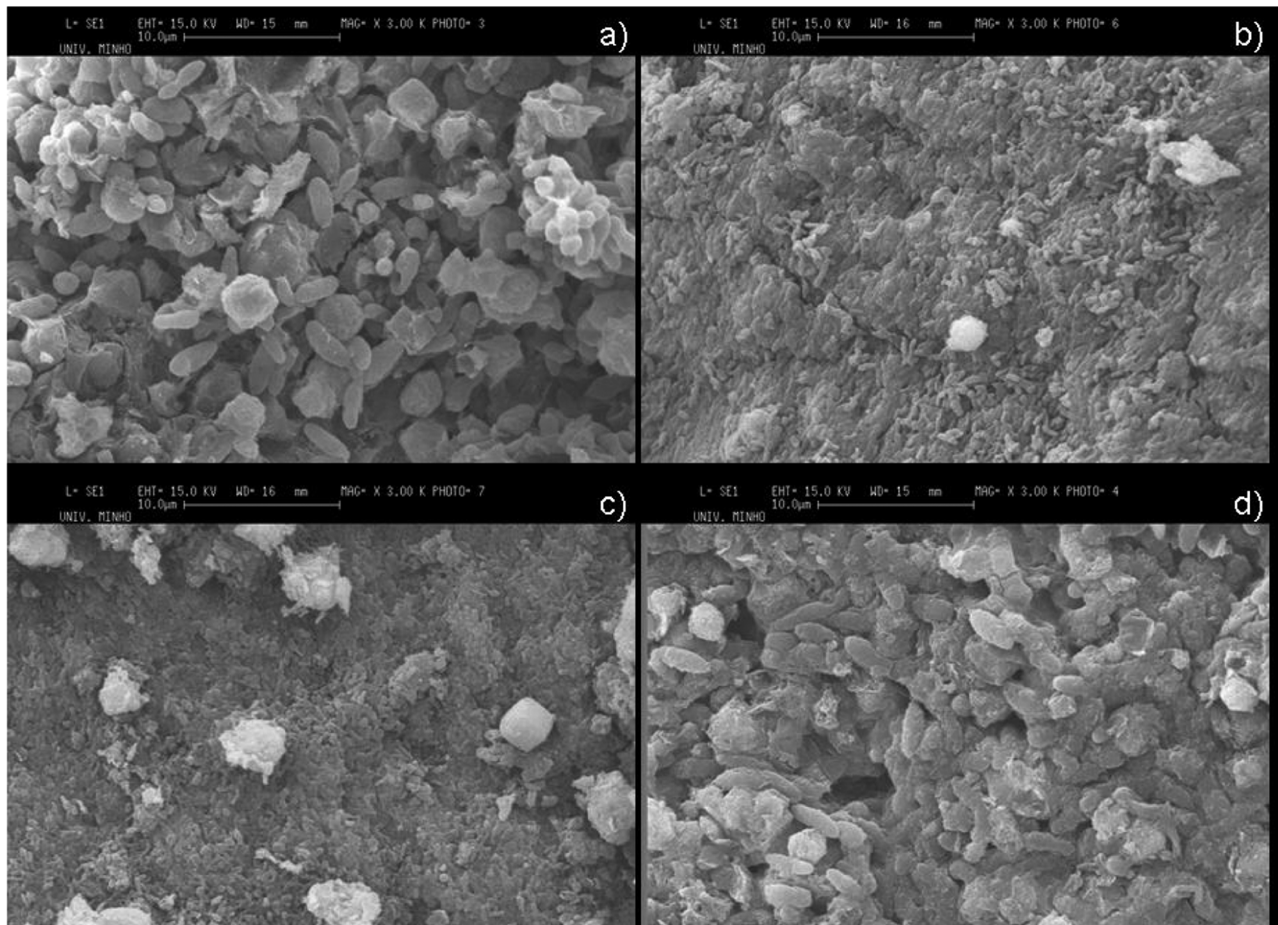

Figure 2. SEM images of several zoomed areas for the loaded Cr biomass. (a) assay A, (b) assay B, (c) assay C, (d) assay D.

procedure allowed better results than those obtained in previous studies with the same bacteria [12,13], proving the positive effect of this optimization on the biosorption process.

The scanning electron micrographs of EPS of the biofilm of *A. viscosus* supported on 13 X zeolite reveal their porous nature along with depressions and grooves on the surface, indicating availability of a large number of binding sites for fixation of chromium ions (Figure 2). These pictures show several white incrustations that, according to Sharma et al. [38], represent the binding of chromium ions on the surface of EPS, and are evidence of the biosorption of chromium by the biofilm.

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