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Preliminary investigation on biosorption mechanism of ^{241}Am by *Rhizopus arrhizus*

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As an important radioisotope in nuclear industry and other fields, ^{241}Am is one of the most serious contamination concerns due to its high radiation toxicity and long half-life. Encouraging biosorption of ^{241}Am from aqueous solutions by free or immobilized *Rhizopus arrhizus* (*R. arrhizus*) has been observed in our experiments. In this study, the preliminary evaluation on the mechanism was further explored via chemical or biological modification of *R. arrhizus* using europium as a substitute for americium. The results indicated that in approximately 48 hours *R. arrhizus* was able for efficient adsorption of ^{241}Am. The pH value of solutions decreased gradually with the uptake of ^{241}Am by *R. arrhizus*, implying that H^+ was released from *R. arrhizus* via ion-exchange. The biosorption of ^{241}Am by the decomposed cell wall of *R. arrhizus* was as efficient as by the intact fungus. The adsorption ratio for ^{241}Am by deacylated *R. arrhizus* dropped, implying that carboxyl functional groups of *R. arrhizus* play an important role in the biosorption of ^{241}Am. Most of the investigated acidic ions have no significant influence on the adsorption of ^{241}Am, while saturated EDTA can strongly inhibit the biosorption of ^{241}Am by *R. arrhizus*. When the concentrations of coexistent Eu^{3+}, Nd^{3+} were 300 times more than that of ^{241}Am, the adsorption ratios would decrease to about 86% from more than 99%. It could be noted by transmission electron microscope (TEM) analysis that the adsorbed Eu is scattered almost in the whole fungus, while Rutherford backscattering spectrometry (RBS) indicated that Ca in *R. arrhizus* have been replaced by Eu via ion-exchange. The change of the absorption peak structure in the IR spectra implied that there was complexation between metals and microorganism. The results implied that the adsorption mechanism of ^{241}Am by *R. arrhizus* is very complicated involved ion-exchange, complexation process as well as nonspecific adsorption in the cell wall by static electricity.

Introduction

For decades, the biosorption technology has been recognized as an attractive potential for removal of heavy metals and degradation of organic chemicals from wastewaters due to good performance, low cost and large available quantities.[1–5] In fact, as early as 1950s, there were some attempts to accumulate precious metals, such as gold and silver by different microorganisms. Until 1980s or later, growing interest was shown in the removal of toxic and harmful materials from wastewaters for environmental protection.[6–9] The sewage purification and the treatment of industrial wastewaters by biosorption technology have been got practical application in China and elsewhere.[10–12] Meanwhile, accumulation of some natural radionuclides, such as uranium, thorium, and radium by different microorganisms has been observed,[13–18] and the biosorption behavior of some artificial radionulides have been investigated.[19–21]

However, the biosorption mechanism for metals has not been clearly understood until now, especially the biosorption mechanism of radioactive elements, even though much effort has been invested in exploring the mechanism for many years. Most investigations demonstrated that biosorption mechanism for metals is very complicated, and involved in many processes, such as surface complexation,[22–25] ion exchange,[26–28] oxidation-reduction,[29–32] adsorption induced by static electricity or enzyme,[33] co-precipitation,[34,35] etc. Additionally, many methods or techniques were used for the investigation in biosorption mechanism of metals,[36–40] such as infra-red spectrum (IR), nuclear magnetic resonance (NMR), transmission electron microscope (TEM), electron energy loss spectrometer (EELS), electron dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), extended X-ray absorption fine structure (EXAFS), time-resolved laser-induced fluorescence spectroscopy (TRLFS), even particle induced X-ray emission (PIXE) analysis. Compared with stable elements or isotopes, the experiments involved in radioactive elements or isotopes are much more difficult, due to the concerns for radioactive contamination of instruments or materials, especially for some radioactive isotopes of interest without stable isotopes including ^{241}Am.

As a transuranium element, americium has no stable isotope although has about 20 radioisotopes or isomers. Among them, ^{241}Am is generally used as target material in nuclear industry or excitation source in some scientific instruments.[41] Also, it has widespread use in other fields. Unfortunately, ^{241}Am is one of the most serious concerns due to its long half-life and α-particle emission, especially, the tendency to deposit on several key tissues or organs, such as skeleton and liver, if it enters the human body. More recently, in order to find a

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feasible method for disposal of the low-medium radioactive wastewater produced in the process of preparing ^{241}Am fire alarms, the biosorption of ^{241}Am from solution by free or immobilized *R. arrhizus* has been investigated in our Institute.[42,43] The preliminary results showed that *R. arrhizus* is a very efficient biosorbent and the biosorption process could be described by the Freundlich adsorption isotherm. An average of more than 99% of the total ^{241}Am could be removed by free *R. arrhizus* from ^{241}Am solutions. Moreover, the immobilized *R. arrhizus* not only can accumulate ^{241}Am as efficiently as free *R. arrhizus,* but also can be used repeatedly or continuously.

In this study, the biosorption mechanism via chemical or biological modification of *R. arrhizus* was further explored. Especially, Eu was used as a model for Am and the Eu-adsorbed *R. arrhizus* instead of the ^{241}Am-adsorbed *R. arrhizus* was analyzed by infra-red spectrum (IR), transmission electron microscope (TEM) and Rutherford backscattering spectrometry (RBS) to avoid possible radioactive contamination when ^{241}Am is involved in the analysis, since europium has similar chemical characters to americium and has several stable isotopes.

Experimental

Reagents and experimental solutions

^{241}Am [^{241}Am(NO$_3$)$_3$] in aqueous solution was provided by the Institute of Nuclear Physics and Chemistry, CAEP (Mianyang, P. R. China). Stock solutions containing ^{241}Am of 555 MBq/l (4.38 mg/l) and diluted solutions were prepared in distilled water at pH 2. All the other chemical reagents were of analytical grade or chromatographic grade and were used without further purification.

All glassware for the biosorption experiments was routinely rinsed with 0.5 mol/l HNO$_3$ and washed extensively with distilled water to prevent interference by contaminants. The pH of each solution used for adsorption was measured by a digital pH meter and adjusted by the addition of 0.2 mol/l HNO$_3$ or 0.2 mol/l NaOH solution.

Strains and culture

R. arrhizus was obtained as a gift from College of Life Science, Sichuan University (Chengdu, P. R. China). The cultivation of *R. arrhizus* was completed as described previously.[42] Culture medium for growing the fungi contained glucose (1%) and (NH$_4$)$_2$SO$_4$ (50 g/l), at pH 6.0. In order to investigate the effect of culture time on ^{241}Am adsorption, the cultured fungi were collected at definite time by centrifugation. The fungi were washed several times with deionized distilled water and centrifuged at 4000 rpm for 15 minutes before the experiments.

Cell wall of R. arrhizus

The cell wall of *R. arrhizus* was obtained by the following procedure: 4 g suspended fungus in 50 ml distilled water at an ice-bath was treated with ultrasonic for 15 minutes at 400 W, 20 kHz. The ultrasonic-treated microorganism was centrifuged at 4000 rpm to remove impurity and untreated fungi. Then the supernatant was centrifuged at 15,000 rpm and the cell wall of *R. arrhizus* was got for further adsorption experiments.

Chemical treatment of R. arrhizus

In order to investigate the biosorption behavior of protein, carboxyl functional and other chemical components of microorganism for ^{241}Am, 200 mg *R. arrhizus* was treated by the following procedures, respectively: (1) deproteinization: mixed with 2 mol/l NaOH at room temperature for 24 hours; (2) defatting: treated with ethanol–chloroform solution (1:3) at room temperature for 24 hours; (3) deacetylation: refluxed with 40% NaOH at 112 °C for 4 hours. After then, the residues were washed with de-ionized water to neutral and filtered under vacuum for further adsorption experiments.

Adsorption experiments for ^{241}Am

The adsorption experiments were performed using static procedure. In brief, sorbents such as wet *R. arrhizus* or decomposed cell wall, or other components of fungi were added to ^{241}Am solutions of definite radioactive concentrations and of desired pH. The mixture was shaken on a rotary shaker at room temperature for 2 hours, except as described otherwise. Then, the mixture was centrifuged at 4000 rpm for 15 minutes. The supernatant liquid was removed, and assayed for radioactivity of residual ^{241}Am by means of an automatic counter with a NaI well detector.

For all the adsorption experiments, the results were expressed as the adsorption ratio (*R*, %):

$$R = (1 - C/C_0) \times 100\%$$

where C_0 is the initial ^{241}Am concentration (MBq/l), C is the final ^{241}Am concentration after adsorption. The conversion between mass and radioactivity for ^{241}Am was expressed as: 1 mg = 126.54 MBq.

Analysis of the Eu-adsorbed R. arrhizus by IR, RBS and TEM

In order to further explore the biosorption mechanism, and observe the metal-adsorbed fungi directly and conveniently, Eu was used as the substitute for ^{241}Am due to its similar chemical behavior to americium and availability as stable isotope. So, the Eu-adsorbed *R. arrhizus* can be analyzed by IR, RBS and TEM without worry about radioactive contamination.

The adsorption of Eu by *R. arrhizus* was performed likewise the adsorption of ^{241}Am. The centrifuged Eu-adsorbed *R. arrhizus* as well as virgin fungus was dried at 80 °C, and then the dried Eu-adsorbed tissues were pressed with KBr into approximate 0.5-mm thick small slide for infrared spectrometry (IR) or pressed into slide directly for Rutherford backscattering spectrometry (RBS). The IR spectrum was recorded on a Perkin-Elmer 983G IR spectrometer (UK). The RBS analysis was performed at the Institute of Nuclear Science and Technology, Sichuan University by an electrostatic accelerator with maximum terminal voltage of 2.5 MeV providing the ^{4}He^{+} ions of 2 MeV. The incident ions were impacted vertically on the samples. The backscattered ions were detected at a scattering angle of 150° by a Si surface-barrier detector with a depletion depth of 100 μm. The RBS spectra were analyzed using SIMNRA computer code.[44]

For transmission electron microscope (TEM) analysis, after centrifuged at 12000 rpm for 20 minutes, the Eu-adsorbed *R. arrhizus* and intact fungus was pre-fixed with glutaraldehyde of 3% first, then fixed with OsO_4, dehydrated with acetone step by step, embedded with Epon 812 and sectioned into ultra-thin samples. The samples were double-dyed with uranium acetate and sodium citrate for TEM analysis. The TEM micrographs were analyzed by a H-600IV spectrometer (UK).

Results and discussion

Change of the pH value of solutions in biosorption process

Many previous reports have shown that the pH or acidity was an important factor influencing the biosorption of heavy metals by microorganism.[7,45–47] In our previous experiments,[42] it was also noted that ^{241}Am uptake on the *R. arrhizus* is a pH-dependent process. In this study, the change of pH value of the solutions from the original pH 6.5 with the uptake of ^{241}Am by *R. arrhizus* was investigated. As summarized in Table 1, the pH value of the solutions decreased gradually with the uptake of ^{241}Am by *R. arrhizus*, implying that H^{+} released from *R. arrhizus*, possibly via ion-exchange.

Effect of culture time of microorganism on ^{241}Am adsorption

The effect of culture time of *R. arrhizus* on ^{241}Am adsorption is shown in Fig. 1. It could be noted that the adsorption ratio for ^{241}Am increases rapidly with the culture time of *R. arrhizus* and came up to 95% at 42 hour. After then, the adsorption ratio goes up to 98% gradually and tended to an equilibrium. The reason maybe that there existed some chemical or biologic substances in *R. arrhizus*, which were involved in biosorption of ^{241}Am and whose contents changed with the culture time. Obviously, the culture time of 48 hours is suitable for *R. arrhizus* to the uptake of ^{241}Am.

Biosorption of ^{241}Am by pretreated R. arrhizus

In order to investigate the biosorption behavior of protein, carboxyl functional and other group of microorganism for ^{241}Am, *R. arrhizus* was chemically or biologically pretreated by ultrasonic-treat, deproteinization, defatting as well as deacetylation.

As summarized in Table 2, the adsorption ratio of the cell wall was as efficient as that of intact fungi, while the adsorption ratio of ^{241}Am by the deacylation *R. arrhizus* was much less than that by intact fungus, implying that the acyl may play an important role in the biosorption of ^{241}Am, and *R. arrhizus* has high acyl content. However, the fatty group has no considerable contribution to the adsorption. Additionally, after deproteinization, the adsorption ratio of *R. arrhizus* also decreased obviously, showing the protein has obvious effect on the adsorption. All these results indicated that the protein and acyl functional groups of *R. arrhizus* are involved in the biosorption process of ^{241}Am, possibly by means of the complexation with the metals.

Table 1. The change of the pH value during the biosorption process of ^{241}Am by *R. arrhizus*

Time, min	0	5	10	20	30	40	50	60	90	120	180	240
pH	6.50	6.36	6.33	6.31	6.30	6.26	6.22	6.20	6.18	6.17	6.16	6.13

C_0 = 1.08 MBq/l, $m_{R.\ arrhizus}$ = 200 mg (wet weight).

Effect of Eu and Nd on ^{241}Am adsorption by R. arrhizus

As rare earth elements, europium or neodymium has similar chemical characters to americium and has stable isotopes. Sometimes, Eu is used as a substitute for Am when the chemical behavior of ^{241}Am, its chemical speciation, translation or migration-sedimentation should be investigated. In this experiment, the effect of Eu^{3+} and Nd^{3+} on adsorption of ^{241}Am by *R. arrhizus* was investigated in the solutions containing Eu^{3+} or Nd^{3+} with concentrations of 10–300 times more than that of ^{241}Am. The result is presented in Fig. 2. It can be seen that the adsorption ratio for ^{241}Am decreased with the increase of the concentration of Eu or Nd ions. When the ion concentration added was 300 times more than that of ^{241}Am, the adsorption ratio for ^{241}Am dropped from 99% to about 86–88%. This result could be explained as that ^{241}Am and Eu or Nd ions would compete for adsorption on *R. arrhizus*, when they coexisted in a solution. In other words, Eu and Nd would inhibit the adsorption of ^{241}Am on *R. arrhizus*, leading to the decrease of adsorption ratio for ^{241}Am.

RBS analysis of the Eu-adsorbed R. arrhizus

The Rutherford backscattering spectrometry (RBS) analysis spectra of the virgin and Eu-adsorbed *R. arrhizus* are shown in Fig. 3. As shown in Fig. 3, there existed a new europium peak in the RBS spectrum of the Eu-adsorbed *R. arrhizus*, but no calcium peak was determined any more, which existed in the virgin fungus. Other elements have no significant change in the Eu-adsorbed *R. arrhizus* in comparison with the intact fungus. The element components of virgin *R. arrhizus* and the Eu-adsorbed *R. arrhizus* were calculated from the RBS spectra using the computer code SIMNRA. The results are summarized in Table 3.

IR spectra and TEM micrograph of Eu-absorbed R. arrhizus

The IR spectra and TEM micrograph are shown in Figs 4 and 5, respectively. In Fig. 4, it could be seen that of some IR peaks, at 1630 cm^{-1}, 1557 cm^{-1}, 1383 cm^{-1}, 1230 cm^{-1}, etc., had changed after biosorption of Eu compared with that of the virgin organism. It implied that maybe some organic groups form complex with metal ions and this interaction resulted in the change of vibration strength or frequency.

The TEM micrograph shows that the electron density is well-distributed in the Eu-adsorbed *R. arrhizus* by comparison with virgin *R. arrhizus,* implying that the adsorbed Eu is almost scattered in the whole fungus, even in the cell surface. This result is consistent with the adsorption behavior of ^{241}Am by cell wall as described above. Furthermore, since the cell wall usually emerges with negative electric charge, biosorption of metals on microorganism should be related to nonspecific adsorption in the cell surface because of electric attraction besides complexation process.

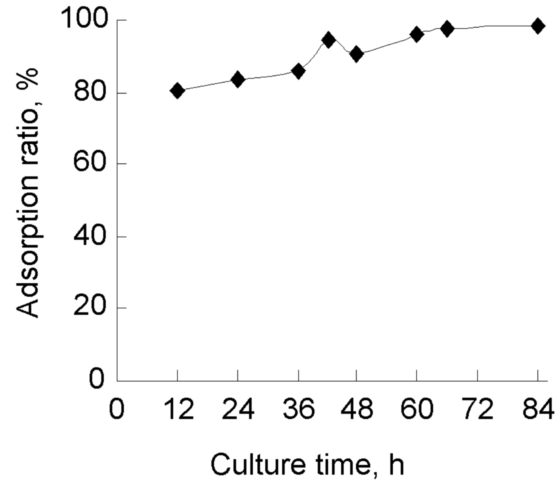

Fig. 1. Effect of culture time of *R. arrhizus* on adsorption of ^{241}Am ($C_0 = 1.08$ MBq/l, $m_{arrhizus} = 30$ mg, pH 3)

Table 2. Adsorption ratio of ^{241}Am by pretreated *R. arrhizus*

Pretreated cell	Adsorption ratio, %
Control	98.1
Cell wall	99.4
Deproteinization	94.1
Defatting	99.6
Deacylation	16.9

$C_0 = 1.08$ MBq/l, $m_{R.\ arrhizus} = 200$ mg (wet weight), pH 3.

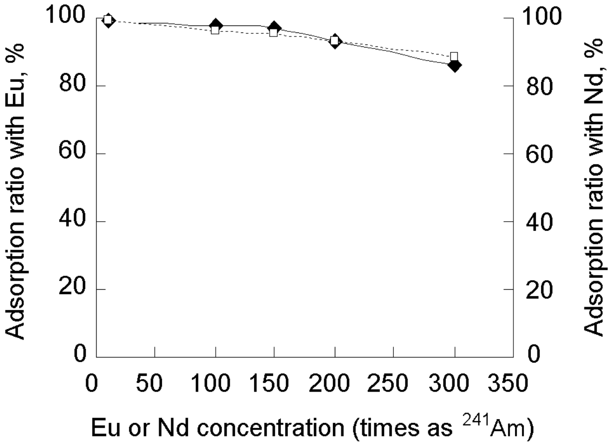

Fig. 2. Effect of co-ions for *R. arrhizus* on adsorption of ^{241}Am ◆ Eu; ■ Nd ($C_0 = 1.08$ MBq/l, $m_{arrhizus} = 200$ mg (wet weight), pH 3)

Effect of several acids on the adsorption

Since pH or acidity has obvious influence on ^{241}Am adsorption by *R. arrhizus* as described above, the effect of several anions on ^{241}Am adsorption by *R. arrhizus* was investigated while the pH value of solution was maintained approximate pH 3.

The results are summarized in Table 4. It can be seen that among the investigated acids, only the saturated EDTA can strongly inhibit the biosorption of ^{241}Am on *R. arrhizus*, resulting in the drop of the adsorption ratio from 98% to 64.6%. Since EDTA (ethylene diamine tetraacetic acid) has four carboxyl groups, usually can be coordinated with metal ions and result in complex compounds. So, this result may be explained as EDTA challenges to *R. arrhizus* via complexation with Am(III) and the resulted Am–EDTA complex is difficult to be adsorbed by *R. arrhizus*. In contrast, the other investigated acids have no significant influence on the ^{241}Am adsorption, since they are weak acids and have no strong ability to complex with Am(III). However, it would be favorable to maintain the pH value of the solutions within the optimum pH range (pH 1–3) for biosorption of ^{241}Am after these weak acids were added.

Table 3. Element components of virgin and the Eu-adsorbed *R. arrhizus* by RBS

Fungi	Element component					
	C	N	O	P	Ca	Eu
Virgin *R. arrhizus*	0.7222	0.08	0.19	0.004	0.004	–
Eu-adsorbed *R. arrhizus*	0.748	0.06	0.15	0.006	–	0.0005

C_{Eu} = 100 mg/l.

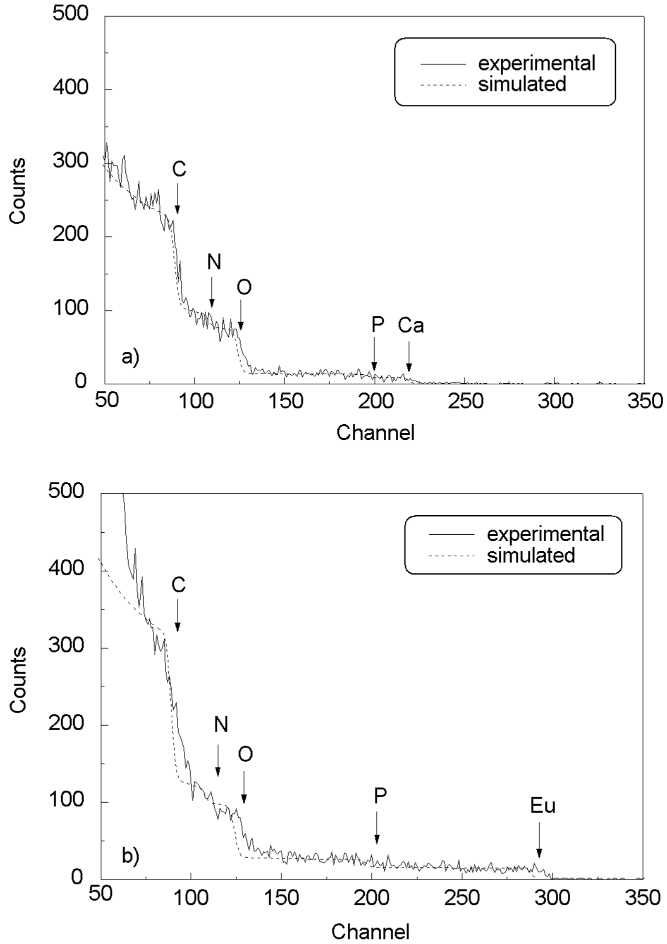

Fig. 3. RBS spectra of *R. arrhizus* before and after adsorption of Eu; (a) virgin *R. arrhizus*; (b) Eu-adsorbed *R. arrhizus*

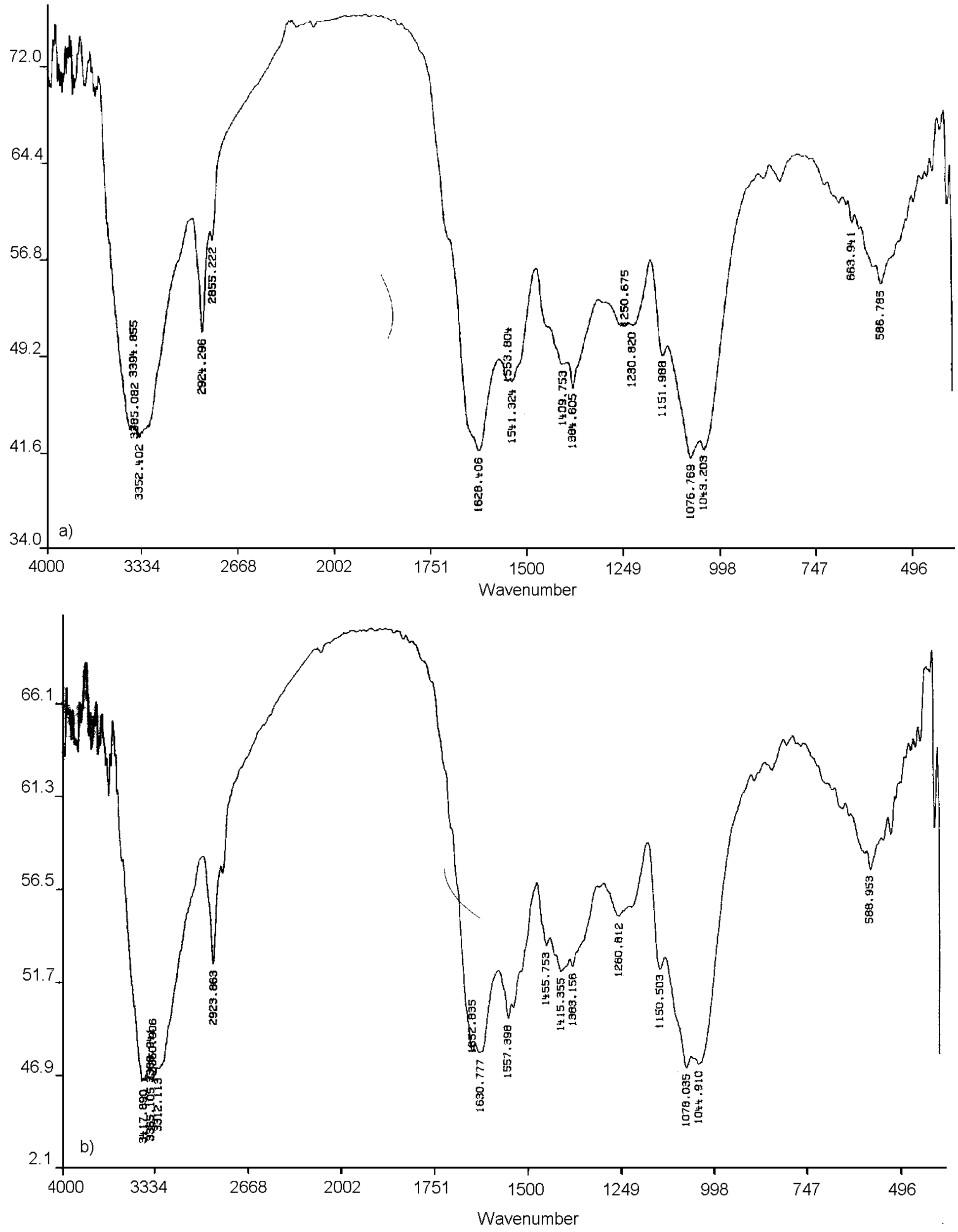

Fig. 4. IR spectra of *R. arrhizus* before and after adsorption of Eu; (a) virgin *R. arrhizus*; (b) Eu-adsorbed *R. arrhizus*

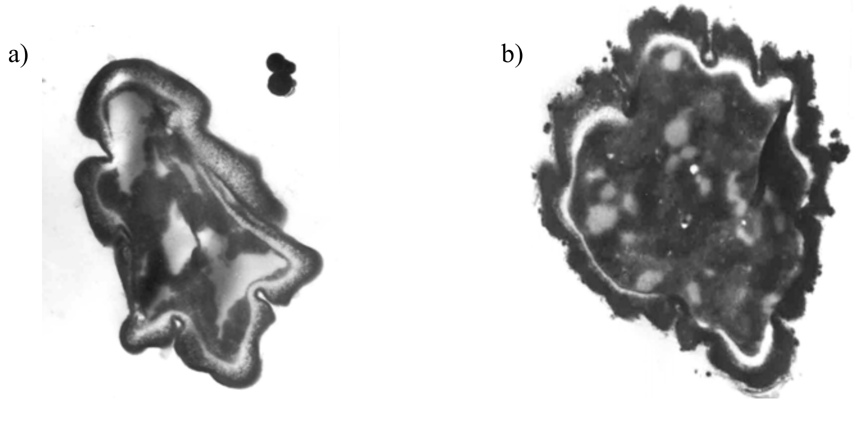

Fig. 5. TEM micrograph (1.4×4000) of virgin and the Eu-adsorbed *R. arrhizus* (a) virgin *R. arrhizus*; (b) Eu-adsorbed *R. arrhizus*

Table 4. Effect of several acid radical ions on ^{241}Am adsorption by *R. arrhizus*

Acid	Concentration	Adsorption ratio, %
Control	–	98.1
Oxalic acid	0.05M	99.5
Phosphoric acid	0.05M	~100
Acetic acid	0.5M	~100
Citric acid	0.1M	99.7
Saturated acid EDTA	–	64.6

C_0 = 1.08 MBq/l, $m_{R.\ arrhizus}$ = 200 mg (wet weight), pH 3.

Conclusions

The results of the adsorption experiments indicated that the biosorption process was strongly dependent on pH value and there was no significant difference in the adsorption ratios for ^{241}Am by the cell wall and intact *R. arrhizus*, because the adsorbed metals was almost scattered in the whole fungus as observed in the TEM micrograph. In the meantime, it can be concluded that protein or carboxyl functional groups play an important role in the biosorption of metals possibly via a complexation process, as shown in Table 2 from adsorption experiments by the chemically pretreated *R. arrhizusce*. Moreover, the first attempt by RBS analysis indicated that calcium in *R. arrhizus* has been replaced by europium via ion exchange.

In summary, the first attempt to explore the adsorption process of ^{241}Am by *R. arrhizus* implies that the adsorption mechanism of ^{241}Am on *R. arrhizus* is very complicated, involved in ion-exchange, complexation process as well as nonspecific adsorption on the cell wall because of static electricity. However, some questions still remained uncertain, such as how much processes are involved in biosorption of ^{241}Am by *R. arrhizus*, complexation, ion-exchange or more, whether the adsorption of ^{241}Am by *R. arrhizus* is related to oxidation-reduction or co-precipitation process, and others. All these questions should be further investigated in future experiments.

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