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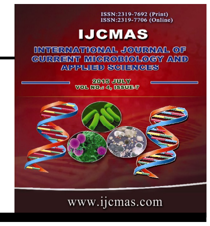

Original Research Article

Aspergillus nidulans Biomass as Biosorbent for cadmium Removal: Effects of Treatment and pH

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Keywords

Biosorption, Cadmium, Biosorbent, *Aspergillus nidulans*

A B S T R A C T

This work evaluated the potential of *Aspergillus nidulans* biomass in cadmium removal process. The biomass obtained by heat inactivation, and treated with formaldehyde was used to remove cadmium in aqueous solutions, at concentrations of 1mM, 2mM and 3mM, and at pH values of 4, 5 and 6. The results indicate that the isolated displays potential for metal removal. The efficiency depended of pH and treatment. The highest removal rates were obtained at pH 6.0 and with biomass submitted to heat inactivation. The results showed the possibility of application of the isolated biomass in cadmium remediation processes even in high concentrations.

Introduction

Currently, the treatment of polluted by heavy metals environments is one of the most important goals of the industries and Governments, mainly because such elements are a problem of growing importance for ecological, environmental and nutritional evolutionary reasons (Chey and Buchanan, 2008; Jarup and Akesson, 2009). Cadmium is a metal that has been spread in the environment and causing serious damage, for example, the local disappearance of several species of organisms which are sensitive to their effects. The toxicity of cadmium may be caused even at low concentrations and the biological effects of metal, as well as its toxicity are not yet clearly understood, despite already knowing that the ion can interact with various enzymes, interfering in different metabolic pathways (ATSDR, 2008; Jarup and Akesson, 2009).

Due to the effects of this metal to the ecosystem, it is vitally important to use efficient processes in its removal (Lazaridis *et al.,* 2003). According to Volesky (1990), the process of heavy metals removal from industrial effluents may be made with the use of neutralization, precipitation chemistry processes, oxy-electrochemical techniques, reduction, ion exchange (using organic solvents or synthetic resins), activated carbon and sophisticated technologies with use of membranes. However, these conventional methods are not effective or cost-effective, especially when used for the reduction of metal ions at low concentrations. Thus, the search for new methods of removal is required to reduce concentrations of metals to environmentally acceptable levels.

The biological removal tends to use biomass, cultivated or obtained as co-product of fermentation, subjected to drying, and detention, generating minimal operating costs, minimum volume production of wastes and tailings and high efficiency in removing metals in very diluted effluents (Klimmek and Stan, 2001). Such advantages are the primordial incentive for the development of biological processes for the heavy metals removal from contaminated solutions. In this way, biotechnology is a new possibility for the minimization of environmental aggression induced by xenobiotics by combining low costs, increased efficiency and effectiveness in removing heavy metals.

A wide variety of biological materials have been developed and marketed for controlling pollution from various sources around the world (Kar and Misra, 2004; Gadd, 2010; Opeolu *et al.*, 2010), including sewage sludge ammonium anaerobically digested (Soltani *et al.,* 2009), bacteria (Vijayaraghavan and Yun, 2008), fungi (Mashita *et al.*, 2008; Gadd, 2010), shells (Cochrane *et al.,* 2006), algae (Brinza *et al.*, 2007), agriculture wastes derived from rice, soybeans, cotton, among others.

In order to avoid the effects of toxicity on the living biomass, the use of dead biomass is of particular interest, since the biomaterial is used the same way as the synthetic adsorbents or ion exchangers systems, and in this process repeated regeneration is possible (Gadd, 2010; Opeolu *et al.*, 2010). Additionally, the biomass may be subject to modification processes that result in greater removal efficiency in aqueous solutions of heavy metals.

In this way, the goal of this work was to evaluate the potential of *Aspergillus nidulans*, isolated from sediment of mangrove swamp, as biosorbent for cadmium removal in aqueous solution by using alive and pre-treated biomass, as well as to evaluate the biosorbent ultrastructure by scanning electron microscopy. Such results can be used for understanding and improving the processes of metallic ion removal of contaminated environments.

Materials and Methods

Production and Processing of biomass for removal Kinetics in aqueous solution

Aspergillus nidulans was obtained from the Culture Collection of the Nucleus of Research in Environmental Sciences and Biotechnology – Catholic University of

Pernambuco – Brazil, included in the Rede Nordestina de Microorganisms do Norte e Nordeste (RENEBRA) and registered in the World Federation Culture Collection (WFCC). The isolate was maintained in Sabouraud medium at 5°C. The fungus was cultivated in Sabouraud medium for the production of pre-inoculum. Erlenmeyer flasks of 300 mL of capacity, containing 150 mL of liquid Sabouraud were inoculated with 10^7 spores/mL and incubated at 28 °C, in an orbital shaker (250 Hertz), for 15 days. Biomass produced was collected and filtered and rinsed thoroughly with distilled water. The living biomass thus obtained is referred as biossorbent BL. The others biossorvents were produced as following:

a) 1 gram of biomass washed was treated for 30 min at 121^{o}C autoclaved at 18 psi, then subjected to drying at 60^{o}C for 12 hours – biosorbent BA;
b) 1 gram of biomass was incubated in a formaldehyde 15% (vol/vol) solution, without boiling, during 15 min – biosorbent BF.

After each treatment the biomass was washed with distilled water and subjected to drying. Cadmium chloride solutions, used in this work, were prepared with distilled deionized water in concentrations of 1mM, 2mM and 3mM, pH 5.0, adjusted using 1N sodium hydroxide solution and acetic acid 10% (v/v). Samples of 1g of biosorbents were incubated for 12 hours at 28 ºC in solutions containing cadmium at 1mM, 2mM and 3mM. After that, samples of supernatants were collected and submitted to atomic absorption spectrophotometry for cadmium residual determination. A standard curve was produced. Experiments were conducted in triplicates and the average values were used in the analysis. The cadmium removal rate was calculated using the following relationship: concentration of cadmium removed/initial concentration of cadmium x 100. The heavy metal removal efficiency, q (mg metal/g dry biomass) was calculated using the following equation:

$$q = (C_0 - C_f)/m$$

Where, C_0 and C_f are the beginning and ending of metal concentration (mg/L), respectively and m dry biomass (g).

Evaluation of the effect of pH on removal of Cadmium in aqueous solution

To assess the effect of pH on the removal process in aqueous solution, the pH of the solution used in kinetic assays of metal removal has been adjusted with HCl or NaOH to the following values: 4.0, 5.0 and 6.0.

Ultrastrucutural Analysis

Samples of the biosorbents produced from *Aspergillus nidulans* mycelium, grown in cadmium, pretreated and have undergone the removal kinetics were processed for the routine technique for scanning electron microscopy. Samples were washed twice in PBS, pH 7.2, for 10 min. Then they were fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.4, for 1 hour at room temperature. After the stage-setting, all samples were again washed twice with phosphate buffer, for 10 min. This procedure was followed by the post-fixing with osmium tetroxide 1% in phosphate buffer, for 1 hour at room temperature, in absence of light. Then the samples were once again washed with 0.1M phosphate buffer, and submitted to the process of dehydration. The dehydration of the samples was done with ethanol, in concentrations of 50%, 70%, 90% (5 min for each exchange) until the proportion of 100% (three times, 10 min each exchange). After this step, the samples were submitted to the critical point, followed by the assembly in support of aluminum and subsequent gold metallization. Once prepared, samples were examined and photographed in the Scanning Electronic Microscope, JEOL LV 5600, operating at 20KV.

Results and Discussion

Removal kinetics in aqueous solution and pH effect

Removal tests with biomass from *Aspergillus nidulans* subjected to different treatment processes were investigated in pH values corresponding to 4.0, 5.0 and 6.0. Cadmium percentage removal was used to reflect the metallic ion removal efficiency according to the initial concentration of metal in aqueous solution.

The results obtained for the removal of cadmium by biomass from *Aspergillus nidulans* in the concentration of 1mM under different pH are presented in figure 1.

The results revealed that 53%, 56.8% and 66.9% of 1mM of cadmium was removed at pH values of 4.0, 5.0 and 6.0, respectively by native washed biomass. For autoclaved biomass 60.76%, 74.3% and 84.9% of cadmium removal were obtained for pH 4.0, 5.0 and 6.0. Biomass submitted to formaldehyde treatment removed 55.8%, 64.7% and 75% at pH 4.0, 5.0 and 6.0.

The results obtained for the removal of cadmium in the test concentration of 2mM are presented in figure 2. Removal percentages of 28.2%, 35.9% and 41.2% were determined respectively for the living washed biomass under pH 4.0, 5.0 and 6.0. Biomass autoclaved, subjected to pH 4.0, 5.0 and 6.0 removed 34.5%, 50.5% and 56.9, respectively. On the other hand, biomass treated with formaldehyde presented removal percentages of 32.2%, 42.3% and 50.33% at pH 4.0, 5.0 and 6.0.

Figure 3 shows the results for the study of cadmium removal at concentration of 3mM.

Living washed biomass exhibited 13.5%, 16.98% and 21.62% of cadmium removal at pH 4.0, 5.0 and 6.0, respectively. Autoclaved biomass removed 19.2%, 23.3% and 31.9% when subjected to pH 4.0, 5.0 and 6.0. For biomass treated with formaldehyde, at pH 4.0, 5.0 and 6.0, removal percentages of 16.96%, 24.0% and 19.28% were determined, respectively.

Species of the genus *Aspergillus* are cosmopolitan and display multiple biochemical, physiological and morphological abilities that put them among the fungi most studied since its isolation. The species exhibit great biotechnological potential

Fungi exhibit advantages such as materials for removal of metals due to their characteristics of mycelial growth and abundant high surface area volume ratio, and can bemorphologically and genetically manipulated to produce a high amount of biomass. The fungal biomass has been generally used in native form, showing mechanical force and biological stability, which may hinder the process of remediation and recovery of biomass. Although living and dead cells, fungi are able to accumulate metals, there may be differences in the mechanisms involved in each case, depending on the metabolic extension, and thus, the preference for living organisms depends on the nutritional conditions and the cellular age. Additionally, as shown the living biomass is subject to the toxic effects of metals in high concentrations. In this way, it has been proposed the use of non viable, dead or the insertion of biomass treatment methods through different methods for obtaining a greater ability to remove waste metal (Gadd, 2010; Opeolu *et al.*, 2010).

The use of filamentous fungi has been reported in remediation of heavy metals from wastewater and effluents in different parts of the world (Soares *et al.*, 2002; Saeed *et al.,* 2009; Aksu and Dönmez, 2006; Han *et al.,* 2006; Melgar *et al.,* 2007).

Studies show that the waste biomass derived from biotechnological importance fungi, particularly species of *Fusarium*, *Aspergillus*, *Penicillium*, *Mucor* and *Saccharomyces* can be used as heavy metal biosorbents (Kapoor *et al.,* 1999; Jianlong *et al.*, 2001; Göksungur *et al.*, 2005; Park *et al.*, 2005). The walls contain a lot of protein and polysaccharides, which have various functional groups such as carboxyl, amine and hydroxyl groups; sulphates, able to quelates metal ions. Thus, they are preferably used, including in the form of waste/by-products of industrial fermentation processes.

Many studies on fungi as biological sorbents on removal of metals from aqueous solutions (Kapoor et al., 1999; Ahmad et al., 2004; Slaba & Dlugonski, 2004; Goksungur et al., 2005; Melgar et al., 2007; Paraszkiewicz et al., 2007). The possibility of increasing the removal of heavy metals as a result of physical and/or chemical pretreatments is pointed to by many authors (Kapoor *et al.,* 1999; Göksungur *et al.*, 2005; Saeed *et al.,* 2009).

In this way, the living cells can be inactivated through the use of physical pretreatments with the use of heat (Galun *et al.*, 1983; Siegel *et al.*, 1986; Townsley *et al.*, 1986), autoclaving and vacuum drying (Tobin *et al.,* 1984; Huang *et al.*, 1988) or where the organic sorbent mass with chemical agents such as acids, alkalis, detergents, organic solvents, aldehydes (formaldehyde, glutaraldehyde) (Muzzarelli *et al.,* 1980; Huang *et al.*, 1988; Azab and Peterson, 1989; Rao *et al.,* 1993; Brady and Ducan, 1994) or through application of mechanical disruption (Tsezos and Volesky, 1981).

The improvement of metal-binding properties in biological sorbents modified is usually explained by increasing access of the metal ions binding sites. Such an improvement in metal binding to biological sorbents can be explained by the increased access of ions to the binding sites assigned to the effect "cleaning" of the agents used, modification of binding sites or changes in cell surface loads (Kapoor *et al.*, 1999; Göksungur *et al.*, 2005).

Thus, the production of biomass and their subsequent use is an independent process of metabolism, and therefore do not suffer physiological constraints, and that can generate extreme biosorbents for different xenobiotics, removing potentials including heavy metals. The present study also assessed the process of removal of cadmium by biomass of *Aspergillus nidulans* in aqueous solution.

Removal of heavy metals including cadmium, using various fungal biomass has been studied. Tobin *et al.* (1984) investigated the removal of cadmium, copper, zinc and mercury with the biomass of *Rhizopus arrhizus.* Fourest and Roux (1994) analyzed the removal of cadmium, copper and zinc from aqueous solutions using *Rhizopus arrhizus*, *Mucor miehei* and *Penicillium chrysogenum*. These studies indicated that fungal biomass has a positive potential for the economic development of biological sorbents. Malik (2004) in a literature review reported that the cadmium sorption maximum efficiency for several fungi was 184mg/g for the species *Gliocladium roseum*.

Massaccesi *et al.* (2002) reported that the maximum efficiency of removal of cadmium by 7 species of fungi was little more than 180 mg Cd/g. Removal of cadmium by *Aspergillus* was determined in 154.8 mg/g of biomass (Doyle *et al.,* 1975).

In these studies, researchers have suggested that the two primary mechanisms used for removal of heavy metals are: (1) reacting ion exchange with the active chemical groups such as hydroxyl, Carbonyl, carboxyl, sulphydryl compounds, sulphonate, thioether, amine, imine and phosphonate present in cellular surfaces and (2) dependent inorganic interactions from the physicochemical properties of metals, directed by adsorption phenomena. It is noticeable that the first is a critical mechanism for removal of most heavy metals.

These mechanisms determine the importance of controlling experimental parameters. Several crucial parameters may influence the process of removing heavy metals by microbial biomass, such as pH, pretreatment methods, fungal species, metal species, contact time and initial concentration of metal. Such parameters that provide information about the effectiveness of metal-biosorbent can and should be obtained from a greater number of experiments *in vitro* (Kapoor *et al.,* 1999; Jianlong *et al.,* 2001; Göksungur *et al.,* 2005; Park *et al.,* 2005).

Several authors report the skills of removing heavy metals such as cadmium, zinc, copper, cobalt, uranium, nickel chromium by biomass, non-viable or dead of filamentous fungi, particularly species of *Aspergillus* and *Rhizopus* (Bai and Abraham, 2001; Teskova and Petrov, 2002; Ahmad *et al.,* 2005; Iram *et al.,* 2009)

Biomass of *Curvularia lunata* displays high removal efficiency for Pb^{2+}, Zn^{2+} and Cd^{2+}. Similar data have been reported by Yan and Viraraghavan (2003) for dead mycelium *Mucor rouxii.* The work point out that the concentrations of metals are variables and the answers depend on their concentration and type of biomass.

In studies of removing there is a lack of information on the adsorption sites and mechanisms responsible for capturing metal ions by biomass. Different species exhibit variations in relation to its potential for removal depending on age, type and concentration of the metal. The fungal surface displays a wide range of chemical groups that can attract and hijack the metals. The cell wall is made up of structural polysaccharides, proteins and lipids that offer functional groups for binding of metal ions (Gupta *et al.,* 2000).

In the present study, the pre-treatment of biomass from *Aspergillus nidulans*, using autoclaving and formaldehyde have resulted in an increase in the removal of cadmium compared to biomass grown in cadmium and biomass removed from middle and matured to wash in distilled water.

The data presented in this work revealed that the treatments of mycelium of *Aspergillus nidulans*, both with autoclaving and formaldehyde, increased efficiency of removal of cadmium over living biomass and biomass cultivated in presence of the metal ion.

The increased exposure of binding sites for cadmium in biomass surfaces also occurs with treatment with acids and alkalis. Huang and Huang (1996) demonstrated the positive effect of treatment of biomass from *Aspergillus oryzae* with oxalic acid phosphoric and in the process of removing cadmium. On the other hand, the treatment with glacial acetic acid decreased the potential removal of *Aspergillus niger* (Kapoor and Viraraghavan, 1998). In this case, the phenomenon reflects that the polymer structure of cell surface displays negative charge due to ionization of inorganic and organic groups. The higher electronegativity of biomass, the greater attraction and adsorption of heavy metal cations. Thus, the remaining hydrogen ions with the acid treatment can modify the electronegativity of biomass, resulting in the reduction of metal ions removal capacity.

The literature reveals that the biomass treatment with organic solvents such as methanol, dimethylsulfoxide and formaldehyde increased the removal of cadmium. Kapoor and Viraraghavan (1998) reported the effects of this type of treatment for *Aspergillus niger*. However, Huang and Huang (1996) had suggested that the treatment of biomass with formaldehyde reduces the efficiency of removal because biomass is boiled during treatment with formaldehyde, which results in the methylation of amino acid present in the cell wall grouping.

The ability to remove heavy metals by biosorbents is strongly pH sensitive. Removal under the dependency of the pH can be related in large part to several functional groups in fungal cell surface and also about the chemistry of metal solution. The functional groups involved in connecting/removing heavy metals are weak acids, in general, such as the carboxylic groups, which are protonated at low pH values. At low pH values, the concentration of hydrogen ions is also high, which results in the possibility of competition of hydrogen ions by binding sites, resulting in a reduced ability to remove (Kapoor and Viraraghavan, 1995; 1998; 1999).

Interactions between metal cations and electron-rich functional groups on biomass can be very sensitive to the pH of the solution. However, the manner in which the pH value plays in the removal of metal ions depends on the type of biomass and the type of metal.

Removal of cadmium by the mycelium of *Aspergillus nidulans* was influenced by the initial pH of the solution, metallic and increased due to an increase in the pH value. The data presented in this study show that when the initial pH corresponds to 4.0 the ability to remove cadmium dramatically decreases, which indicates the availability of competing hydrogen ions with the binding sites on the biomass of *Aspergillus nidulans*. Such a process is reduced by the rise in pH to pH 5.0, where the removal rate is already larger and larger removal occurred where 6.0.

The results of many studies of metal removal vary widely because of different parameters used by the authors in search of suitable materials. Some researchers have used biomass types easily available, others use isolated and specific lineage and some use gross biomass processed in order to improve the properties of removal (Kapoor and Viraraghavan, 1995; 1998; 1999; Barros Jr. *et al.*, 2003). Thus, in the absence of a uniform methodology, results are reported in many different units of research, making it impossible for a quantitative comparison.

It is clear from the discussion so far that every inexpensive adsorbent has its specific physical and chemical characteristics such as surface porosity, and physical strength, as well as the inherent advantages and disadvantages in wastewater treatment. In addition, the adsorption capacities of sorbent mass also vary according to the experimental conditions. Therefore, the comparison of sorption performance becomes complex.

However, of course, that the literature reveals that many microbial systems may have potential as a readily available, cheap and sorbents effective for removal of heavy metals. Such materials, especially those isolates obtained from natural environments and environments contaminated with heavy metals exhibit advantages for environmental purposes, such as adsorption rate and high capacity, and high selectivity for different concentrations of metals and also show faster removal kinetics. It is therefore necessary to seek organic adsorbents, viable and cost-effective solution to meet growing demand due to high concentration of heavy metals in the environment, despite the number of published data laboratory (Paraszkiewicz *et al.*, 2009; Gadd, 2010).

In this experiment, the biomass of *Aspergillus nidulans* was simply mixed with formaldehyde and not boiled, which resulted in increased cadmium removal capacity for biomass. The data suggest that the biomass of isolated from *Aspergillus nidulans*, obtained from sediment of mangrove swamp, physically or chemically treated was able to remove cadmium ions from aqueous solution.

The results showed that the maximum removal was achieved with pH value equal to 6.0. So it can be affirmed that the pH of the solution the electrostatic binding of ions influence the functional groups. Species of *Aspergillus* exhibit abundance of chitin and chitosan units, a reasonable amount of histidine-rich proteins and other aminoacids which serve as array of COOH NH_2 groups (Mukhopadhyay *et al.*, 2006a, b), which in turn are involved in the binding of metal ions (Tsezos, 1983). Thus, the cadmium interaction matrix is probably determined by the extent of protonation of the functional groups of the cell wall, which depend on the pH of the solution.

Ultrastrucutural analysis

With a view to identify possible changes in hyphae in response to the pretreatment and exposure to cadmium, samples of biomass produced for removal kinetics assays were processed for ultrastructural analysis. Figure 4 shows electronmicrographs of *A. nidulans* biomass.

In control samples the mycelia displays hyphae densely compressed, with reduced electron density (Figure 4A, 4B and 4C).

On the other hand, the samples submitted to contact with cadmium on 1mM, 2mM and 3mM, autoclaved and exposed to formaldehyde exhibit highly ultrastructure of diverse observed for sample control. The variations depend on the concentration of the metal and the treatment of the sample. The changes appear as a modification to the electron density, thickness of hyphae and the emergence of precipitates on the surface of mycelium were noted (Figure 4D-I).

A search in the literature indicated the absence of data on the ultrastructural studies of fungal biomass, natural and/or pretreated subjected to heavy metals removal kinetics. In this way, a comparison with the data obtained in this study is not feasible. Currently, the data submitted for the first time in literature.

Figure.1 Effect of pretreatment and pH on removal of cadmium at 1mM by *Aspergillus nidulans*. **BL**- living biomass washed with distilled water; **BA**- autoclaved biomass and **BF**- biomass treated with formaldehyde

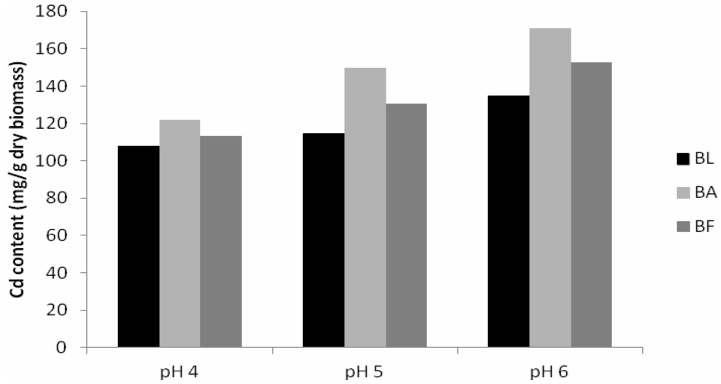


Figure.2 Effect of pretreatment and pH on removal of 2mM of cadmium by *Aspergillus nidulans*. **BL**- living biomass washed with distilled water; **BA**- autoclaved biomass and **BF**- formaldehyde treated biomass

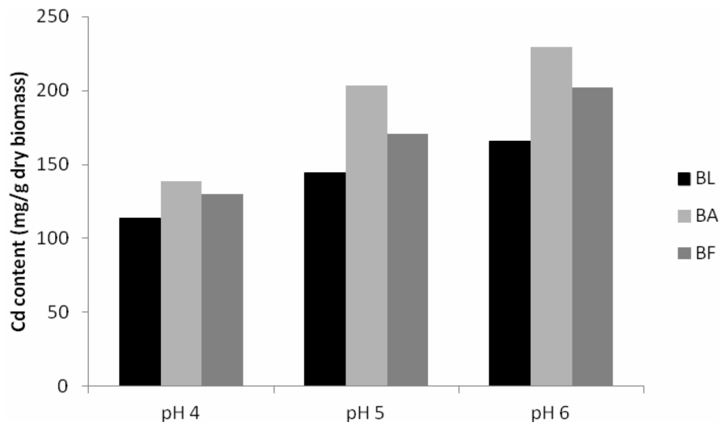


Figure.3 Effect of pretreatment and pH on removal of 3mM of cadmium by *Aspergillus nidulans*. **BL**- living biomass washed with distilled water; **BA**- autoclaved biomass and **BF**- biomass treated with formaldehyde

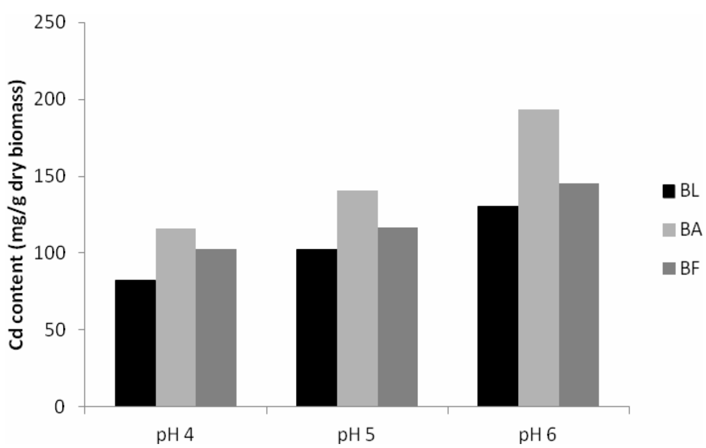
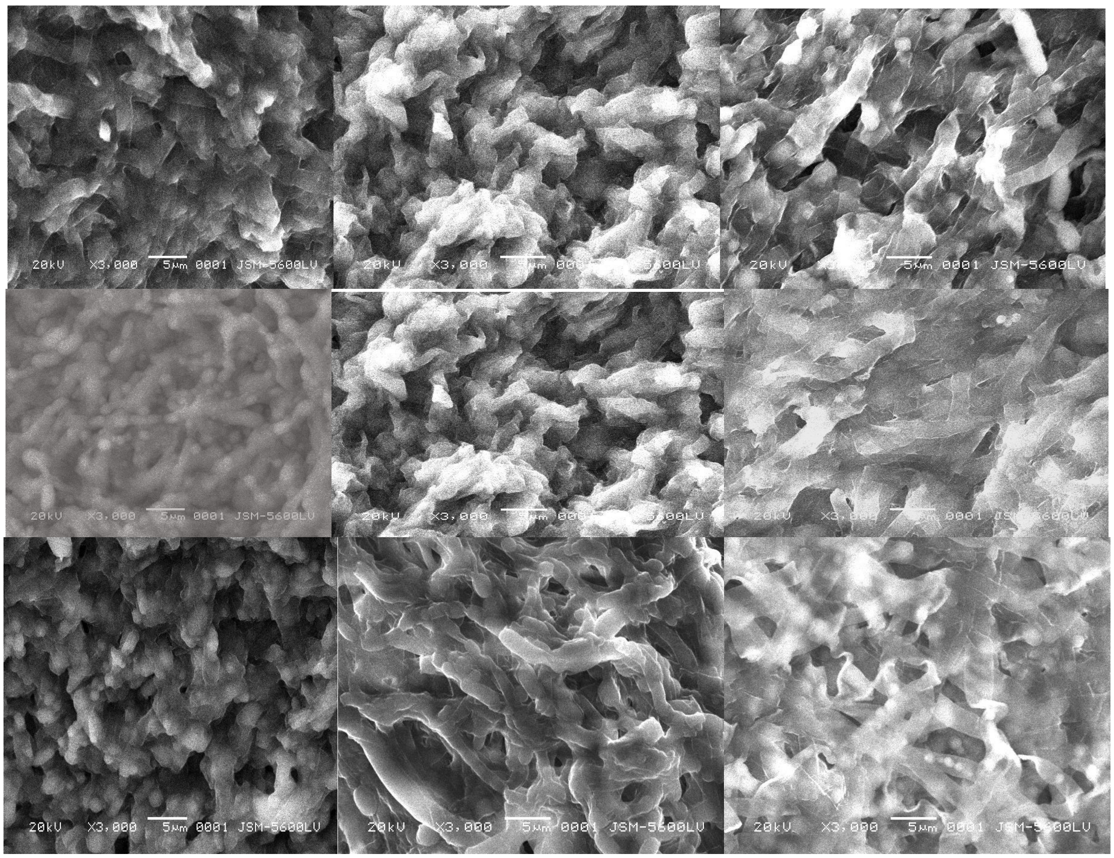


Figure.4 Electronmicrographs of *Aspergillus nidulans* mycelia used on cadmium removal kinetics. A-B-C living biomass submitted to removal kinetics, exposed to cadmium 1mM, 2mM and 3mM, respectively; D-E-F-biomass treated with formaldehyde subjected to removal kinetics, exposed to cadmium 1 mM, 2 mM and 3 mM; G-H-I-autoclaved biomass and subjected to removal kinetics exposed to cadmium 1 mM, 2 mM and 3 mM. 3.000 X



From the results obtained in this study concluded that in this study, an isolated from *Aspergillus nidulans* metabolically active was used successfully for the removal of cadmium in a high concentration, being able to remove the metal ion of the culture medium. The mycelia submitted to formaldehyde treatment and autoclaved were efficient, increasing the speed and efficiency of the process of cadmium removal in each concentration. The pH influences the removal process, being the one who led the largest removal efficiencies, in this study pH 6.0 was the best for all treatments used.

Acknowledgments

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