Journal of Radioanalytical and Nuclear Chemistry, Vol. 260, No. 3 (2004) 659–663

Sorption of ^{241}Am by *Aspergillus niger* spore and hyphae

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(Received October 14, 2003)

Biosorption of ^{241}Am by a fungus *A. niger*, including the spore and hyphae, was investigated. The preliminary results showed that the adsorption of ^{241}Am by the microorganism was efficient. More than 96% of the total ^{241}Am could be removed from ^{241}Am solutions of 5.6–111 MBq/l (C_o) by spore and hyphae of *A. niger*, with adsorbed ^{241}Am metal (Q) of 7.2–142.4 MBq/g biomass, and 5.2–106.5 MBq/g, respectively. The biosorption equilibrium was achieved within 1 hour and the optimum pH range was pH 1–3. No obvious effects on ^{241}Am adsorption by the fungus were observed at 10–45 °C, or in solutions containing Au^{3+} or Ag^{+}, even 2000 times above the ^{241}Am concentration. The ^{241}Am biosorption by the fungus obeys the Freundlich adsorption equation. There was no significant difference between the adsorption behavior of *A. niger* spore and hyphae.

Introduction

For recent years, biosorption technology has been considered as attractive potential for removal of heavy metals and degradation of organic chemicals from wastewaters.[1–7] In the 1950s, there were some attempts to accumulate precious metals, such as gold, silver and copper by microorganisms.[8] Until the 1980s or later, growing interest was shown in the removal of toxic and harmful materials from wastewaters for environmental protection.[9–13] Meanwhile, accumulation of some natural radionuclides, including uranium, thorium, and radium by different microorganisms has been investigated.[14–17] However, few reports dealt with the treatment of radioactive wastewaters from the nuclear industry by biosorption technology.[18]

As an important radionuclide in nuclear industry, scientific research and other fields, ^{241}Am is one of the most serious contamination concerns due to its long half-life ($T_{1/2}$ = 433 y) and its high radiation toxicity with α-particle emission (E_α = 5.468 MeV, 86.6%; 5.443 MeV, 12.3% E_γ = 0.0596 MeV, 35%). It mainly stays in several key tissues or organs, such as skeleton and liver, if it gets into a human body. Recently, in order to find a feasible method for disposal of the low and medium radioactive wastewaters produced in the process of preparing ^{241}Am fire alarm, we made the first attempt to adsorb ^{241}Am from a solution by a fungus *Saccharomyces cerevisiae*.[19] The preliminary results showed that the biosorption technology might be an encouraging way for the removal of ^{241}Am from solutions. The goal of these experiments is to further evaluate other fungus *A. niger*, including the spore and hyphae as biosorbent for ^{241}Am from solutions. The various factors that affect the biosorption were investigated.

Experimental

Reagents and experimental solutions

^{241}Am solution in nitrate salt form [^{241}Am$(NO_3)_3$] was provided by the Institute of Nuclear Physics and Chemistry, CAEP (Mianyang, P.R. China).

Stock solution containing ^{241}Am of 1.11 GBq/l (8.76 mg/l) and diluted solutions were prepared in redistilled water. All the other chemical reagents were of A.P. or chromatographic grade and were used without further purification.

All glassware for the biosorption experiments was routinely rinsed with 0.5N 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 0.2N HNO_3 or 0.2N NaOH solution.

Strains and culture

Aspergillus niger (A. niger) spore and hyphae was obtained as a gift from the College of Life Science, Sichuan University (Chengdu, P.R. China). The culture medium for growing *A. niger* spore and hyphae contained 1% glucose and 0.5% $(NH_4)_2SO_4$ in exudate of potato, and its pH was 6.5. The maintained *A. niger* spore and hyphae spores were inoculated in a test tube and incubated for 7 days at 25 °C. The spores were eluted by sterile water and were transferred to the culture medium, then cultivated for 7–10 days at 25 °C.

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0236–5731/2004/USD 20.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

The cultured *A. niger* spore and hyphae were collected by centrifugation and were directly applied to the adsorption experiments. In order to calculate the mass of the adsorbed metal (Q), the *A. niger* spore and hyphae was heated in an oven for 2 hours at 100–120 °C, and the dry to wet weight ratio was calculated. In this experiment, the dry to wet weight ratio of *A. niger* spore and hyphae was 1 : 13.3 and 1 : 9.7, respectively.

Adsorption experiments

To the ^{241}Am solutions of definite radioactive concentrations and pH value precultured wet *A. niger* spore or hyphae were added. The mixture was shaken on a rotary shaker at 180 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 for ^{241}Am by means of an automatic counter with a NaI well detector.

The results were expressed as the adsorption rate (R, %) or adsorbed metal. The adsorption rate and adsorbed metal were calculated as:

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

and

$$Q = V(C_o - C)/m \quad (2)$$

where Q is the adsorbed metal (μg/g or MBq/g dry sorbent), V is the volume of solution in the contact batch flask (ml), C_o is the initial ^{241}Am concentration (MBq/l, or μg/l), C is the final ^{241}Am concentration in the solution (MBq/l, or μg/l), and m is the sorbent concentration in dry form (g/l). The conversion relationship between mass and radioactivity for ^{241}Am is: 1 mg = 126.54 MBq.

Results and discussion

Effect of pH or acidity on ^{241}Am adsorption

The effect of pH or acidity on the adsorption of ^{241}Am from solutions by *A. niger* spore and hyphae is shown in Fig. 1. It can be seen that pH or acidity has considerable influence on the biosorption of ^{241}Am by both *A. niger* spore and hyphae, as the biosorption of heavy metals by other microorganisms is reported.[20,21] The optimum pH range for ^{241}Am adsorption ranged from 1 to 3. Within this range, the adsorption rate could be up to 99%. When the pH was above 3, the adsorption ability of *A. niger* for ^{241}Am decreased gradually with increasing pH. However, the adsorption rate was still as high as 40% at pH 5. In contrast, when the acidity increased beyond the optimum, the adsorption ability markedly decreased. For example, at 0.5 mol/l HNO_3, the adsorption rate of ^{241}Am by *A. niger* spore or hyphae was 79.6% or 54.3%, respectively, while at 2.0 mol/l HNO_3, it dropped to 6.27% or 0.8%, respectively. The result implied that the adsorption of ^{241}Am on *A. niger* possibly involved cations, ternary complexes, or hydroxyanions, onto a negatively charged surface of *A. niger*. However, in order to explain the results about the effect of pH on ^{241}Am adsorption, the adsorption mechanism of ^{241}Am on *A. niger* should be further investigated.

Effect of contact time on ^{241}Am adsorption

The effect of contact time on ^{241}Am adsorption by *A. niger* spore and hyphae is presented in Fig. 2. It is shown that the ^{241}Am adsorption is closely related to contact time. The adsorption rate increased rapidly with time and came up to 96% at 1 hour. After then, the adsorption rate grew very little and appeared to be at equilibrium. Based on this result, the contact time was set to 2 hours for the other adsorption experiments, except as otherwise described.

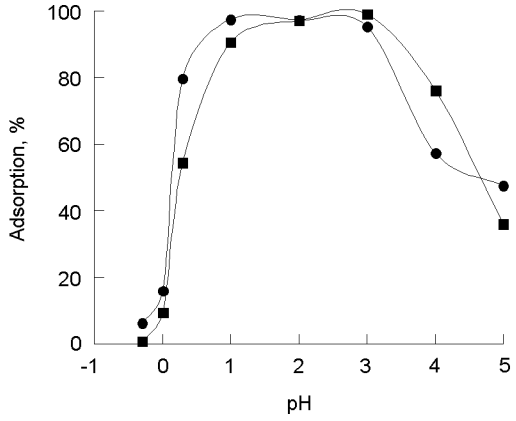

Fig. 1. Effect of pH on the adsorption by • *A. niger* spore, ■ *A. niger* hyphae; C_o = 5.6 MBq/l, $m_{(spore)}$ = 0.75 g/l, $m_{(hyphae)}$ = 1.03 g/l

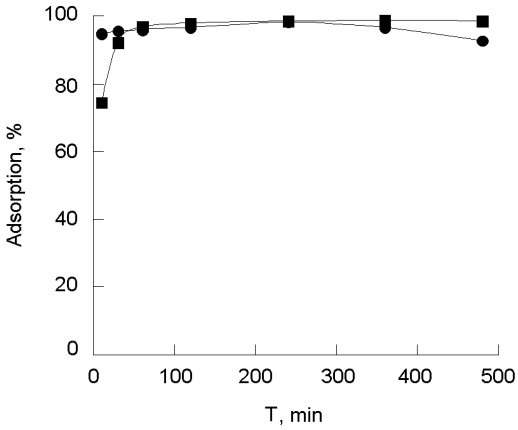

Fig. 2. Effect of contact time on the adsorption of ^{241}Am by • *A. niger* spore, ■ *A. niger* hyphae; C_o = 5.6 MBq/l, $m_{(spore)}$ = 0.75 g/l, $m_{(hyphae)}$ = 1.03 g/l, pH 2

Effect of temperature on ^{241}Am adsorption

As shown in Table 1, no significant differences in ^{241}Am biosorption were observed between 10–45 °C. Even until 45 °C, the adsorption rate was still 97%. This result indicated that the temperature was not a primary factor to influence the ^{241}Am adsorption. Since the microorganism is almost in a stagnant state at about 45 °C, the result implied that the "dead" microorganism still has strong ability in adsorption for ^{241}Am. This is similar to the results reported by others.[21–23] For convenience, most of the other adsorption experiments were performed at room temperature.

Effect of microorganism concentration on ^{241}Am adsorption

The effect of *A. niger* concentration on ^{241}Am adsorption is shown in Fig. 3 and Fig. 4. It can be seen that the ^{241}Am adsorption by *A. niger* spore and hyphae was considerably efficient and the adsorption rate grew slowly with increasing biomass concentration and eventually, appeared to be at equilibrium, while the adsorbed ^{241}Am per unit biomass decreased with increasing biomass concentration. The most probable reason for the effect of biomass concentration on ^{241}Am adsorption is that increasing biomass density leads to interference between the binding sites. This factor, therefore, should be taken into account in any application of fungal biomass as an adsorbent.

Effect of ^{241}Am concentration on the adsorption

The effect of ^{241}Am concentration on the adsorption is presented in Fig. 5 and Table 2. As is shown within the range of initial ^{241}Am concentration (C_o) of 5.6–111 MBq/l (44.3–877.2 μg/l), the amount of ^{241}Am adsorbed by *A. niger* spore of 1.03 g/l grew with increasing ^{241}Am concentration, same as the result by *A. niger* hyphae of 0.75 g/l, while the adsorption rate almost kept constant with an average value of 96% for *A. niger* spore and of 97% for *A. niger* hyphae. It can be seen that the adsorbed amount of ^{241}Am had not become saturated even at 111 MBq/l ^{241}Am concentration. So, the ^{241}Am biosorption process by *A. niger* spore and hyphae may be described by a Freundlich isotherm equation:

$$q = kC_o^{1/n}$$

where k and n are Freundlich constants related to the degree of adsorption and adsorption strength, q is the amount of the metal adsorbed per biological cell, C_o is the metal-ion concentration of the solution. Taking the logarithmic form of the equation should give a right line, and so the constant k and n may be evaluated. The result is shown in Fig. 6, with fitting curve for *A. niger* spore:

$$\log q = 0.999 \log C_o + 0.110$$

its correlation coefficient: $r = 0.9999$.

For equation:

$$q = kC_o^{1/n}$$

where $k(^{241}\text{Am}) = 1.29$, $n(^{241}\text{Am}) = 1.00$, we got:

$$q = 1.29C_o^{1/1.00}$$

or for *A. niger* hyphae:

$$\log q = 1.009 \log C_o - 0.0370$$

with $r = 0.9999$ ($k(^{241}\text{Am}) = 0.92$, $n(^{241}\text{Am}) = 0.99$:

$$q = 0.92C_o^{1/0.99}$$

Effect of coexistent ions on ^{241}Am adsorption

Because wastewaters produced from the preparation and recovery processes of ^{241}Am fire alarms contained mainly two coexisting ions, Au^{3+} and Ag^{+}, the effect of Au^{3+} and Ag^{+} ions on ^{241}Am adsorption by *A. niger* should be studied as well. The adsorption rate of ^{241}Am by *A. niger* spore and hyphae was between 96.0% and 98.5% in the presence of Au^{3+} and Ag^{+}. This implied that at $C_o(^{241}\text{Am}) = 44.3$ μg/l, $m_{(A.\ niger\ \text{spore})} = 1.03$ g/l or $m_{(A.\ niger\ \text{hyphae})} = 0.75$ g/l, Au^{3+} and Ag^{+} were not found to have a distinct influence on ^{241}Am adsorption, even though the concentrations of Au^{3+}, Ag^{+} were 2000 times more than that of ^{241}Am. At this condition, the adsorption rate of ^{241}Am by *A. niger* spore and hyphae was still up to 96%. It may be explained that the adsorption amount adsorbed by *A. niger* spore and hyphae was far from saturated adsorption. Although the ^{241}Am adsorption was challenged with Au^{3+} or Ag^{+}, no significant differences were observed.

Table 1. Effect of the temperature on the adsorption of ^{241}Am by *A. niger* spore and hyphae*

Temperature, °C	15	25	35	45
Adsorption by *A. niger* spore, %	96.5	97.2	97.4	96.5
Adsorption by *A. niger* hyphae, %	97.8	98.7	98.9	96.6

* The adsorption experiments were performed in the conditions as C_o (^{241}Am) = 5.6 MBq/l, $m_{(\text{spore})} = 0.75$ g/l, $m_{(\text{hyphae})} = 1.03$ g/l, pH 2.

Table 2. Effect of ^{241}Am concentration on the adsorption by *A. niger* spore and hyphae*

^{241}Am concentration, MBq/l	5.6	11.1	27.8	55.5	82.3	111.0
Adsorption by *A. niger* spore, %	97.4	94.7	97.8	96.2	96.7	96.2
Adsorption by *A. niger* hyphae, %	97.1	97.2	97.4	97.5	97.9	98.8

* The adsorption experiments were performed in the conditions as $m_{(spore)} = 0.75$ g/l, $m_{(hyphae)} = 1.03$ g/l, pH 2.

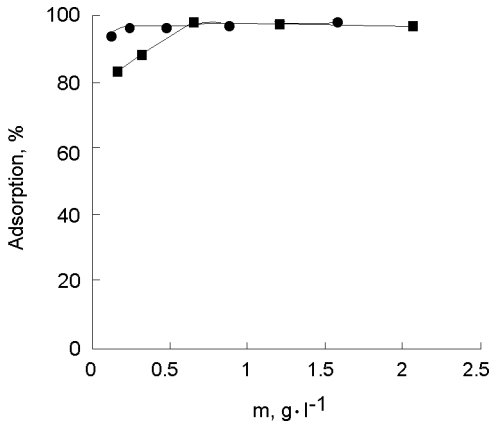

Fig. 3. Effect of microorganism concentration on the adsorption of ^{241}Am; • *A. niger* spore, ■ *A. niger* hyphae; $C_o = 5.6$ MBq/l, pH 2

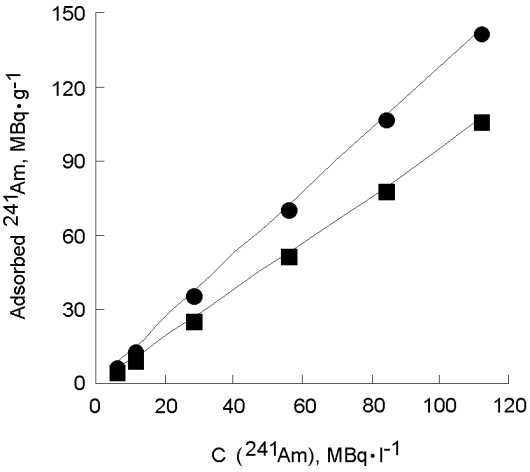

Fig.5. Effect of ^{241}Am concentration on the adsorbed metal; • *A. niger* spore, ■ *A. niger* hyphae; $m_{(spore)} = 0.75$ g/l, $m_{(hyphae)} = 1.03$ g/l, pH 2

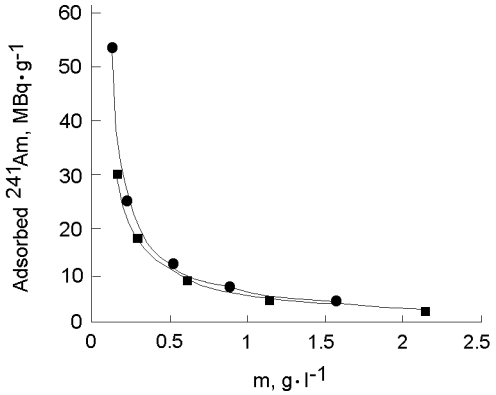

Fig. 4. Effect of microorganism concentration on ^{241}Am adsorbed; • *A. niger* spore, ■ *A. niger* hyphae; $C_o = 5.6$ MBq/l, pH 2

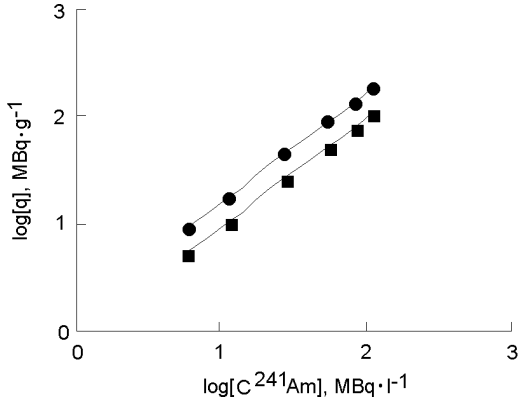

Fig.6. Freundlich adsorption curve of ^{241}Am by • *A. niger* spore, ■ *A. niger* hyphae; $m_{(spore)} = 0.75$ g/l, $m_{(hyphae)} = 1.03$ g/l, pH 2

Conclusions

It has been shown that *A. niger*, in spore as well as hyphae is considerably efficient biosorbent for ^{241}Am. The adsorbed ^{241}Am amounts (Q) could be up to 142.4 MBq/g dry weight for *A. niger* spore and 106.4 MBq/g dry weight for *A. niger* hyphae from ^{241}Am solution of 111 MBq/l (877.2 μg/l) (C_o). There was no significant difference between the adsorption behavior of *A. niger* spore and hyphae. These preliminary results indicated that *A. niger* spore and hyphae can be used for the absorption of ^{241}Am from wastewaters. The immobilized microorganism and continuous chromatography was considered as a promising way for practical treatment.

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