The Use of the Biomass of a Macromycete Fungus for the Bioremediation of Chromium (VI) in Solution

Ismael Acosta Rodríguez¹, Erika Enriquez Domínguez¹, Adriana Rodríguez Pérez², Juan Fernando Cárdenas González², Víctor Manuel Martínez Juárez³ and Juana Tovar Oviedo¹

¹Laboratorio de Micología Experimental, Facultad de Ciencias Químicas Universidad Autónoma de San Luis Potosí, S.L.P., México.
²Unidad Académica Multidisciplinaria, Zona Media, Universidad Autónoma de San Luis Potosí, S.L.P., México.
³Instituto de Ciencias Agropecuarias, Área Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de Hidalgo, Tulancingo de Bravo, México.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Recently, the removal capacity of different heavy metals from sites contaminated by low-cost materials has been studied, with promising results. These adsorbents include dead microorganisms, clay minerals, agricultural waste, industrial waste, and other materials. The objective of this work was studying the removal capacity of Cr (VI) by a commercial mushroom, the macromycete Agaricus bisporus (white strain), by the Diphenylcarbazide colorimetric method. It was found that the biomass removal 100 mg/L of the metal at 21 minutes, pH 1.0, 28°C, and 100 rpm. On the other hand, if the concentration of the metal is increased, the removal capacity for the analyzed biomass decreases at 28°C. 200 mg/L are removal at 60 minutes, while with 1 g/L of the metal, its removal 90.3%. If the concentration of the bioadsorbent is increased, the removal of the metal also increases, and the presence of other heavy metals does not influence in the removal of...
the metal, and this was desorbed 70.4%, with NaOH 0.5 N. Finally, it was observing that after 7 days of incubation, 76.2%, and 66.1%, of Cr (VI) present in naturally contaminated earth and water, were removal, respectively.

Keywords: Removal; biomass; heavy metals; Agaricus bisporus.

1. INTRODUCTION

Fungi are a very important component of the biological diversity of forest ecosystems and play a fundamental ecological role in the way they obtain their nutrients. They are formed by branched hyphae, which are grouped into mycelial cords and reproductive bodies, visible and measurable in centimeters [1]. They are saprobic organisms that absorb dead organic matter from the substrates where they grow, are parasites of trees, or associate their hyphae with the roots of trees (mycorrhizae) with various plant species; there are edible and poisonous ones. Saprobic fungi and symbionts contribute to the recycling of organic matter; pathogens can modify the composition and structure of a plant community [2]. Although macrofungi constitute one of the taxonomic groups with great diversity, knowledge about their richness and presence at the local level is very scarce [3]. It is estimated that in Mexico there are between 140,000 and 200,000 fungal taxa [1,4], while in the world their number exceeds 1,500,000 [5]. Approximately 10% of them are macrofungi and the rest are micromycetes [1]. The conservation of this biodiversity is relevant, given the current global trend towards its loss. One of the strategies that have been used for this conservation includes the mycetobiota, which is the establishment of protected natural areas, such as national parks, biosphere reserves or protected areas of flora and fauna [6].

On the other hand, fungi have a long association with humanity and have a profound biological and economic impact. Since ancient times, man has consumed wild mushrooms probably delicately, due to their pleasant taste [7]. They have a great nutritional value with a high content of proteins, vitamins, minerals, fibers, trace elements and low/no calories and cholesterol [8]. Mushrooms have been described as a rich source of different bioactive substances such as antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory, antiproliferative, anticancer, antioxidant, cytotoxic, anti-HIV, hypcholesterolemic, antidiabetic, anticoagulant and hepatoprotective compounds, among other [7,8,9]. Of the approximately 14,000 known species, 2,000 are safe for human consumption and about 650 possess medicinal properties [5]. In developing countries such as India and Mexico, with great biodiversity, mushrooms are a boon for progress in the field of food, medicine, and unemployment because they contain various nutraceuticals, there are also many medicinal mushrooms that are very useful for the development of human health as food, medicine, minerals, and drugs among others [4,7].

In Mexico there are reports of fungal populations in different places such as the population of macrofungi in the “Las Palomas” station, Guanajuato [10], fungi from urban areas of Mexico City and the State of Mexico [11], different species of Agaricus in Mexico [6], the study of fungi of biocultural importance in communities of Oaxaca [12], arbuscular mycorrhizal fungi associated with corn plants in Guasave, Sinaloa [13], edible wild fungi from the Yucatán peninsula [14], boletal mushroom of a tropical holm oak grove in the southeast of Mexico [15], and the record of Ganoderma subincrustatum in Sonora [16].

On the other hand, the great industrial growth has produced a progressive increase in wastewater discharges from the same and, therefore, a deterioration in water quality. Pollutants pose a danger to both human and environmental health. Some pollutants are of organic origin, such as hydrocarbons and pesticides, and inorganic, such as heavy metals, which play a fundamental role due to their importance and potential danger [17]. Some metals that are of great toxicological and exotoxicological importance are: mercury, chromium, lead, cadmium, nickel, and zinc, which, once released into the environment, accumulate, and concentrate in the soil and sediments, where they can remain for hundreds of years, affecting ecosystems. Therefore, it is more feasible to control the problem from the source and source of emission before they reach the environment [17]. Therefore, it is very important to try to eliminate the greatest number of pollutants from the different contaminated ecological niches to reduce said contamination and reduce the risks to human health. The elimination of different heavy metals and other contaminants by this type of fungi has been
reported, such as: The biosorption of Cadmium, Lead and Copper by organic carbon of *A. bisporus* and *Pleurotus ostreatus* [18,19], the accumulation of Mercury, Cadmium, Lead and Arsenic by *Amanita ponderosa*, *Boletus edulis*, *Marasmius oreades* and *Tricholoma georgii* [20], the accumulation of different heavy metals by the edible fungi *Melanoleuca cognata* and *Melanoleuca stridula* [21], the removal of Copper (II) by different macromycetes [22], the removal of Cu (II), Zn (II) and Cd (II) by a mineral-rich compound of *A. bisporus* [23], the removal of Mercury, Lead, Cadmium and Chromium by *P. ostreatus* [24], the removal of Lead and Cadmium in water by biochar from *Ganoderma lucidum* [25], the phytoremediation of soils contaminated with heavy metals by *P. ostreatus* and *Megathyrsus maximus* [26], and the removal of heavy metals from coal wash effluents with the fungus *P. ostreatus* [27]. On the other hand, the adsorption efficiency of other pollutants such as: paracetamol and α-ethynyl estradiol (EE2) by *A. bisporus* and *Lentinula edodes* has also been reported [28], the removal of sulfonamides by *P. ostreatus* [29], acid red 97 and crystal violet by *A. bisporus* [30], malachite green by different macromycetes [31], and textile dyes using *Trametes versicolor*, *P. ostreatus* and *A. bisporus* [32]. This bioadsorption of contaminants by this biomass, has been suggested to be due to some compounds of the cell wall of the fungus, which is made up mainly of neutral polysaccharides (made up mainly of glucose and to a lesser percentage of galactose, mannose, and xylose) and amino acids (N-acetylglucosamine in the form of chitin), with a lower proportion of proteins and lipids, in amounts that they differ significantly depending on whether it is the vegetative or aggregate mycelium. The polysaccharides formed are mainly glucans together with galactans, mannanes, xylan, and often mixed polysaccharides (heteropolysaccharides) such as mannoxylans, glycoxyllans etc., with different types of bonds, relatively branched and with both α and β configurations, as well as a chitin complex embedded in a β-glucan matrix [30,33,34, and [35]. Too, the fungal cell wall of the fungi is composed of functional groups containing amide, carboxyl and phosphate, and these structures can facility the adsorption of this metal and other compounds as colorants [30,35, and [36].

Therefore, the objective of this work was to evaluate the adsorbent capacity of the biomass of a commercial *A. bisporus* mushroom in the removal of Chromium (VI) in aqueous solution.

### 2. MATERIALS AND METHODS

#### 2.1 Bioadsorbent Used

The *A. bisporus* (white) mushroom was obtained from a supermarket in San Luis Potosí, Mexico, in May 2020, and was classified considering that when the people of the region collect wild mushrooms, they generally collect different non-toxic species of the same genus, assuming they are the same fungus as: commercial mushrooms Champignon and/or Champignon c: white *A. bisporus* strain and Portabella: brown *A. bisporus* strain (Fig. 1a). It was washed 24 hours with EDTA at 10% (w/v), one week with trideionized water with constant agitation, and water changes every 24 hours. Subsequently, it was boiled for 60 minutes to removal the dust and adhering organic components, and it was washed again under the same conditions for 24 hours. It was dried at 80°C for 24 hours in a bacteriological oven, ground in a blender and stored in an amber bottle until use (Fig. 1b).

![Fig. 1. *Agaricus bisporus*. a. Commercial strain. b. Stored and ground biomass](image-url)
2.2 Chromium (VI) Solutions

We worked with 100 mL of a 100 mg/L Chromium (VI) solution obtained by diluting a 1.0 g/L standard solution prepared in trideionized water from K₂Cr₂O₇. The pH of the dilution to be analyzed was adjusted with 1 M HNO₃ and/or 1 M NaOH, before adding it to the cell biomass.

2.3 Removal Studies

1.0 g of A. bisporus biomass, (previously sterilized at 15 pounds and 120°C, in 250 mL Erlenmeyer flasks) was mixed with 100 mL of a 100 mg/L Chromium (VI) solution [at different pH values, temperatures, concentrations of Cr (VI) and biomass and 200 mg/L of other metals] and were incubated at 28°C and 100 rpm, taking aliquots of 5 mL each at different times, which were centrifuged at 3000 rpm (5 min), and in the respective supernatant was determined the metal concentration in solution, using the Diphenylcarbazide colorimetric method [37]. All experiments were performed a minimum of 2 times in duplicate.

3. RESULTS AND DISCUSSION

The optimal time and pH for the removal of Chromium (VI) by the biomass of the fungus A. bisporus, was 100% at 21 minutes, pH 1.0, 100 rpm, 28°C and 1.0 g/100 mL of biosorbent, with a concentration initial metal of 100 mg/L (Fig. 2), using a Corning Pinnacle 530 model pH meter and 1 M HNO₃ to keep the pH value constant, since the capture rate is controlled by the rate at which the adsorbate is transported from the outside to the inside of the biosorbent particles [38]. In this regard, a time of 240 minutes has been reported for the biosorption of Cadmium (II) and Zinc (II) by the biomass of A. bisporus [39], an optimum time of 2 hours for the solutions of 100 mg/L and 500 mg/L of Chromium (VI) with the modified biomass of Pleurotus cornucopiae [40], 15 minutes for the biosorption of Lead, Chromium and Copper by the palm pod of peach modified and colonized by Agaricus blazei [41], 20 hours of incubation with 0.59 mM Chromium (VI) for Hypocrea tawa [42], 240 minutes for 96.4% elimination Lead by Agaricus campestris [43], 60 minutes with 8 g/L of biodosorbent for Copper biosorption for a chitosan compound from A. bisporus [44], 2 hours for elimination of Chromium (VI) by Pleurotus sajor-cajor, Ganoderma lucidum and Agaricus bitorquis [45], 120 minutes for the adsorption of Copper (II), Zinc (II), and Mercury (II), for residues of Flammulina velutipes, Auricularia polytricha, Pleurotus eryngii and P. ostreatus [46], and a removal of 93% from 100 mg/L of Lead (II) with the biomass of P. ostreatus [18]. Changes in cell permeability of unknown origin could partly explain the differences found in the incubation time, providing greater or lesser exposure of the functional groups of the cell wall of the analyzed biomass [47].

On the other hand, the highest metal adsorption was observed at a pH of 1.0 with the analyzed biomass (Fig. 2), which is similar to that reported for P. sajor-cajor, G. lucidum and A. bitorquis, with an optimal pH of 2.0 and 2.5 for the elimination of Iron (III), Nickel and Cobalt [45], but some authors report different pH values optimal for the removal of this and other metals, such as A. bisporus biomass, in which the maximum removal efficiencies were 79.82%, and 67.30% at pH 7.5 and pH 5.5 for Cadmium (II) and Zinc (II), respectively [39], for the biosorption of Cadmium, Lead, and Copper by organic carbon of A. bisporus and P. ostreatus, it was observed that an increase in pH increased the adsorption capacity [18], a pH of 5.0 for the biosorption of different heavy metals by peach pod modified and colonized by A. blazei [41], different pH values (3.5, 4.5, 6.0 and 6.5), for H. tawa [42], a pH of 8.0 for A. campestris used as a biosorbent in the treatment of wastewater containing Copper and Lead ions in a dynamic process [43], an initial pH of 6.0 for the biosorption of cupric ions for a chitosan compound from A. bisporus [44], and for different mushrooms [45], a pH between 5.0 and 7.0 for removal of Chromium, Copper, Lead and Mercury by different macromycetes [22,24,45] and [48]. This is probably since the dominant species (CrO₄²⁻ and Cr₂O₇²⁻ of Cr ions in solution, interact more strongly with the ligands carrying positive charges [47,49].

On the other hand, at low concentrations of the metal (200 mg/L and 28°C), the biomass studied showed the best removal responses, adsorbing 100% at 60 minutes while with 1.0 g/L of Cr (VI), removal was 90.3% (Fig. 3a). These results are consistent for the removal of Mercury and Lead contained in water effluents by P. ostreatus, since at 60 minutes 90% of the metal is removed in solution (50 mg/L) [18], for modified P. cornucopiae, with a removal efficiency of 75.91% and 48.01% with 100 mg/L and 500 mg/L of Cr (VI, respectively [40], for H. tawa, in which, the initial concentration of Cr (VI) is increased from...
0.59 to 4.13 mM, the biomass yield decreases [42], for A. campestris and other macrofungi, in which at an initial concentration of metal ions lower than 50 ppm, the biosorbent exhibited the highest adsorption efficiencies [43]. While the gradual increase in the concentration of Copper and Lead in the medium culture, gradually decreased the adsorption efficiencies of a chitosan compound from A. bisporus, because the maximum percentage Lead removal rate was 93.5% at an initial concentration of 20 mg/L, while for high concentrations of the metal the removal is lower [44], for the Agrocybe cylindracea fungus treated with Fe3O4, because when increasing the concentration of Cr (VI) from 20 to 1 000 mg/L, decreased the removal from 98.28% to 7.94% [45]. But, these results are different for the biomass of A. bisporus, in which the removal of Cd (II) and Zn (II) increases at a higher concentration of heavy metals [39], for removal of Cr (III) and Cr (VI) by P. sajor-cajor, G. lucidum and A. bitorquis, since the biosorption of both metal ions increases, when increasing their concentration from 25 to 200 mg/L [45], which may be due to the increase in the number of ions that compete for the functional groups available on the surface of the biomass [46]. But they are different for the removal of Copper (II) by different macrofungi in which the optimum removal temperature was 25°C [50]. While, at 60°C, the biomass studied removal 100% at 90 minutes with 1 000 mg/L of the metal (Fig. 3b).

With respect to other biomasses, these results are similar for A. bisporus, in which by increasing the temperature from 25 to 35 and 45°C, the biosorption of Cd (II) and Zn (II) increases significantly [39], for chitosan from A. bisporus, Lead absorption increases from 92.76% to 94.1%, when the temperature increases from 10°C to 30°C [51], and in Pleurotus eryngii, the elimination of 90% to 96% of Cd (II), when the temperature was increased from 25 to 50°C [52].

![Fig. 2. Effect of pH and incubation time on the bioadsorption of Cr (VI) in aqueous solution by the biomass of A. bisporus. 100 mg/L. 1 g of biomass, 28°C 100 rpm](image)

![Fig. 3. Effect of initial concentration of Cr (VI) on the bioadsorption of Cr (VI) in aqueous solution by the biomass of A. bisporus. 1 g of biomass. 100 rpm. pH 1.0](image)
In the Fig. 4, the influence of the biomass concentration for the removal of 1.0 g/L of Cr (VI) is shown. If the amount of biomass is increased from 1 to 5 g, the removal of the metal in solution also increases, with 90.3%, with 1 g of biomass at 160 minutes, while with 5 g of biomass, the removal is 100% at 100 minutes, at pH 1.0, 28°C and 100 rpm, because there are more bioadsorption sites of it, since the amount of bioadsorbent added determines the number of binding sites available for the bioadsorption of heavy metals [47,49]. Similar results have been reported for three brown algae and one fungus (Cystosiera compressa, Sargassum vulgare, Turbinaria sp. and A. campestris), since by gradually increasing the amount of bioadsorbent from 0.1 to 1 g, the removal of metal ions Cu²⁺ and Pb²⁺ is increased [43], for the removal of Lead using the eggshell (Dromaius novaehollandiae) and a chitosan compound from A. bisporus, with an increase in removal from 88.6% to 94.1% and concentrations of the bioadsorbent from 3 to 7 g/L [44], for the biomass of Lentinula edodes modified with MgCl₂, the elimination of Cd (II) and Cu (II) increases by increasing the amount of the bioadsorbent of 1 at 5 g/L [53], for the elimination between 80% and 98% of Chromium, Copper, Manganese, Zinc, Aluminum, Iron (III), Nickel and Cobalt, from wastewater with 5 kg of biomass of A. bisporus [48], for the removal of Mercury and Lead from aquatic effluents by P. ostreatus, which increases from 70% to 96%, by increasing the biomass concentration from 1 to 5 g [18]. But they are different for P. ostreatus nanoparticles, where the adsorption capacity of Mn (II) in aqueous solution decreases with increasing adsorbent dose [54], and for the elimination of Zirconium by G. lucidum [55].

On the other hand, industrial effluents frequently contain more than one type of metal ion, which can interfere in the elimination/recovery of the metal of interest by the biomass to be studied [47,49]. In this work, the presence of other metals in solution such as Lead, Mercury, Cobalt and Copper (200 mg/L), does not interfere significantly with the removal of Cr (VI) in solution, although removal takes between 10 and 30 minutes more (Fig. 5), which may be due to the optimal removal pH found (1.0), and coincides with some reports in the literature for other biomasses where it is reported that the presence of other metals (Na⁺, K⁺, Ca²⁺, Mg²⁺, SO₄²⁻, NO₃⁻, PO₄³⁻, Cl⁻) does not interfere significantly in the adsorption of Cd (II) by P. eryngii [52], for the biosorption of Copper in the presence of Cl⁻, SO₄²⁻, NO₃⁻ and HCO₃⁻, using chemically modified chitosan beads [54], for the removal of Cr (VI) by the pink shrimp of the Gulf of Mexico (Farfantepenaeus duorarum) [56], for Zhihengliuella sp. ISTPL4 does not interfere with the removal of different heavy metals in the presence of others [57]. But, they are different for the biomasses of P. sajor-cajor, G. lucidum and A. bitorquis, because in the presence of the cations: Na⁺, Ca²⁺, and Al³⁺, and the anions Cl⁻, NO₃⁻, SO₄²⁻, and CH₃COO⁻, the removal of Cr (III) and Cr (VI) is affected in presence of co-metallic cations and not by the presence of anions [45], for the adsorption of Pb (II) ions and Cu (II) in natural chitosan and chitosan treated with H₂SO₄, increasing the concentration of NaCl and NaNO₃ decreases the elimination of Pb (II) and Cu (II) [58], and for the yeast Saccharomyces cerevisiae, in which the presence of heavy metals interferes with their removal [59].

Fig. 4. Effect of initial concentration of the fungal biomass on the bioadsorption of Cr (VI) in aqueous solution.1 g/L of Cr (VI). 100 rpm. pH 1.0
Fig. 5. Effect of the presence of different heavy metals (200 mg/L) on the biosorption of Cr (VI) in aqueous solution. 200 mg/L of Cr (VI). 28°C. 100 rpm. pH 1.0

Also, the capacity of different solutions to desorb the metal (1g/L) of the commercial strain of the macrofungi was analyzed, obtaining a high efficiency with NaOH 0.1 N and 0.5 N (62.3% and 70.4%) at 7 days of incubation, respectively (Fig. 6). These results are similar for the desorption of Cr (III) and Cr (VI) with P. sajor-cajor, G. lucidum and A. bitorquis, in presence of EDTA, CH₃COOH, H₂SO₄, HCl and NaOH, which desorb between 80% to 100% of the metals studied [45], for the desorption of 95% of Pb (II) and Cd (II) by Lactarius scrobiculatus, with 1 M HNO₃ [58]. Also, the desorption of Pb (II) and Cu (II) by natural chitosan and chitosan treated with H₂SO₄, increased with increasing eluent concentration [60], for the increase in arsenic desorption using chitosan, when increasing the initial pH from 3.5 to 5.0 [61], for the biomass of P. osteratus, a reduction in the biosorption efficiency of 14.21% was observed for Cr (VI), 8.37% for Cu (II), 6.48% for Ni (II) and 1.84% for Zn (II), respectively, after four adsorption cycles [62], a high desorption efficiency of 100 mg/L of Lead (II) after five adsorption/desorption cycles with P. ostreatus biomass [19], for the desorption of Cd²⁺, Cu²⁺, and Pb²⁺ by the fruiting body of the gelatinous fungus Auricularia polytricha, with 85-100% desorption of the heavy metals analyzed, being more effective solutions of HNO₃ 0.05 and 0.1 M/L than those of NaCl 0.1 and 0.2 M/L, while water deionized exhibited an insignificant desorption capacity [63], the 20 cycles reported for the removal of 25 µg/L of Lead (II) and Chromium (VI) by P. ostreatus [19,64], and an efficient desorption of Lead and less for Cadmium by biochar of G. lucidum [65].

Fig. 6. Desorption of 1.0 g/L of Cr (VI) by different solutions. 28°C. 100 rpm. pH 1.0
Fig. 7. Bioremediation of Cr (VI) from soil and water contaminated with 297 mg/g soil (pH 6.8), and 400 mg/L Cr (VI) (pH 8.2) (5 g of fungal biomass. 28°C, 100 rpm

In order to analyze the possible use of A. bisporus biomass for the removal of metal from industrial waste, a bioremediation test was adapted in aqueous solution, incubating 5 g of biomass with 20 g of non-sterile soil, contaminated with 297 mg Chromium (VI)/g of soil, pH 6.8, and 100 mL of water contaminated with 400 mg of Chromium (VI)/L, pH 8.2, resuspending the soil in trideionized water to a final volume of 100 mL, at 28°C, and 100 rpm, observing that after 7 days of incubation the Cr (VI) concentration of the soil and water samples decreased between 66.1% and 76.2% (Fig. 7), without significant changes in the total Chromium content (data not shown). In the control of the experiment (without biomass), the metal concentration of the samples decreased between 7% and 14% (data not shown), which may be caused by the autochthonous microflora and (or) reducing components present in the samples [46]. The chromium removal capacity from wastewater by these biomasses is equal or better than others analyzed, for example: The removal by A. bisporus of aromatic hydrocarbons, Pyrene, Cadmium and Lead from contaminated soils [66,67], the elimination of Cu (II) (46.01%), Ni (II) (59.22%), Zn (II) 9.1% and Cr (VI) (9.4%) from real effluents by P. osteratus [59], the remediation in soils co-contaminated with cadmium and endosulfan using Pleurotus eryngii and Coprinus comatus [68], the bioremediation of wastewater by A. bisporus [39], the elimination of paracetamol and α-ethynyl estradiol (EE2) from waters contaminated by biomass from the stem of A. bisporus and L. edodes [28], the bioremediation of colored effluents by A. bisporus [32], the removal of Mercury and Lead in water effluents [19], the elimination of water contaminated with mercury [68], and the bioremediation of wastewater contaminated with Aluminum by A. bisporus [35]. Finally, the fungal biomass used in this work was classified taking into account that when the people of the region collect wild mushrooms, they generally collect different non-toxic species of the same genus, assuming they are the same fungus and call them: Champignon (Champignon c.): A. bisporus white strain and Portabella: A. bisporus strain brown [69,70], and there are few studies with these strains, such as: the evaluation of the composition, total phenols and antioxidant activity of wild edible mushrooms (Agaricus sp., Boletus sp., and Macrolepiota sp.) and two commercial edible mushrooms (A. bisporus strain white or Portabella, and A. bisporus strain brown) from the State of Chihuahua, in northern Mexico, finding that wild mushrooms had higher phenol content and antioxidant capacity than commercial mushrooms [71].

4. CONCLUSION

Recently, the removal capacity of different heavy metals from sites contaminated by low-cost materials has been studied, with promising results. These adsorbents include dead microorganisms, clay minerals, agricultural waste, industrial waste, and other materials. In this work, the biomass of a commercial strain of A. bisporus was analyzed for the removal of Chromium (VI) in aqueous solution, with the following conclusions:

1. The biomass of the commercial strain of A. bisporus (white) eliminates 100 mg/L of Cr (VI) at 21 minutes of incubation, with 1 g of biomass, 28°C, pH 1.0 and 100 rpm.
2. If the temperature is increased, the removal efficiency is increased.
3. To lower metal concentration, is higher the removal efficiency.
4. To higher the biomass concentration, the removal efficiency increases.
5. The presence of other metals does not interfere in the elimination of Chromium (VI) by the analyzed biomass.
6. In bioremediation tests, it was found that biomass efficiently removes metal from soil and waters contaminated with Chromium (VI), therefore, their application is viable for its treatment, in addition, the biomass used is natural, of easy obtained, handling, and affordable cost.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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