Mechanism of hexavalent chromium detoxification using *Cyberlindnera fabianii* yeast isolated from contaminated site in Fez (Morocco)

Wifak Bahafid, Nezha Tahri Joutey, Hanane Sayel, Naïma EL Ghachtouli*

Microbial Biotechnology Laboratory, Faculty of Sciences and Technology,
Sidi Mohammed Ben Abdellah University, Fez, Morocco

Abstract

Bioremediation of metal pollution remains a major challenge in environmental biotechnology. In this study, the objective was to investigate mechanism of Cr(VI) removal from aqueous solutions using *Cyberlindnera fabianii* strain. The changes in chromium content in the medium and in the cells during incubation with different chromate concentrations were investigated. Results showed that this strain has remarkable capacity to completely remove Cr(VI) at a concentration of 25 mg/L in 48h under aerobic conditions, however any change in total content of chromium in the culture liquid was detected, which can be explained by the formation of Cr(III). Fractionation of the cell-free extracts shows that reduction of chromate ions takes place by chromate reductase activity of cell-free extracts of *C. fabianii* and the mechanism of Cr(VI) removal by this strain is "adsorption-coupled reduction". The surface sequestration of Cr(VI) by *C. fabianii* was investigated with a scanning electron microscope equipped with an energy dispersive X-ray analysis (EDXA). The nature of possible cell–metal ions interactions was also evaluated by infrared spectroscopy. The results indicate that the binding process of chromium ions involves the active participation of functional groups present in the external surface of biomass, such as CH_2 asymmetric stretch, amide I, amide II, amide III, and phosphate. Comprehension of chromium elimination mechanism by *C. fabianii* is important for targeting the process of chromium bioremediation and also minimizing treatment costs.

Keywords: Bioremediation; yeast; *Cyberlindnera fabianii*; Cr(VI) removal; mechanism.

1. Introduction

Pollution by toxic metals is a global environmental problem. Release of metals without proper treatment poses a serious threat to public health because of the persistence, biomagnification, and accumulation of some of them in the food chain [1]. A number of studies have established the effects of metals on animals, plants, and human health [2]. Chromium is one of the toxic metals; it is dissipated into the environment as a result of various industrial activities [3] such as electroplating, leather tanning, metal finishing and chromate preparation. Two stable oxidation states persist in the environment, i.e. Cr(III) and Cr(VI). Cr(VI) is the most toxic of both species which have contrasting toxicities, motilities and bioavailability while Cr(III) is an essential element because it regulates the glucose metabolism in the human body [4].

Cleaning up of chromium-contaminated sites is a challenging task because removal of Cr(VI) in aqueous solution is difficult [5]. Hence, proper treatment of tannery wastewater is essential before releasing it into the recipient environment. Many conventional techniques including chemical precipitation, membrane separation, ion exchange, reverse osmosis, and solvent extraction have been employed for the treatment of metal bearing industrial effluents [6]. However, these methods consume high amounts of energy and large quantities of chemical reagents. Furthermore, these are not economically viable because of high operating cost or difficulty in treating the solid wastes generated [7]. These constraints have caused the search for alternative, cost-effective technologies for metal sequestering to environmentally acceptable levels. In regards of its simplicity and high-efficiency characteristics even for a minute amount of metals, biological methods such as microbiological detoxification of polluted water are looked upon as a better technology [8].

Several types of biomasses have been investigated for their use in wastewater treatment for metal removal, such as yeast, bacteria and fungi [9]. One of the most ubiquitous biomass types available for bioremediation of metals at low pH is yeast. Yeast biomass is inexpensive and readily available. Furthermore, yeast cells retain their ability to remove a broad range of metals under a wide range of external conditions [10]. In particular, they are the convenient objects for such studies since some strains were found to be capable of growing at high concentrations of chromium and some of adsorbing or accumulating significant quantities in the cells and transforming them into chelated, less toxic forms [11].

The present work elucidates the mechanisms of Cr(VI) removal by *C. fabianii*. Preliminary studies were carried out to evaluate the Cr(VI) reduction capacity of the isolate. The Cr(VI) reductase activity in a soluble crude cell-free extract (CFE) and adsorption capacity of the yeast was also investigated. EDXA and SEM analyses were carried out to determine the localization of chromium on the surface of cells and FTIR was used to determine the functional groups involved in the interaction with chromium.

2. Materials and methods

2.1. Micro-organisms and growth conditions

Strain of *C. fabianii* used in the present work HE650139 has been isolated from chromium contaminated site located in Oued sebou, Fez (Morocco) [12]. Stock culture of the strain was maintained on YPG solid medium (2% glucose, 2% peptone, 1% yeast extract and 2% agar) and sub-cultured at monthly intervals. The liquid culture medium was prepared by mixing (2% glucose, 0.2% peptone and 0.2% yeast extract). The growth temperature for HE650139 was 30 °C. A stock solution of chromium was prepared by dissolving potassium dichromate ($K_2Cr_2O_7$) in distilled water and diluted to get the desired concentration.

2.2. Evaluation of chromium resistance and other metals tolerance

Contaminated habitats are generally characterized by the co-existence of a large number of toxic cations and, therefore, it is necessary to find out if the isolate will be capable of tolerating one or more of the metals. The minimum inhibitory concentration (MIC) was determined by plating 100 µL of culture on YPG agar amended with concentrations of Cr(VI) ranging from 100 to 2000 mg/L and of other metals such as Ni, Zn, Hg, Pb, Co, Cu and Hg, ranging from 100 to 4000 mg/L supplemented as ($NiCl_2$, $ZnSO_4$, $Pb(NO_3)_2$, $CoCl_2$, $CuSO_4$ and $HgCl_2$). The lowest concentration of metal that inhibit visible growth of the isolate was taken as the MIC.

All metals solutions were prepared in bi-distilled water and filter-sterilized.

2.3. Cr(VI) removal experiment

To test the ability of yeast to remove Cr(VI), a sterile solution of chromate (25, 50 and 100 mg/L) was added to the yeast culture with cell concentration 2 g/L (an early exponential phase of the growth with the optical density 1,9) and incubated with aeration at 30°C under continuous shaking (150 rpm). Media without cells served as control. Aliquots (200 µL) were taken at regular time interval and analyzed for chromium reduction. Chromate-reducing activity was determined as decrease of chromate over time.

The sum of chromium species remaining in the extra-cellular liquid, as well as the total chromium up-taken/absorbed by the cells after their mineralization was also determined. Yeast cells cultured in the presence of 25 and 50 mg/L of Cr(VI) for 48 hours, were harvested by centrifugation 6000 g for 20 min at 4°C. The supernatant (the extracellular fraction) was collected and then sterilized by filtration through a nitrocellulose filter of 0.45 µm, while the pellet was washed and mineralized by the hot nitric acid [13].

2.4. Cell fractionation

To prepare various fractions of cells, the yeast culture was grown overnight in 100 mL of YPG medium and harvested by centrifugation 6,000 g for 20 min at 4°C. The supernatant (the extracellular fraction) was collected and then sterilized by filtration through a nitrocellulose filter of 0.45µm, while cells (pellet) were washed twice with 20 ml of 10 mmol Tris HCl buffer pH 7.2 and suspended in 5 mL of Tris-HCl 25 mmol/L (pH 7.2). These cells suspensions were placed in ice and lysed using an ultrasonic probe (Sonics Vibra Cell 500, USA) for 15 min at a temperature of 0°C [14]. The efficiency of sonication was verified by microscopic observation and by cells plating on YPG agar plate. The sonicated material is then centrifuged at 12,000 g for 40 min at 4°C. The membrane fraction of sonicated cells (pellet) were separated on the intracellular fraction (supernatant) and immersed in the same volume of Tris-HCl (pH 7.2).

10 mg/L of Cr (VI) was added to the three fractions thus obtained (the extracellular, the intracellular (cytoplasmic) and the membrane fraction) to test their ability to reduce Cr(VI), The reaction mixture was incubated for 12h at 30°C. After centrifugation, the residual concentration of Cr(VI) was determined by using the diphenylcarbazide method. All fractions that were heated at 100°C for 30 min act as controls.

2.5. Scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDXA)

To understand the mechanism of complex metal–microbes interactions, it is important to determine the location of the chromium relative to the yeast cells. SEM (scanning electron microscopy) is frequently used to visualize the surface of a biosorbent and EDXA (energy dispersive X-ray analysis) is a useful instrument to evaluate the compositional characteristics before and after the biosorption of heavy metals.

Batch experiment using 50 mL of culture medium with 50 mg/L of Cr(VI) was conducted to visualize the change of the cell wall of the biosorbent caused by the sorption of Cr(VI). The pH condition of the solution was adjusted to pH 4. The mixture was harvested by centrifugation at 6000 g for 15 min. After two washes with phosphate buffer, cells were fixed in glutaraldehyde (2% final concentration), filtered onto nitrocellulose filters (0.2 μm), dehydrated in a graded series of ethanol concentrations (60% followed by 70%, 80%, 90% and 100%) and dried under a CO_2 atmosphere [15]. The dried filters were then examined with a Quanta 200 FEI scanning electron microscope. Energy dispersive X-ray analysis (EDXA) was performed to detect Cr and its compounds that had been adsorbed onto the cell surface.

2.6. FTIR analysis

Infrared spectra for *C. fabianii* cells grown in YPG broth supplemented with 50 mg/L of Cr(VI) and without Cr(VI) were obtained using a Fourier Transform Infrared Spectrometer (Bruker vertex 70 FT-IR spectrometer). Control and test cells loaded with Cr(VI) were pelleted by centrifugation at 6000 g, 4°C for 30 min, displayed over glass and dried at 50°C for 8 h. The FTIR spectra were collected at resolution of 4 cm^{-1} in the transmission mode (4000–400 cm^{-1}).

2.7. Analytical techniques

2.7.1. Cell concentration

The cell concentration was estimated by dry cell measurements. Absorbance of cells from overnight grown was measured at 600 nm using a spectrophotometer. Sample of cells culture with known cell concentrations were centrifuged at 6,000 g for 10 min, and the cell pellet was washed with distilled water and dried at 100°C to find out the dry weight of cells. Corresponding absorbance at 600 nm was converted to dry weight of cells.

2.7.2. Chromium analysis

Chromate removal was estimated by measuring remaining Cr(VI) concentration in the supernatant during the assay. A colorimetric method using the diphenylcarbazide (DPC) reaction was used. The pink colored complex, formed from 1,5-diphenylcarbazide (0.25% prepared in acetone) and Cr(VI) in acidic solution (H_2SO_4), was spectrophotometrically analyzed at 540 nm [16]. For determination of the total chromium up-taken/absorbed by the cells, aliquots of the washed cells were mineralized by burning in 3 mL of concentrated nitric acid (10 N). Samples were put in the test-tubes and heated at 120 °C for 2 hours. The solution was cooled, filtered, and diluted to 10 mL with distilled water. The resulting solution was analyzed for total chromium [13]. Total chromium concentration in the supernatant (extracellular) and in the mineralized cells was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

2.7.3. Statistical analysis

All the experiments were carried out in triplicate. The results were subjected to statistical analyzes and standard error of the means (S.E.M.) were calculated [17].
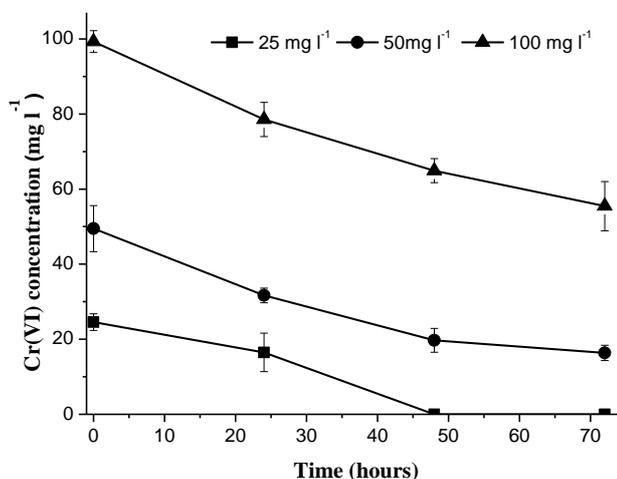
3. Results and Discussion

3.1. Metals yeast resistance

As various metals can be present in the industrial effluents, resistance of the isolate to various metallic salts has been determined. *Cyberlindnera fabianii* was found to be resistant to chromium up to a concentration of 1500 mg/L. The yeast strain was also checked for its resistance to various other metals, it was able to resist Co(II) (100 mg/L), Hg(II) (100 mg/L), Cu(II) (1000 mg/L), Pb(II) (4000 mg/L), Ni(II) (200 mg/L) and Zn(II)

(700 mg/L). A similar level of metal resistance was shown for filamentous fungi such as *Fusarium* sp. and *Penicillium* sp. isolated from polluted sites [18]. The results indicate that yeast isolate from heavy metal-contaminated sites have the ability to resist higher concentrations of metals than those from natural environments and indicate the microorganisms' adaptation capacity in stress conditions of hostile environments. Two major strategies can be used by microorganisms to protect themselves against heavy metal ion toxicity [11]. The first, avoidance, restricts metal ion entry into the cell, either by a reduced uptake/active efflux or by the formation of complexes outside the cells. The second, sequestration, reduces the levels of free ions in the cytosol, either by the synthesis of ligands to achieve intracellular chelation/reduction or by compartmentalization into vacuoles. Anyway, Cr(VI) contaminated environment may be a good original place for screening out micro-organisms with the Cr(VI) resistant and Cr(VI) reducing ability [8].

3.2. Chromium reduction studies in liquid medium

The ability of *C. fabianii* to remove Cr(VI) in liquid medium was examined. Chromate removal was monitored at different initial chromium concentrations ranging from 25 to 100 mg/L in aerobic conditions at 30°C. Figure 1 shows that 25 mg/L were reduced to zero in 48 h, while for 50 mg/L and 100 mg/L, about 50% were reduced in 72h.

Figure 1. Effect of initial Cr(VI) concentration (25-100 mg/L) on Cr(VI) removal

It can be observed that the time required for complete removal of Cr(VI) increased with the initial Cr(VI) concentrations. Similar results have been reported by several authors [19,20]. These results can be explicated by the mutagenic and toxicity of chromium for culture metabolism [21].

The calculation of the sum of the total chromium in the cultural liquid and in the mineralized cells close to the initial level of Cr(VI) (Table 1), which suppose the adsorption of the highly toxic and soluble hexavalent chromium and its transformation to the less toxic and less mobile trivalent form by this yeast. The chromium accumulated by *C. fabianii* cells was 2.6 mg/L (10%) and 5 mg/L (10%) in the presence of 25 mg/L and 50 mg/L of Cr(VI), respectively. Cr(III) concentration in the final solution may be calculated as the difference between the total chromium, soluble Cr(VI) and Cr concentration uptaken by the cells ([Cr]initial = [Cr(VI)]soluble + [Cr(III)]soluble +[Cr]up-taken/absorbed by the cells).

The capacity of Cr(VI) reduction by *C. fabianii* in our experiments was much greater than those found with *C. maltosa* who reduced only 35% of 50 μg/Lof Cr(VI) after 70 h of incubation [22]. However, it is important to take into account that the microbial chromate-reduction parameters are correlated with the medium composition and the cell density [4].

Table 1: Chromium content in the medium and in C. fabianii cells after incubation of the cells for 48 hours in YPG medium supplemented with 25 and 50 mg/L Cr(VI)

	25 mg L^{-1}	50 mg L^{-1}
Initial Cr(VI) in the medium	24.85 ± 0.21	49.94 ± 0.32
Total chromium in the medium	22.20 ± 0.16	44.60 ± 0.52
Total chromium content in the cells	02.60 ± 0.04	05.00 ± 0.07
Cr(VI) remaining in the medium	00.10 ± 0.01	20.90 ± 0.23

3.2. Biosorption potential of the cell wall and cytoplasm for removal of Cr(VI)
To our knowledge, it's the first report of the mechanism of Cr(VI) removal by *C. fabianii* strain. Thus with the objective of clarifying the mechanism of chromium removal by this strain, experiments to localize such activity in the yeast cells were carried out. Cell extracts were separated into extracellular, intracellular and membrane fractions and Cr(VI) reduction activity was assayed in vitro. Table 2 shows the activity at 30°C under aerobic conditions.

Table 2: Chromium content in the medium after incubation of heated and natural cellular fractions for 12 hours in medium supplemented with 10 mg/L Cr(VI)

	Concentration of Cr(VI) remaining in the medium (mg L^{-1})	
	Fraction heated at 100 °C	Natural fraction
Extracellular fraction	9.83 ± 0.21	9.86 ± 0.32
Intracellular fraction	9.78 ± 0.36	5.18 ± 0.19
Membrane fraction	4.11 ± 0.52	4.10 ± 0.36

Results indicate that the intracellular fraction could reduce about 48% of 10 mg of Cr(VI) in 4 hours, while membrane fractions could reduce 60% of 10 mg of Cr(VI) in the same time. No significant reduction was observed with the extracellular fraction. Intracellular fraction heated at 100°C for 30 min did not cause a decrease in Cr(VI), indicating that the reducing activity was heat labile, while for membrane fraction heated, a reduction of 60% of chromium was detected. These results may indicate that the mechanism of Cr(VI) removal by *C. fabianii* is "adsorption-coupled reduction", suggesting that after initial surface binding, chromate ions entered into the cytosol, and the intracellular fraction of this yeast remove chromium mainly through its reduction into less toxic Cr(III) compound via enzymatic interaction. Since the chromate reductase existing in the intracellular fraction lost its activity after being heated. Similar result was reported using *Thermoanaerobacter* [14].
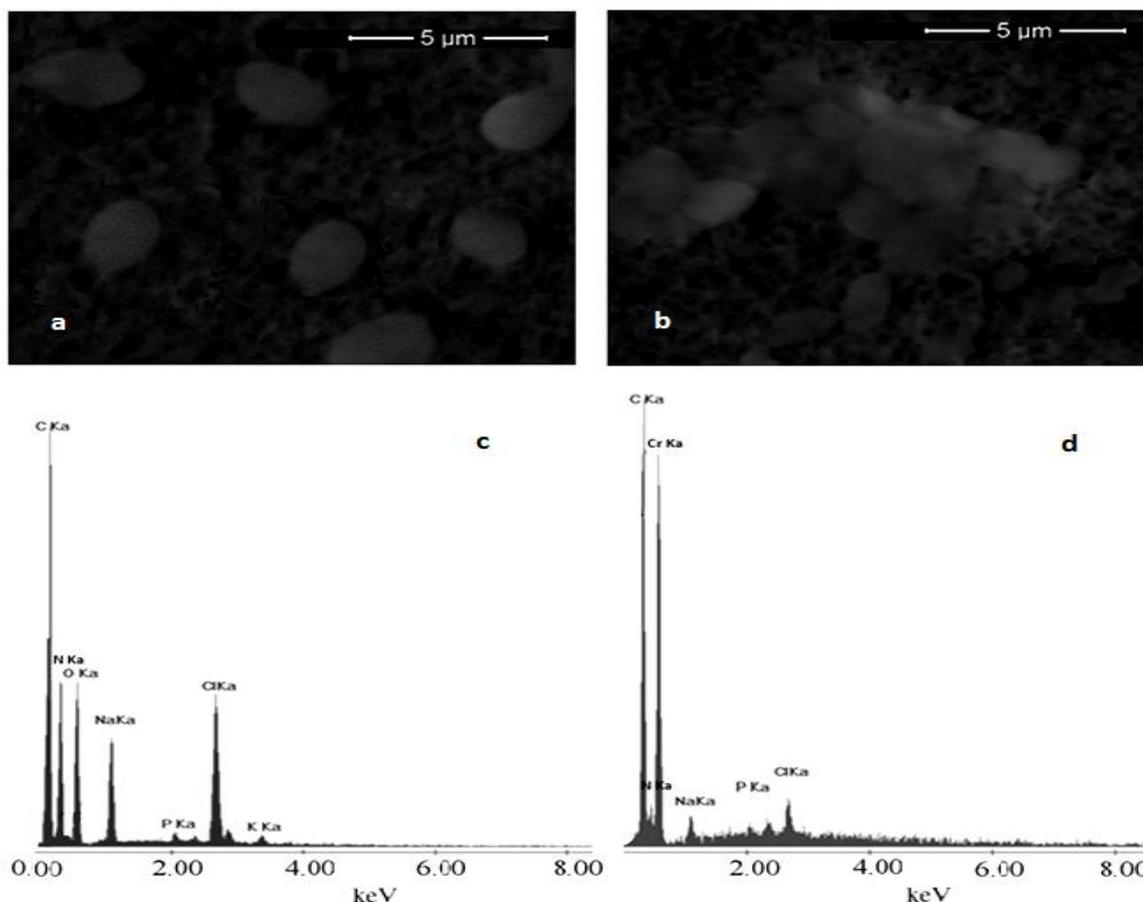
3.4. SEM and EDXA analysis
To determine the interactions between biosorption and morphological characteristics of C. fabianii, and to investigate the toxicity of Cr(VI), the cells were visualized by SEM. SEM micrographs for *C. fabianii* grown with and without Cr(VI) on YPG medium are presented in Figure 2(a,b).
There is a slight difference in the morphology of the two cultures. The cells grown with Cr(VI) were rounder, however, upon cultivation in 50 mg/L of Cr(VI) for 24 hours, the cells lost their shape and became irregular. This indicates that Cr(VI) has toxic effects on the cells. *Acinetobacter* sp. was also reported to undergo morphological changes upon contact with Cr(VI) such as the appearance of distinct ridges in the cell wall region [23]. Irregular surface with appearance of wrinkles were also observed for *A. haemolyticus* exposed to 30mg/L of Cr(VI) [24].
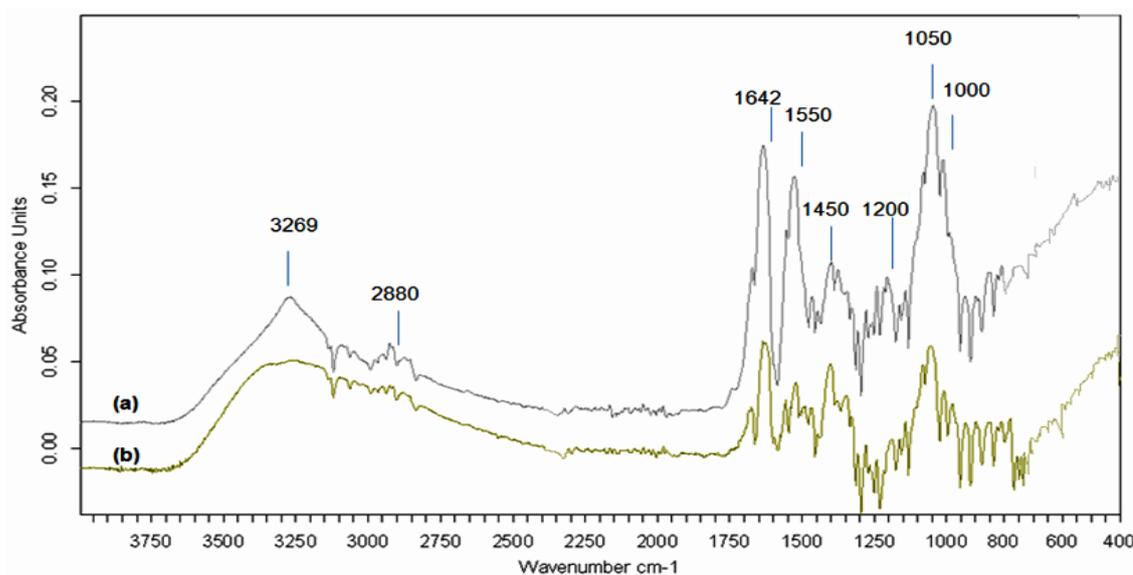Figure 2(c,d) shows a representative energy dispersive X-ray analysis (EDXA) spectrum of metal loaded biomass. The spectra recorded in spot profile mode, indicate the presence of N, C, O , Na, K, P and Cl (Figure 2c). We can suggest that these signals are due to Xray emissions from the polysaccharides and proteins present on the cell wall of the biomass [25]. In addition, elemental analysis as provided by EDXA showed specific peaks for chromium on surfaces of cells grown with Cr(VI) (Figure 2d) as opposed to those without Cr(VI) (Figure 2c), indicating the binding of metal ions on the biomass. Simultaneously, Figure 3b showed that the peaks of K and O were disappeared, while the peaks of N, Na and Cl were reduced, which suggested that an ion exchange mechanism might be involved [26,27]. Similar results were observed by other authors [25-27].

3.5. FTIR analysis
Biomass cell walls are made of large molecules (peptidoglycan) linked with teichoic acid and polysaccharides which can participate in chromium ions biosorption [28]. In order to determine the functional groups involved in the metal binding, FTIR analysis was carried out. In order to determine the functional groups involved in the metal binding, FTIR analysis was carried out. Figure 3 represent the FTIR spectra of control biomass and chromium treated biomass.

Figure 2. Scanning electron microscopy and representative energy dispersive X-ray spectrum of *C. fabianii* cells grown without (a, c) and with Cr(VI) (b, d) in aqueous solution. The pH of the medium was 4 and the contact time was 24 h.

Figure 3. FTIR spectra of *C. fabianii* cells grown without Cr(VI) (a) and with 50 mg/L of chromium (b)

The FTIR spectra of the control cells showed a number of peaks reflecting a complex nature of the yeast cell surfaces. There was a change in the intensity of the bands at different regions such as 1650–900 and 3500–2800 cm^{-1}, after interaction with Cr (VI). , these changes indicate that the metal binding process on the surface of the cells involves certain functional groups [27]. It is apparent that there were changes in the absorption

peak frequencies between 3250 and 3450 cm^{-1} which are attributed to –OH of glucose and –NH stretching of the protein and acetamido group [29, 30]. There was also a slight change in the intensity of the bands at peak 2950 cm^{-1}. This peak could be assigned to the CH$_2$ asymmetric stretch [31, 32]. Changes were also detected in the absorption peak shifted at 1642, 1550 and 1400 cm^{-1} corresponding to amide I (protein C–O stretching), amide II (protein N–H bend, C–N stretch) and amide III (protein C–N stretch) respectively [33, 34]. The presence of CH$_2$ asymmetric stretching band is mainly due to the lipids, while the existence of amide I, amide II and amide III largely owes to proteins. Another characteristics peak at 1,100–1,030 and 1,050– 910 cm-1 relate to phosphate functional groups such as orthophosphate (PO$_4^{3-}$), and P–OH respectively [35]. However, the peaks occur around the same position as C–N stretching and C–O of polysaccharides which is between 1,350–1,000 cm^{-1} [36] and 1065–1110 cm^{-1} [37] respectively. These peaks confirmed the presence of bonded hydroxyl group, CH$_2$ asymmetric stretch, amide and phosphate functional groups in the external surface of the adsorbent. Similar observations were made with *C. miniata* and *A. haemolyticus* [35, 38].

3. Conclusion

Kinetics study reveals that *C. fabianii* isolated from chromium contaminated site can effectively remove Cr(VI) with concentrations ranging between 25 and 100 mg/L. The removal of chromium by this strain is due to adsorption of Cr(VI) and its reduction into less toxic Cr(III) compound. Scanning electron microscopy equipped with energy dispersive X-ray analysis reveal that the chromium localizes in the cell wall. FTIR study indicates that hydroxyl group, CH$_2$ asymmetric stretch, amide I, amide II, amide III and phosphate were the functional groups for binding Cr(VI) by this strain. *Cyberlindnera fabianii* offers excellent potential for Cr(IV) removal from contaminated sites and thus, the comprehension of chromium elimination mechanism by *C. fabianii* is important for targeting the process of chromium bioremediation and also minimizing treatment costs.

References
1. Rehman, A., Anjum, M.S., *Water, Air, Soil Pollut*. 205 (2010) 149–159.
2. Chisti, Y., *Biotechnol. Adv*. 6 (2004) 431–432.
3. Papp, J.F., Knoerr, A.W., Washington, DC Bureau of Mines bulletin 675 U, S: Government Printing Office. (1985) 139.
4. Wang, Y., Washington: American Society for Microbiology Press. (2000) 225–235.
5. Singanan, M., Abebaw, A., Singanan, V., Shoa, W., Town, A., *Electronic J. Environ. Agr. Food Chem*. 11 (2007) 2557-2564.
6. Alguacil, F.J., Alonso, M., Lopez, F., Lopez-Delgado, A., *Chem*. 72 (2008) 684–689.
7. Silver, S., London: Kluwer Academic Publishers (1991) 265–287.
8. Shakoori, A.R., Rehman, A., Haq, R.U., *Bull. Environ. Contam. Toxicol*. 72 (2004) 1046–1051.
9. Padma, S.V., Bajpai, D., *Desalination* 222 (2008) 255–262.
10. Villegas, L.B., Amoroso, M.J., De-Figueroa, L.I.C., *J. Basic Microbiol*. 45 (2005) 381–391.
11. Ksheminska, H., Honchar, T.M., Gayda, G.Z., Gonchar, M.V., *Cent. Eur. J. Biol*. 1 (2006) 137–149.
12. Bahafid, W., Tahri, J.N., Sayel, H., Iraqui, H.M., EL Ghachtouli, N., *Geomicrobiol. J*. (2012) DOI:10.1080/01490451.2012.705228.
13. Guillen-Jimenez, F.D.M., Morales-Barrera, L., Morales-Jimenez, J., Hugo Hernandez Rodrıguez, C., Cristiani-Urbina, E., *J. Ind. Microbiol. Biotechnol*. 35 (2008) 1277–1287.
14. Bhowmick, D.C., Bal, B., Chatterjee, N.S., Ghosh, A.N., Pal, S., *J. App. Microbiol*. 106 (2009) 2006–2016.
15. Baldi, F., Vaughan, A.M., Olson, G.J., *App. Environ. Microbiol*. Vol. 56 (1990) 913-918.
16. Pattanapipitpaisal, P., Brown, N.L., Macaskie, L.E., *App. Microbiol. Biotechnol*. 57 (2001) 257–261.
17. Steel, R.G.D., Torrie, J.H., London: McGraw Hill International Book Company (1981).

18. Ezzouhri, L., Castro, E., Moya, M., Espinola, F., Lairini, K., *Afr. J. Microbiol. Res.*, 3 (2009) 035–048.
19. Cardenas-Gonzalez, J.F., Acosta-Rodriguez, I., *Bioinorg. Chem. Appl*. (2010) doi: 10.1155/2010/676243.
20. Sayel, H., Bahafid, W., Tahri, J.N., Derraz, K., Fikri, B.K., Ibnsouda, K.S., El Ghachtouli, N., *Annal Microbiol*. 62 (2012) 1269–1277
21. Wang, Y.T., Shen, H., *Water Res*. 31 (1997) 727–32.
22. Ramirez-Ramirez, R., Calvo-Méndez, C., Avila-Rodriguez, M., Lappe, P., Ulloa, M., Vazquez-Juarez, R., Gutiérrez-Corona, J.F., *Antonie van Leeuwenhoek*. 85 (2004) 63–68.
23. Srivastava, S., Thakur, I.S., *Biodegradation* 18 (2007) 637–646.
24. Pei, Q.H., Shahir, S., Raj, A.S.S., Zakaria, Z.A., Ahmad, W.A., *World. J. Microbiol*. Biotechnol. 25 (2009) 1085–1093.
25. Das, K. S., Guha, K. A., *Colloids Surf.* B. 60 (2007) 46–54.
26. Huang, L., Zeng, G., Huang, D., Li, L., Du, C., Zhang, L., *Environ. Earth Sci*. 60(8) (2009) 1683–1691
27. Bueno, B.Y.M., Torem, M.L., Molina, F., De Mesquita, L.M.S., *Miner. Eng*. 21 (2008) 65–75.
28. Baysal, Z., Cinar , E., Bulut, Y., Alkan, H., Dogru, M., *J. Hazard. Mater*. 161 (2009) 62–67.
29. Ting, Y.P., Deng, S., *Water Res*. 39 (2005) 2167–2177.
30. Abraham, T.E., Bai, R.S., *Water Res*. 36 (2002) 1224–1236.
31. Park, D., Yun, Y.S., Park, J.M., *Chem*. 60 (2005) 1356–1364.
32. Kapoor, A., Viraraghavan, T., *Bioresource Technol*. 61 (1997) 221–227.
33. Garip, S., Gozen, A.C., Severcan, F., *Food Chem*. 113 (2009) 1301–1307.
34. Jain, M., Garg, V.K., Kadirvelu, K., *J. Hazard. Mater*. 162 (2009) 365–372.
35. Quek, H.P., Shafinaz, S., Santhana, R., Zainul, A.Z., Wan, A.A., *World. J. Microbiol. Biotechnol*. 25 (2009) 1085–1093.
36. Mungasavalli, D.P., Viraraghavan, T., Jin, Y.C., *Colloids Surf. A* 301 (2007) 214–223.
37. Mishra, S., Doble, M., *Ecotoxicol. Environ. Saf*. 71 (2008) 874–879.
38. Han, X., Wong, Y.S., Wong, M.H., Tam, N.F.Y., *J. Hazard. Mater*. 146 (2007) 65–72.

(2013) ; http://www.jmaterenvironsci.com/