

ABSTRACT

Cr (VI) is a toxic metal, which belongs to the list of priority pollutants due to its mutagenic and carcinogenic properties, defined by the US EPA. Contamination comes from electroplating, leather tanning, textile dyeing and metal finishing industries. Recently, a variety of low cost materials has been studied for their ability to removal this metal from aqueous solution with promising results. We studied the removal capacity of Cr (VI) in solution by the *Opuntia ficus-indica* biomass, using the Diphenylcarbazide method to evaluate the metal concentration. Therefore, the highest biosorption of the metal (50 mg/L) occurs within 30 minutes, at pH 1.0, and 28°C. According to temperature, the highest removal was observed at 60°C, in 9 minutes, when the metal (50 mg/L) is completely adsorbed. At the concentrations of Cr (VI) and biomass analyzed, we observe an excellent removal capacity, besides, it removal efficiently the metal *in situ* (100% removal, at 6 and 7 days of incubation, 5 g of biomass, for water and earth contaminated), and after of 80 minutes of incubation, the studied biomass reduces 1.0 g of Cr (VI) with the simultaneous production of Cr (III), so it can be used to eliminate it from industrial wastewater.

KEYWORDS: Chromium (VI), Removal, Biomass, *Opuntia Ficus-indica*

INTRODUCTION

Chromium is regarded as an environmental pollutant due to its wide use in various industrial activities, such as electrolytic plating, leather tanning, explosives manufacturing etc. The stable forms of chromium in the environment are trivalent (Cr (III)) and hexavalent chromium (Cr (VI)). Further, Cr (VI) is highly soluble, making it mobile in soil and aquatic environments, with consequent toxicity ecosystems. Chromium in their different forms can be use in the production of steel alloys and other metals chromed, for dyes and pigments, and the preservation of leather and wood. It can also be find naturally in the soil [1]. The primary forms of chromium found in nature are Cr (III) and Cr (VI) and these forms are converted to each other depending on environmental conditions [2]. Cr (VI) is consider the most toxic form of chromium, and is usually associated with oxygen as chromates (CrO_4^{2-}) and dichromate ($\text{Cr}_2\text{O}_7^{2-}$) [3], which due to its high solubility are highly mobile in soil environments and water [2]. Moreover, Cr (III) is in the form of oxides, hydroxides or poorly soluble sulfates, by which it is much less mobile, and there joined organic matter in the soil and aquatic environments [4,5]. Cr (VI) is a strong oxidizing agent, and in the presence of organic matter is reduced to Cr (III); this transformation is faster in acidic environments [3]. However, high levels of Cr (VI) may exceed the reducing capacity of the environment and thus can persist as a contaminant. It has been established now that various chromium compounds as oxides, chromates and dichromate, are environmental contaminants in water, soil, and industrial effluents, because this metal is widely used in various manufacturing, such as electrolytic plating, explosives manufacturing, leather tanning, metal alloy, dyes and pigments manufacturing, etc. [1, 5]. There are studies of many methods for removal of chromium ion present in water industrial waste, for example: ion exchange on resins, coagulation-flocculation, adsorption on activated carbon, reduction, chemical precipitation, sedimentation, etc., [6], which in most cases are expensive or inefficient, especially when the concentration of these ions is very low [6]. Therefore arise emerging technologies such as biosorption, the process of attracting various chemical species by biomass (live or dead), by physicochemical mechanisms as adsorption

or ion exchange [6]. Recently, varieties of low cost materials have been studied for their ability to remove Cr (VI) from aqueous solution and promising results are shown. Among these low cost adsorbents are dead microorganisms, clay minerals, agricultural wastes, industrial wastes and various other low cost materials [1, 7]. Thus, there is a need to develop or find innovative low cost adsorbents with an affinity towards metal ions for the removal of Cr (VI) from aqueous solution, which leads to high adsorption capacity [6-9]. The objective of this study was to analyze in vitro biosorption of Cr (VI) by *Opuntia ficus-indica* biomass.

MATERIALS AND METHODS

Biosorbent used

The *Opuntia ficus-indica* biomass was obtained from the store offered in the market place Republic, in February 2016, of the capital city of San Luis Potosí, S.L.P. México. To obtain the biomass, the product was washed with trideionized water 72 hours under constant stirring, with water changes every 12 hours. Subsequently, boiled 1 hour to remove traces of dust, and were dry at 80°C for 12 hours in the oven, ground in blender and stored in amber vials until use.

Biosorption studies and determination of hexavalent chromium

In these studies, was used 1 g of dried biomass mixed with 100 mL, containing 50 mg/L of the metal bearing solution in an Erlenmeyer flask at the desired temperature and pH. The flasks were agitated on a shaking bath Yamato BT-25 model at different times. Samples of 5 mL were taken at different times and centrifuged at 3000 rpm for 5 min. The supernatant liquid was separated and analyzed for Cr (VI) ions. Hexavalent Chromium and trivalent Chromium were quantify by a Spectrophotometric method employing Diphenylcarbazide and chromazurol S, respectively [10, 11], total Chromium was determine by electrothermal atomic absorption spectroscopy [10]. The information shown in the results section are the mean from three experiments carried out by triplicate. The information shown in the results section are the mean from three experiments carried out by triplicate.

RESULTS AND DISCUSSION

The optimum time and pH for Cr (VI) removal for *Opuntia ficus-indica* biomass was 30 minutes and pH 1.0, at constant values of biosorbent dosage (1 g/100 mL), with an initial metal concentration (50 mg/L), and temperature of 28°C (Figure 1). It was used a pH meter Corning Pinnacle 530 model and we use nitric acid 1M to maintain the pH. Adsorption efficiency of Cr (VI) was observe a maximum at pH 1.0 with the biomass analyzed. As the initial pH values increased from 1.0 to 4.0, the removal efficiencies of Cr (VI) with the biomass decreased from 100% to 42.9%, respectively (Figure 1). It has been reported, an optimum time of 48 h for the removal of Cr (VI) by the fruiting body of *Auricularia polytricha*, which is one of the most common edible mushrooms in China and other places in the world [12], 48 min for the removal of Cr(VI) from aqueous solutions using powder of potato peelings [13], 30 minutes for the removal of Cr (VI) by the *Citrus reticulata* shell [14], 70 minutes for *Citrus paradise* shell [15], and 24 h for modified Russian knapweed flower powder [16]. Changes in the permeability of unknown origin, could partly explain the differences founded in the incubation time, providing greater or lesser exposure of the functional groups of the cell wall of the biomass analyzed [1,6]. Adsorption efficiency of Cr (VI) was observe a maximum at pH 1.0 with the biomass analyzed, and this is like to some reports: *C. reticulata*, *C. Paradise*, *Mammea americana* L. and *Litchi chinensis* shells [7,8,12,13], and for the fruiting body of *A. polytricha*, [12], but are different for the removal of the same metal with powder of potato peelings [13], who report an optimum pH of 2.5, a pH of 2.0 for modified Russian knapweed flower powder [16], a pH of 4.0 using chitosan [17], for the removal of lead (II) in solution with african palm bagasse [18], and a pH of 2.0 with granular activated charcoal prepared of *Cordia macleodii* tree bark [9]. This was due to the dominant species (CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$) of Cr ions in solution, which were expect to interact more strongly with the ligands carrying positive charges [1].

Temperature was found to be a critical parameter in the bioadsorption of Cr (VI) (Figure 2). To maintain constant the temperature in all experiments, we use a shaking bath Yamato BT-25 model. The total removal was observed at 60°C with 9 minutes of incubation, and our results are coincident for tamarind shell with 95% of removal at 58°C and 3 h [19], for the adsorption of the same metal with *C. reticulata*, *C. Paradise*, *M. americana* L., shells [7,8,12, and 15], and for the granular activated charcoal prepared of *C. macleodii* tree bark [9], but, are different for the mandarin waste [20], *Caladium bicolor* (wild cocoyam) biomass [21], and *Saccharomyces cerevisiae* [22]. The increase in temperature increases the rate of removal of Chromium (VI) and decrease the contact time required for complete removal of the metal, to increase the redox reaction rate [19].

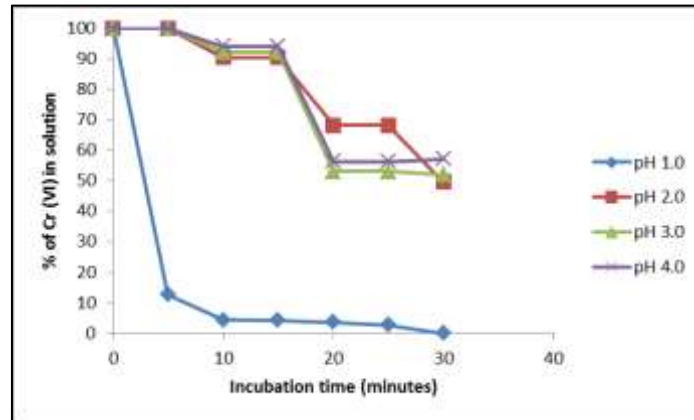


Figure 1. Effect of incubation time and pH on Chromium (VI) removal by the biomass of *Opuntia ficus-indica*. 50 mg/L Cr (VI), 100 rpm, 28°C. 1 g of biomass.

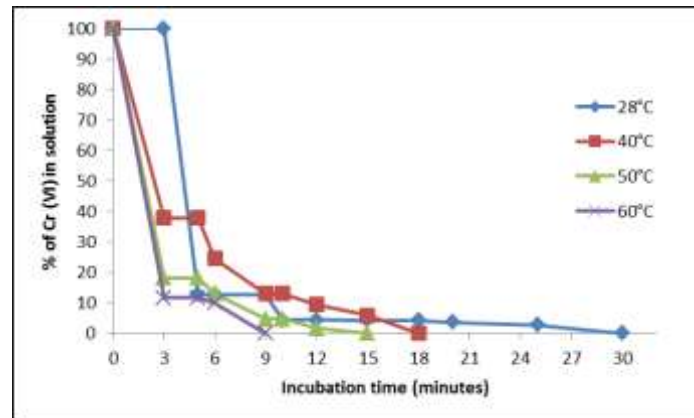


Figure 2. Effect of the temperature on Chromium (VI) removal by the biomass of *Opuntia ficus-indica*. 50 mg/L Cr (VI), 100 rpm, pH 1.0, 1 g of biomass.

It was found that the concentration of the metal does not influence in the removal of the same, since the analyzed concentrations (200-1000 mg/L) are eliminated at 70 minutes (Figure 3). This results are like for sawdust pine tree [8], and different natural biomasses [23], but are different for the granular activated charcoal prepared of *C. macleodii* tree bark [9], for *C. reticulata*, [14], *Tamarindus indica* shell [19], the fruiting body of *A. polytricha* [12], for modified *Acroptilon repens* (Russian knapweed) flower powder [16], for the removal of Cr (VI) from aqueous solutions using powder of potato peelings [15], and chitosan [17]. With respect to other biomasses, most authors report lower removal efficiencies of metal, for example: 45 mg/L for eucalyptus bark [24], 13.4 and 17.2 mg/L for bagasse and sugar cane pulp, 29 mg/L coconut fibers, 8.66 mg/L for wool [25], 25 and 250 mg/L of chitin and chitosan [26], and 1 mg/L for cellulose acetate [27]. The increase in initial concentration of Cr (VI), results in the increased uptake capacity and decreased in the percentage of removal of the metal. This was due to the increase in the number of ions competing for the available functional groups on the surface of biomass [19]. In addition, we observe the development of a blue-green and white precipitate, which changes more rapidly at higher concentration, and indicate the production of Cr (III) (Figure 4), which are similar for the adsorption of the same metal with *C. reticulata*, *C. Paradise*, *M. americana* L., shells [7,8,12, and 15],

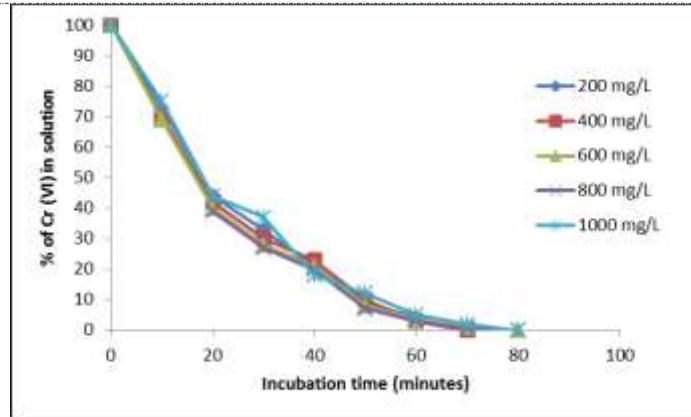


Figure 3. Effect of the initial concentration of metal on Chromium (VI) removal by the biomass of *Opuntia ficus-indica*, 100 rpm, 28°C, pH 1.0, 1 g of biomass.

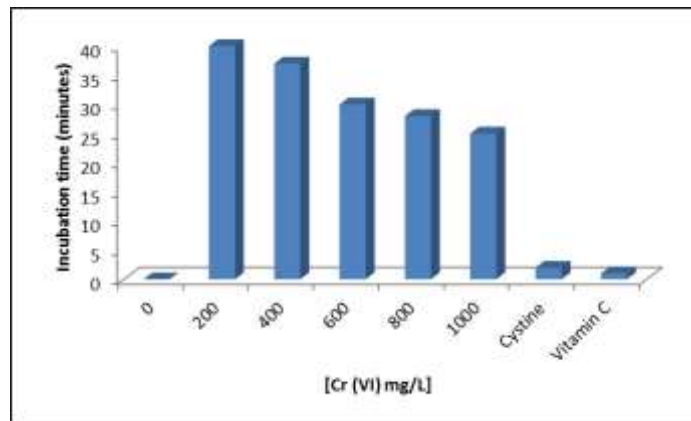


Figure 4. Formation of green color by different concentration of Chromium (VI) by the biomass of *Opuntia ficus-indica*, 100 rpm, 28°C, pH 1.0, 1 g of biomass.

On the other hand, the influence of biomass concentration on the removal capacity of Cr (VI) is depict in Figure 5. If we increase, the amount of biomass also increases the removal of the metal in solution, with more biosorption sites of the same, because the amount of added biosorbent determines the number of binding sites available for metal biosorption [29]. similar results have been reported for modified corn stalks [29], *C. reticulata*, *C. Paradise M. americana* L. and *L. chinensis* shell [7,8, 14, 23], which, removal efficiency increased from 88% to 100%, 56.1% to 91.7% and 45% to 79.2% at concentrations of 2, 10 and 15 mg/L, respectively, and for the removal of Cr(VI) from aqueous solutions using powder of potato peelings [13], which a complete removal is attained after only 48 minutes with a dosage of 4 g/L, whilst a dosage of 0.5 g/L requires 120 minutes to attain $96 \pm 2\%$ removal. But they are different from those reported for the fruiting body of *A. polytricha*, for modified *A. repens* (Russian knapweed) flower powder [16], with a removal amount of Cr (VI) reached the highest at the dosage of 4 g/L and then declined with the dosage, and with wastes biomass of mandarin (gabasse), with an optimal concentration of biomass of 100 mg/L [20].

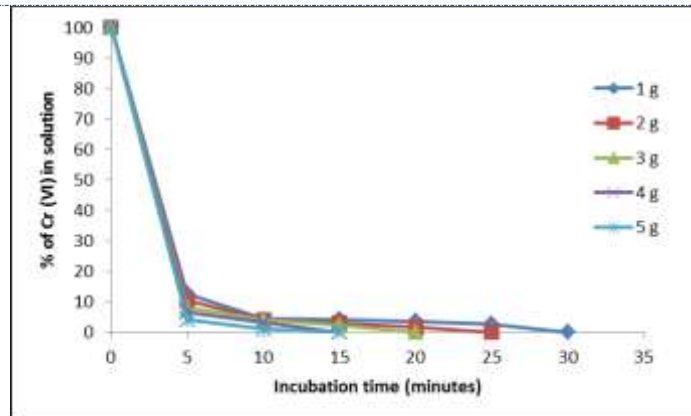


Figure 5. Effect of the initial biomass concentration on the removal of 200 mg/L of Chromium (VI), by the biomass of *Opuntia ficus-indica*, 100 rpm, 28°C, pH 1.0.

The ability of this biomass of to lower the initial Cr (VI) of 1.0 g/L and Cr (III) production in solution was analyzed. Figure 6 shows that the biomass exhibited a remarkable efficiency to diminish Cr (VI) level with the concomitant production of Cr (III) in the solution (indicated by the formation of a blue-green color and a white precipitate, and his determination for Cromazurol S, date not shown). Thus, at 80 min of incubation, the biomass analyzed, caused a drop in Cr (VI) from its initial concentration of 1.0 g/L to almost undetectable levels, and the decrease level occurred without change significant in total Chromium content. As expected, total Chromium concentration remained constant over time, in solution control. These observations indicate that this biomass is able to reduce Cr (VI) to Cr (III) in solution. Furthermore, as this biomass contains vitamin C and some carbohydrates, we found that vitamin C and cystine reduce faster Cr (VI) to Cr (III), and could be very important part in the metal reduction (date not shown), confirming some reports in the literature [14,31,32, and 33].

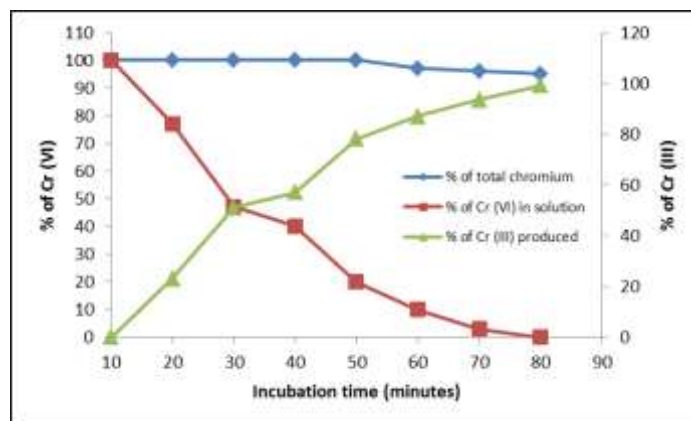


Figure 6. Time-course of Cr (VI) decrease and Cr (III) production in solution with 1.0 g/L Cr (VI). 28°C, pH 1.0, 100 rpm.

Finally, we adapted a water-phase bioremediation assay to explore possible usefulness of this biomass for eliminating Cr (VI) from industrial wastes, the biomass (5 g), was incubate with non-sterilized contaminated earth and water containing 297 mg Cr (VI)/g, and 200 mg Cr(VI)/L, suspended in trideionized water. It was observe that in 6 and 7 days of incubation with the biomass, the Cr (VI) concentration of water and earth samples decrease totally (Figure 7), and the decrease level occurred without change significant in total Chromium content during the experiments. In the experiment carried out in the absence of the biomass, the Cr (VI) concentration of the earth samples decreased by about of 18% (date not shown); this might be caused by indigenous microflora and (or) reducing components present in the soil. In the literature, was reported the ability of the yeast *Candida tropicalis* to remove Cr was tested in artificially contaminated soils in a microcosm system

to simulate natural environmental conditions, showing reduction of 72.2% of 40 mg/L Cr (VI) [34]. An *A. niger* strain isolated from a tannery was used in an airlift reactor for the treatment of tanning wastewater, leading to maximum removal efficiency by adsorption of 88 % of an initial Cr (III) concentration of 1300 mg/L [35]. These results are even better than those obtained (removal efficiency of 72%) with the same organism using a stirred tank reactor [36]. The use of a consortium of Cr-resistant fungi immobilized in a support material in a stirred tank bioreactor filled with wastewater from a tannery achieved an overall removal efficiency of 99.9 % of the total Cr present.

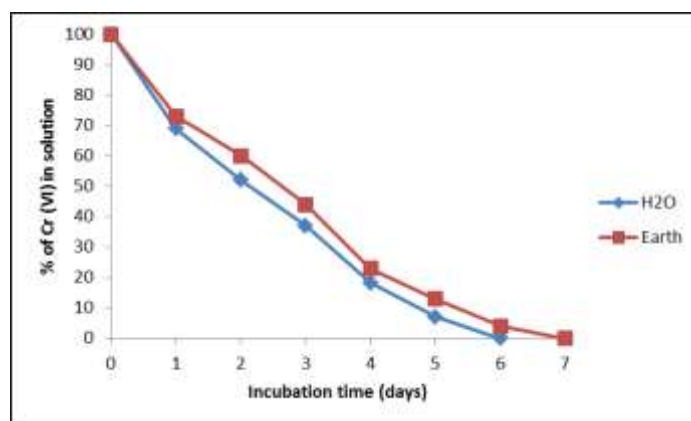


Figure 7. Removal of Cr (VI) in industrial wastes incubated with 5 g of biomass. 28°C, 100 rpm, 20 g and 100 mL of contaminated earth and water (297 mg Cr (VI)/g, pH 8.2, and 200 mg Cr(VI)/L, pH 6.2).

CONCLUSION

The biomass analyzed, showed complete capacity of biosorption of 50 g/L Cr (VI) in solution at different time of incubation, at 28°C, 100 rpm with 1 g of biomass, besides this removal the metal *in situ* (6 and days of incubation, 5 g of biomass), in water and earth contaminated. These results suggest their potential applicability for the remediation of Cr (VI) from polluted soils in the fields.

REFERENCES

- [1] M. Ahemad, "Bacterial mechanisms for Cr(VI) resistance and reduction: an overview and recent advances". FOLIA MICROBIOLOGICA, VOL. 59, 321–332, 2014.
- [2] J.F. Gutiérrez-Corona, P. Romo-Rodríguez, F. Santos-Escobar, A.E. Espino-Saldaña, and H. Hernández-Escoto, "Microbial interactions with chromium: basic biological processes and applications in environmental biotechnology", WORLD JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, VOL. 32, No. 1-9, 2016.
- [3] H. Al-Battashia, S. J. Joshib, B. Pracejusc, and A. Al-Ansaria, "The Geomicrobiology of Chromium (VI) Pollution: Microbial Diversity and its Bioremediation Potential", THE OPEN BIOTECHNOLOGY JOURNAL, Vol. 10 (Supl-2, M10), 379-389, 2016.
- [4] O. F. Artunduaga Cuellar, "Tratamientos para la remoción de Cromo (VI) presente en aguas residuales", REVISTA NOVA COLOMBIA, VOL. 1, No. 1, 66-73, 2015.
- [5] F. Vendruscolo, G.L. da Rocha Ferreira, N.R. Antoniosi Filho, "Biosorption of hexavalent chromium by microorganisms", INTERNATIONAL BIODETERIORATION & BIODEGRADATION, XXX, 1-9, 2016. In press.
- [6] C. Tejada-Tovar, A. Villabona-Ortiz y L. Garcés-Jaraba. "Adsorción de metales pesados en aguas residuales usando materiales de origen biológico". TECNO LÓGICAS, VOL. 18, No. 34, 109-123, 2015.
- [7] I. Acosta Rodríguez, J.F. Cárdenas González, M.G. Moctezuma Zárate, J. Tovar Oviedo, M.Z. Acosta Nava, V.M. Martínez Juárez, and F. Navarro Castillo, "Biosorption of Chromium (VI) by *Cucumis melo* Shell", JOURNAL OF MULTIDISCIPLINARY ENGINEERING SCIENCE AND TECHNOLOGY (JMEST), VOL.2, No. 5, 988-993, 2015.

- [8] I. Acosta-Rodríguez, K.C. Ruíz-Trujillo, J.F. Cárdenas González, M.G. Moctezuma-Zarate, and V.M. Martínez-Juárez, "Chromium (VI) removal by Sawdust of pine tree", INTERNATIONAL JOURNAL OF LATEST RESEARCH IN SCIENCE AND TECHNOLOGY, VOL. 4, No. 2, 124-128, 2015.
- [9] A. K. Wanjari, and U. E. Chaudhari, "Removal of Cr (VI) from aqueous solution using granular activated charcoal prepared from *Cordia Macleodii* tree bark and surface modified granular activated charcoal: a comparative study via spectroscopic characterizations", IJESRT, VOL. 5, No. 10, 304-316, 2016.
- [10] A.E. Greenberg, L.S. Clesceri y A.D. Eaton. Standard methods for the examination of water and wastewater. 18a. ed. American Public Health Association, Washington, D.C. 3, pp. 58-3.60, 187-190 (1992).
- [11] R.P. Pantaler, and I.V. Pulyaeva, "A spectrophotometric study of complexation between chromium and chromazurof S", JOURNAL OF ANNALS OF CHEMISTRY (Moscow), VOL. 40, 1634-1639, 1985.
- [12] S. Zheng, H. Huang, R. Zhang, and L. Cao, "Removal of Cr(VI) from aqueous solutions by fruiting bodies of the jelly fungus (*Auricularia polytricha*). APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, VOL. 98, 8729-8736. 2014.
- [13] F. Mutongo, O. Kuipa, and P.K. Kuipa, "Removal of Cr(VI) from aqueous solutions using powder of potato peelings as a low cost sorbent", BIOINORGANIC CHEMISTRY AND APPLICATIONS, VOL. Vol. 2014, Article ID 973153, 7 pages. <http://dx.doi.org/10.1155/2014/973153>. 2014.
- [14] I. Acosta, E., Coronado, J.F., Cárdenas, J. Tovar, and V.M. Martínez, "Hexavalent chromium removal by *Citrus reticulata* Shell". JOURNAL OF NATURAL SCIENCES, VOL. 1, No. 1, 29-39. 2013.
- [15] I. Acosta-Rodríguez, J.F. Cárdenas-González, M.G., Moctezuma-Zarate, A. Rodríguez-Pérez, E. Enríquez-Domínguez, J. Tovar-Oviedo, V.M. Martínez-Juárez, and M.Z. Acosta-Nava, "Biosorption of Chromium (VI) by *Citrus paradise* Shell". JOURNAL OF MULTIDISCIPLINARY ENGINEERING SCIENCE AND TECHNOLOGY, (JMEST), VOL. 3, No. 6, 5056-5061, 2016.
- [16] M.T., Ghaneian, M. Dehvari, M. and B. Jamshidi, "A batch study of hexavalent chromium removal from synthetic wastewater using modified Russian knapweed flower powder". INTERNATIONAL JOURNAL OF ENVIRONMENTAL HEALTH ENGINEERING, VOL. 2, No. 5, 38-46, 2013.
- [17] S. Sobhanardakani, R., Zandipak, M., Bonyadi, H., Parvizimosaed, M. Moslemi, M. Tahergorabi, and S.M. Hosseini, "Evaluation of removal efficiency of Cr (VI) ions from aqueous solution using chitosan". JOURNAL OF CHEMICAL HEALTH RISKS, VOL. 5, No. 1, 29-38. 2015.
- [18] C. Tejada Tovar, E. Ruiz Paternina, J. Gallo Mercado, and J. Moscote Bohorquez, "Evaluación de la biosorción con bagazo de palma africana para la eliminación de Pb (II) en solución". PROSPECT, VOL. 13, No. 1, 59-67, 2015.
- [19] G.S., Agarwal, H. Kumar, and S. Chaudari, "Biosorption of aqueous chromium (VI) by *Tamarindus indica* seeds". BIORESOURCE TECHNOLOGY, VOL. 97, 949-956, 2006.
- [20] A. Zubair, H.N. Bhatti, M.A. Hanif, and F. Shafqat, "Kinetic and equilibrium modeling for Cr(III) and Cr(VI) removal from aqueous solutions by *Citrus reticulata* waste biomass". WATER AIR SOIL POLLUTION, VOL.191, 305-318, 2008.
- [21] M.H. Jnr, and A.I. Spiff, "Effects of temperature on the sorption of Pb²⁺ and Cd²⁺ from aqueous solution by *Caladium bicolor* (wild cocoyam) biomass". ELECTRONICAL JOURNAL OF BIOTECHNOLOGY, VOL. 8, No. 2, 162-169, 2005.
- [22] A. Ozer, and D. Ozer, "Comparative study of the biosorption of Pb (II), Ni (II) and Cr (VI) ions onto *Saccharomyces cerevisiae*: Determination of biosorption heats". JOURNAL OF HAZARDOUS MATERIALS, VOL. 100, 219-229, 2003.
- [23] I. Acosta Rodríguez, J.F. Cárdenas González, M.E. Torre Bouscoulet, A. González Estrada, S.E. Guerrero Martínez, C.Y. Salazar Salazar, V.M. Martínez Juárez y L.E. Frago Morales, "Biosorption of chromium (VI) by different natural biomasses". JOURNAL OF MULTIDISCIPLINARY ENGINEERING SCIENCE AND TECHNOLOGY (JMEST), VOL. 2, No. 7, 1736-1741, 2015.
- [24] V. Sarin, and K.K. Pant, "Removal of chromium from industrial waste by using eucalyptus bark". BIORESOURCE TECHNOLOGY, VOL.97, 97: 15-20, 2006.
- [25] M.M. Dakiky, M. Khamis, A. Manassra, and M.Mereb, "Selective adsorption of chromium (VI) in industrial wastewater using low-cost abundantly available adsorbents". ADVANCES IN ENVIRONMENTAL RESEARCH, VOL. 6, 533-540, 2002.
- [26] Y. Sag, and Y. Aktay, "Kinetic studies on sorption of Cr (VI) and Cu (II) ions by chitin, chitosan and *Rhizopus arrhizus*". BIOCHEMICAL ENGINEERING JOURNAL, VOL. 2, 143-153, 2002.

- [27] G. Arthanareeswaran, P. Thanikaivelan, N. Jaya, D. Mohan, and M. Raajenthiren, "Removal of chromium from aqueous solution using cellulose acetate and sulfonated poly (ether ketone) blend ultrafiltration membranes". *BIOCHEMICAL ENGINEERING JOURNAL*, VOL. 12: 43-153, 2002.
- [28] R.P. Pantaler, and I.V. Pulyaeva, "A spectrophotometric study of complexation between chromium and chromazurol S", *JOURNAL OF ANNALS OF CHEMISTRY (Moscow)*, VOL. 40, 1634-1639, 1985.
- [29] C. Cervantes, J. Campos, J., J.S. Devars, F. Gutiérrez, H. Loza, J.C. Torres, and R. Moreno, "Interactions of chromium with microorganisms and plants". *FEMS MICROBIOLOGY REVIEW*, VOL.25, 335-347, 2001.
- [30] S. Chen, Q. Yue, B. Gao, Q. Li, and X. Xu, "Removal of Cr(VI) from aqueous solution using modified corn stalks: Characteristic, equilibrium, kinetic and thermodynamic study". *CHEMICAL AND ENGINEERING JOURNAL*, VOL.168, 909- 917, 2011.
- [31] W.A. Smith, W.A. Apel, J.N. Petersen, and B.M. Peyton, "Effect of Carbon and Energy Source on Bacterial Chromate Reduction", *BIOREMEDIATION JOURNAL*, VOL. 6, No. 3, 205-215, 2002.
- [32] X.R. Xu, H.B. Li, J.D. Gu, and X.Y. Li, "Kinetics of the reduction of Chromium (VI) by Vitamin C", *ENVIRONMENTAL TOXICOLOGICAL CHEMISTRY*, VOL. 24, No. 6, 1310-1314, 2005.
- [33] L. Yong, X. Xin, and H. Ping, "Remediation of Cr (VI) in solution using vitamin C", *JOURNAL OF ZHEJIANG UNIVERSITY SCIENCE*, VOL. 6B, No. 6, 540-542, 2005.
- [34] W. Bahafid, N. Tahri Joutey, H. Sayel, I. Boularab, and N. El Ghachtouli, "Bioaugmentation of chromium polluted soil microcosms with *Candida tropicalis* diminishes phytoavailable chromium". *JOURNAL OF APPLIED MICROBIOLOGY*, VOL. 115, 727-734, 2013.
- [35] M.N. Sheper, S. Nasser, M.Zarrabi, M.R. Samarghandi, and A. Amrane, "Removal of Cr(III) from tanning effluent by *Aspergillus niger* in airlift bioreactor". *SEPARATION AND PURIFICATION TECHNOLOGY*, VOL. 96, 256-262, 2012.
- [36] M.N. Sepehr, M. Zarrabi, and A. Amrane, "Removal of Cr (III) from model solutions by isolated *Aspergillus niger* and *Aspergillus oryzae* living microorganisms: equilibrium and kinetic studies". *JOURNAL OF TAIWAN INSTITUTE OF CHEMISTRY*, VOL. 43, 420-427. 2012.