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## Biosorption of Heavy Metals by *Candida albicans*

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### Abstract

The objective of this work was to study the resistance and removal capacity of heavy metals by the yeast *Candida albicans*. The resistance of some heavy metals was analyzed: the yeast grows in 2000 ppm of chromium, zinc, lead, and copper, 1500 ppm of arsenic (III), 500 ppm of silver, and little bit in cobalt (300 ppm) and mercury and cadmium (200 ppm). Analyzing its potential to remove heavy metals, it can efficiently remove is as follows: Cr(VI) (76%), lead (57%), silver (51%), cadmium (46%), fairly arsenic(III) (40% with the modified biomass), cobalt (37%), mercury (36%), copper (31%), little bit zinc (22%), and fluoride (10%). We determine the optimal characteristics for chromium(VI) removal in living cells and death biomass. The ideal conditions for the removal of 50 mg/L of Cr(VI) in living cells were 28°C, pH 7.0, and  $10 \times 10^6$  yeast/mL, with glycerol-like carbon source. In dead yeast biomass, the ideal conditions for removal of metal are 200 mg/L of Cr(VI), 60°C, pH 1.0, 20 h, and 5 g of biomass.

**Keywords:** biosorption, heavy metals, *Candida albicans*, bioremediation, microorganism

## 1. Introduction

Heavy metals are grouped in the category of 53 elements with a specific weight greater than 5 g/cm<sup>3</sup>. Some elements like zinc, copper, manganese and cobalt are considered as micronutrients, while others like cadmium, lead, mercury or chromium have no biological functions in plants. Heavy metals do not biodegrade in soil, in which matrix they are as free metal ions, interchangeable metal ions, soluble metal complexes, metals bound in organic materials, precipitates or in insoluble compounds like oxides, carbonates, and hydroxides or also may

form part of the silicates [1]. Human-induced pollution can result from mining, industrial, agricultural, military, and nuclear activities, which can induce high concentrations of heavy metals that can enter the food chain and represent a long-term risk potential for the environment and human health [2].

At present, there is a great concern in the world due to the considerable increase in the indices of contamination of industrial effluents by heavy metals such as chromium, nickel, cadmium, lead, and mercury [2]. These toxic substances tend to persist indefinitely in the environment, compromising the well-being and balance not only of the fauna and flora existing in the ecosystem but also the health of people living in the surrounding communities, through their accumulation and entry into the food chain [3]. Among the various effects produced by heavy metals in plants are necrosis at the tips of leaves, inhibition of root growth, and at worst the total death of the plant. In humans, heavy metals can become very toxic when introduced into the organism. At high concentrations, these can cause skin rashes, stomach upset (ulcers), respiratory problems, weakening of the immune system, damage to the kidneys and liver, hypertension, alteration of genetic material, cancer, neurological disorders, and even death [4]. World Health Organization (WHO) established that the maximum concentration of heavy metal ions in water should be in the range of 0.01–1 ppm [5]; however, concentrations of heavy metal ions are up to 450 ppm in effluents [6].

Among the main industrial sectors that are sources of contamination of heavy metals are mining, cement industry, dye industry, tanning, electroplating, steel production, photographic material, corrosion paints, energy production, textile fabrication, wood preservation, aluminum anodizing, water cooling, and others [1, 2]. The environmental impact generated by these toxic substances has led the scientific community to develop different methods for the treatment of industrial effluents contaminated with these substances, which are precipitation, oxidation-reduction, exchange ionic, filtration, electrochemical treatment, membrane technologies, and recovery by evaporation. However, these methods have been quite costly and inefficient, especially when the metal concentration is very low, as well as the formation, disposal, and storage of sludge and wastes, originating during the processes, which become a major problem to solve [7].

Adsorption is the preferential accumulation of a substance on the surface of a normally porous solid. The substance that is adsorbed is called adsorbate and may be an ion or molecule. Furthermore, the solid on which the adsorption occurs is known as an adsorbent [8], and biosorption is a phenomenon widely studied in the bioremediation of sites impacted by pollution. The study of microorganisms and bioadsorbent material is constantly growing with the use of microbial consortia, which would increase yield's uptake of certain specified metals or mixtures thereof [7, 8]. There is evidence of isolation of resistant microorganisms to heavy metals and the use of microbial biomass for the removal of heavy metals from industrial wastewater and/or contaminated water: the isolation and characterization of a variant manganese-resistant strain of *Saccharomyces cerevisiae* [9]; *Pichia guilliermondii* resistant to heavy metal [10]; *Candida albicans* resistant to crude oil [11]; *Pichia anomala*, *Candida krusei*, and *Cryptococcus laurentii* tolerated high concentrations of zinc (up to 20 mM) [12]; the yeast *S. cerevisiae* partly retains heavy metals (Cu, Fe, Pb, Zn, Ba) and arsenic from soil extracts [2]; hydroxyapatite (HAp)/yeast biomass composites for the removal of  $Pb^{+2}$  [13]; removal of zinc by *Pichia kudriavzevii* A16 [14]; copper(II) and phenol adsorption by *Candida tropicalis* [15]; the removal of copper(II) by *Candida krusei* [16]; the application of bifunctional *Mangifera indica* L.-loaded *Saccharomyces cerevisiae* as efficacious biosorbent for bivalent cobalt and nickel cations [17]; the biosorption of Cr(VI) from aqueous solutions by *Candida albicans* and *Cryptococcus neoformans*

isolated from leather works [18]; and Cr(VI) reduction in a chromate-resistant strain of *Candida maltosa* [19], with highly satisfactory results. This chapter reports the removal of different heavy metals in an aqueous solution by a strain of *Candida albicans*, which is highly resistant to this metal.

## 2. Materials and methods

### 2.1. Microorganism and heavy metal-resistant tests

A yeast strain was isolated from the Bancote River in the Huasteca Potosina (Ciudad Valles, SLP, México) [20], and this was used for the screening. The strain was grown on a petri dish containing modified Lee's minimal medium (LMM) (with 0.25%  $\text{KH}_2\text{PO}_4$ , 0.20%  $\text{MgSO}_4$ , 0.50%  $(\text{NH}_4)_2\text{SO}_4$ , 0.50% NaCl, 0.25% glucose, and 2% agar). The pH of the medium was adjusted and maintained at 5.3 with 100 mmol/L of citrate phosphate buffer. The plates were incubated at 28°C for 7 days.

Yeast cultures grown in thioglycolate broth were used as primary inoculums. Heavy metal-resistant tests of the isolated strain, yeast *C. albicans*, were performed on liquid LMM containing the appropriate nutritional requirements and different concentrations of heavy metals (as salt), and the dry weight was determined.

### 2.2. Identification of yeast

The strain was identified based on its macroscopic characteristics and microscopic observations [21]. Germ tube induction test was performed as follows:  $1 \times 10^6$  yeast/mL is taken, seeded into LMM (added with proline and biotin, 0.5 and 0.001 g/L, respectively), and incubated at 37°C for 3 h. Subsequently, a small sample was taken to analyze in a microscope, the formation of a germinal tube without constriction in its source of origin and with the characteristic shape of hand mirror [21].

Moreover, to examine the formation of chlamydo spores, yeast ( $1 \times 10^6$  yeast/mL) was grown in corn flour agar medium and incubated at 48–72 h at 28°C, observing under microscope the formation of asexual, thick-walled, and refringent spores, called chlamydo spores, which may be intercalated or in terminal position of the hyphae partitions or septate [21].

### 2.3. Resistance test

Petri dishes were prepared with Sabouraud Dextrose Agar and added with different salts of heavy metals. The prepared plates were inoculated with  $1 \times 10^6$  yeast/mL, uniformly spread throughout the dishes, and incubated at 28°C for 7 days, and the growth of the plates was compared with a control.

### 2.4. Preparation of biomass

The yeast cells were grown at 28°C in a stirred and aerated liquid media containing thioglycolate broth at a concentration of 8 g/L (w/v). After 7 days of incubation, the cells were recovered by centrifugation (3000 rpm, 10 min) and washed three times in the same conditions with deionized water, and, subsequently, they were dried (80°C, 24 h) in an oven.

Iron oxide-coated biomass was also prepared; 80 mL of 2 M  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  was prepared, and 1.0 mL of 10 M NaOH was added to this solution and mixed thoroughly. Twenty grams of the yeast biomass powder was taken in a porcelain pot, and a mixture of iron oxide and NaOH solution was added to the porcelain pot, homogenized, and kept in an oven for 3 h at 80°C. After 3 h, the oven temperature was raised to 110°C and continued for 24 h. The coated biomass powder was separated by crushing with mortar and pestle [22].

### 2.5. Biosorption tests of chromium(VI) by using dry yeasts

Solutions of Cr(VI) for analysis were prepared by diluting 71.86 mg/L of stock metal solution. The concentration range of Cr(VI) solutions was 50–1000 mg/L. The pH of each solution was adjusted to the required value by adding 1 M  $\text{H}_2\text{SO}_4$  solution before mixing with the microorganism. The biosorption of the metal by yeast dry cells was determined at different concentrations of 100 mL Cr(VI) solution, with 1 g of yeast biomass, at 100 rpm, and the sample was filtered. The filtrate containing the residual concentration of Cr(VI) was determined spectrophotometrically. For the determination of the rate of metal biosorption, the solution of Cr(VI) was used at concentrations of 200, 400, 600, 800, and 1000 mg/L. The supernatant was analyzed for residual Cr(VI) at different times after a contact period. For the determination of factors such as pH and temperature, seven solutions were analyzed, which included pH 1.0, 2.0, 3.0 and temperatures of 28°C, 40°C, 50°C, and 60°C. Moreover, biosorption to the contaminated earth and water was examined. Four Erlenmeyer flasks containing 5 g of fungal biomass, 20 g of contaminated earth and 20 mL of water (297 mg Cr(VI)/g earth or 155 mg Cr(VI)/L water) from tannery (Celaya, Guanajuato, Mexico), were calibrated to 100 mL with tridesionized water, were incubated during 7 days, stirred at 120 rpm, and filtered in whatman filter paper No. 1. The concentration of Cr (VI) was determined by the 1,5-diphenylcarbazide method.

### 2.6. Reduction of Cr(VI) by living yeasts

Reduction efficiency of Cr(VI) by living yeasts was examined. To examine the living yeasts, cultures in 100 mL of LMM were inoculated with  $1 \times 10^6$  yeasts/mL (28°C, 7 days), the cells were centrifuged (3000 rpm, at 4°C, 10 min) and washed three times with sterile trideionized water, and the pellet was resuspended in 3 mL of the same solution and was transferred on a fresh LMM (100 mL with 50 mg/L Cr(VI)). At different times, 1 mL aliquots were removed and centrifuged (3000 rpm, 10 min), and the concentration of Cr(VI) or total Cr in the supernatant was determined [23].

### 2.7. Removal by different heavy metals by using dry yeasts

Solutions of heavy metals for analysis were prepared by diluting 1 g/mL of stock metal solution. The concentration range of heavy metal solutions was 1–200 mg/L. The pH of each solution was adjusted to the required value by adding 1 M  $\text{H}_2\text{SO}_4$  solution before mixing with the microorganism. The biosorption of the metals by yeast dry cells was determined at different concentrations of 100 mL heavy metal solution, with 1 g of yeast biomass, at 100 rpm, and the sample was filtered. The concentration of heavy metals was determined in the filtrate with the following methodologies: Cr (VI) spectrophotometrically with dhyphenylcarbazide, Zn, Pb, Hg, Cd, with dithizone, Co by methyl isobutyl ketone, F by specific ion, and Cu, As (III), As (V), Ag by Atomic absorption [23].

### 3. Results and discussion

#### 3.1. Isolation and identification of a yeast strain tolerant to heavy metals

The microorganism collected from Bancote River was grown on the LMM agar plates containing different concentrations of heavy metals, and the largest colony of yeast was isolated. The colony of the isolated strain grew rapidly within 3–5 days. They were creamy, white-yellowish, glossy, slightly raised colonies with well-defined borders [Figure 1a]. Blastoconidia are formed with 3–6  $\mu\text{m}$  in diameter singly in chains or in small loose clusters (Figure 1b), and in certain conditions, they form germ tube (Figure 2) and chlamydoconidia (Figure 3). We designated the strain as *Candida albicans*.

The isolated cells grew in medium on LMM supplemented with different concentrations of heavy metals, about 62.2, 53, and 22% with 2 g/L of Cr(VI) and Pb(II) and 600 mg/L of silver, respectively; growth relative to control (32.3 mg of dry weight without metal) was obtained (Figure 4) and, therefore, probably is resistant to the metals. As well, in plate-resistant testing, the yeast grew in 2 g/L of Zn(II), Pb(II), Cu(II), and Cr(VI), 1.5 g/L of As(III), and 200 mg/L of Hg(II) and Cd(II) (Table 1). Several microorganisms that are heavy metal resistant have been isolated from different contaminated sites. An important example is a mutant IM3 strain of *S. cerevisiae*, spontaneously grown on solid yeast extract peptone dextrose, medium with highly concentrated Mn (10 mM) [9], *P. guilliermondii* resistant to 400 mM of Mn(II), Zn(II), and Co(II) [10], *C. krusei* and *C. laurentii*, isolated from water, soil, and plant environments, which tolerated high concentrations of Zn(II) (up to 20 mM) [12], *Pichia pastoris* genetically engineered, it grows in 6 mM of  $\text{AgNO}_3$  (corresponding to 647.2  $\mu\text{g/ml}$  of elemental silver) and 4 mM of  $\text{SeO}_2$  (corresponding to 315.8  $\mu\text{g/ml}$  of elemental selenium) [24], *Rhodotorula mucilaginosa* planktonic cells showed the tolerance in the presence of Hg(II) (0.08 mM), Cu(II) (6.40 mM), and Pb(II) (3.51 mM) [25], and the ability of cadmium uptake by metal-resistant yeast, *Candida tropicalis*, isolated from wastewater from industrial area of Sheikhpura, a small town located 40 km central west of Lahore, Pakistan, known for its industry. This yeast grew in 2.5 g/L of Cd(II), 1.4 g/L of Zn(II), 1 g/L of Ni(II), 1.4 g/L of Hg(II), 1 g/L of Cu(II), 1.2 g/L of Cr(VI), and 1 g/L of Pb(II) [26]. The resistance of *Candida albicans* to heavy metals

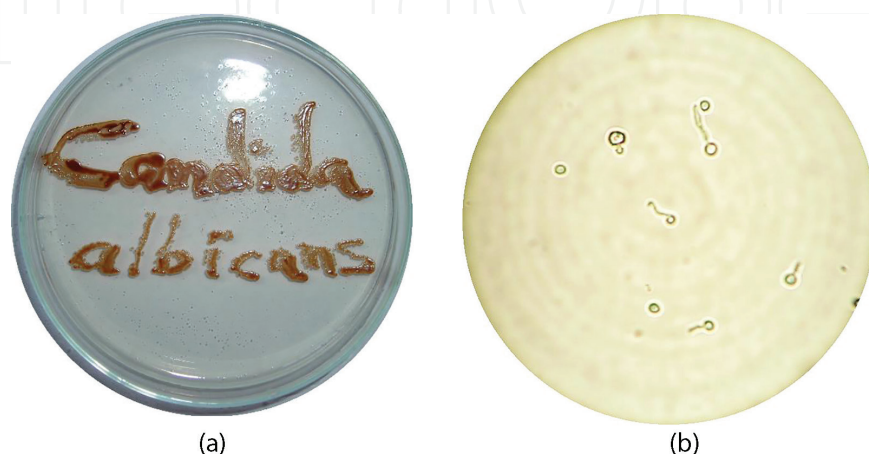
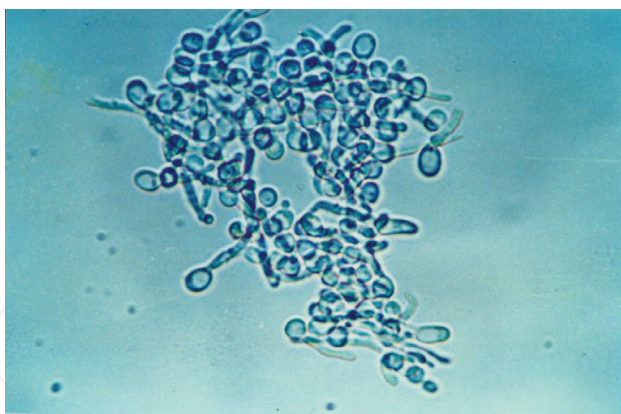


Figure 1. (a) Macroscopic and (b) microscopic morphology of the yeast *Candida albicans*.



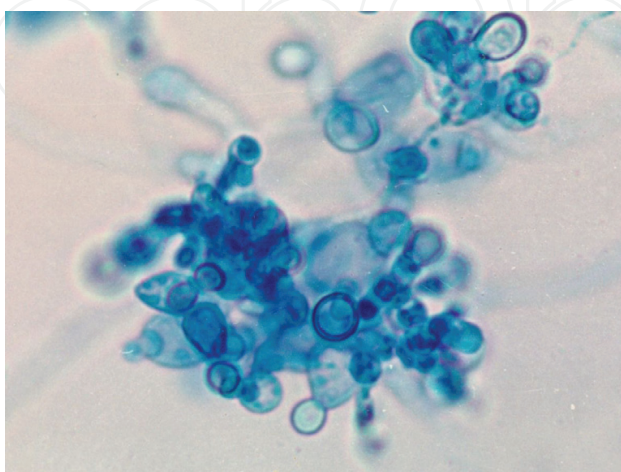
**Figure 2.** Germ tube induction by *Candida albicans*.

is sufficiently high compared to other yeasts. Therefore, we used the yeast for the following biosorption assays.

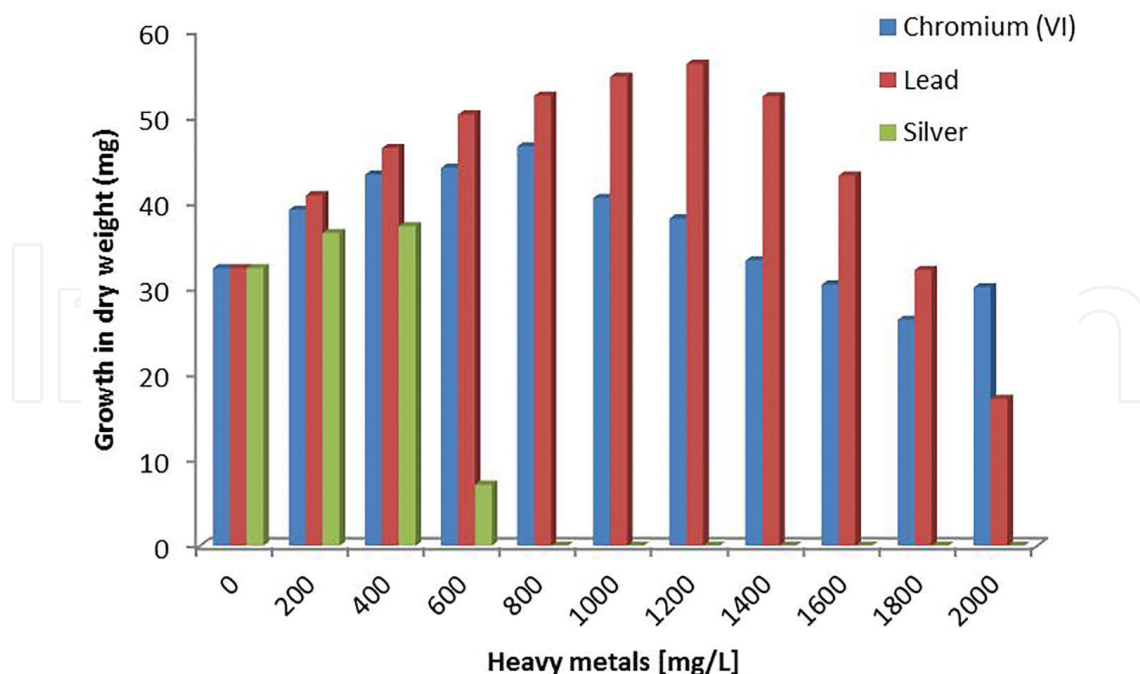
### 3.2. Removal of chromium(VI) by dry cells of *Candida albicans*

#### 3.2.1. Effect of pH

The effect of pH on biosorption by using dry cells was examined. First, the capacity of biosorption of heavy metals in dry cells of *Candida albicans* was examined. **Figure 5** shows the effect of incubation time and pH on the biosorption of 50 mg/L of Cr(VI) by the biomass of *A. C. albicans*. It was found that a higher removal, which is proportional to the biosorption, occurs at 2 days and at a pH of 1.0. It was reported a time of 24 h *Cyberlindnera fabianii*, *Wickerhamomyces anomalus*, and *Candida tropicalis*, at a pH range between 2 and 4 for the three species [27]; the removal of Cr(VI) (100%) by *Cyberlindnera fabianii* at 48 h [28]; *Candida tropicalis* isolated from chromium-contaminated site removal 50 mg/L of the metal at 48 h [29]; and *Candida intermedia* in the biosorption of Cr(III) and Cr(VI) were reported [30]. Permeability and porosity of the cell wall can affect the incubation time of each



**Figure 3.** Formation of chlamydoconidia *Candida albicans*.



**Figure 4.** Growth in dry weight of *Candida albicans* with different heavy metal concentrations.  $1 \times 10^6$  yeast/mL, 28°C, 7 days of incubation, 100 rpm.

microorganism, giving greater or lesser exposure of the functional groups in the cell wall of the biomass analyzed [31]. With acid pH used in these experiments, Cr(VI) has negative charge, and the removal by protonation of the biosorbent surface is favored, which induces a strong attraction to these anions, increasing biosorption and, therefore, the removal of the solution. However, if the pH increases, the concentration of  $\text{OH}^-$  ions increases, and the

Heavy metals	Growth of heavy metal concentration (mg/L)
Zinc	2000
Lead	2000
Copper	2000
Chromium(VI)	2000
Arsenic(III)	1500
Silver	500
Fluor	400
Cobalt	300
Mercury	200
Cadmium	200

**Table 1.** Growth in LMM in plate of *Candida albicans* with different heavy metals.  $1 \times 10^6$  yeast/mL, 28°C, 7 days of incubation.



positive positions are reduced in the adsorbent surface, releasing the ions of Cr(VI) to the solution. This explains why at higher pH, removal of Cr(VI) [32] decreases, though not always desorption of anions of Cr(VI), is observed with an increase of pH, so biosorption is not the only mechanism occurring, but the reduction of Cr(VI) by organic matter also occurs, due to the high oxidation potential of these species, forming Cr(III), which is an insoluble species in basic medium.

### 3.2.2. Effect of the temperature

Temperature is also a critical parameter in the removal of Cr(VI) (Figure 6); at higher temperatures, we observe greater removal: at 60°C, 100% of the metal is removed in 20 h, and 76% is removed in 24 h at 28°C. These results are similar to those reported for *Pichia jadinii* M9 and *Pichia anomala* M10. More rapidly with an increment in temperature, with an optimum value of 30°C [33], in the removal of copper for encapsulated *Candida krusei* [34], and for *C. neoformans*, with a maximum adsorption capacity for Cr(VI) ion was observed at 28°C [18]. When the temperature increases, the rate of removal of Cr(VI) increases and the contact time required for complete removal of the metal decreases, increasing the redox reaction rate [35].

### 3.2.3. Effect of the initial concentration of chromium(VI) at 28 and 60°C

The effects of initial concentration of Cr(VI) was examined. At room temperature, the concentration influences the removal of the metal; at lower concentration of the same, removal is faster (at 10 days, 200 ppm, the removal is 100%, and with 1 g/L only, removal is 20% at same time) and at 180 min, 800 and 1000 ppm, are removed, respectively (Figure 7a). At 60°C, we observed a removal of 100% at 8 days with 200 mg/L, and only 48% with 1 g/L of the metal

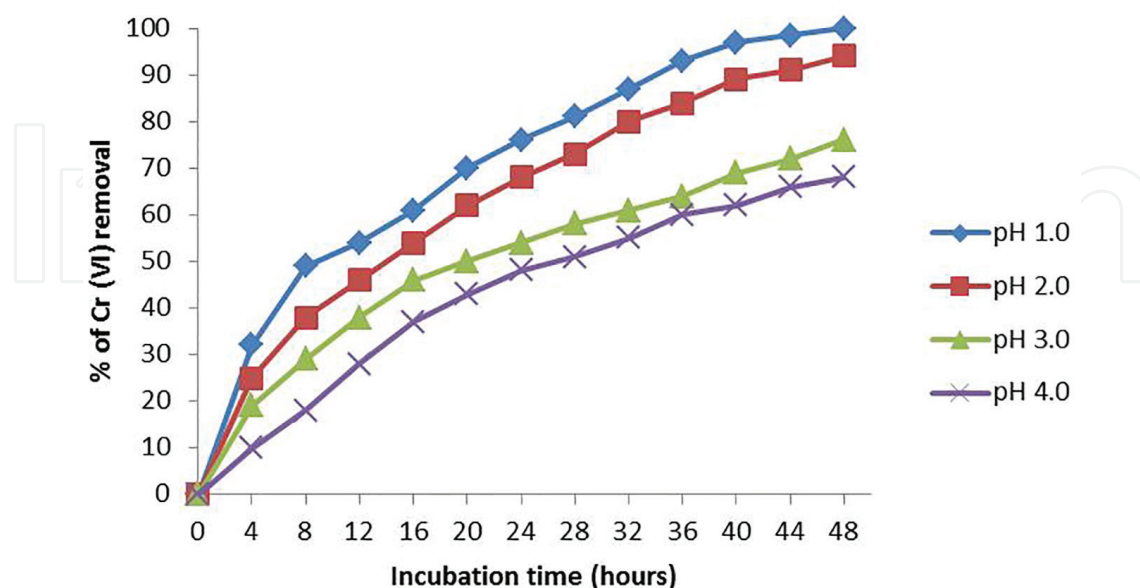
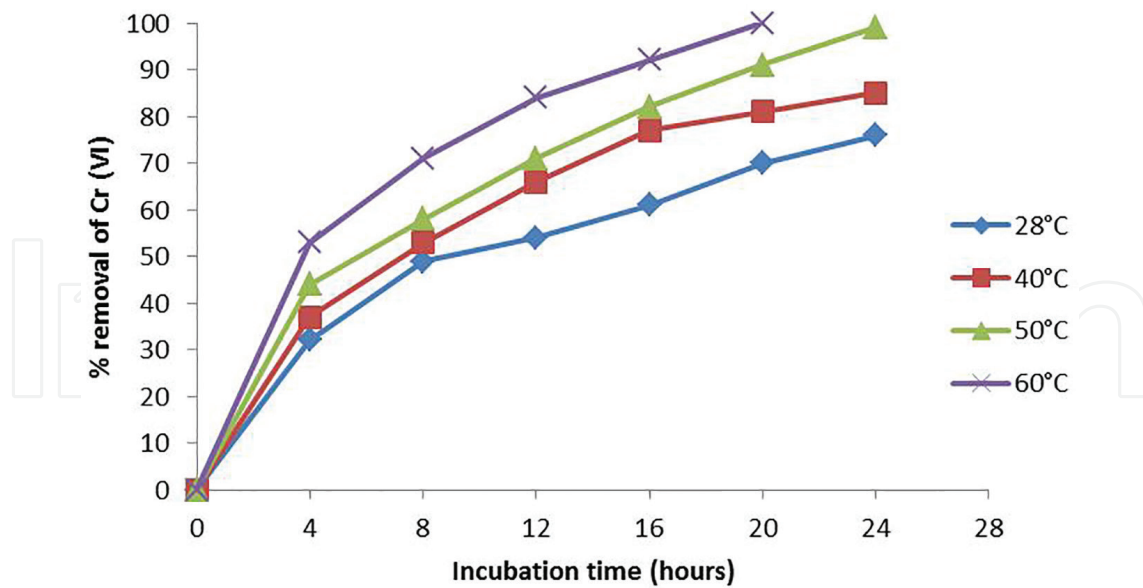
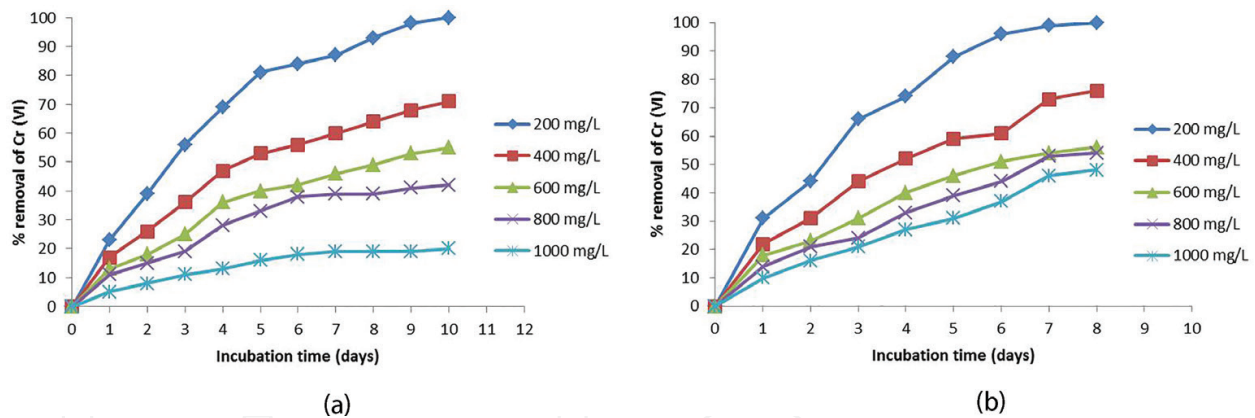


Figure 5. Effect of incubation time and pH on Cr(VI) removal by *C. albicans*. 50 mg/L Cr(VI), 100 rpm, 28°C, and 1.0 g of yeast biomass.



**Figure 6.** Effect of the temperature on Cr(VI) removal by *C. albicans*. 50 mg/L Cr(VI), 100 rpm, pH 1.0, and 1.0 g of yeast biomass.



**Figure 7.** Effect of initial metal concentration on chromium(VI) removal by *C. albicans*. (a) 28°C and (b) 60°C, pH 1.0, 100 rpm, and 1 g of yeast biomass.

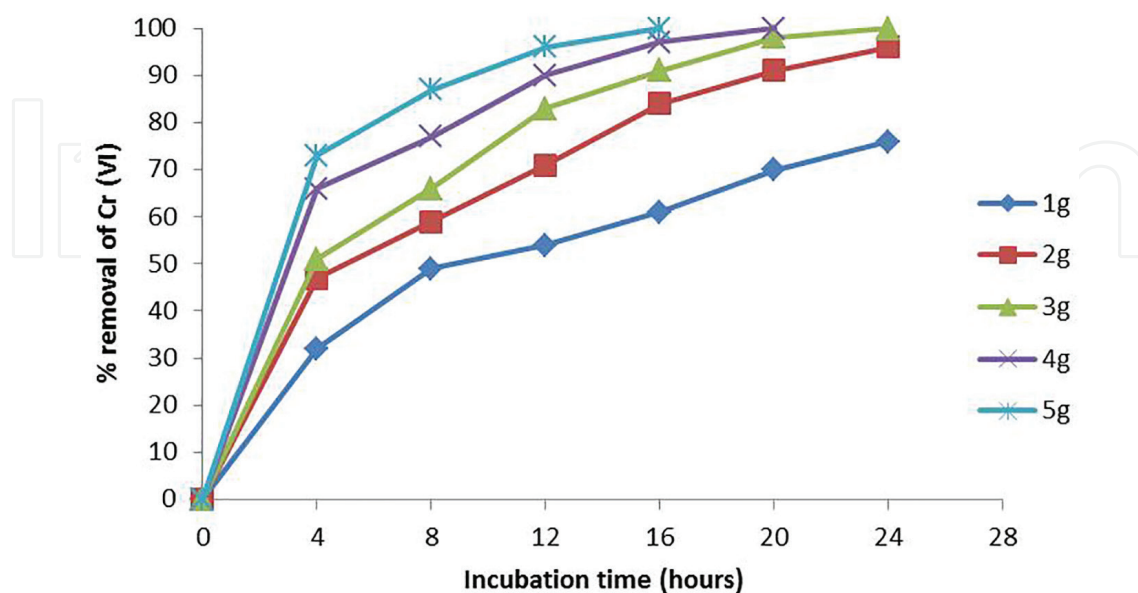
(Figure 7b), which may be due to sorption happening at low concentrations, but at higher concentrations, possibly when positive positions were saturated, precipitation occurs (which is a slower process) [36]. Some yeasts are not affected by the concentration of the metal, like the three yeast strains isolated from sediments in Morocco [27], and in other yeasts, the removal of metal increases in direct proportion to the increase of the concentration of Cr(VI) in the solution like *Rhodotorula mucilaginosa* isolated from the effluent of Chittaranjan locomotive workshop effluent samples [25], for *Pichia jadinii* M9 and *Pichia anomala* M10 [33], metal-resistant yeast, *Candida tropicalis* [26]. *C. neoformans* showed a higher biosorption capacity at low concentrations of metal ions (0.2 mg/L) [18], *Rhodotorula mucilaginosa* for the removal of copper [37], and *Yarrowia* strains isolated from sediments of mercury-polluted estuarine water [38].

### 3.2.4. Effect of the initial concentration of biosorbent

The effect of the initial concentration of biosorbent on 50 mg/L of Cr(VI) was examined. It was found that the higher the concentration of the latter, the greater and faster the removal of Cr(VI). One gram of biomass with the removal of 76% was observed at 24 h, whereas for 5 g, the removal time was 16 h (**Figure 8**). These observations explain that the amount of added bioadsorbent determines the number of sites available for biosorption load of chrome anions or any metal contaminant [39]. Similar results were reported for the removal of different heavy metals by *S. cerevisiae*, which showed an increase in removal efficiency on increasing biomass from 0.01 to 0.1 g [40], and for the removal of Pb(II) by *S. cerevisiae* CCTCC AY92003 [41]. However, Zn removal rate of the yeast *Pichia kudriavzevii* A16 was not significantly improved when the initial biomass concentration was raised from 0.05 to 1 g/L [14] and is different for *S. cerevisiae* (BCRC23331), which the biosorption capacity of Ni(II) decreasing with increasing adsorbent dose.

### 3.2.5. Removal of chromium(VI) in industrial wastes with yeast biomass

To analyze the possible use and the ability of *C. albicans* biomass to remove chromium(VI) from sediments and effluents, a removal assay was mounted in an aqueous solution in the presence of 5 g biomass, with nonsterile soil contaminated with 297 mg of Cr(VI)/g/L and 100 mL of contaminated water with 155 mg of Cr(VI), resuspending the land in trideionized water at 28°C and stirring at 100 rpm. It was observed that after 7 days of incubation, 74 and 69% of Cr(VI) present in the contaminated water and soil were removed, respectively (**Figure 9**). The ability to remove by biomass is equal to or greater than the other biomass that have been studied, *Candida maltose* RR1 [19], *C. tropicalis* was observed to remove 40% Cd (II) from the wastewater after 6 days and was also able to remove 78%



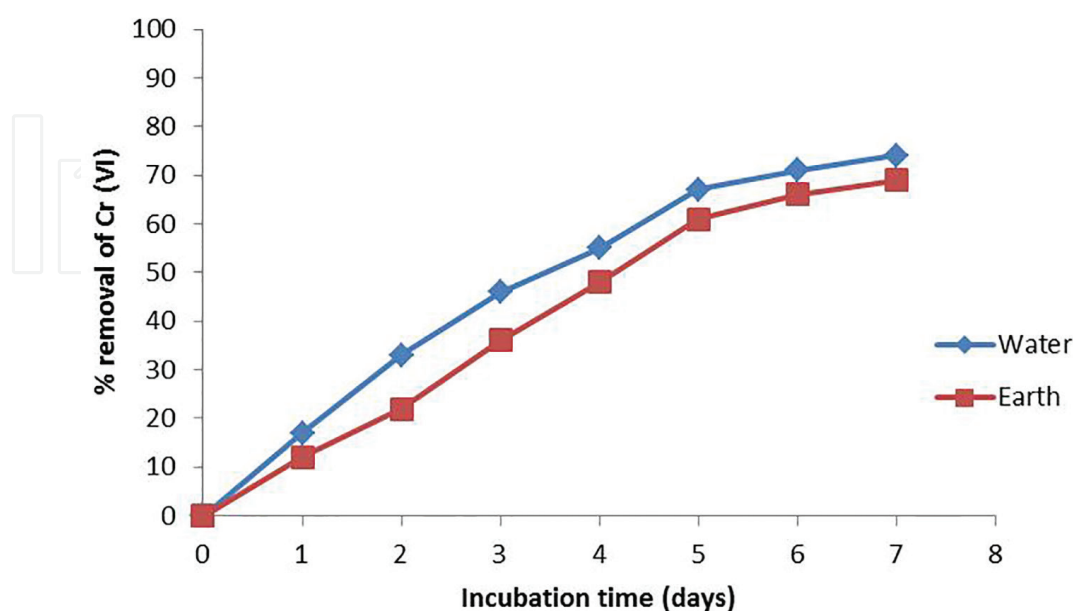
**Figure 8.** Effect of biomass concentration on chromium(VI) removal by *C. albicans*. 50 mg/L Cr(VI), 28°C, pH 1.0, and 100 rpm.

from the wastewater after 12 days [26], *S. cerevisiae* and *Torulaspora delbrueckii* decrease in 98.1, 83.0, 60.7, 60.5, and 54.2% for turbidity, sulfates, BOD, phosphates and COD, respectively, of the tannery effluent [42], *S. cerevisiae* “wild-type” (WT) parental strain BY4741 very efficient in removing Mn(II), Cu(II), Co (II) from synthetic effluents containing 1–2 mM cations [43].

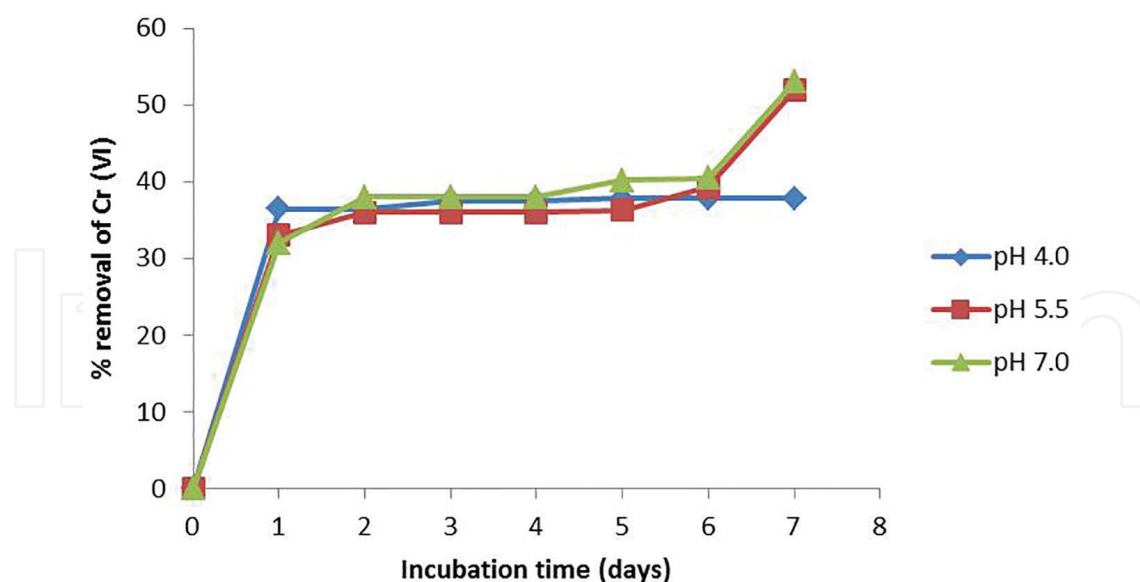
### 3.3. Removal of Cr(VI) by the living cells of *Candida albicans*

#### 3.3.1. Effect of incubation time and pH

Next, we will discuss the characteristics of adsorption in the living cells of *C. albicans*. **Figure 10** represents the effect of different pH (4.0, 5.5, and 7.0, maintained at phosphate-citrate buffer 100 mM/L) on the removal of Cr(VI). The speed and rate of metal removal increase as pH goes up. The maximum removal was observed at pH 5.5 and 7.0 (52 and 53% after 7 days of incubation at 28°C and 100 rpm). The ability to remove by living yeast biomass were found were found at pH 4.0 for *C. fabianii* HE650139 and *W. anomalus* HE648168; at pH 3.0 for *C. tropicalis* HE650140, with a percentage removal of 100%, by all living microorganisms [27]; at pH of 5.0–6.5 for the Hg(II) bioremoval by *Yarrowia* strains [38]; at an optimum pH for the strains *P. jadinii* M9 and *P. anomala* M10 of 7.0 and 3.0, respectively, for Cr(VI) reduction [33]; and a pH between 1 and 2 for the removal of Cr(VI) by *Candida utilis* [44]. The decrease of pH causes protonation of the adsorbent surface by attracting ions of Cr(VI) in the solution, so it increases the acidity of the solution, and the biosorption is favored for some microorganisms. As much as the pH increases, the concentration of OH<sup>-</sup> ions increases too, favoring the presence of Cr(VI) ion valence. This stimulates changes in the biosorbent and prevents metal biosorption [3].



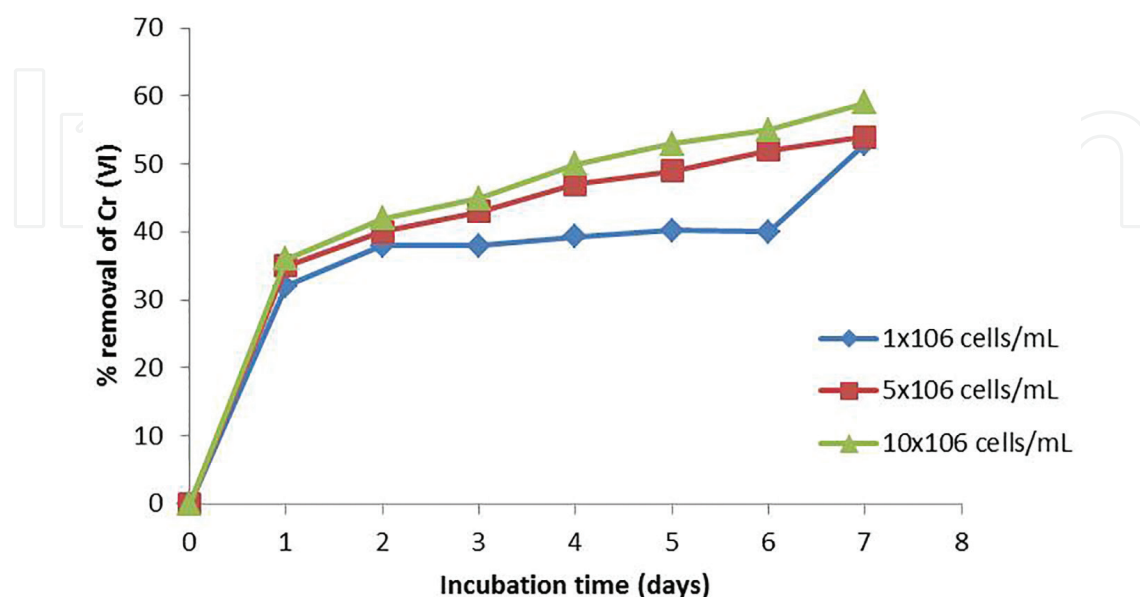
**Figure 9.** Removal of Cr(VI) from industrial wastes incubated with 5 g of yeast biomass, 100 rpm, 28°C, 20 g, and 100 mL of contaminated soil and water (297 mg Cr(VI)/g earth and 155 mg Cr(VI)/L), respectively.



**Figure 10.** Effect of pH on Cr(VI) removal by biomass of *C. albicans*. 50 mg/L Cr(VI), 100 rpm, and 28°C.

### 3.3.2. Effect of the initial concentration of the inoculum

The effect of the concentration of cell biomass for the removal capacity of Cr(VI) in the solution (**Figure 11**) was analyzed. At the concentrations tested ( $1 \times 10^6$ ,  $5 \times 10^6$ , and  $10 \times 10^6$ ), the removal capacity was similar (53, 54, and 59%, respectively) for the strains *P. jadinii* M9 and *P. anomala* M10 with pH 7.0 and 3.0, respectively, for Cr(VI) reduction [33] and the removal of Cr(VI) by *Candida utilis* [44]. In contrast to our observations, most reports in the literature indicate that the higher amount of biomass increases the percentage of removal: the Hg(II) (6.0, 12.0, 24.0, 48.0, 96.0, and 192.0 mg/L of  $\text{HgCl}_2$ ) bioremoval by *Yarrowia* strains [38]. So the greater the amount of the inoculum, the more binding sites for complexing metal (e.g., ions and  $\text{HCrO}_4^-$   $\text{Cr}_2\text{O}_7^{2-}$ ) [1].



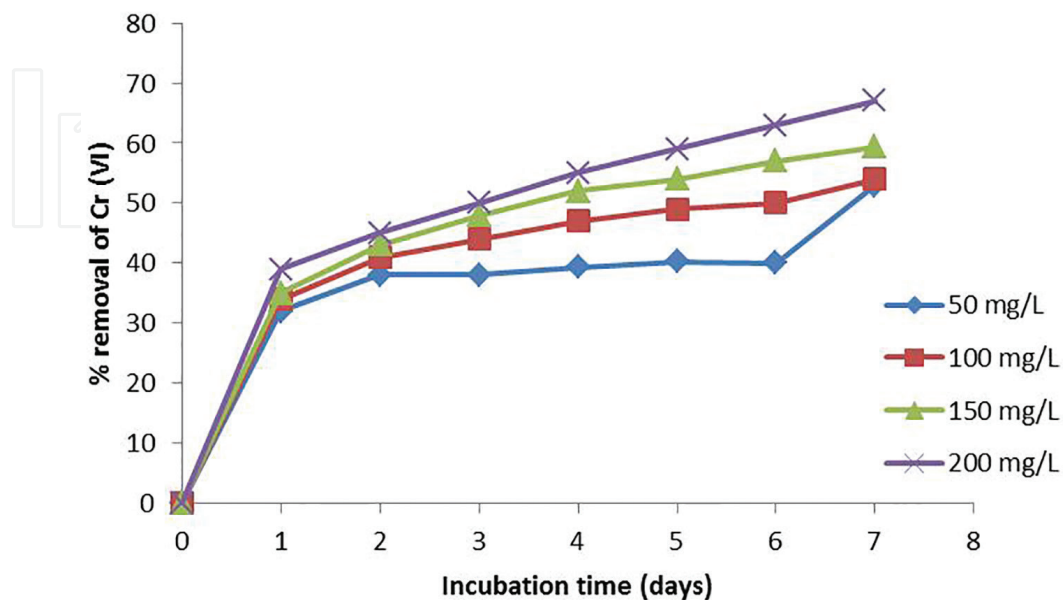
**Figure 11.** The effect of yeast concentration on the removal of Cr(VI), 50 mg/L Cr(VI), 100 rpm, 28°C, and pH 7.0.

### 3.3.3. Effect of the initial concentration of Cr(VI)

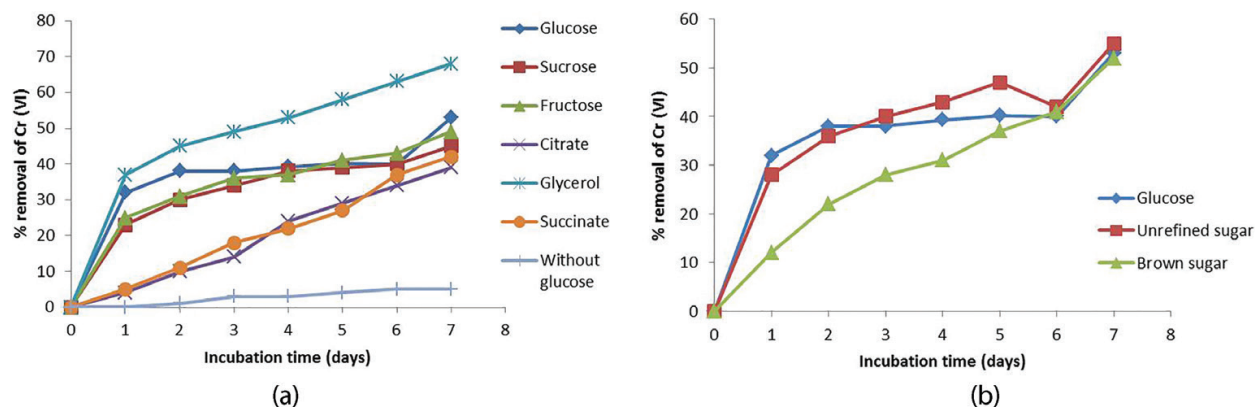
The effect of initial concentration of living cells was examined (**Figure 12**); it was observed that metal removal is more efficient in high concentration of living cells, showing that the removal was 67, 59, 54, and 53% for 200, 150, 100, and 50, mg/L, respectively. The results may be due to the increased amount of ions competing for free functional groups on the surface of the biomass of *C. albicans*. These observations are consistent for the removal of Cr(VI) by *Candida utilis* [44], for contaminated soil for bioremediation of Cr(VI) [45], for the strains *P. jadinii* M9 and *P. anomala* M10 for Cr(VI) reduction (26–104 µg/mL) [33], and for *Candida* sp. isolated from a sewage treatment plant for removal of Cr(VI) [46] and are different from the removal of Cr(VI) by *C. tropicalis* [29].

### 3.3.4. Removal capacity of Cr(VI) with different carbon sources

Moreover, the effect of carbon sources on the removal was examined. In **Figure 13**, we showed the efficiency of the yeast in the removal of Cr(VI) using different carbon sources such as fermentable (glucose, sucrose, and citrate), nonfermentable (succinate), oxidized (glycerol), and commercial (unrefined sugar and brown sugar). We found out that the reduction of Cr(VI) is higher when the medium contains fermentable carbon sources (53% glucose, 97.2% sucrose, and 45% citrate), and removal is high with oxidizable carbon source (68% glycerol), unlike the nonfermentable (42% succinate), while with the other sources of commercial and economic carbon as unrefined (55%) and brown sugar (52%), the removal of Cr(VI) is very similar at 7 days. If we incubate the fungal biomass without a carbon source, there are no changes in the initial Cr(VI) concentration during the experiment (data not shown), suggesting that a carbon source is required to decrease Cr(VI) concentration in the growth medium. Our studies are similar with those reported for *Aspergillus niger* [47], in which the reduction of Cr(VI) is higher when the medium contains fermentable carbon sources (100% glucose, 97.2% sucrose, and 93.35% citrate), and removal is high with oxidizable carbon source (89.9% glycerol), unlike the



**Figure 12.** The effect of initial concentration of Cr(VI) on the removal of 50 mg/L Cr(VI), 100 rpm, 28°C, and pH 7.0.



**Figure 13.** The effect of different carbon sources on the capability of *C. albicans* to decrease Cr(VI) levels in the growth medium, 100 rpm, 28°C, and pH 7.0.

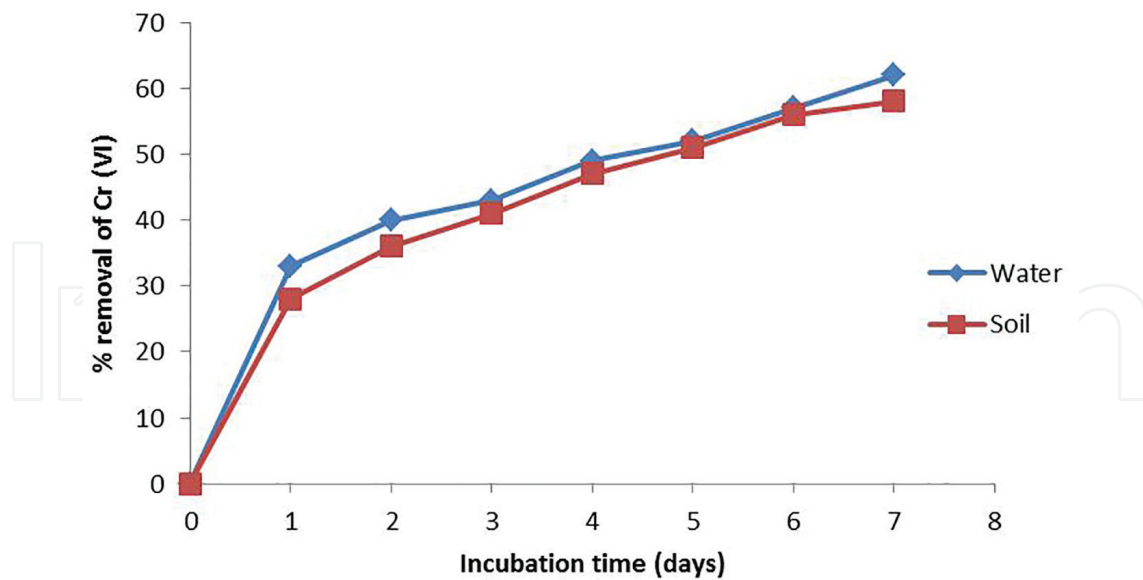
nonfermentable (41.80% succinate), while with the other sources of commercial and economic carbon as unrefined (86.50%) and brown sugar (100%) [47], with *Penicillium lilacinum* using chromate-resistant strains of filamentous fungi indigenously [48], for *Aspergillus foetidus* [49], and the strain Ed8 of *A. niger* chromium resistant, all with glucose as the carbon source [50], but are different from the observations with *Aspergillus* sp., for which the observation was that sodium acetate was the carbon source that induced a greater removal of Cr(VI) [51].

### 3.3.5. Analysis of the possible use of the yeast *C. albicans* to the removal of Cr(VI) in nonsterile earth and water contaminated with the same metal

Bioremediation study using soil was also carried out, which was inoculated in  $1 \times 10^6$  yeast/mL and 100 mL of LMM (pH 5.3) and then 20 g of nonsterile earth and 10 mL of water, contaminated with 50 mg Cr(VI)/g and 50 mg/L of earth and water, respectively, obtained from a factory from the city of Celaya, Guanajuato, Mexico, were added and incubated at 28°C and 100 rpm, observing that after 7 days of incubation, the removal of the metal in the solution was 58 and 62% in earth and water samples, respectively (**Figure 14**), unchanged significantly in the total Cr content. In an experiment conducted in the absence of the yeast, the concentration of Cr(VI) of the samples decreased by about 14% in earth and 6% in water (data not shown), which may be caused by native microflora and reducers present in contaminated samples or components. The removal capacity of Cr(VI) by the fungus is equal or better than that for other yeasts reported such as *C. maltose* RR1 [19], with the removal of Cr(VI) by *C. tropicalis* [29]; the strains *P. jadinii* M9 and *P. anomala* M10, for Cr(VI) reduction [33]; for contaminated soil for bioremediation of Cr (VI) [45]; for *Aspergillus niger* [47]; and different yeasts [52].

### 3.3.6. Removal of different heavy metals by yeast biomass of *C. albicans*

Finally, we analyzed the capacity of heavy metal removal by dry cell of the yeast. The results are shown in **Table 2**. The efficiency of yeast removal is shown as follows: Cr(VI) (76%), Pb(II) (57%), Ag(II) (51%), and little bit Zn(II) and F(I) (10%). Both living and dead yeast cells can be effective metal accumulators, and there is evidence that some biomass-based cleanup processes are economically viable [2]. The tolerance of some yeast species to heavy metals, as well



**Figure 14.** Bioremediation of Cr(VI) from contaminated earth and water (50 mg Cr(VI)/g soil and/or 50 mg/L of water), 28°C, pH 5.3, and 100 rpm.

Heavy metal	pH	Initial concentration (mg/L)	Removal (%)
Chromium(VI)	1.0	50	76
Lead(II)	4.0	100	57
Silver(I)	6.0	100	51
Arsenic(V)*	6.0	1	48
Cadmium(II)	6.0	5	46
Arsenic(III)*	6.0	1	40
Cobalt(II)	4.0	200	37
Mercury(II)	5.5	100	36
Copper(II)	5.0	100	31
Zinc(II)	5.0	100	22
Fluor(I)	6.0	10	10

\*Yeast biomass modified with  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ .

**Table 2.** Removal of different heavy metals by yeast biomass of *Candida albicans*. 28°C, 1 g of yeast biomass, 100 rpm, 24 h.

as the physiological response to them, has also been determined [3]. The removal of heavy metal ions, using yeast as biosorbents, was previously investigated [1–3, 7]. Our results confirm the capacity of the microorganism biomass to remove heavy metals with different effectiveness, like yeast-based microbiological decontamination of heavy metal-contaminated soils of Tarnita [2], for bacteria and fungi resistant to crude oil [11], yeasts isolated from water, soil,



and plant environments [12], with yeast biomass adsorption for lead (II) [13], removal of zinc by yeast *Pichia kudriavzevii* A16 [14], copper(II) and phenol adsorption by *C. tropicalis* cells in aqueous suspension [15], *C. krusei* for the removal of copper(II) [16], biosorption of cadmium by *C. tropicalis* [26], removal of Cr(VI) by indigenous *Pichia* sp. [33], and Ni(II) biosorption by *S. cerevisiae* [53].

#### 4. Conclusion

We isolated a *C. albicans* yeast, which grew with different heavy metals in LMM and which is probably resistant to the metals. With dead biomass, the removal efficiently 50 mg/L of Cr(VI), 60°C, pH 1.0, 24 h, with 1 g of yeast biomass, with the living cells of *C. albicans*, showed an efficient capacity of reduction (53%) of 50 mg/L Cr(VI) in the growth medium after 7 days of incubation, at 28°C, pH 7.0, 100 rpm and with an inoculum of  $10 \times 10^6$  yeast/mL, and removal efficiently Cr(VI) (76%), lead (57%), silver (51%), and cadmium (46%). Finally, these results suggest the potential applicability of *C. albicans* for the remediation of Cr(VI) from polluted soils and waters.

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