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ORIGINAL PAPER

Modulation of tolerance to Cr(VI) and Cr(VI) reduction by sulfate ion in a *Candida* yeast strain isolated from tannery wastewater

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Abstract The main aim of this study was to investigate the influence of the sulfate ion on the tolerance to Cr(VI) and the Cr(VI) reduction in a yeast strain isolated from tannery wastewater and identified as *Candida* sp. FGSFEP by the D1/D2 domain sequence of the 26S rRNA gene. The *Candida* sp. FGSFEP strain was grown in culture media with sulfate concentrations ranging from 0 to 23.92 mM, in absence and presence of Cr(VI) [1.7 and 3.3 mM]. In absence of Cr(VI), the yeast specific growth rate was practically the same in every sulfate concentration tested, which suggests that sulfate had no stimulating or inhibiting effect on the yeast cell growth. In contrast, at the two initial Cr(VI) concentrations assayed, the specific growth rate of *Candida* sp. FGSFEP rose when sulfate concentration increased. Likewise, the greater efficiencies and volumetric rates of Cr(VI) reduction exhibited by *Candida* sp. FGSFEP were obtained at high sulfate concentrations. Yeast was capable of reducing 100% of 1.7 mM Cr(VI) and 84% of 3.3 mM Cr(VI), with rates of 0.98 and 0.44 mg Cr(VI)/L h, with 10 and 23.92 mM sulfate concentrations, respectively. These results indicate that sulfate plays an important role in the tolerance to Cr(VI) and Cr(VI) reduction in *Candida* sp. FGSFEP. These findings may have significant implications in the biological treatment of Cr(VI)-laden wastewaters.

Keywords *Candida* · Cr(VI) reduction · Hexavalent chromium [Cr(VI)] · Modulation · Sulfate

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Introduction

Chromium compounds are environmental pollutants occurring in soil, water and industrial effluents because they are widely used in electroplating, metal finishing, magnetic tapes, pigments, dyes, photographic film, automotive parts, leather tanning, wood protection, chemical manufacturing, electrical and electronic equipment, catalysis, among many other industrial activities [25, 32].

Chromium exists in nine oxidation states, from −2 to +6 [23]; however, the most stable states in the environment are the trivalent [Cr(III)] and hexavalent [Cr(VI)] forms [34]. The hexavalent form of chromium is highly soluble in water, mobile in the environment, toxic, mutagenic, carcinogenic [18, 54], and it is the most frequently used in industrial processes [55]. The trivalent form is less soluble and mobile [54], 100 times less toxic [10] and 1,000 times less mutagenic [31] than the hexavalent form. In addition, Cr(III) is an essential trace element required for glucose and lipid metabolism, as well as for amino acid utilization [59]. Considering its potential for hazardous toxicity and exposure, Cr(VI) has been designated as a priority pollutant in many countries [7, 30, 59].

Conventional methods of Cr(VI) removal from industrial wastewaters are chemical reduction to Cr(III) followed by precipitation under alkaline conditions

(mainly as chromium hydroxide), as well as ion exchange, reverse osmosis and adsorption [20, 37], which demand large amounts of chemicals or energy, and generate toxic sludge or other residues that are difficult to manage and treat [9, 37]. It is, therefore, important to develop more economic, safe and environmental friendly methods to remove Cr(VI) ions from industrial wastewaters. A potential method is microbial biotransformation (i.e., bioreduction) of the highly toxic, water-soluble and mobile Cr(VI), to the less toxic, insoluble and immobile Cr(III). This process has been considered as an economically feasible alternative for the treatment of wastewaters contaminated with Cr(VI) [6].

Many bacterial species capable of reducing Cr(VI) to Cr(III) under aerobic and/or anaerobic conditions have been reported [5, 6, 11–16, 21, 46, 47, 51, 53]; in contrast, there are only meager reports on fungi with this ability. Among the reported fungi are the yeasts of the genus *Candida* [24, 41, 52] and filamentous fungi of the genera *Aspergillus* [1, 17], *Penicillium* [1, 48], *Phanerochaete* [43], *Trichoderma* [38, 39], and *Hypocrea* [40].

Microbial reduction of Cr(VI) is affected by several environmental conditions such as: type of electron donor, type of final electron acceptor, Cr(VI) concentration, pH, temperature, dissolved oxygen, oxidation-reduction potential, as well as the presence of other metals and/or toxic organic compounds in the microorganism growth medium [28, 29, 35, 56, 59]. Therefore, any study regarding the influence of significant factors of the culture medium on Cr(VI) reduction by microbial cells is highly desirable, since Cr(VI) reduction processes can be improved, and times and costs of treatment of industrial effluents contaminated with Cr(VI) can be reduced.

In this work, the effect of the sulfate ion on the tolerance to Cr(VI) and on its reduction is reported in a *Candida* yeast strain isolated from leather tannery wastewater.

Material and methods

Microorganism

A yeast strain capable of reducing Cr(VI) was isolated from leather tannery wastewater by batch enrichment culture techniques. By morphological and biochemical techniques, it was determined that the yeast belongs to the genus *Candida*.

The amplification, sequencing, and neighbor-joining tree by distance analysis of the 26S rRNA D1/D2 domain of the isolated yeast revealed that it is related to Trichomonascaceae family, specifically to members of *Candida santjacobensis* and *Sugiyamaella* clades (Fig. 1). However, a relatively small nucleotide similitude of the sequence problem and of the members of both clades was observed (about 90% of the 584 nucleotides analyzed), which suggests that the D1/D2 domain partial sequence has not been

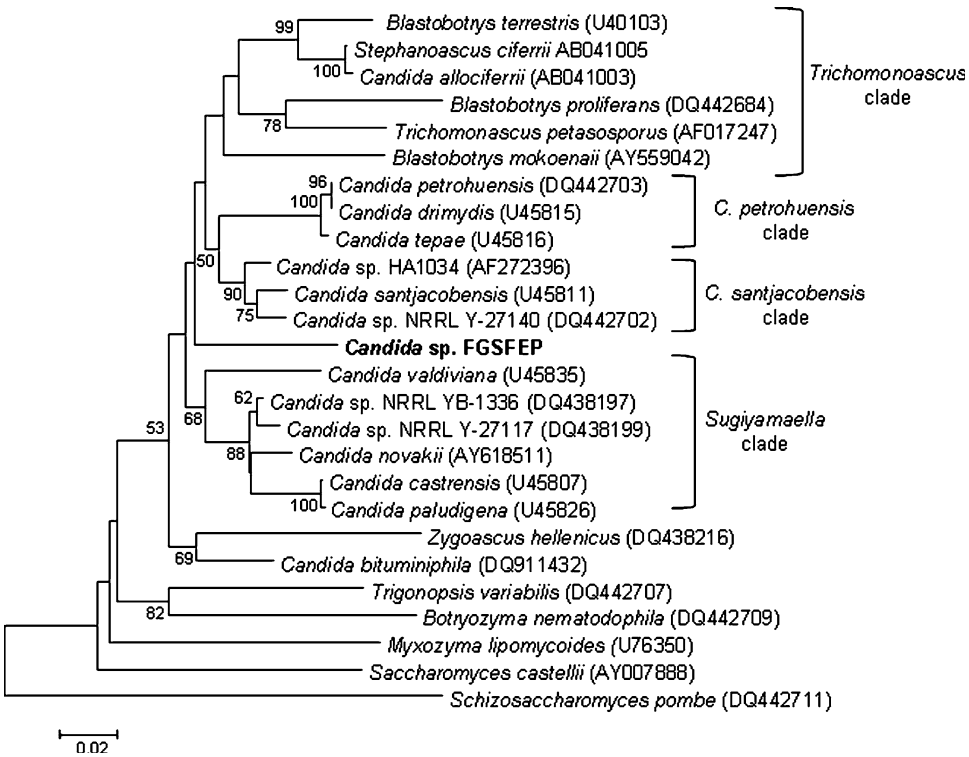

Fig. 1 Phylogenetic relationships between the 26S rRNA gene sequence of *Candida* sp. FGSFEP and those of members of the Trichomonascaceae yeast family. *Schizosaccharomyces pombe* was used as yeast outgroup. Bootstrap values >50% are indicated in the main nodes in a bootstrap analysis of 1,000 replicates. The *scale bar* represents the expected number of substitutions averaged all sites analyzed

submitted to the nucleotide databases as an independent sequence. Although it is quite evident from the results displayed in Fig. 1 that the isolated yeast strain belongs to the genus *Candida*, it is not possible to assign a species designation due to the small similarity between the known partial sequences of the 26S rRNA gene and that of the isolated yeast strain. It has been proposed that yeast ribosomal gene sequences (D1/D2 domain sequence of the 26S rRNA or 18S rRNA gene) with similarities higher than 99% belong to the same microbial species [27], and the similarity of the nucleotide sequence of the 26S rRNA D1/D2 domain of the isolated yeast strain was only of about 90%.

Furthermore, phylogenetic tree topology suggests that the yeast strain used in our work belongs to a new species; thus, it was designated as *Candida* sp. FGSFEP. The nucleotide sequence was submitted to GenBank (accession number: EU563853). Yeast was maintained on YPG agar slants (2% dextrose, 1% casein peptone, 1% yeast extract, and 2% agar), at 4 °C.

Development of the inoculum

The chemical composition of the basal culture medium used in this study for inoculum propagation was as follows: glucose 10 g/L; NH_4Cl 2.43 g/L; KH_2PO_4 1 g/L; $MgCl_2{\cdot}6H_2O$ 0.247 g/L; KCl 0.1 g/L; yeast extract 0.1 g/L; $CaCl_2$ 0.05 g/L, and $FeCl_3$ 1 mg/L. The initial culture medium pH was 6.0 ± 0.1. The *Candida* sp. FGSFEP inoculum was grown in 1,000 mL Erlenmeyer flasks containing 200 mL of culture medium, and incubated in a shaker at 54 cycles/min, at 28–30 °C, for 40–42 h. Then, cells were aseptically separated by centrifugation at 1,500*g* (4 °C) for 15 min, and washed twice with sterile distilled water to eliminate culture medium components and cell debris. The cell pellet was resuspended in a small volume of sterile distilled water by shaking in a vortex mixer for 30 s. The resulting cell suspension was used as inoculum for every experiment performed in this study.

Culture conditions

The effect of sulfate concentration on *Candida* sp. FGSFEP cell growth, glucose consumption and Cr(VI) removal was assessed in batch cultures. For these experiments, the NH_4Cl present in the basal culture medium was partially or completely replaced with $(NH_4)_2SO_4$ in order to obtain different initial sulfate concentrations, but maintaining the same initial nitrogen concentration (45.4 mM). The assayed sulfate concentrations were 0.0, 1.217, 2.5, 5, 10, 15, 20, and 23.92 mM. It should be mentioned that sulfate concentration in the basal culture medium was 2.3 mg/L (0.02 mM); this concentration was labeled "0 mM sulfate" in this work. The sulfate present in the basal culture medium may stem from the yeast extract and/or from impurities contained in the inorganic salts added to the culture medium.

Three series of 1,000 mL Erlenmeyer flasks were prepared containing 200 mL of culture medium with different initial sulfate concentrations. To the first series, no Cr(VI) was added in order to determine the effect of sulfate on yeast cell growth and glucose consumption in absence of Cr(VI) (control cultures). The flasks of the second and third series were added with a volume of a sterile stock solution of K_2CrO_4 (20 g/L) to obtain initial Cr(VI) concentrations of 1.7 and 3.3 mM, respectively. The flasks were inoculated with a volume of the *Candida* cell suspension to obtain an initial biomass concentration of the yeast cultures of 1 mg (dry weight)/mL. Flasks were incubated with constant shaking at 54 cycles/min, at 28–30 °C. Samples were collected at different incubation times, and biomass, residual glucose, Cr(VI) and total chromium concentrations were determined. The maximum specific growth rate (μ_{max}) of every yeast culture was estimated as follows:

$$\mu_{max} = [(\ln X_2 - \ln X_1)/(t_2 - t_1)] \quad (1)$$

where X_1 and X_2 are biomass concentrations at time 1 (t_1) and time 2 (t_2) in the exponential growth phase, respectively.

To determine whether the observed Cr(VI) removal was biological or abiotic, experiments without biomass (cell-free controls) and with heat-killed biomass (heat-killed cell controls) were conducted with all sulfate concentrations (0, 1.217, 2.5, 5, 10, 15, 20, and 23.92 mM) and Cr(VI) concentrations (1.7 and 3.3 mM) assayed in this study, as well as with different pH values (2.5, 3, 4, 5, and 6). Heat-killed cell controls were autoclaved twice at 121 °C for 20 min. Cell-free and heat-killed cell controls were maintained under the same incubation conditions as the cultures with viable *Candida* sp. FGSFEP cells. Periodically, culture samples were collected from every cell-free and heat-killed cell control, and Cr(VI) and total chromium concentrations were determined.

Hexavalent chromium removal performance of the yeast strain was evaluated according to two criteria: removal efficiency (E, %) and volumetric rate (R_v, mg Cr(VI)/L h) of Cr(VI) removal, which were calculated as follows [39, 40]:

$$E(\%) = [(Cr_o - Cr_f)/Cr_o] \times 100 \quad (2)$$

$$R_v = [(Cr_o - Cr_f)/(t_f - t_o)] \quad (3)$$

where Cr_o is initial Cr(VI) concentration at time $t_o = 0$ h, Cr_f is residual Cr(VI) concentration at time t_f, and t_f is the

cultivation time at which Cr(VI) was completely removed or the total incubation time for those experiments in which Cr(VI) was not completely removed.

All experiments in this study were performed in triplicate and average values are reported herein. The maximum variation coefficient of the three replicates was 4.2%.

Analytical techniques

Biomass concentration

Biomass concentration was determined by measuring dry cell weight. Culture samples were filtered through preweighed 1.6 μm filters (Whatman GF/A), which were washed twice with sterile distilled water and subsequently dried at 90 °C until constant weight was attained. The obtained filtrates were used to determine residual glucose, Cr(VI) and total chromium concentrations.

Glucose concentration

Glucose concentration of culture samples was enzymatically determined (glucose oxidase and peroxidase) using a Sigma glucose assay kit.

Cr(VI) and total chromium concentration in aqueous solution

Cr(VI) concentration was determined using the 1,5-diphenylcarbohydrazide method according to the procedures described in the Hach Water Analysis Handbook [19]. Total chromium concentrations in solution were measured by atomic absorption spectroscopy (SpectrAA220 FS, Varian, Inc.), following the procedures of method 3111B of the Standard Methods for the Examination of Water and Wastewater [8].

Total chromium contents in yeast biomass

To determine total chromium uptake by the yeast biomass, aliquots of the washed cells were pretreated according to the procedures described in method 3030E of the Standard Methods for the Examination of Water and Wastewater [8]. Briefly, aliquots of the washed cells were mineralized by burning in concentrated nitric acid, then cooled and made up with deionized water to a final volume of 50 mL. The resulting solution was analyzed for total chromium by electrothermal atomic absorption spectroscopy (SpectrAA220 FS, Varian, Inc.) with a graphite furnace and a chromium hollow cathode lamp, according to the procedures described in method 3113B of the Standard Methods for the Examination of Water and Wastewater [8].

Results and discussion

Influence of sulfate on tolerance of *Candida* sp. FGSFEP to Cr(VI)

The effect of eight different sulfate concentrations (0, 1.217, 2.5, 5, 10, 15, 20, and 23.92 mM) on *Candida* sp. FGSFEP cell growth was investigated in batch cultures without Cr(VI) and with initial Cr(VI) concentrations of 1.7 and 3.3 mM.

Figure 2 depicts the *Candida* sp. FGSFEP cell growth curves obtained at different sulfate and Cr(VI) concentrations. In this figure, a horizontal straight line was drawn at a biomass concentration of 1 g/L in order to show if the yeast was capable or not of growing at the assayed concentration of sulfate and/or of Cr(VI). Points lying on the straight line indicate that there was not a net yeast growth; points above the line show that the yeast was able to grow, and points below the line indicate that cell lysis did occur.

In culture media without Cr(VI), yeast cell growth was very similar at all assayed sulfate concentrations (Fig. 2a), which suggests that the sulfate ion had no stimulating or inhibiting effect on *Candida* sp. FGSFEP cell growth. In addition, the amount of sulfate (0.02 mM) present in the basal culture medium proved to be enough to allow adequate growth of the yeast.

In culture media with 1.7 mM Cr(VI), *Candida* sp. FGSFEP cell density decreased as culture time increased when a sulfate concentration of 0 mM was used, which indicated the presence of cell lysis (Fig. 2b). With 1.217 mM sulfate concentration, biomass concentration was almost constant along the incubation period indicating that, under these conditions, yeast net growth was practically negligible. In contrast, starting at a sulfate concentration of 2.5 mM, *Candida* sp. FGSFEP cell concentration gradually increased as sulfate concentration increased; the maximum cell density values were obtained within the sulfate concentration interval of 10–23.92 mM (Fig. 2b). Biomass concentration profiles obtained within this sulfate concentration interval were similar to those in cultures without Cr(VI) (Fig. 2a), which suggests that toxic effects of Cr(VI) on yeast cell growth are practically null at these sulfate concentrations.

Initial 3.3 mM concentration of Cr(VI) produced lysis of *Candida* sp. FGSFEP cells when sulfate concentrations in the culture medium were lower than 10 mM (Fig. 2c); in contrast, as sulfate concentration increased from 10 to 23.92 mM, cell concentration also increased. Within this sulfate concentration interval, cell lysis was observed at incubation times longer than 150 h. Cell density in culture media with 3.3 mM Cr(VI) was lower than in cultures without Cr(VI) (Fig. 2a) or with 1.7 mM Cr(VI) (Fig. 2b)

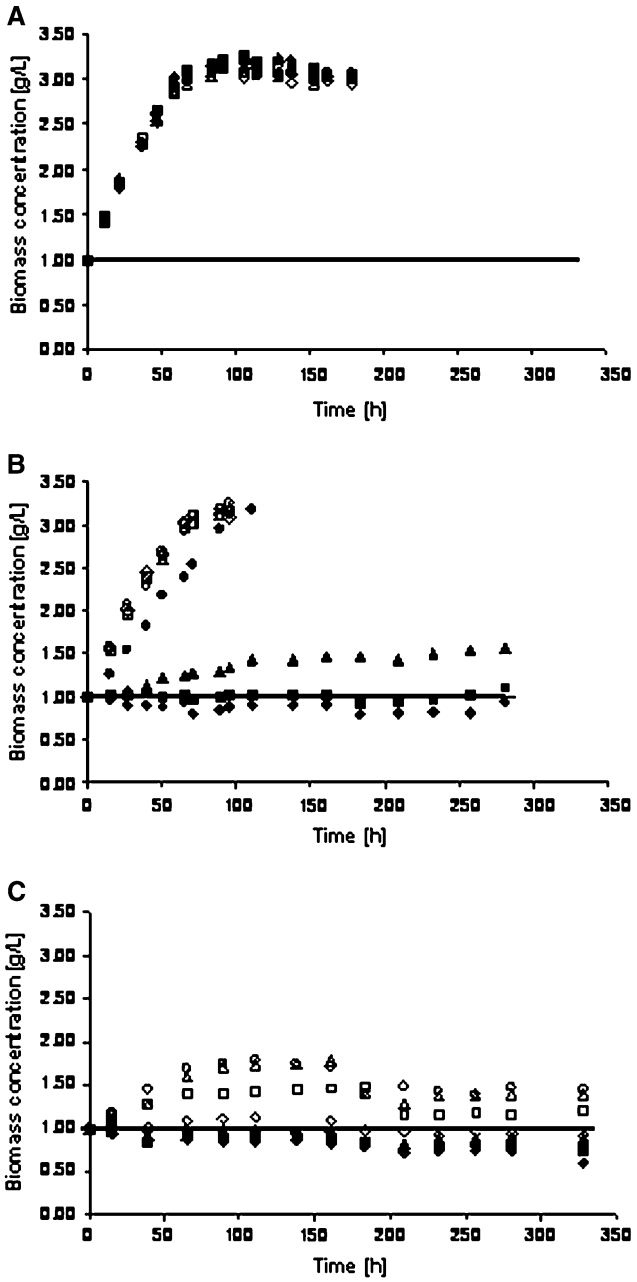

Fig. 2 Changes in biomass concentration during batch cultures of *Candida* sp. FGSFEP at different initial sulfate and Cr(VI) concentrations [**a** without Cr(VI); **b** initial Cr(VI) concentration = 1.7 mM; **c** initial Cr(VI) concentration = 3.3 mM; sulfate concentration: (*filled diamond*) 0 mM; (*filled square*) 1.217 mM; (*filled triangle*) 2.5 mM; (*filled circle*) 5 mM; (*open diamond*) 10 mM; (*open square*) 15 mM; (*open triangle*) 20 mM; (*open circle*) 23.92 mM]

at all assayed sulfate concentrations, which may be the result of more toxic culture media.

Figure 3 shows variations of *Candida* sp. FGSFEP specific growth rate (μ) with respect to sulfate concentration and Cr(VI) concentrations (0, 1.7 and 3.3 mM). When culture media contained no Cr(VI), specific growth rate was practically constant along the assayed sulfate concentration

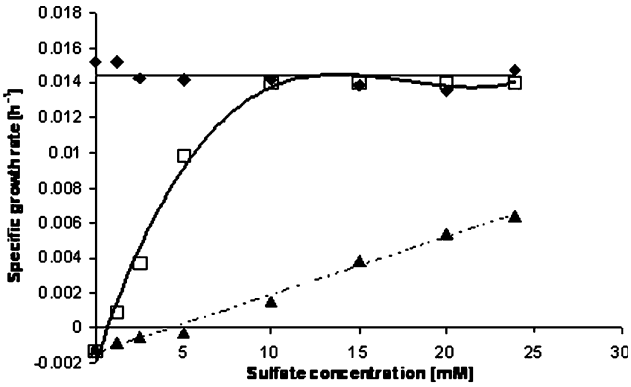

Fig. 3 Dependence of yeast specific growth rate on sulfate concentration [*filled diamond* without Cr(VI); *open square* initial Cr(VI) concentration = 1.7 mM; *filled triangle* initial Cr(VI) concentration = 3.3 mM]

interval, with a mean value of 0.0144/h. Thus, in absence of Cr(VI), the sulfate ion had no effect on yeast growth rate.

In contrast, the *Candida* sp. FGSFEP specific growth rate was dependent on sulfate concentration in presence of Cr(VI). When Cr(VI) was added to an initial concentration of 1.7 mM, the specific rate of cell lysis (k_d) was 0.0013/h ($\mu = -0.0013$/h) at 0 mM sulfate concentration. The specific growth rate of the yeast gradually increased as sulfate concentration increased, to an almost constant maximum value of approximately 0.0142/h, observed from an initial 10 mM sulfate concentration. This specific growth rate was similar to that obtained in control cultures [without Cr(VI)].

With an initial concentration of 3.3 mM Cr(VI), cell lysis specific rates (k_d) went from 0.0013 to 0.0003/h (μ values from -0.0013 to -0.0003/h) in the sulfate concentration interval from 0 to 5 mM; at higher sulfate concentrations, the specific growth rate (μ) was positive (above 0). A linear relationship was observed between specific rate and sulfate concentration ($r^2 = 0.99$) along the assayed sulfate concentration interval, in which μ values increased from -0.0013 to $+0.0064$/h as the sulfate concentration increased from 0 to 23.92 mM. The steady increase in the specific growth rate at 3.3 mM Cr(VI) suggests that the yeast tolerance to Cr(VI) increased proportionally as the sulfate concentration increased; a similar trend was observed at 1.7 mM Cr(VI) in the sulfate concentration range of 0–5 mM. It can be observed in Fig. 3 that the specific growth rates at 3.3 mM Cr(VI) were significantly lower than those obtained in cultures without Cr(VI) or with an initial concentration of 1.7 mM Cr(VI).

Present results suggest that sulfate plays an important role in *Candida* sp. FGSFEP tolerance (resistance) to the toxic effects of Cr(VI). The higher the sulfate concentration, the greater the tolerance of this yeast to the inhibitory

effects of Cr(VI), and, thus, the less effect on cell growth. Likewise, results reveal that the "protective effect" of sulfate on cell growth of the yeast kept in Cr(VI)-containing media depended on initial Cr(VI) concentration.

Ohtake et al. [42] reported increased cell growth in two *Pseudomonas fluorescens* strains (LB300 and LB303) in the presence of CrO_4^{2-} and high sulfate concentrations. Also, the *P. fluorescens* tolerance level to CrO_4^{2-} depended on the sulfur source, since bacterial cells growing in culture medium with added cysteine were more resistant to CrO_4^{2-} than cells growing on sulfate. Since chromate is carried into the cells by the sulfate transport system, the ability of sulfate to protect *P. fluorescens* LB300 cells from the inhibitory effects of Cr(VI) was attributed to the fact that sulfate competitively inhibits chromate uptake by bacterial cells [42].

Likewise, a study carried out by Pepi and Baldi [49] with a *Candida* sp. strain, using varying sulfate (4–160 mM) and Cr(VI) (0.04–0.2 mM) concentrations, revealed that sulfate ion increases yeast tolerance to Cr(VI). Maximum growth of this *Candida* sp. strain was observed in Yeast Nitrogen Base medium without amino acids, at initial Cr(VI) concentrations of 0.04 and 0.08 mM, and 50 mM sulfate. It was also found that if *Candida* sp. is grown in the presence of S-amino acids, especially methionine, it is more resistant to Cr(VI) than if the sulfur source is sulfate [49]. The general mechanism of resistance to chromate in *Candida* sp. was also attributed to a reduced chromium uptake [49]. In contrast, resistance of *Ochrobactrum tritici* strain 5bvI1 to Cr(VI) was not dependent on sulfate concentration [4].

Effect of sulfate on glucose consumption in *Candida* sp. FGSFEP

Figure 4 shows the variation profiles of residual glucose concentration in relation with incubation time. Glucose was completely consumed (overall efficiency of consumption = 100%) by *Candida* sp. FGSFEP cells in approximately 83 h of incubation when the yeast was grown in culture media without Cr(VI) (Fig. 4a). No significant difference was observed in residual glucose levels at the different sulfate concentrations assayed. These results show that, in the absence of Cr(VI), glucose utilization was not affected by sulfate. In contrast, in the presence of Cr(VI), glucose consumption was affected by sulfate in a concentration-dependent manner (Fig. 4b, c).

With an initial Cr(VI) concentration of 1.7 mM, yeast consumed between 30 and 35% of the initially added glucose with sulfate concentrations of 0 and 1.217 mM (Fig. 4b), in spite of the fact that cell lysis did occur at 0 mM SO_4^{2-} and yeast cell growth was negligible at 1.217 mM SO_4^{2-} (Fig. 2b). The yeast consumed all of the glucose in culture media at sulfate concentrations higher than 1.217 mM (overall efficiency of monosaccharide consumption = 100%); however, the time required for this decreased as sulfate concentration increased. The *Candida* sp. FGSFEP glucose consumption profiles were similar with sulfate concentrations ranging from 10 to 23.92 mM (Fig. 4b), and these profiles were similar to those obtained in cultures without Cr(VI) (Fig. 4a). This could be the result of similar cell density in the cultures (Fig. 2a, b).

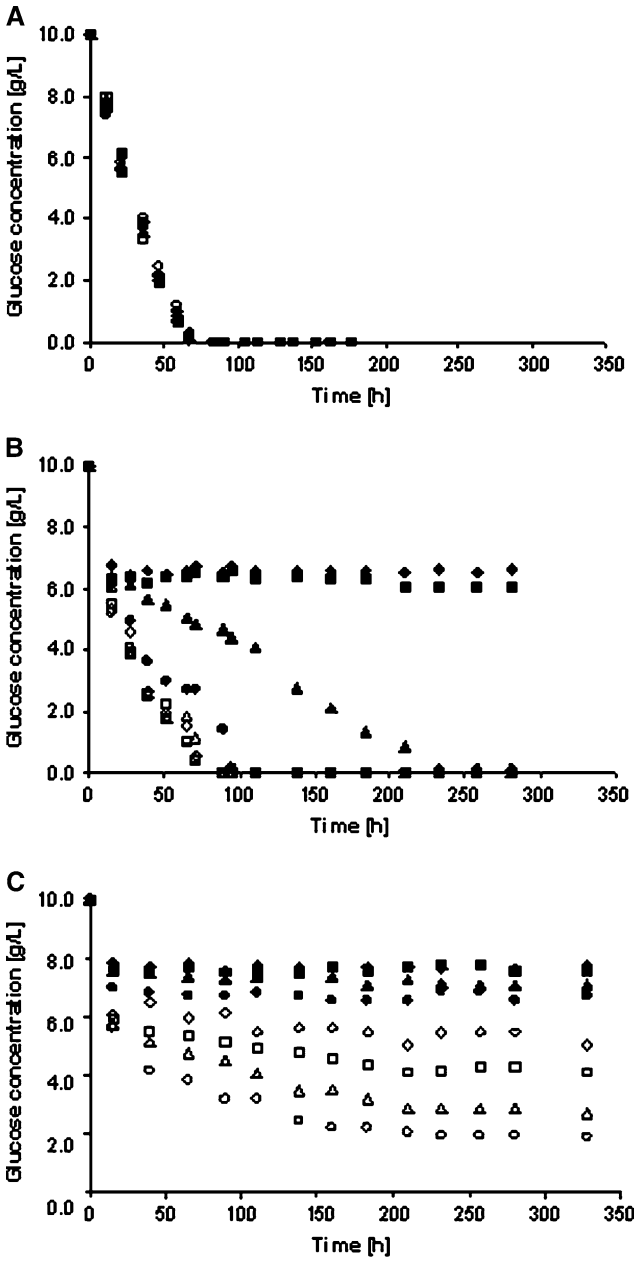

Fig. 4 Glucose consumption curves for batch cultures of *Candida* sp. FGSFEP at different sulfate and Cr(VI) concentrations [**a** without Cr(VI); **b** initial Cr(VI) concentration = 1.7 mM; **c** initial Cr(VI) concentration = 3.3 mM; Sulfate concentration: (*filled diamond*) 0 mM; (*filled square*) 1.217 mM; (*filled triangle*) 2.5 mM; (*filled circle*) 5 mM; (*open diamond*) 10 mM; (*open square*) 15 mM; (*open triangle*) 20 mM; (*open circle*) 23.92 mM]

At initial concentration of 3.3 mM Cr(VI), residual glucose levels decreased as sulfate concentration increased (Fig. 4c); however, residual glucose levels were higher than those obtained with 1.7 mM Cr(VI) (Fig. 4b), at all assayed sulfate concentrations. The highest glucose consumption efficiency was about 80% and was obtained at sulfate concentration of 23.92 mM. With the two assayed Cr(VI) concentrations (1.7 and 3.3 mM), some glucose was consumed during the first hours of incubation by yeast cultures which also showed cell lysis [0 mM SO_4^{2-}, 1.7 mM Cr(VI); and 0–5 mM SO_4^{2-}, 3.3 mM Cr(VI)]. The glucose was probably used to maintain the living cells.

As expected, *Candida* sp. FGSFEP cultures with the highest cell density at 1.7 and 3.3 mM Cr(VI) consumed more glucose, and this happened when high concentrations of sulfate were used. Taken together, the results obtained in the presence of Cr(VI) indicate that glucose utilization was dependent on the sulfate concentration, which in turn affected the intensity of *Candida* sp. FGSFEP growth.

Influence of sulfate on Cr(VI) removal by *Candida* sp. FGSFEP

Figure 5 shows the variation profiles of residual Cr(VI) concentration with respect to incubation time at different sulfate concentrations, and at the two initial Cr(VI) concentrations assayed. Although residual Cr(VI) concentrations decreased as incubation progressed, the level of Cr(VI) removal by *Candida* sp. FGSFEP cultures depended on the sulfate and Cr(VI) concentrations (Fig. 5a, b).

At the two initial Cr(VI) concentrations, yeast cultures showing cell lysis [0 mM SO_4^{2-}, 1.7 mM Cr(VI); and 0–5 mM SO_4^{2-}, 3.3 mM Cr(VI)] were able to remove a small quantity of Cr(VI). These results are in agreement with the report of Wang and Shen [57], who found that cell density of *Escherichia coli* and *Bacillus* sp. cultures decreased continuously during Cr(VI) removal.

The present study reveals that with an initial Cr(VI) concentration of 1.7 mM, residual Cr(VI) concentrations decreased as sulfate concentration increased from 0 to 10 mM (Fig. 5a). From this last sulfate concentration (10 mM) on, the variation profiles of residual Cr(VI) concentration remained similar, probably because the cell density in these cultures also continued to be practically the same (Fig. 2b). Additionally, no residual Cr(VI) was detected with sulfate concentrations within the interval from 10 to 23.92 mM at incubation times longer than 89 h. In contrast, at 3.3 mM initial Cr(VI) concentration, the yeast was unable to completely remove the Cr(VI) initially present in the culture media, at any of the assayed sulfate concentrations (Fig. 5b); however, residual Cr(VI) levels decreased as sulfate concentration increased.

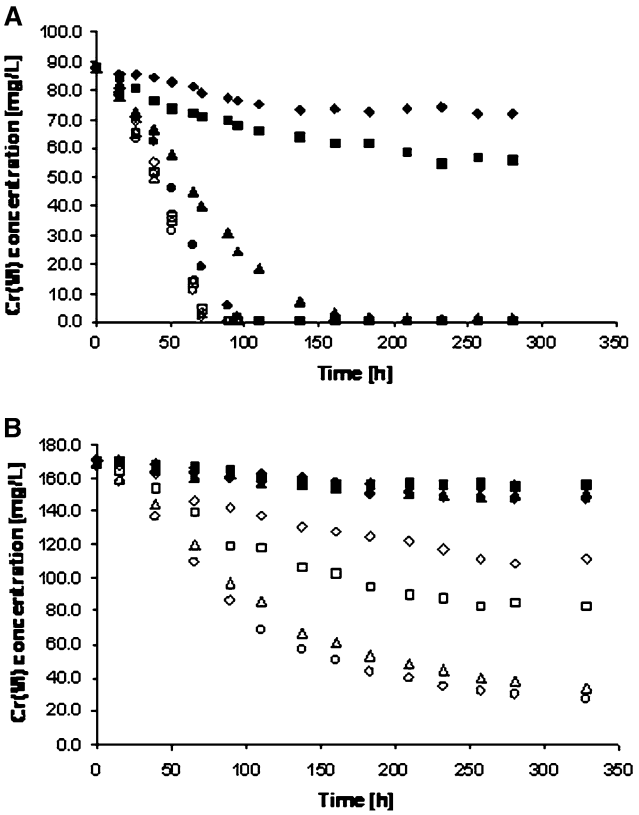

Fig. 5 Cr(VI) variation profiles in batch cultures of *Candida* sp. FGSFEP at different sulfate and Cr(VI) concentrations [**a** initial Cr(VI) concentration = 1.7 mM; **b** initial Cr(VI) concentration = 3.3 mM; Sulfate concentration: (*filled diamond*) 0 mM; (*filled square*) 1.217 mM; (*filled triangle*) 2.5 mM; (*filled circle*) 5 mM; (*open diamond*) 10 mM; (*open square*) 15 mM; (*open triangle*) 20 mM; (*open circle*) 23.92 mM]

Though residual Cr(VI) concentrations in yeast cultures decreased as incubation progressed, no significant changes in total chromium concentration in solution were detected throughout the incubation time of *Candida* sp. FGSFEP yeast cells in culture media containing different initial Cr(VI) and sulfate concentrations. In fact, it was found that about 97% of the chromium initially added to the culture media was always present in solution. Likewise, at all the sulfate and Cr(VI) concentrations assayed in this study, the total chromium accumulated by *Candida* sp. FGSFEP cells was only about 3% of the Cr(VI) which they were able to remove. Moreover, the mass balance of chromium in batch culture experiments with *Candida* sp. FGSFEP fully confirmed the above results.

In this study, the pH of culture media decreased as incubation increased, as a result of the metabolic activity of growing cells. The lowest pH was 2.5 and corresponded to culture media with no Cr(VI) content, as well as to media with initial Cr(VI) concentration of 1.7 mM and sulfate concentrations ranging from 5 to 23.92 mM. At initial

3.3 mM Cr(VI) concentration, the lowest pH was 3.6 in culture media with sulfate concentrations of 20 and 23.92 mM. As expected, the lowest pH values were observed in yeast cultures with the highest cell concentrations and highest glucose consumption.

As it is known that low pH's favor Cr(VI) reduction by organic matter [54], experiments with two different abiotic controls (cell-free and heat-killed cell controls) were carried out in this work at different pH values (2.5, 3, 4, 5, and 6 ± 0.1) and initial Cr(VI) (1.7 and 3.3 mM) and sulfate concentrations (0, 1.217, 2.5, 5, 10, 15, 20 and 23.92 mM), in order to determine if the pH influences chromium removal in the absence of living biomass. It was found that the Cr(VI) and total chromium concentrations remained constant along 340 h, in both the cell-free and heat-killed cell controls at pH values ranging from 3.0 to 6.0. At pH 2.5, no measurable change in total chromium concentration was observed in the cell-free and heat-killed cell controls; in contrast, the Cr(VI) concentration decreased by 2.7–3% and 8–10% in the cell-free and heat-killed cell controls, respectively. The loss of Cr(VI) content in the cell-free and heat-killed cell controls at pH 2.5 are attributable to Cr(VI) reduction by medium components and by medium components and dead biomass, respectively. The results obtained in the heat-killed cell controls at pH 2.5 are in agreement with those reported by Park et al. [44, 45], who showed that dead biomass of *Rhizopus oryzae*, *Aspergillus niger*, *Penicillium chrysogenum, Saccharomyces cerevisiae* [44] and *Ecklonia* sp. [45] is capable of reducing Cr(VI) when the biomass is brought into contact with a chromate solution at pH 2.0.

Taken together, the above observations indicate that the primary mechanism of Cr(VI) removal by viable cells of *Candida* sp. FGSFEP was the transformation (reduction) of Cr(VI) to forms of lower valence. As the stable forms of chromium are the trivalent and the hexavalent [34], it seems most likely that the yeast was capable of transforming the highly toxic and soluble hexavalent chromium to the much less toxic and less mobile trivalent form. Furthermore, the incubation of viable *Candida* sp. FGSFEP cells in culture media without carbon and energy source (media without glucose and yeast extract, but with sulfate and chromate at the different concentrations assayed in this study) produced no changes in Cr(VI) and total chromium concentrations, which indicates that a carbon source is required in the yeast growth medium in order to provide the necessary reducing power for Cr(VI) reduction and, therefore, that the observed Cr(VI) reduction was mainly due to the metabolic activity of *Candida* sp. FGSFEP cells.

At initial 1.7 mM Cr(VI) concentration, overall efficiency of Cr(VI) reduction exhibited by *Candida* sp. FGSFEP increased from 17.9% to 98.6% as sulfate concentration increased from 0 to 2.5 mM, and at higher sulfate concentrations, Cr(VI) reduction efficiency was 100%. Likewise, at 3.3 mM Cr(VI), reduction efficiency increased from 8.3 up to 84% as sulfate concentration increased from 0 to 23.92 mM.

These results clearly show that *Candida* sp. FGSFEP has a remarkable ability to reduce very high Cr(VI) concentrations in the presence of sulfate. The Cr(VI) concentrations that were reduced by *Candida* sp. FGSFEP were much higher than concentrations commonly found to be reduced by bacteria [2, 5, 6, 16, 28, 36, 60], yeasts [41, 52] and filamentous fungi [1, 38] in the presence of sulfate.

Overall volumetric rate of Cr(VI) reduction, which is a measure of Cr(VI) reduction per unit volume of culture medium per hour, was also affected by initial sulfate and Cr(VI) concentrations (Fig. 6). At initial 1.7 mM Cr(VI) concentration, the highest volumetric rate of reduction was 0.98 mg Cr(VI)/L h, and it was obtained within the sulfate concentration interval ranging from 10 to 23.92 mM. In contrast, the highest Cr(VI) reduction rate at 3.3 mM Cr(VI) was 0.44 mg Cr(VI)/L h, and was obtained at the maximum assayed sulfate concentration (23.92 mM).

At all assayed sulfate concentrations, both the overall efficiencies and volumetric rates of Cr(VI) reduction obtained at 1.7 mM Cr(VI) were higher than those obtained at 3.3 mM Cr(VI), which is probably due to the fact that Cr(VI) had toxic effects on the yeast, which increased as Cr(VI) concentration increased.

Present results demonstrate that sulfate affects aerobic Cr(VI) reduction but that it does not have a significant

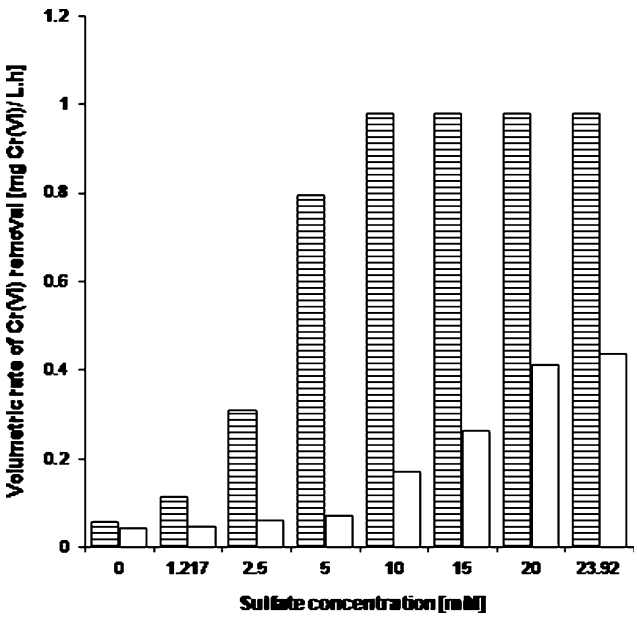

Fig. 6 Dependence of volumetric rate of Cr(VI) reduction on sulfate concentration [*dotted square* initial Cr(VI) concentration = 1.7 mM; *open square* initial Cr(VI) concentration = 3.3 mM]

effect on total chromium removal by *Candida* sp. FGSFEP. These results differ from other reports which find no inhibitory or stimulating effect of sulfate on Cr(VI) reduction in aerobic microbial cultures. Sulfate concentration of up to 1 mM was reported to have no effect on chromate reduction by *Pseudomonas putida* cells [22]. Sulfate at 10 mM concentration did not affect Cr(VI) reduction by *Bacillus* sp. [58]. Liu et al. [28] reported that sulfate concentrations of 40 and 80 mg/L (0.416 and 0.83 mM) did not affect aerobic Cr(VI) reduction by *Bacillus* sp. XW-4. Likewise, Cr(VI) reduction by *Acinetobacter haemolyticus* was not altered in the presence of 10 mM sulfate [60]. In contrast, under anaerobic conditions, sulfate does usually have effects on chromate bacterial reduction; this may be due to the fact that sulfate competes with chromate as final electron acceptor [16, 28]. In anaerobic cultures of *Escherichia coli* ATCC 33456, Cr(VI) reduction rate was not affected by up to 41.6 mM sulfate; however, the rate decreased at 83 mM sulfate [53]. Sulfate concentrations as high as 50 mM did not inhibit Cr(VI) reduction by *Desulfovibrio vulgaris* [33]. Philip et al. [50] reported that the presence of up to 1,000 mg/L (10.4 mM) of sulfate did not affect Cr(VI) reduction by *Bacillus coagulans*. However, Cr(VI) reduction by *Enterobacter cloacae* HO1 under anaerobic conditions showed 32% inhibition in the presence of only 25 μM of sulfate [26].

As mentioned above, sulfate increases tolerance to Cr(VI) in the *Candida* sp. strain isolated by Pepi and Baldi [49]; however, this yeast strain was not capable to reduce Cr(VI). Likewise, even if *Pseudomonas fluorescens* LB300 is capable of reducing Cr(VI) to Cr(III) [3] and sulfate increases its cell growth in the presence of chromate [42], to the best of our knowledge no effect of sulfate on Cr(VI) reduction by this microorganism has been reported.

Results obtained in the present study clearly demonstrate that sulfate affected tolerance of *Candida* sp. FGSFEP to Cr(VI) and its ability to reduce it. It is evident that, as sulfate concentration increased, yeast growth as well as the overall Cr(VI) reduction efficiency and rate increased. These results also reveal that Cr(VI) reduction by *Candida* sp. FGSFEP depends on culture cell density, in such a way that the larger the quantity of biomass produced, the higher the Cr(VI) reduction. Even if the *Candida* sp. FGSFEP strain is sensitive to the toxic effects of Cr(VI) in the absence of sulfate, this yeast has a remarkable ability to reduce very high concentrations of Cr(VI) in presence of sulfate.

The present work proves the important role of sulfate for both, tolerance to Cr(VI) and aerobic Cr(VI) reduction by *Candida* sp. FGSFEP. These findings may have important implications in the biological treatment of Cr(VI) contaminated wastewaters.

Conclusions

This work shows that the tolerance of *Candida* sp. FGSFEP to the toxic effects of Cr(VI) rose as sulfate concentration increased; thus, yeast cell growth, glucose consumption, overall efficiency and volumetric rate of Cr(VI) reduction remarkably increased at high sulfate concentrations. The results obtained in this work could be useful to improve the biological treatment of industrial effluents contaminated with Cr(VI).

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