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RESEARCH ARTICLE

Bioremediation of water contaminated with uranium using *Penicillium piscarium*

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Abstract

Penicillium piscarium can be indicated as promising in the treatment of sites contaminated with uranium. Thus, this research aimed to analyze the *P. piscarium* dead biomass in uranium biosorption. This fungus was previously isolated from a highly contaminated uranium mine located in Brazil. Biosorption tests were carried out at pH 3.5 and 5.5 in solutions contaminated with concentrations of 1 to 100 mg/L of uranium nitrate. Our results showed that the dead biomass of *P. piscarium* was able to remove between 93.2 and 97.5% uranium from solutions at pH 3.5, at the end of the experiment, the pH of the solution increased to values above 5.6. Regarding the experiments carried out in solutions with pH 5.5, the dead biomass of the fungus was also able to remove between 38 and 92% uranium from the solution, at the end of the experiment, the pH of the solution increased to levels above 6.5. The analysis of electron microscopy, Energy-dispersive spectroscopy, and X-ray fluorescence demonstrated the high concentration of uranium precipitated on the surface of the fungal biomass. These results were impressive and demonstrate that the dead biomass of *P. piscarium* can be an important alternative to conventional processes for treating water contaminated with heavy metals, and we hope that these ecofriendly, inexpensive, and effective technologies be encouraged for the safe discharge of water from industrial activities.

KEYWORDS
bioremediation, *Penicillium piscarium*, uranium, wastewater

1 | INTRODUCTION

Bioremediation is a promising technology that uses the metabolic potential of microorganism for the removal or reduction to acceptable levels of heavy metal ions in contaminated sites.[1,2] This technology may be performed ex situ or in situ. Ex situ treatment usually requires a higher cost, with the removal and transport of the contaminants to be treated at other locations. In situ treatment is carried out at the site of contamination, that is, it does not require transportation or excavation of the site.

Bioremediation requires a simplified logistic that reduce the cost of the process, which makes it more feasible and with minimal interference at the site. Bioremediation usually presents a lower cost than conventional treatment methods.[3] There are four main types of bioremediation with the use of microorganisms, which are: biosorption; bioreduction; bioaccumulation; and biomineralization.

Bioreduction: In the absence of O_2, during the anaerobic fungi respiration, uranium is the final electron acceptor, thus, uranium VI (soluble) is transformed into IV (insoluble).[4]

Bioaccumulation: Uranium's own toxicity changes the membrane permeability of fungi, so the accidental capture of U inside of fungal cell occurs.[4]

Biomineralization: Mainly through enzymes produced by fungi (e.g., phosphatase) uranium ions can be precipitated from waste water as minerals, such as uranium phosphates.[5]

In biosorption, chemical or physical binding of the heavy metal to biomass occurs.[6] This process demands a solid and liquid phase, the solidphase is composted by biological material (biosorbent), while in the liquidphase water is normally used as a solvent containing a dissolved species to be sorbed, as metal ions. The attracted and bound of sorbent to sorbate involves complex mechanisms, the process continues till equilibrium between the amount of solid-bound sorbate species and its portion remaining in the solution.[7-10]

The use of fungi as bioremediation agents of soil and water contaminants has been studied before, finding that fungi have high metabolic capacities that can be applied to radionuclides. The high metabolic capacity of the fungi causes them to be chosen as the best microorganisms to grow and develop in acidic environments and contaminated by radionuclides.[11-13] The fungal cell surface presents chitin and chitosan, which are considered as excellent biosorbents of heavy metal ions.[14]

The fungi *Penicillium* spp., *Aspergillus* spp., *Rhizopus* spp., *Mucor* spp., *Saccharomyces* sp., and *Fusarium* sp. have been shown to be excellent biosorbents of metal ions.[15,16] The biomass of *Penicillium* sp., *Rhizopus* sp., and *Saccharomyces* sp. can biosorb toxic metals, such as U, Th, Sr, Ni, Zn, Pb, Cr, and As.[17-19]

Uranium ion can cause several risks to human health, the main ones are effects on the kidneys (inflammation) and change in urine color, consumption of water and food contaminated with high concentrations of uranium increases the risk of lung and bone cancer.[20]

Studies on uranium uptake of fungal biomass have focused on biosorption mechanisms, but current studies have shown that fungi can use other pathways to reduce the contamination of radionuclides in the environment.[21-23] Under field conditions, variations in the physical–chemical, climatic conditions, and wastewater composition of the soil and water in each location can interfere with the process. Therefore, there is a growing need for bioremediation process, including fungal-mediated remediation, to be adapted to site-specific conditions.[24,25]

Studies using dead biomass of *Penicillium* species for heavy metal remediation are very scarce, specifically for sites contaminated by uranium. We verified its ability to remove uranium from the water in conditions of temperature and pH close to find in the area study. Thus, our investigation on indigenous fungi aims future applications in field based on site conditions from which the microorganisms were isolated. We highlighted that the knowledge of the relationship between fungi dead biomass and uranium in the environment is fundamental to develop an economic and ecofriendly tool for bioremediation. In our biosorption study, dead biomass of *P. piscarium* was used, the most frequent species isolated from the contaminated uranium mine Osamu Utsumi in previous study.

2 | MATERIAL AND METHODS

P. piscarium strain used in this research was previously isolated and identified from a uranium mine located in Brazil.[26] The *P. piscarium* dead biomass was tested for its ability to remove uranium in a contaminated solution.

2.1 | Biosorption tests

2.1.1 | Production of dead biomass

P. piscarium strain was inoculated in petri dishes with potato dextrose agar, then, kept in an incubator at 25°C for 7 days. After growth, 5 mm diameter plugs were removed from *P. piscarium* cultures using a cork borer.[27] The plugs were added in 500 ml flasks containing 150 ml yeast extract peptone dextrose broth.[28]

The conical flasks were then kept under agitation on a horizontal orbital shaker at 25°C in 150 rpm for up to 10 days for the production of fungal biomass. After the growth of the fungal biomass, the flasks were autoclaved and centrifuged, the biomass was separated from the liquid medium by filtration, using a paper filter, the biomass was washed five times with Milli-q water, and then it was dried by the lyophilization technique and ground for subsequent use.[29]

2.1.2 | Biomass contact with uranium

After obtaining the biomass, a stock solution of 100 mg/L of uranium nitrate was prepared, the pH of the solution was adjusted to 3.5 and 5.5, using sodium hydroxide (0.1 N) and hydrochloric acid (0.1 N), and the measurement was performed with a digital pH meter (Kasvi). Both solutions were diluted in the following concentrations: 1, 10, 25, 50, and 100 mg/L.

The dead biomass was weighed (0.2 g) and added to 50 ml sterile conical tubes with 20 ml of the stock solutions with different pH and concentrations. The conical tubes were placed on a horizontal orbital shaker at 150 rpm for 1 hr at 25°C. After, 5 ml of the solution was vacuum filtered using a 0.2 μm PTFE syringe filter (Allcrom) and then mixed to a 5 ml of 2% nitric acid solution, this acid solution was used for the digestions of inorganic and organic samples improving the inductively coupled plasma atomic emission spectroscopy (ICP OES) analysis.[30,31] All tests were performed in triplicate.

2.1.3 | Uranium measurement

The uranium concentration was determined by the analytical technique of ICP OES at the Institute of Chemistry and Environment/IPEN.

2.2 | Scanning electron microscopy and X-ray fluorescence

After the biosorption, the dead biomass was analyzed by scanning electron microscopy (SEM + energy-dispersive spectroscopy [EDS])

and energy dispersive X-ray fluorescence (ED-XRF) analysis at the Institute of Physics, University of São Paulo, Brazil, to identify uranium precipitated on the fungal cell surface.

SEM analysis was performed with a JEOL® model 6460LV and ED-XRF uses a portable Amptek® setup composed with a mini X-ray tube (silver [Ag] target) and a Si Drift X-ray semiconductor detector ($25mm^2 \times 500\ \mu m$ 0.5 mil^{-1}) with a thin beryllium end window of 3.8 cm and energy resolution of 125 eV FWHM at @ 5.9 keV (55Fe). The XRF measurements were carried out with 30 kV voltage and 50 μA current and an excitation/detection time of 300 with a fixed distance of ~3(1) mm. In the U measurements, a filter of W (25 μm) and Al (250 μm) foil is used in the X-ray tube exit.

3 | RESULTS

3.1 | Biosorption

The results of biosorption showed a high reduction in the concentration of uranium and increase in the pH of the water after 24 hr of contact with 0.2 g of dead biomass of *P. piscarium* in 20 ml of solution with concentrations of 1 to 100 mg/L of uranium nitrate.

The percentage of uranium reduction in water contaminated with 1 to 100 mg/L of uranium at pH 3.5 ranged from 93.2 to 97.5%. In solutions with initial concentrations of 1 mg/L the concentrations were lower than quantification limits of ICP OES technique (0.1 mg/L). At all concentrations, the pH of the solution increased to values between 5.6 and 5.9 (Table 1).

TABLE 1 Results of uranium biosorption in water (pH 3.5) using dead biomass of *P. piscarium*

Control (pH 3.5) mg/L	After the treatment with 0.2 g of dead biomass (mg/L)	Percentage of uranium reduction (%)	Final pH
1	<0.1	—	5.7
10	0.6 ± 0.05	93.2	5.6
25	1.5 ± 0.01	93.6	5.8
50	0.7 ± 0.04	98.5	5.6
100	2.8 ± 0.08	97.1	5.9

TABLE 2 Results of uranium biosorption in water (pH 5.5) using *P. piscarium* dead biomass

Control (pH 5.5) mg/L	After the treatment with 0.2 g of dead biomass (mg/L)	Percentage of uranium reduction (%)	Final pH
1	0.6 ± 0.04	38.0	7.1
10	1.8 ± 0.05	81.8	6.5
25	7.2 ± 0.05	70.9	6.8
50	11.0 ± 0.1	78.0	6.6
100	7.8 ± 0.1	92.2	6.8

In water contaminated with uranium with an initial pH of 5.5, there was also an increase in the pH of the solution to values between 6.5 and 7.1 and a high reduction in the concentration of uranium after treatment with dead *P. piscarium* biomass, the percentage of reduction was from 38.0 to 92.2% in all tested concentrations (1 to 100 mg/L of uranium) (Table 2).

3.1.1 | SEM of *P. piscarium* dead biomass

After the biosorption tests, SEM analysis was performed and the image showed that the dead biomass before contact with contaminated water, demonstrated a "smooth" surface with a compact aspect, (Figure 1a,b at ×600 and ×1,500 magnification). After the use in the treatment of water contaminated with uranium, the biomass image appeared more spread (not compact) and with the heavy metal covering its surface, showing the uranium precipitation, (Figure 1c,d at ×1,500 and ×3,000 magnification).

EDS confirmed the presence of uranium in fungal biomass after biosorption of uranium. Comparing EDS spectra before (Figure 2) and after (Figure 3) uranium biosorption, it was possible to identify a strong peak of uranium in Figure 3, which confirmed uranium biosorption onto *P. piscarium* dead biomass.

The quantitative results obtained with EDS, Figure 2 (after biosorption) confirmed the mass of uranium by weight of 2.91% on the fungal biomass.

3.1.2 | Energy dispersive X-ray fluorescence

The ED-XRF analysis confirmed the high concentration of uranium precipitated onto *P. piscarium* dead biomass after contact with water contaminated with the toxic metal.

In Figure 4a, the ED-XRF spectrum of the control (biomass before being used for uranium biosorption), no uranium peaks were observed. However, comparing Figure 4b (uranium standard) with Figure 4c (fungus biomass after uranium biosorption), we identified three similar uranium peaks, which confirms the strong presence of the metal in the dead fungus biomass after the biosorption treatment.

The IAEA quality assurance reference material RGU-238 (uranium standard) was measured in the same geometry and conditions of the sample and were used to confirm the U L-lines (Figure 4b), ED-XRF spectrum of Figure 4c clearly shows the three peaks for U, Lα, Lβ, and Lγ lines in *P. piscarium* dead biomass, confirming this way, the presence of precipitated uranium on the surface of *P. piscarium* biomass.

4 | DISCUSSION

Acid mine drainage (AMD) is a major problem in mines contaminated with heavy metals. AMD generates cationic metals (+) which are toxic for organisms and cause ecological damage. Most biosorption research is on metallic and related pollutants, including radionuclides.

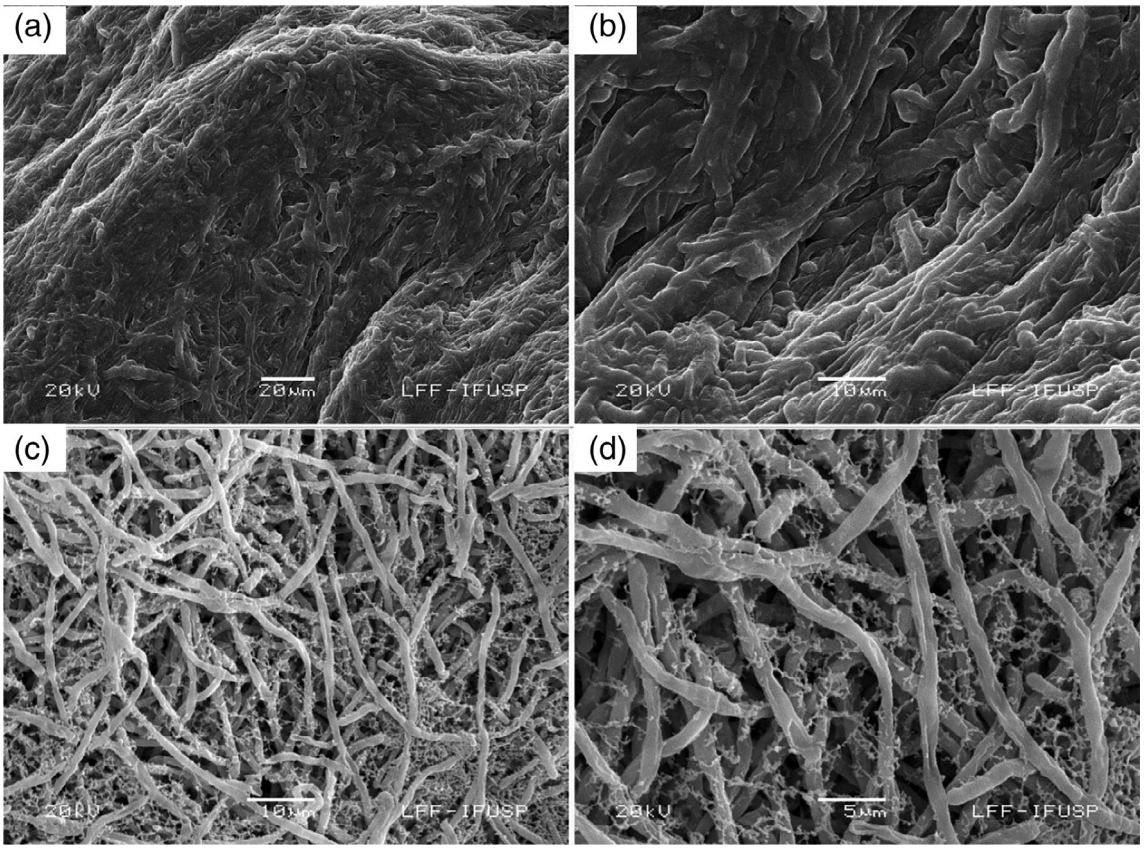

FIGURE 1 Scanning electron microscopy shows the *P. piscarium* dead biomass before and after the contact with uranium solution. (a) Dead biomass before the treatment; (b) enlarged image of Figure, A; (c) *P. piscarium* dead biomass after contact with uranium solution; and (d) enlarged image of Figure, C

Biosorption is described as a fast and reversible process of binding of ions from aqueous solutions onto functional groups present on the surface of a biomass; the process is independent of cellular metabolism and can be carried out through mechanisms, such as surface complexation, ion exchange, and precipitation. This is a characteristic of living and dead organisms and has been described as promising biotechnology for removing pollutants from the solution.[32,33]

In our findings, the pH increased during the biosorption processes, this result was similar to the Cu biosorption studies carried out by Verma.[34] The pH is considered a very important environmental factor that will determine the interaction between the fungal mycelium and uranium since it is known that the concentration of H+ or OH– changes the fungal cell's polarization and thus the biosorption of the metal. This phenomenon occurs, because the H+ (protons) compete with the heavy metal for the binding sites of the fungus biomass, in many cases, this competition can decrease the biosorption of heavy metals.[16] In our experiments, even with an increase in pH, the dead biomass of *P. piscarium* was able to remove high concentrations of uranium from contaminated acid water.

In biosorption processes, pH can affect the binding sites and functional groups on the fungal cell surface,[35] components of the fungal cell surface, such as mannoproteins, chitin, glucans and minerals, such as carbonates, are examples of functional groups that serve for the binding and precipitation of uranium ions.[36,37]

The decrease in uranium biosorption at pH 5.5 compared with the pH 3.3 experiment, may be related to the repulsion between the negative charge of anionic species in solution and the negative surface charge of the fungal cell binding sites. In addition, precipitation of metallic (insoluble) hydroxides can occur in the solution and thus reduce the biosorption process. High pH also causes a decrease in uranium solubility and consequently a decrease in biosorption rate.[31]

In our research, SEM and ED-XRF analysis confirmed uranium precipitation on the *P. piscarium* dead biomass. After biosorption, micrographs show uranium precipitates cover the surface of the dead fungal cell (Figures 1, 2 and 3). Fungal biomass presents porosity, and this formation with pores is important because it offers more sites of attachment to the metal, increasing the contact area and the diffusion during biosorption.[29]

The filamentous fungi biomass has been described as an important cost-effective for biosorption of heavy metals. PO_3, —NH, —CH, —OH, C═O, and C—O have been reported as important functional groups in the adhesion of heavy metals, which are part of polysaccharides, mainly chitin, but also proteins and lipids of the fungal cell.[23,38,39] Our EDS analyses showed the presence of some of these components present in the fungal cell biomass, such as C, Na, and K, these structural components can help the adhesion of the metal onto the dead biomass.

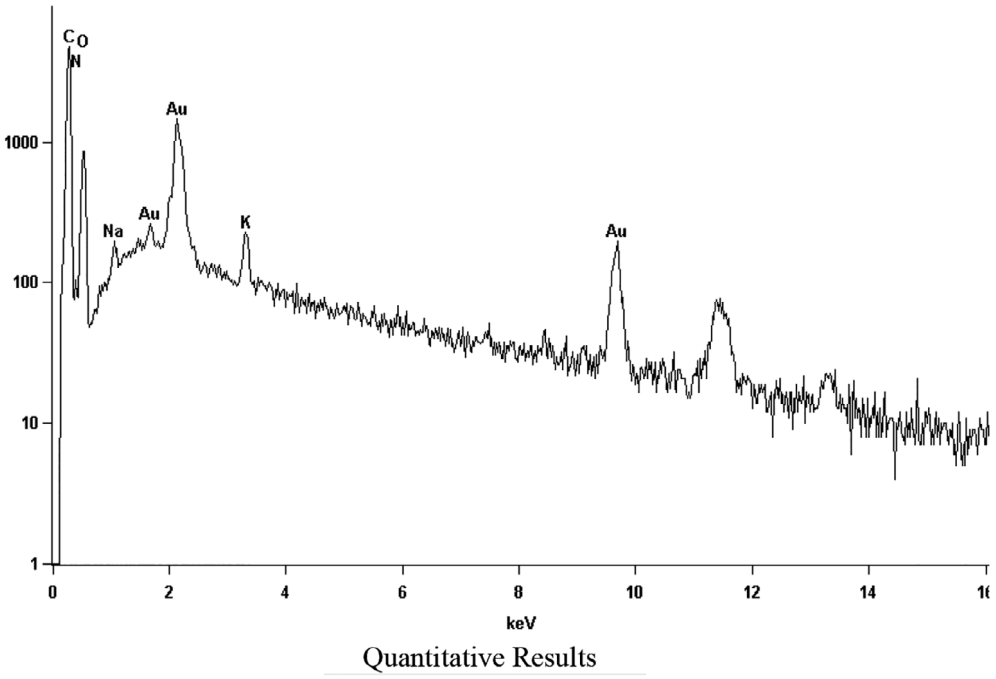

FIGURE 2 Energy-dispersive spectroscopy spectrum and quantitative results of *P. piscarium* dead biomass before biosorption

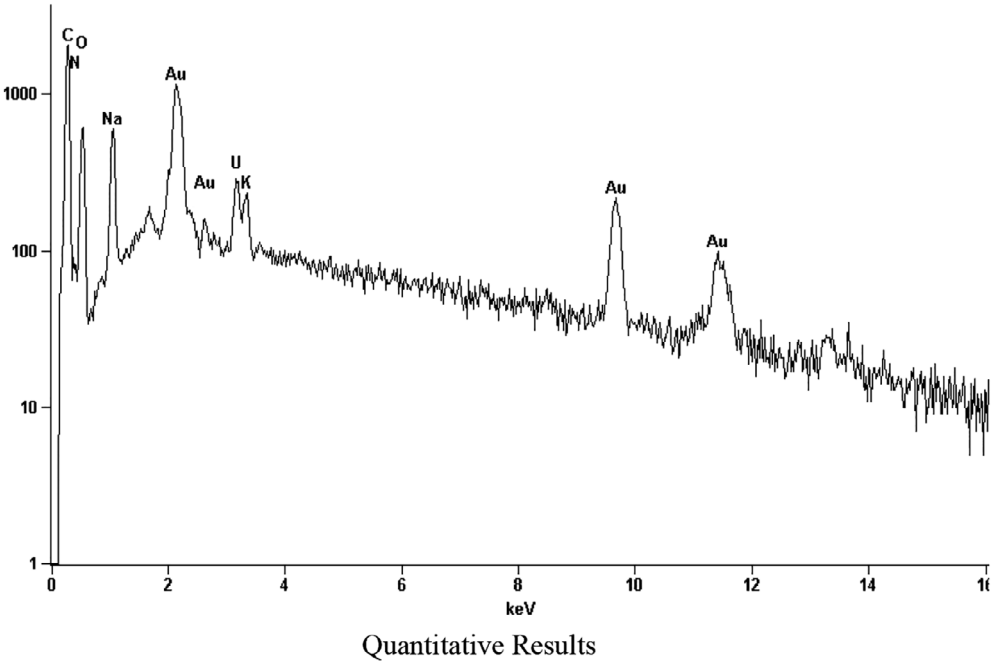

FIGURE 3 Energy-dispersive spectroscopy spectrum and quantitative results of *P. piscarium* dead biomass after uranium biosorption

ED-XRF analysis is based on the fact that the X-rays emitted from an ionized atom have energies that are specific to the element involved in the analysis. X-ray intensity is proportional to both the elemental concentration and the strength of the ionizing source.[40] Radioactive substances produce three types of dangerous radiation: alpha particles, beta particles, and gamma rays. These types of radiation cannot be seen with the naked eye and, therefore, you do not see a light or anything else, this makes radionuclides dangerous. Thus, the identification of these components in materials indicated the presence of radionuclides, such as uranium.[41,42]

Fungi are currently described as excellent biosorbents for removing heavy metals from contaminated environments, both live and dead biomass have the advantage of being resistant to other factors that impair remediation processes, such as the pH and toxicity of heavy metals.[43-45]

In some studies, macrofungi are described as great biosorbents for uranium,[46] algae biomass is also cited by several authors as promising for use in the biosorption of heavy metal.[47,48] Filamentous fungi are also described in the literature for removal of diluted uranium, as species of *Aspergillus niger*,[49] this demonstrates a constant search to find new materials that are cheap and effective in removing uranium ions from water that are present in low concentrations.

The removal of toxic metals by biological processes has the following benefits: low-operating costs, high efficiency, biosorption

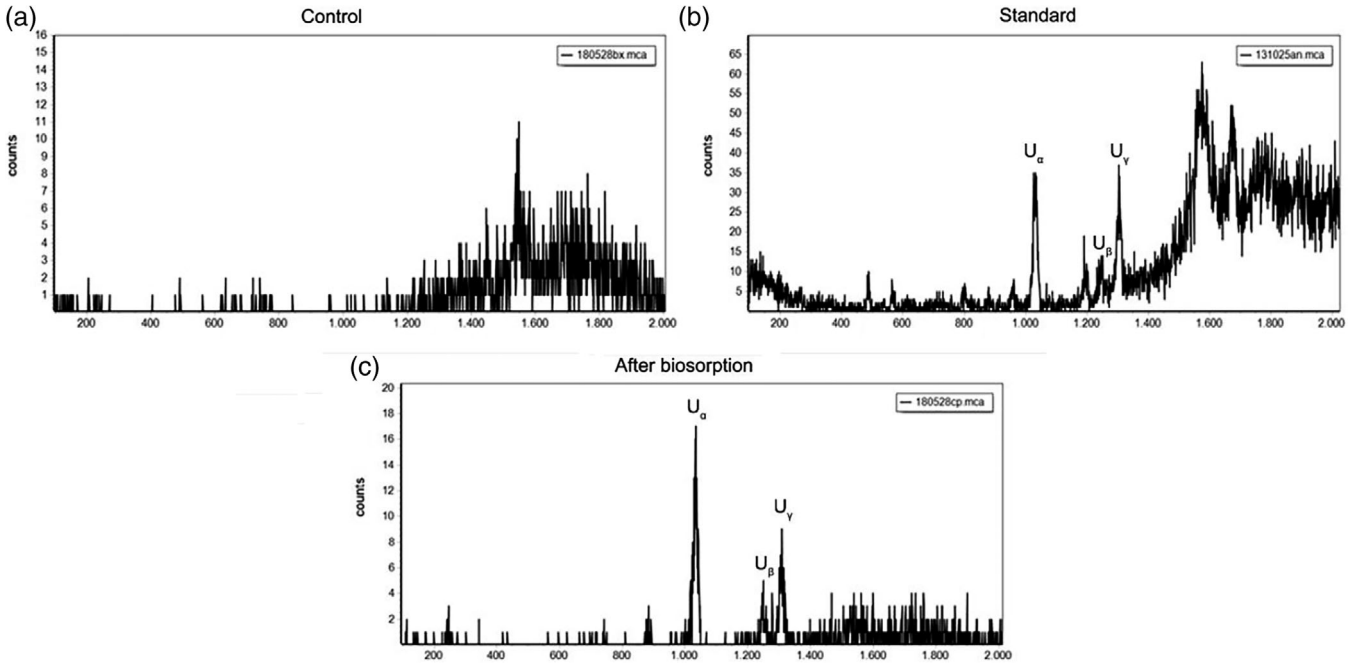

FIGURE 4 Energy dispersive X-ray fluorescence spectra obtained for (a) control biomass, (b) uranium standard (powder reference material RGU-238), and (c) biomass after biosorption

capacity of various types of heavy metals, and low production of tailings (does not produce alkaline sludge like chemical treatments).[50,51]

Penicillium species are widely distributed and are present throughout the environment. *Penicillium* are widely studied in the biosorption of heavy metals in contaminated environments, and there are several studies with Fe, Cu, Pb, Cd, and Zn but studies related to uranium biosorption are still scarce.[34,52]

The removal of uranium from solutions by *Penicillium* species has been studied by some authors, but each species (despite having the same name) has its effectiveness in removing heavy metal, this is because each fungus, depending on where it grew and developed, has its own characteristics, for example, more binding structures on the cell surface when compared to those that are not isolated from extreme environments and did not need to adapt to stress, as chitin or melanin. In addition, the presence of these structures will give more resistance to microorganisms in extreme environments.[29,53-55]

Our results using *P. piscarium* were impressive, as conventional methods of treating water contaminated with heavy metals are not effective in removing heavy metal ions when it is in low concentrations, less than 100 mg/L.[56,57] We believe that soon this new biotechnology, using dead fungal biomass, will be important to the treatment of water contaminated with uranium.

5 | CONCLUSION

P. piscarium dead biomass showed a high capacity to remove uranium from aqueous solution even in concentrations below 100 mg/L. This characteristic is important in the field of biotechnology, since obtaining dead fungi biomass occurs by a simple and inexpensive process. The technology can also be carried out assisting conventional processes, reducing the cost of treating water contaminated with heavy metals. In addition, the dead biomass increases the pH of contaminated water, as most of the time these mining effluents have an acidic pH, which makes their safe disposal difficult. Our findings can assist scientific studies about the use of *P. piscarium* in bioremediation processes of contaminated water and we hope that new effective, economic, and eco-friendly alternatives be encouraged.

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E. C. and B. C. conceived the experiments. E. C., T. A. R., M. C., and M. R. carried out experiments. E. C. and B. C. did modeling. E. C., T. A. R., and B. C. wrote the manuscript. All authors discussed the data and the results, and commented the manuscript. B. C. supervised the project.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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