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Biosorption of ^{241}Am by *Saccharomyces cerevisiae*: Preliminary investigation on mechanism

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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. The encouraging biosorption of ^{241}Am from aqueous solutions by free or immobilized *Saccharomyces cerevisiae (S. cerevisiae)* has been observed in our previous experiments. In this study, the preliminary evaluation on mechanism was further explored via chemical or biological modification of *S. cerevisiae*, and using europium as a substitute for americium. The results indicated that the culture times of more than 16 hours for *S. cerevisiae* was suitable and the efficient adsorption of ^{241}Am by the *S. cerevisiae* was able to achieve. The pH value in solutions decreased gradually with the uptake of ^{241}Am in the *S. cerevisiae*, implying that H^+ released from *S. cerevisiae* via ion-exchange. The biosorption of ^{241}Am by the decomposed cell wall, protoplasm or cell membrane of *S. cerevisiae* was same efficient as by the intact fungus. However, the adsorption ratio for ^{241}Am by the deproteinized or deacylated *S. cerevisiae* dropped obviously, implying that protein or carboxyl functional groups of *S. cerevisiaece* play an important role in the biosorption of ^{241}Am. Most of the investigated acidic ions have no significant influence on the ^{241}Am adsorption, while the saturated EDTA can strong inhibit the biosorption of ^{241}Am on *S. cerevisiae*. When the concentrations of coexistent Eu^{3+}, Nd^{3+} were 100 times more than that of ^{241}Am, the adsorption ratios would decrease to 65% from more than 95%. It could be noted by transmission electron microscope (TEM) analysis that the adsorbed Eu is almost scattered in the whole fungus, while Rutherford backscattering spectrometry (RBS) analysis indicated that Ca in *S. cerevisiae* have been replaced by Eu via ion-exchange. All the results implied that the adsorption mechanism of ^{241}Am on *S. cerevisiae* is very complicated and at least involved in ion exchange, complexation process as well as well as nonspecific adsorption in cell wall because of static electricity.

Introduction

As a transuranium element without stable isotopes, americium has about 20 radioisotopes or isomers. Among them, ^{241}Am ($T_{1/2}$ = 433 years, E_α = 5.468 MeV, 86.6%; 5.443 MeV, 12.3%; E_γ = 0.0596 MeV, 35%) is the most important one. Generally used as target material in nuclear industry or excitation resource in some scientific instruments.[1] Also, it has widespread use in other fields. For example, most of the fire alarms in hotels, hospitals and service centers contain a ^{241}Am source. 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 human body. The maximum permissible quantity for ^{241}Am in human body is 11.1 kBq ($8.77\cdot10^{-8}$ g) and the maximum permissible concentration in water is 1.48 Bq/ml ($1.17\cdot10^{-6}$ g/l).[2] For those reasons, all countries over the world have paid considerable attention to disposal or treatment of wastewater containing ^{241}Am.[3–6] However, the most studies were focused on the solvent extraction of ^{241}Am from highly radioactive liquids,[7–9] while few reports were involved in the treatment for radioactive solutions containing ^{241}Am using biosorption technology except our recent experiments.[10,11]

For decades of years, biosorption technology has been recognized as attractive potential for removal of heavy metals and degradation of organic chemicals from the wastewater due to the good performance, low cost and large available quantities.[12–16] In fact, as early as 1950s, there were some attempts to accumulate precious metals, such as gold, silver and copper by different microorganism. Until the 1980s or later, growing interest was put in the removal of toxic and harmful materials from wastewater for environmental protection,[17–20] and the sewage purification and the treatment of industrial wastewater by the biosorption technology have been got the practical application in China and elsewhere.[21–23] Meanwhile, accumulation of some natural radionuclides, such as uranium, thorium, and radium by different microorganism has been screened.[24–31] In contrast with chemical treatment, the biosorption of radioactive nuclides was characterized by great efficiency and commercial availability of biomaterials, especially, no further environmental contamination. However, the biosorption technology is more suitable for the low or medium radioactive aqueous solutions.

As a kind of yeast with commercial availability and extensive uses in winery or brewery, *Saccharomyces cerevisiae* has been also used in accumulation of several metals from solutions elsewhere.[20,28,32–35] The involved

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metal elements included Cu, Sr, V, Th, Cd, Pb, U, Au, and etc. Of which, the adsorbed Pb^{2+} or U by *S. cerevisiae* came up to as great as 189–270 mg/g or 180 mg/g, respectively. More recently, in order to find a 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 *S. cerevisiae* has been investigated in our Institute.[10,11] The preliminary results showed that *S. cerevisiae* 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 *S. cerevisiae* from ^{241}Am solutions and the adsorption capacities (W) can reach 237.9 MBq/g biomass (dry weight) (1880.0 μg/g). Moreover, the immobilized *S. cerevisiae* not only can accumulate ^{241}Am as efficiently as free *S. cerevisiae,* but also can be used repeatedly or continuously. The goal of this study is to further explore the biosorption mechanism via chemical or biological modification of *S. cerevisiae*. Especially, Eu was used as a model for Am and the Eu-adsorbed *S. cerevisiae* instead of the ^{241}Am-adsorbed *S. cerevisiae* was analyzed by 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 solution in nitrate salt form (^{241}Am $(NO_3)_3$) was provided by 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 redistilled water. All the other chemical regents were of A.P or chromatographic grade and were used without further purification.

All glassware for the biosorption experiments was routinely rinsed with 0.5M HNO_3 and washed extensively with water to prevent interference by contaminants. The pH of each solution was measured by a digital pH meter and adjusted by the addition of 0.2M HNO_3 or 0.2M NaOH solution.

Strains and culture

S. cerevisiae was obtained as a gift from College of Life Science, Sichuan University (Chengdu, P.R. China). The cultivation of *S. cerevisiae* was completed as described previously.[9] Culture medium for growing the fungi contained saccharomycete (1%), protein peptone (2%), glucose (2%) and pH is 6.5. In order to investigate the effect of culture time on ^{241}Am adsorption, the cultured fungi were collected by centrifugation at definite time points.

Cell wall, protoplasm or cell membrane of S. cerevisiae

The cell wall of *S. cerevisiae* was obtained as following procedure: the suspended fungus of 4 g in distilled water of 50 ml at an ice-bath was treated with ultrasonic for 15 minutes at 400 W, 20 kHz. The ultrasonic-treated microorganism was centrifugalized for 15 minutes at 4000 rpm to remove impurity and untreated fungi. The resulted supernatant was centrifuged for 15 minutes at 15000 rpm and the cell wall of *S. cerevisiae* was got for further adsorption experiment.

For protoplasm of *S. cerevisiae,* culture solutions of 10 ml containing fungi with concentration of $7 \cdot 10^7$ cell/ml were centrifuged for 10 minutes at 3000 rpm, washed with sterile water in twice. Then, the fungi was pretreated with 0.1% β-mercaptoethanol of 10 ml for 30 minutes at 28 °C, centrifuged for 5 minutes at 4000 rpm, and washed with PB solution. The settled fungi was suspended in a solution containing 2% cochleozyme (in PBS solution) again and shook at 30 °C for 40 minutes, then centrifuged for 15 minutes at 4000 rpm, washed with PB buffer in twice to achieve the protoplasm of *S. cerevisiae*. The resulted protoplasm was stored in NaCl or sucrose solution and used for further adsorption experiment after centrifugation.

By comparison with cell wall or protoplasm of *S. cerevisiae*, the cell membrane of *S. cerevisiae* could be more easily obtained by centrifugation of the resulted protoplasm above in sterile water at 9000 rpm and 4 °C for 20 min.

Chemical treatment of S. cerevisiae

In order to investigate the biosorption behavior of protein, carboxyl functional and other group of microorganism for ^{241}Am, *S. cerevisiae* of 200 mg was treated as follows procedures, respectively: (1) deproteinization: mixed with 2N 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 every residue was washed with de-ionized water to neutral and filtrated under vacuum for the further adsorption experiments.

Adsorption experiments for ^{241}Am

To the ^{241}Am solutions of definite radioactive concentrations in desired pH values were added wet

S. cerevisiae or decomposed cell wall, protoplasm, cell membrane or other components. The mixture was shaken on a rotary shaker at 200 rpm and room temperature for 2 hours, except as otherwise described. Then, the mixture was centrifuged at 3000 rpm for 15 minutes. The supernatant liquid was removed, and assayed on radioactivity of ^{241}Am by means of an automatic counter with a NaI well detector.

For all the adsorption experiments, the results were generally expressed as adsorption ratio (R, %). The adsorption ratio was calculated as:

$$R = (1–C/C_0)\times 100\%$$

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

Analysis of the Eu-adsorbed S. cerevisiae *by TEM and RBS*

In order to further explore the biosorption mechanism, Eu was used to the substitute for ^{241}Am due to its similar chemical behaviors to americium and the availability of stable isotopes. The latter make the Eu-adsorbed *S. cerevisiae* can be analyzed by TEM and RBS without worry about radioactive contamination.

The adsorption of Eu by *S. cerevisiae* was performed by the procedure as described above for ^{241}Am. After centrifuged for 20 minutes at 12000 rpm, the Eu-adsorbed *S. cerevisiae* as well as intact fungus was solidified, and then was sectioned for small samples for Rutherford backscattering spectrometry (RBS) analysis. The RBS analysis were performed at the Institute of the Nuclear Science and Technology, Sichuan University. An electrostatic accelerator with maximum terminal voltage of 2.5 MeV provided the $^4He^+$ 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 depletion depth of 100 μm. The RBS spectra were analyzed using a computer code SIMNRA.[36]

For transmission electron microscope (TEM) analysis, the Eu-adsorbed *S. cerevisiae* 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-stained with uranium acetate and sodium citrate for TEM analysis. The TEM micrographs were observed on a H-600IV spectrometer (UK).

Results

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

The effect of culture time for *S. cerevisiae* on ^{241}Am adsorption was described in Fig. 1. It could be noted that the adsorption ratio for ^{241}Am increased rapidly with culture time of *S. cerevisiae* and came up to 96% at 16 hour. After then, the adsorption ratio tended to the equilibrium. The reason maybe was that there existed some chemical substances in *S. cerevisiae*, which were involved in biosorption of ^{241}Am and whose contents changed with culture time. Usually, the culture time for *S. cerevisiae* is 48 h. However, for biosorption of ^{241}Am, the *S. cerevisiae* with culture time of 16 h is suitable.

Change of pH value of solutions in biosorption process

Many previous reports have shown that pH or acidity was an important factor influencing the biosorption ability of heavy metals by microorganism.[14,18,25,33] In our previous experiments,[10] it has been also observed that ^{241}Am uptake on the *S. cerevisiae* is a pH-dependent process and the optimum pH ranged pH 1–3. Within this range, the adsorption ratio could be up to 99%. In this study, the change of pH value of solutions with the uptake of ^{241}Am on the *S. cerevisiae* was investigated. As summarized in Table 1, the pH value in solutions decreased gradually with the uptake of ^{241}Am on the *S. cerevisiae*, implying that H^+ released from *S. cerevisiae*, possibly via ion exchange.

Effect of several acids on the adsorption

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

The results are summarized in Table 2. It can be seen that among the investigated acids, only the saturated EDTA can strongly inhibit the biosorption of ^{241}Am on *S. cerevisiae*, resulting in the adsorption ratio from more than 99% drop to 43.7%. Since EDTA (ethylene diamine tetraacetic acid) has four carboxyl groups, it usually can be coordinated with metal ions and result in complex compounds. So, this result may be explained as EDTA challenges to *S. cerevisiae* via complexation with Am(III) and the resulted Am-EDTA complex compound is difficult to be adsorbed by *S. cerevisiae*.

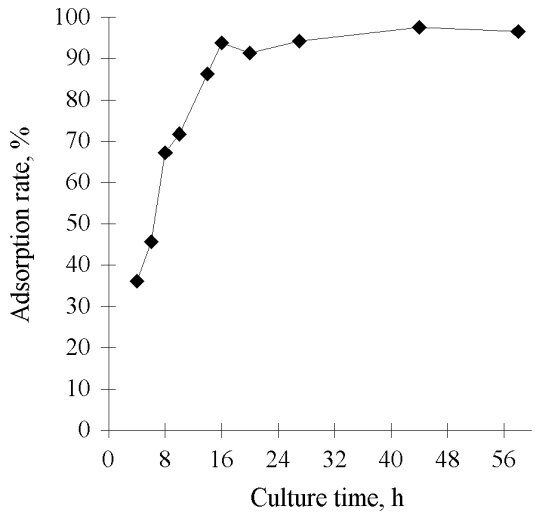

Fig. 1. Effect of culture time for *S.cerevisiae* on adsorption of ^{241}Am. C_0 = 1.08 MBq/l, m = 30 mg, pH 2

Table 1. The change of pH value in the biosorption process of ^{241}Am by *S.cerevisiae*

Time, min	0	5	10	20	30	40	50	60	90	120	180	240
pH	6.60	6.39	6.36	6.34	6.30	6.27	6.23	6.20	6.19	6.21	6.19	6.12

C_0 = 1.08 MBq/l, $m_{S.cerevisiae}$ = 200 mg.

Table 2. Effect of several acid radical ions on ^{241}Am adsorption by *S. cerevisiae*

Acid	Adsorption ratio, %
Control	99.2
Oxalic acid (0.05M)	99.8
Phosphoric acid (0.05M)	99.7
Acetic acid (0.5M)	99.7
Citric acid (0.1M)	99.5
Saturated acid EDTA	43.7

C_0 = 1.08 MBq/l, $m_{S.cerevisiae}$ = 200 mg, pH 2.

In contrast, the other investigated acids have no significant influence on the ^{241}Am adsorption by *S. cerevisiae*, 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 solutions within the optimum pH range (pH 1–3) for biosorption of ^{241}Am after these weak acids were added.

Effect of Eu and Nd on ^{241}Am adsorption by S. cerevisiae

As rare earth elements, europium and neodymium has similar chemical characters to americium. Some times, Eu is used as a substitute for Am when the chemical behavior of ^{241}Am, such as 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 *S. cerevisiae* was investigated in the solutions containing Eu^{3+} or Nd^{3+} with concentrations of 10–300 times more than that of ^{241}Am and the result was presented in Fig. 2. It can be seen that the adsorption ratio for ^{241}Am decreased with increasing concentration of Eu or Nd ions. When the ion concentration added was 100 times more than that of ^{241}Am, the adsorption ratio for ^{241}Am dropped from 96% to about 65%. This result could be explained as that ^{241}Am and Eu or Nd ion would compete for adsorption on *S. cerevisiae*, when they coexisted in a solution. In other words, Eu and Nd would inhibit the adsorption of ^{241}Am on *S. cerevisiae*, leading to the decrease of adsorption ratio for ^{241}Am.

Biosorption of ^{241}Am by different tissues of S. cerevisiae *cell*

As presented above or described in our previous experiments, intact *S. cerevisiae* is an efficient biosorbent for ^{241}Am with adsorption ratio of more than 95%. However, the biosorption of ^{241}Am by the cell wall, protoplasm or cell membrane of *S. cerevisiae* was same efficient as by the intact fungus. As summarized in Table 3, all the investigated parts can remove ^{241}Am of more than 96% from solution. Of which, protoplasm was the left part of intact *S. cerevisiae* whose cell wall was removed by digestion and hydrolysis with cochleozyme. Here, the effect of enzymolysis time on adsorption of ^{241}Am by protoplasm was further

investigated and the results was summarized at Table 4. It could be noted that the adsorption ratio for ^{241}Am by protoplasm increases with enzymolysis time and the resulted protoplasm in sugar solution exhibits better adsorption ability than in NaCl solution.

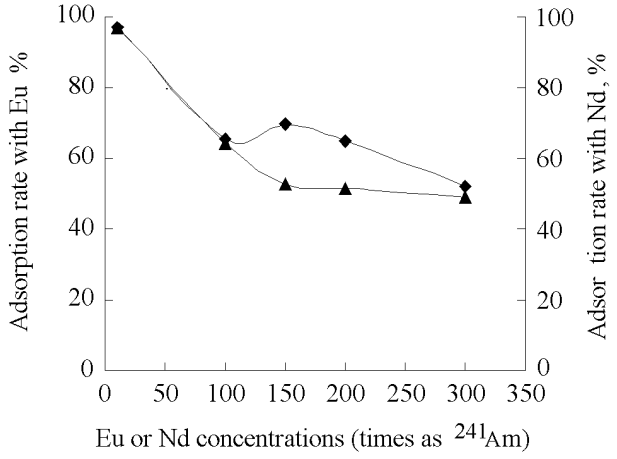

Fig. 2. Effect of Eu and Nd on ^{241}Am adsorption by *S. cerevisiae.* C_0 = 1.08 MBq/l, $m_{S.\ cerevisiae}$ = 200 mg, pH 2, ▲ Eu, ♦ Nd

Table 3. Adsorption ratio of ^{241}Am by different parts of *S. cerevisiae*

Part of cells	Adsorption ratio, %
Intact cell	99.2
Cell wall	96.2
Protoplasm	98.8
Cell membrane	98.7

C_0 = 1.08 MBq/l, m = 30 mg, pH 2.

Table 4. Adsorption of ^{241}Am on protoplasm produced by enzymolysis of *S. cerevisiae* in NaCl or sugar solution

Enzymolysis time, min	Adsorption ratio for ^{241}Am, %	
	NaCl solution 0.55 mol/l	Sugar solution 0.8 mol/l
0	\	92.8
30	92.7	97.5
90	96.7	98.1
180	98.3	98.4

C_0 = 1.08 MBq/l, $m_{S.cerevisiae}$ = 200 mg, pH 2.

Table 5. Adsorption ratio of ^{241}Am by the chemically pretreated *S. cerevisiae*

Pretreatment	Adsorption, %
Control	99.2
Deproteinization	17.8
Defatting	98.3
Deacylation	9.51

C_0 = 1.08 MBq/l, m = 200 mg, pH 2.

Biosorption of ^{241}Am by the chemically pretreated S. cerevisiae

In order to investigate the biosorption behavior of protein, carboxyl functional and other group of microorganism for ^{241}Am, *S. cerevisiae* was chemically pretreated, such as deproteinization, defatting and deacetylation.

As summarized in Table 5, the adsorption ratio of ^{241}Am by the deproteinized *S. cerevisiae* was much less than that by intact fungus, implying that the protein may play an important role in the biosorption of ^{241}Am, and the *S. cerevisiae* has high protein content. In contrast, the fatty group has no considerable contribution to the adsorption. Additionally, after deacylation, the adsorption ratio of *S. cerevisiae* also decreased markedly, showing the carboxyl functional groups has obvious effect on the adsorption. All these results indicated that the protein and carboxyl functional groups of *S. cerevisiae* are involved in the biosorption process of ^{241}Am or Eu, possibly by means of the complexation with the metals. Furthermore, since cell wall usually emerges as negative electric charge, biosorption of metals on microorganism should be related to nonspecific adsorption in cell wall because of electric attraction besides complexation process.

TEM and RBS analysis of the Eu-adsorbed S. cerevisiae

The Eu-adsorbed *S. cerevisiae* was observed by TEM and the results were shown in Fig. 3. It could be seen that the electron density is well-distributed in TEM micrographs of the Eu-adsorbed *S. cerevisiae* by comparison with the intact *S. cerevisiae,* implying that the adsorbed Eu is almost scattered in whole fungus, included the cell face. This result is similar to the adsorption behavior of ^{241}Am by different parts of *S. cerevisiaece* cell as described above.

However, RBS analysis results indicated that calcium in *S. cerevisiae* has been replaced by europium via ion exchange. As described in Fig. 4, there existed a new europium peak in RBS spectrum of the Eu-adsorbed *S. cerevisiae*, but no calcium peak has yet, which existed in the original fungus. Fortunately, other elements have no significant change in the Eu-adsorbed *S. cerevisiae* by comparison with the intact fungus. Moreover, the element components of original *S. cerevisiae* and the Eu-adsorbed *S. cerevisiae* were calculated from RBS spectra using a computer code SIMNRA, and the result was summarized in Table 6. It could be noted that Eu component in the Eu-adsorbed *S. cerevisiae* with Eu solution of 100 mg/l is higher than Eu solution of 25 mg/l, while no calcium was detected in both Eu-adsorbed *S. cerevisiae* sample.

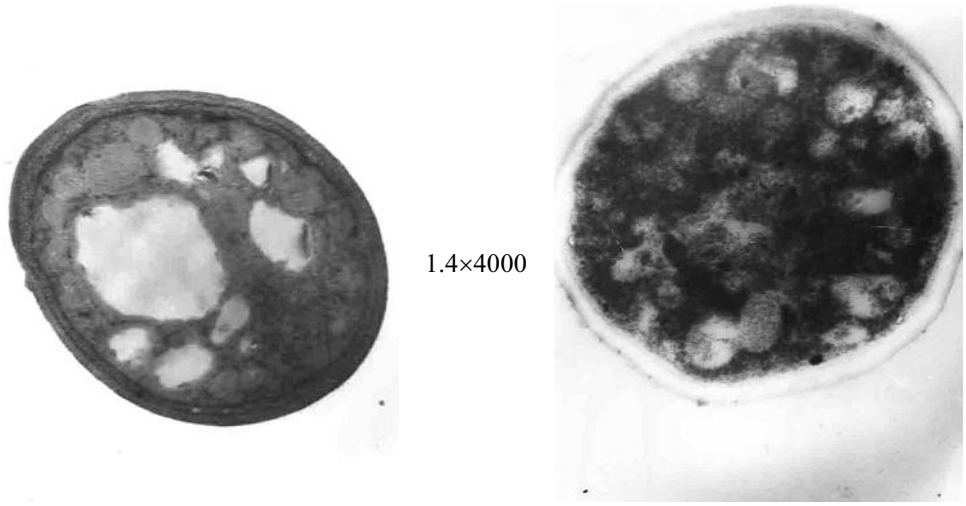

Fig. 3. TEM spectra of original and the Eu-adsorbed *S. cerevisiae.* a) Original *S. cerevisiae*, b) the Eu-adsorbed *S. cerevisiae*

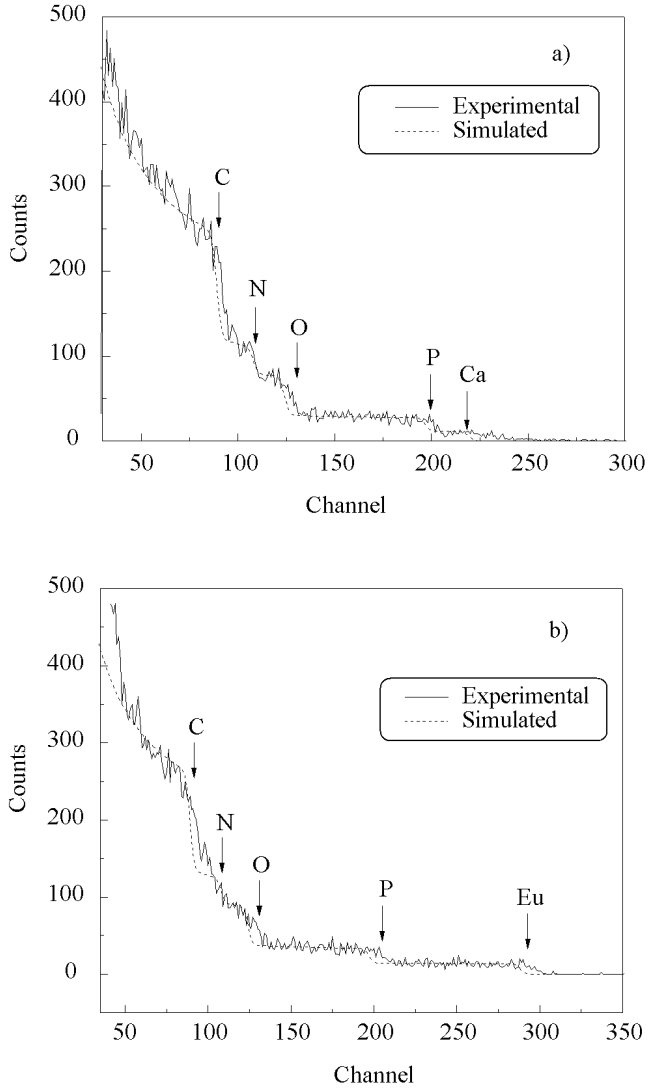

Fig. 4. RBS spectra of original and the Eu-adsorbed *S. cerevisiae.* a) Original *S. cerevisiae*, b) the Eu-adsorbed *S. cerevisiae*

Table 6. Elemental components of original and the Eu-adsorbed *S. cerevisiae* by RBS

S. cerevisiae		Elemental component					
		C	N	O	P	Ca	Eu
Original		0.712	0.13	0.14	0.013	0.005	
Eu-adsorbed, mg/l	25	0.747	0.13	0.11	0.013		0.0002
	100	0.704	0.13	0.15	0.015		0.0006

Discussion

It is considered popularly that the removal of heavy metals or degradation of organic compounds from the wastewater by biosorption technology is feasible for environmental protection. In fact, many results are very encouraging, in that the biosorption is very efficient, and especially, the sewage purification and the treatment of industrial wastewater by the technology have been got the practical application.[21–23] However, the biosorption mechanism for metals has not been clearly understood until now, especially for biosorption mechanism of radioactive elements, even though much effort has been invested in exploring the mechanism for many years. Most investigation demonstrated that biosorption mechanism for metals is very complicated, and involved in so much process, such as surface complexation,[25,36–40] ion-exchange,[41–43] oxidation-reduction,[44–47] adsorption induced by static electricity or enzyme,[33,46–48] co-precipitation,[49–50] etc. Additionally, many methods or techniques were used for the investigation in biosorption mechanism of metals,[15,37,42,51–55] 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. However, there were no or few reports related to analyses of the metal-adsorbed microorganism by Rutherford backscattering spectrometry (RBS).

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 like as ^{241}Am. Fortunately, there exist some stable elements that have similar chemical behavior to other radioactive elements. So, these stable elements can be used as the model for the radioactive elements, especially for study in biosorption mechanism of these isotopes.

Obviously, it is not easy to clearly demonstrate the mechanism for biosorption of radioactive elements or isotopes. However, this paper made the first attempt to try to understand the adsorption process of ^{241}Am by *S. cerevisiae* in more precise terms. For this reason, several questions should be answered by the experiments. It included which tissue or chemical group of *S. cerevisiae* plays more important role in biosorption of ^{241}Am, how much processes are involved in biosorption of ^{241}Am by *S. cerevisiae*, complexation, ion exchange or more, and others?

It can be noted that H^+ released gradually with the uptake of ^{241}Am on the *S. cerevisiae*. Meanwhile, the results from adsorption experiments and TEM micrographs indicated that there was no significant difference in adsorption ratios for ^{241}Am by the cell wall, protoplasm, cell membrane and intact *S. cerevisiae*, and the adsorbed metals was almost scattered in whole fungus. However, it can be conclude that protein or carboxyl functional groups play an important role in the biosorption of metals possibly via a complexation process, as shown in Table 5 from adsorption experiments by the chemically pretreated *S. cerevisiaece*. Moreover, the first attempt by RBS analysis indicated that calcium in *S. cerevisiae* has been replaced by europium via ion-exchange. Unfortunately, there still remained uncertain whether the adsorption of ^{241}Am by *S. cerevisiaece* is related to oxidation-reduction or co-precipitation process. These questions should be further investigated in future experiments.

In summary, the first attempt to explore the adsorption process of ^{241}Am by *S. cerevisiae* has been made in this paper, and all the results implied that the adsorption mechanism of ^{241}Am on *S. cerevisiae* is very complicated and at least involved in ion exchange, complexation process as well as nonspecific adsorption in cell wall because of static electricity.

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