

Biosorption of uranium by *Bacillus* sp.FB12 isolated from the vicinity of a power plant

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(Received June 06, 2013, Revised November 15, 2013, Accepted November 16, 2013)

Abstract. Biosorption represents a technological innovation as well as a cost effective excellent remediation technology for cleaning up radionuclides from aqueous environment. In the present study, a bacteria strain FB12 with high adsorption rate of uranium ion was isolated from the vicinity of the nuclear power plant. It was tentatively identified as *Bacillus* sp.FB12 according to the 16S rDNA sequencing. Efforts were made to further improve the adsorption rate and genetic stability by UV irradiation and UV-LiCl cooperative mutagenesis. The improved strain named *Bacillus* sp.UV32 obtains excellent genetic stability and a high adsorption rate of 95.9%. The adsorption of uranium U (VI) by *Bacillus* sp.UV32 from aqueous solution was examined as a function of metal ion concentration, cell concentration, adsorption time, pH, temperature, and the presence of some foreign ions. The adsorption process of U (VI) was found to follow the pseudo-second-order kinetic equation. The adsorption isotherm study indicated that it preferably followed the Langmuir adsorption isotherm. The thermodynamic parameters values calculated clearly indicated that the adsorption process was feasible, spontaneous and endothermic in nature. These properties show that *Bacillus* sp.UV32 has potential application in the removal of uranium (VI) from the radioactive wastewater.

Keywords: uranium contamination; *Bacillus* sp.; mutagenesis; Biosorption; kinetic

1. Introduction

The depletion, deterioration and exhaustion of non-renewable energy have become serious bottlenecks constraining economic and social development. Development and use of nuclear energy is one solution to this problem because of its high density and low cost, but mining and processing of uranium mineral resources have also brought a large area of U pollution (Pollmann *et al.* 2006, Kryvoruchko and Antonina 2007). Additionally, depleted uranium (DU) weapons have been used in war frequently, leading to DU contaminated soil and water in combat areas (Li and Zhang 2012). Uranium, which exists commonly in UO^{2+} form in waste water, has biologically dynamic toxicity, metabolism toxicity and chemical toxicity, leading to potential long-term harm to mammalian reproduction and development with reduced biological fertility, abnormal and slow embryonic development (Kalin *et al.* 2004, Domingo 2001). Toxicity is closely related to solubility, i.e., the more soluble the uranium compound is, the more toxic it becomes (Gavrilescu

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et al. 2009). Generally, hexavalent uranium, which forms soluble compounds, is more likely to be a systemic toxicant than the less soluble tetravalent uranium.

Therefore, finding a way for remedying uranium contaminated water effectively and thoroughly has become a hot research topic. Several traditional methods including chemical clarification, precipitation, membrane filtration, and reverse osmosis are available for removing uranium from waste water (Vijayaraghavan and Yun 2008, Silva 2009). Biosorption, as a biological method, has become a favorable method of choice for following advantages: (1) biological processes can be carried out in situ at the contaminated site; (2) cost effective; (3) high efficiency to treat low concentrations of heavy metals in wastewater; (4) easy to produce high concentrations of metal ion adsorption active groups (Vijayaraghavan and Yun 2008, Farooq *et al.* 2010, Goyal *et al.* 2003).

Bacterial biomass, with functional groups such as carboxyl, amine and phosphonate in their cell wall, represents an efficient and potential class of biosorbents for decontamination or recovery of radioactive compounds from both the wastewater of nuclear facilities and aquatic environments (Dhami *et al.* 1998, Dabbagh *et al.* 2007). Although several strains capable of sequestering uranium were reported (Dhami *et al.* 1998, Li *et al.* 2012, Choudhary and Sar 2011, Kazy *et al.* 2009, Abolelonas *et al.* 1998), they all, however, had a limited application as the adsorption process is complex and dependent on the chemistry of the metal ions, specific surface properties of the organisms, cell physiology and the physicochemical influence of the environment like pH, temperature and metal concentration (Kedari *et al.* 2001, Fourest *et al.* 1994). The optimal conditions for one strain's performance may be inconsistent with the natural ones. It is essential to isolate more candidate stains with characteristic identified before its further application in decontamination or recovery of uranium. The present work is focused on the isolation of such a candidate stain and characterization of its uranium adsorption process.

2. Materials and methods

2.1 Isolation and bacterial culture conditions

Cell growth (CG) medium (g/L): maltose 10, casein peptone 10, beef extract 5, NaCl 2, pH7.2. Fermentation (FM) medium (g/L): maltose 10, casein peptone 10, beef extract 6, $\text{NH}_4\text{H}_2\text{PO}_4$ 4, K_2HPO_4 1.2, KH_2PO_4 2.5, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.15, MnSO_4 0.012, ZnSO_4 0.008, pH 7.2. Screening (SM) Medium (g/L): K_2HPO_4 1.2, ZnSO_4 0.008, MgSO_4 0.15, $\text{NH}_4\text{H}_2\text{PO}_4$ 4, MnSO_4 0.012, uranium 10 mg/L, pH 7.2. All the above culture mediums were sterilized at 121°C for 20 min. For solid medium agar (2% w/v) was added.

Soil obtained from the vicinity of the nuclear power plant in Fujian, China, was diluted with physiological saline and rubbed onto SM plates. The SM plates were incubated at 32°C for 2-3 days and individual bacterial colonies were further separated and transferred to numbered tubes containing agar slant medium. 63 bacterium stains cultured in tube slants were stored at 4°C for further screening.

Strains maintained in the tube containing agar slant culture-medium were inoculated to 20 ml FM medium in 50 ml silicone stopper plugged flasks and incubated at 32°C in a rotating shaker. The cells were then harvested at a suitable time by centrifugation at $10,000 \times g$ for 10 min at 4°C and washed three times with distilled water. The wet biomass (0.01 g) were added to 100ml solutions containing 100 mg/L uranium. The suspensions were incubated at 30°C on a shaker at

180 rpm for 180 min, and then centrifuged at 10,000 ×g for 10 min. The supernatant was filtered through 0.45 μm cellulose membrane and detected by vanadate titration the residue uranium to determine the absorption rate and quantity.

2.2 Determination of growth curve

The Growth curve of the isolated strain (FB12) with the highest absorption was determined by calculating the cell concentrations of periodically sampled bacterium suspension.

2.3 Identification of the strain

The strain FB12 obtained from logarithmic phase was rubbed to FM plating medium and was incubated at 32°C for 2 days. The morphology of the bacterial cells was observed by gram stain. Genomic DNA of the isolate was extracted according to Chen *et al.* (2011).

2.4 UV irradiation and UV-LiCl cooperative mutagenesis

The strain (FB12) was subjected to UV irradiation and UV-LiCl cooperative mutagenesis as described in our previous report (Chen *et al.* 2011).

2.5 Genetic stability of the mutants

The strains improved by mutagenesis may be susceptible to back mutation or re-screening, which may lead to the decreasing of absorption rate. Two strains, UV32 and UV51 with high absorption rate were subcultured to obtain strains with various passage numbers.

2.6 Effect of uranium on bacterial growth in liquid media

For this purpose, the bacterial cells were grown in FM medium containing a range of uranium concentrations. A culture grown in the absence of uranium served as control. Twenty milliliters of FM medium containing different concentrations of U (VI) were inoculated with 1.6 ml of mid-log phase culture of the stain. The cultures were incubated for 24 h and the bacterial cells growth was followed by measuring the absorbance at 600 nm using a UV-VIS spectrophotometer.

2.7 Biosorption experiments

U solution of various concentration in UO_2^{2+} form was made from U_3O_8 according to Li *et al.* (2012). The biosorption of U on *Bacillus* sp.UV32 cells from aqueous solution was investigated by batch biosorption equilibrium experiments. The effects of pH, initial U concentration, contact time, dosage of cells and medium temperature on the biosorption were studied. All the biosorption were performed using 10 mg (except sorbent mass variation study) of cells suspended in 30 mL uranium solution in a flask at selected pH. The flasks were shaken at different temperatures and mixing times. The solution was separated from the solids by centrifugation. The amount of biosorbed U was calculated from the change of the concentration in solution before and after biosorption. The results were expressed as biosorption rate (%) and biosorption content (mg/g), which were calculated using vanadate titration method (Sanke Gowda and Shakunthala 1978).

2.8 Desorption experiments

The *Bacillus* sp.UV32 cells with adsorbed uranium in them were harvested by centrifugation and incubated with 30ml solution containing 1 mol/L NaHCO₃, HNO₃, Na₂CO₃, HCl, or 0.01 mol/L EDTA at 25°C with an agitation speed of 180 r/min for 180 min. The supernatant obtained by centrifugation was subjected to determination of uranium content to calculate the desorption rates of various desorption reagents by following equation: desorption rate (%) = (desorption content / sorption content) * 100%.

2.9 Adsorption isothermal experiment

The adsorption isotherm was carried out at different temperatures 25, 30, 40 and 50°C with various initial U concentration. To ensure full equilibration, a biomass of 0.01 g, pH 6.0 and a shaking (180 rpm) time of 180 min were used for all concentrations of U in this study.

2.10 Thermodynamic experiment

A biomass of 0.01 g was incubated with U solution at an agitation speed of 180 r·min. Sampling the solution periodically to determine its uranium concentration at different contact time.

3. Result and discussion

3.1 Isolation, characterization and development of absorption strain

Among the 63 strains grown in the SM plate medium, 20 strains could grown in FM medium with high quantity and were subject to absorption experiment. The strain named FB12 with the highest absorption rate was used for further study. The morphological examination by optical microscopy showed that the FB12 was gram-positive and exhibited a rhabditiform shape as showed in Fig. 1. The FB12 growth curve was plotted by time against cell concentration. As can be seen from Fig. 2, with an inoculum concentration of about 0.1 g/L, the strain entered the logarithmic phase at 6 h and reached to stable phase at 18 h.



Fig. 1 FB12 under biological microscopes

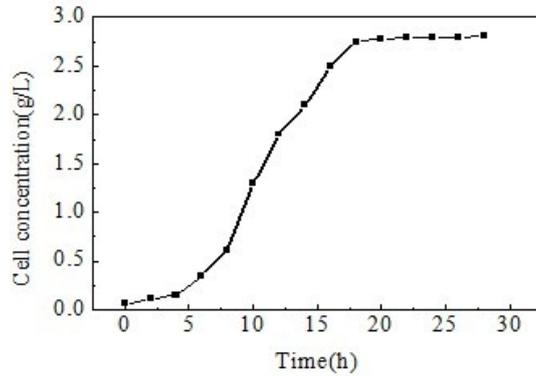


Fig. 2 Growth curve of FB12

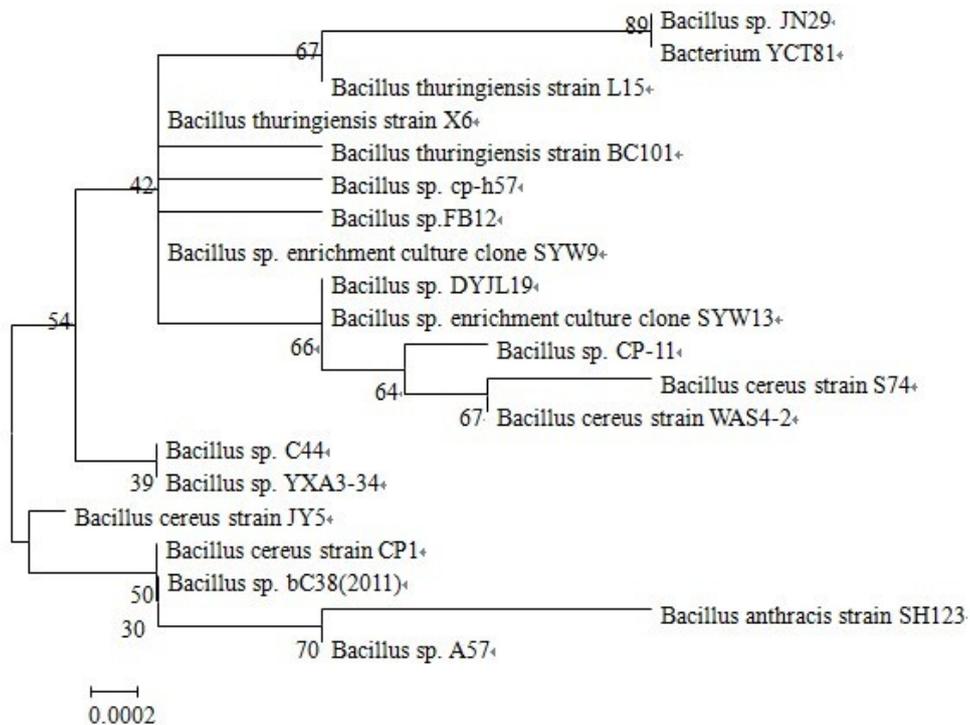


Fig. 3 Phylogenetic tree based on 16S rDNA sequences

The partial 16S rDNA sequence of strain FB12 (comprising 1450 nucleotides) was successfully amplified, determined and submitted to the GenBank database. Performing BLAST analyses, the sequence was most closely related to various *Bacillus* with maximal identity as high as 99% and a phylogenetic tree (Fig. 3) was constructed based on 16S rDNA sequence. Presently, the acceptable positional standard is that those strain sequences with the similarity higher than 96-98% are regarded as belonging to the same genus (Drancourt *et al.* 2000, Mahmood *et al.* 2009). Therefore, the isolate was identified as *Bacillus* sp.B12.

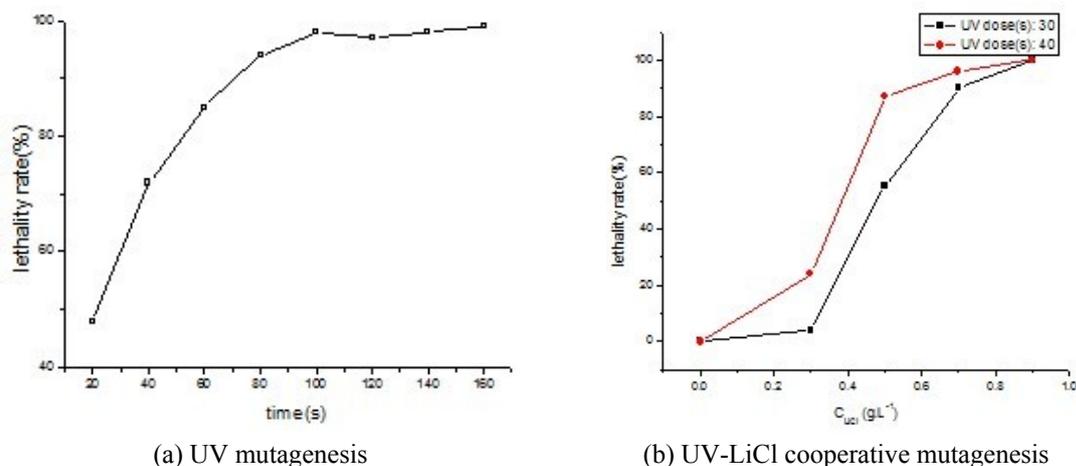


Fig. 4 The lethality rate of mutagenesis on *Bacillus* sp.B12

To enhance biosorption capacity, biomass modifications and immobilization were used (Liu *et al.* 2008, Xie *et al.* 1996). Gene mutation has the potential to improve the strains to the point where they will have intrinsic capability as well as specificity and greater resistance to ambient condition. UV radiation will cause mutations in cells, due to mistakes in repair of the resultant thymine dimers. Characterized by its simple operation and high efficiency, UV induced mutation is extensively used for improvement of strains with higher productivity. LiCl is a metal salt mutagen, and is always being used with other mutagen since it has no effect when used alone. It can be found from Fig. 4(a) that lethality rate of the organism increases drastically with an increase in the duration of UV irradiation. Fig. 4(b) shows that lethality rate of the organism increases sharply with the increase of the duration of UV irradiation (in accordance with Fig. 4(a)) and the doses of LiCl. Finally, the colonies grown in SM medium exhibiting the maximal size in diameter indicating a certain tolerance towards U (VI) were selected for absorption experiments. Two strains, UV32 and UV51, with absorption rate higher than 95%, were obtained successfully.

To obtain the strain with the best genetic stability, the two strains were subjected to absorption experiments with biomass of different passages. It was found that UV32, with no decline after eight passages, possess a far better genetic stability as compared with UV51 which declined after 3 passages (see in Table 1).

The growth responses of *Bacillus* sp.UV32 at different concentrations of U in liquid cultures are given in Fig. 5. The bacteria cells growth was completely hindered at 100 mg U/l. More than 50% of bacterial growth was inhibited in the presence of more than 60 mg U/l. *Bacillus* sp.UV32 performed an excellent tolerance in culture containing U concentration range from 20 to 40 mg/L.

3.2 Biosorption experiments

Solution pH is an important factor controlling the biosorption process. The pH may affect the surface charge of the biosorbent, the degree of ionization, speciation of metal in solution, precipitation of metal ions and ionization of surface functional groups (Arica *et al.* 2003). The change of the absorbed percentage of U with the pH is given in Fig. 6(a). The optimal pH condition

Table 1 Stability of high adsorption rate mutants

Gap	UV32		H16	
	Adsorption rate (%)	q (mg.g ⁻¹)	Adsorption rate (%)	q (mg.g ⁻¹)
G1	95.3	285.9	95.8	287.4
G2	96.1	288.3	96.3	288.9
G3	95.7	287.1	95.1	285.3
G4	96.2	288.6	93.3	279.9
G5	95.5	286.5	86.1	258.3
G6	95.3	285.9	90.7	272.1
G7	95.8	287.4	85.9	257.7
G8	96.1	288.3	76.3	228.9

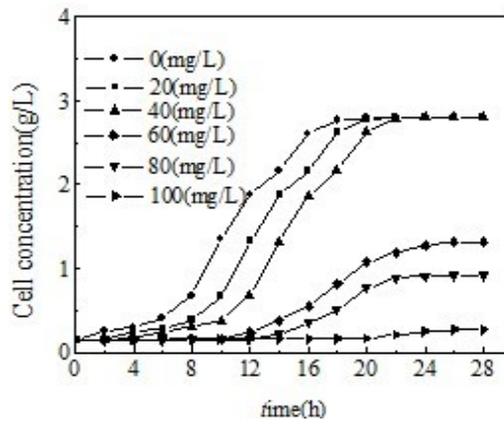


Fig. 5 The tolerance experiments of strain to uranium (VI)

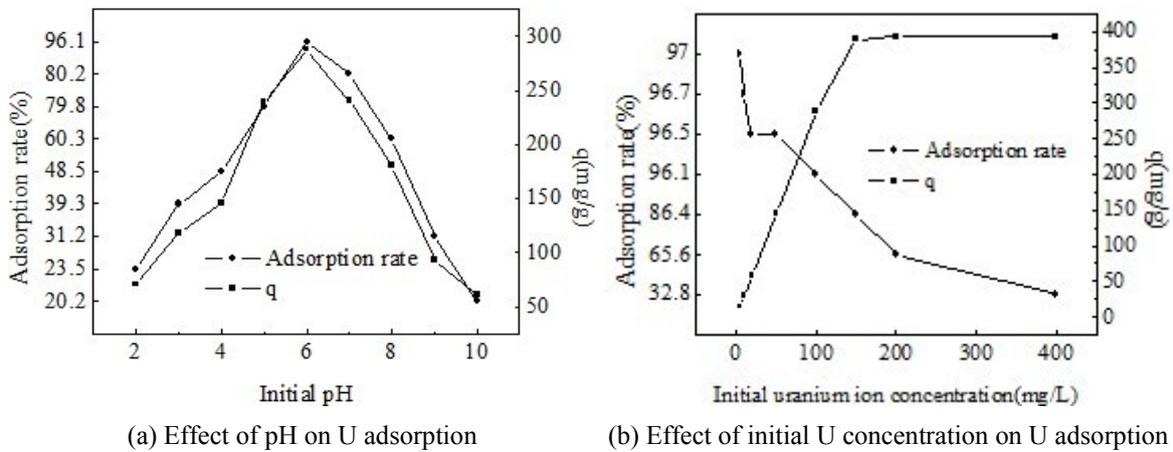


Fig. 6 Effect of initial pH and U concentration on adsorption U adsorption

for U absorption is around 6.0 with approximately 96% of initial amount of U was adsorbed. Either increase or decrease the pH value may likely to result in decline in absorption rate and content. It is favourable that the pH value (6.0) for the optimal absorption process similar to natural Ph.

It is always necessary to identify the maximum saturation potential of the biosorbent, for which experiments should be conducted at a range of initial U concentrations (Genc *et al.* 2003). The amount of U biosorbed on the UV32 cells quickly increased with the increase of the initial U concentration up to 200 mg/L as seen in Fig. 6(b). But, after this concentration, of which the absorption content reached to as high as 393.5 mg/g, the biosorption of uranium maintain constant indicating saturation of the binding sites on UV32 cells. The absorption rates had a negative relationship with the initial concentration of U.

The biosorption of uranium from solution by UV32 biomass was studied as a function of contacting time from 5 to 210 min. As seen from Fig. 7(a), the absorption rate and content increases gradually and consequently reaches to the equilibrium at about 180 min. About 96.1% of the U biosorption was achieved in 180 min, which was selected as optimal contact time.

Fig. 7(b) displays the effect of biosorbent dosages ranging from 0.083 to 1.67 (g/L) on the biosorption rate and content. With the increase of cells dosage, the absorption rate increased. This could be due to the increased functional groups on the cell wall with the increasing of biosorbent surface area. Conversely, the quantity of biosorbed solute per unit weight of biosorbent decrease with the increase of biosorbent dosage, which may be due to the insufficient available solute. A dosage of 0.33 g/L biomass is thought to be the best choice as it can achieve high absorption rate (96.1%) as well as high content (288.3 mg/g).

Temperature can influence the biosorption efficiency both in positive or negative way. Higher temperatures usually enhance sorption due to the increased surface activity and kinetic energy of the solute while the opposite may result from exothermic nature of the absorption processes (Vijayaraghavan and Yun 2008). The result (see in Fig. 8) indicated that the absorption rate and content increased softly when the temperature was increased from 10 to 45°C, suggesting that the process was an energy slightly dependent mechanism. It is always desirable to conduct biosorption in room temperature, saying 25°C, as this temperature is economic and easy to replicate.

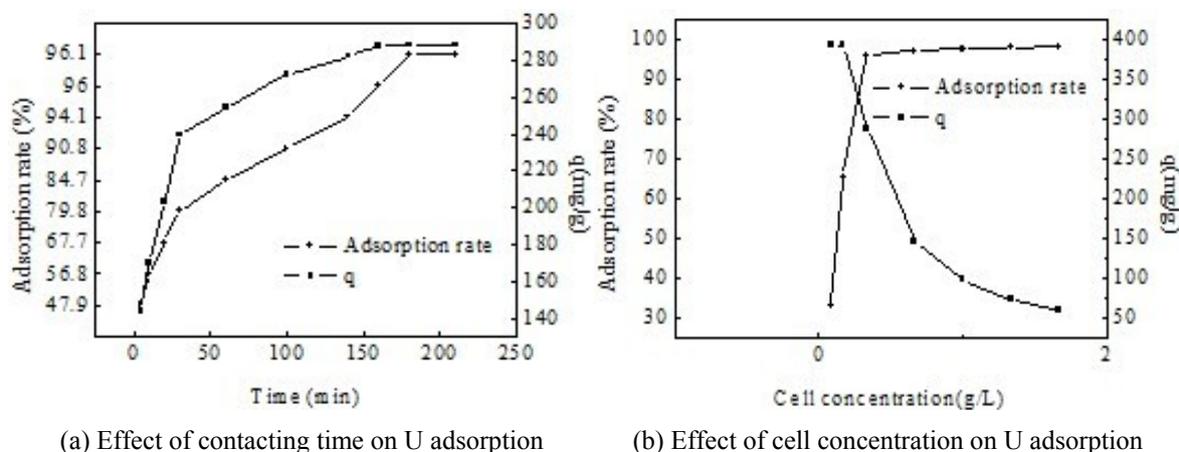


Fig. 7 Effect of contacting time and cell concentration on adsorption of U

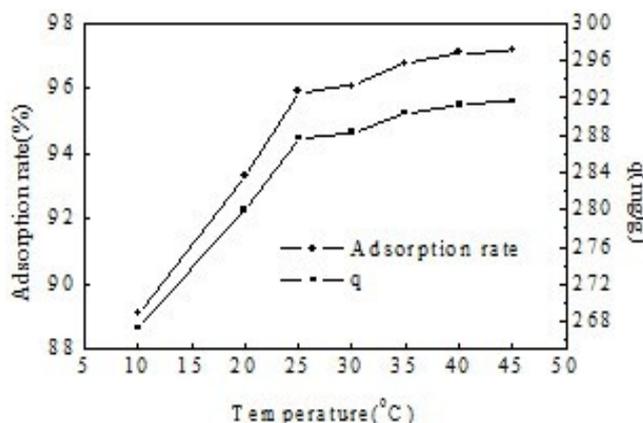


Fig. 8 Effect of temperature on U adsorption

Table 2 Effect of co-ions on U adsorption

Co-existing ion	Adsorption rate (%)	Adsorption content (mg/g)
control	95.9	287.7
Ca ²⁺	93.1	279.3
K ⁺	94.8	288.4
Al ³⁺	20.1	60.3
Fe ²⁺	69.8	209.4
Fe ³⁺	72.3	216.9
Cu ²⁺	89.2	267.6
CO ₃ ²⁻	86.1	258.3
Cl ⁻	95.5	286.5
SO ₄ ²⁻	93.4	280.2
HCO ₃ ⁻	91.8	275.4

As the effluents generate in nuclear fuel reprocessing is expected to contain salt, the sorption U on UV32 in the presence of 0.1 M concentration of various anions and cations at pH 6.0 was studied and the results are presented in Table 2. The influence of Cl⁻ and SO₄²⁻ on biosorption of U can be ignored according to Sar *et al.* (2004). It was found that the presence of these ions, Al³⁺, Fe²⁺, Fe³⁺, Cu²⁺, CO₃²⁻, at 0.1 M concentration decreased the absorption rate and content values for U, with maximum reduction in the presence of CO₃²⁻. Thus, the influence of these ions on absorption should be taken into consideration when practical applications are carried out. CO₃²⁻ can be selected as one of the desorbent candidates for desorption study.

3.3 Desorption experiments

The Fig. 9 shows that both NaHCO₃ and Na₂CO₃ performed an excellent remove effect of U from UV32 cells, with desorption rate above 99%. This may be accounted by the high stability coefficient of the complex resulted from U and CO₃²⁺ reaction (Mormson *et al.* 1995).

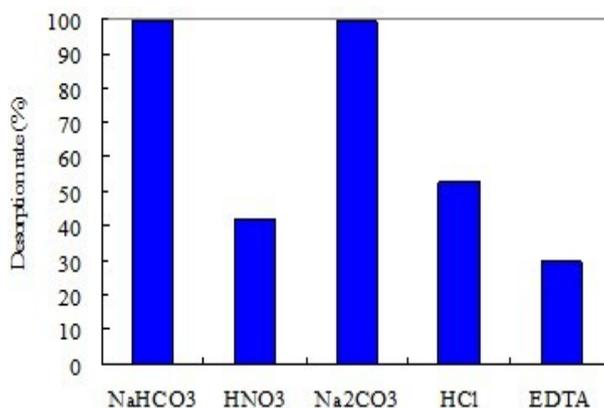


Fig. 9 Effect of different desorbents on desorption

3.4 Absorption isotherms

In order to optimize the design of biosorption system for the removal of uranium, it is important to establish the most appropriate correlation for equilibrium curves. The experimental isotherm data were described by two absorption isotherm models, namely Langmuir and Freundlich, which are most widely used in radionuclide Biosorption (Das 2012).

Langmuir adsorption isotherm, originally developed to describe gas–solid-phase adsorption onto activated carbon, has been traditionally used to quantify and contrast the performance of different adsorbents (Gok *et al.* 2013). This model suggests monolayer sorption on a homogeneous surface without interaction between sorbed molecules. In addition, the model assumes uniform energies of sorption onto the surface and no transmigration of the sorbate (Langmuir 1918). Non-linear form of Langmuir equation can be expressed by the following equation

$$q_e = \frac{bq_{\max}C_e}{1 + bC_e} \quad (1)$$

Likewise, liner form of Langmuir equation can be expressed by the following equation

$$\frac{1}{q_e} = \frac{1}{bq_{\max}} \frac{1}{C_e} + \frac{1}{q_{\max}} \quad (2)$$

where q_e is the amount of metal ions biosorbed onto biosorbent, C_e is the equilibrium concentration of uranium in solution, and q_{\max} and b are Langmuir constants related to maximum biosorption capacity and biosorption energy.

The empirical Freundlich equation based on sorption is as follows

$$q_e = KC_e^{1/n} \quad (3)$$

Its liner form can be rewritten as

$$\ln q_e = \frac{1}{n} \ln C_e + \ln K \quad (4)$$

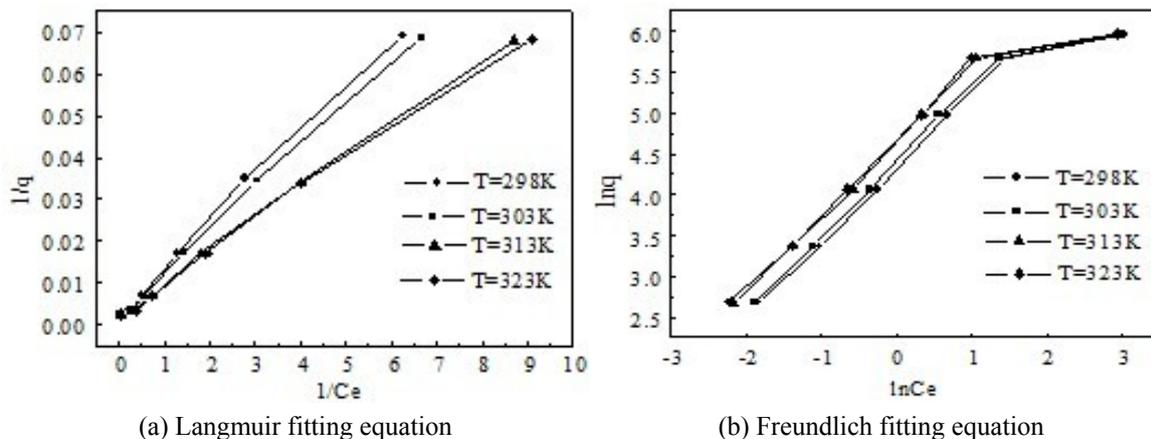


Fig. 10 Fitting equations

Table 3 Constant of Langmuir and Freundlich fitting equation

T/K	Langmuir fitting equation	Constant		R ²	Freundlich fitting equation	Constant		R ²
		b	q _{max}			K	1/n	
298	Y = 0.0109x + 0.0022	0.2018	454.55	0.997	Y = 0.719x + 4.2064	67.085	0.719	0.939
303	Y = 0.0101x + 0.0021	0.2079	476.19	0.998	Y = 0.709x + 4.2656	71.176	0.709	0.935
313	Y = 0.0077x + 0.0021	0.2727	476.19	0.998	Y = 0.682x + 4.4345	84.27	0.682	0.921
323	Y = 0.0074x + 0.0021	0.2837	476.19	0.997	Y = 0.679x + 4.4582	86.29	0.679	0.917

where q_e is the amount of metal ions biosorbed onto biosorbent, C_e is the equilibrium concentration of uranium in solution, K is the Freundlich constant which indicates the absorption ability, n is adsorption equilibrium constant.

Langmuir fitted equation was described by the plot (see in Fig. 10(a)) of $1/C_e$ versus $1/q_e$ according to result data (not showed) while Freundlich fitted equation was described by the plot (see in Fig. 10(b)) of $\ln C_e$ versus $\ln q_e$.

The Langmuir isotherm model can better describe the experimental data according to the compared correlation coefficient (R^2) values given in Table 3. The biosorption of the uranium ions was taken place at the functional groups/binding sites on the biosorbent, which is regarded as monolayer biosorption. The maximum monomolecular biosorption capacity (Q_{max}) was found to be 476 mg/g. The constant (b) of the biosorption equilibrium and Freundlich constant (K) had a positive relationship with the temperature indicating the increasing absorption ability as the temperature rose. The slope ($1/n$) ranging between 0 and 1 is a measure of the ease of the absorption reaction. The $1/n$ values in present experiments are approximately 0.7, which indicates a light difficulty of the absorption reaction.

3.5 Thermodynamic analysis

Thermodynamic parameters can be calculated using the following equations (Froglu *et al.* 2009). The distribution constant can be written as

$$K = q_e / C_e \quad (5)$$

The well known Gibbs free energy change can be expressed as

$$\Delta G = -RT \ln K \quad (6)$$

ΔG is related to the enthalpy, and entropy change as follows

$$\Delta G = \Delta H - T\Delta S \quad (7)$$

The values of ΔH and ΔS can be obtained from the plot of $\ln K$ against $1/T$ (Fig. 11), of which ΔG is calculated using Eq. (7) as showed in Table 4. The negative values of ΔG show that the biosorption is a spontaneous process. The positive value of ΔH shows that the process has an endothermic character; therefore, increasing the temperature increases the absorption rate and content. The positive value of ΔS indicates the increasing entropy in the absorption process.

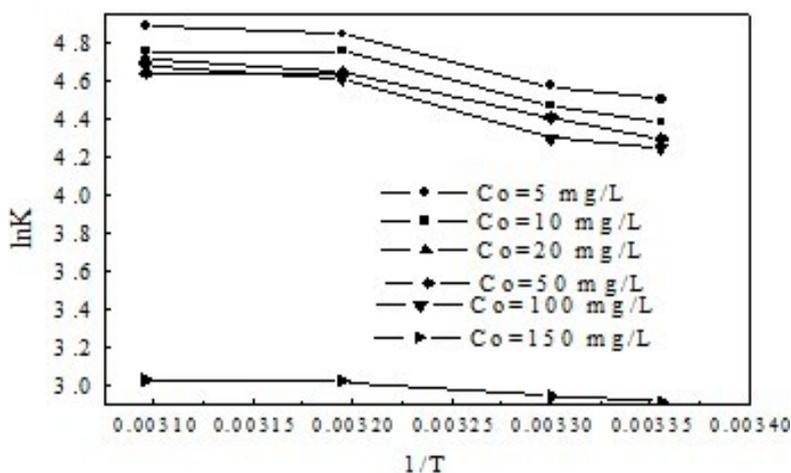


Fig. 11 $\ln K$ - $1/T$ in different initial U concentration

Table 4 Confirmation of thermodynamic constants

C_o (mg/L)	ΔH (kJ mol ⁻¹)	ΔS (J mol ⁻¹)	ΔG (kJ mol ⁻¹)			
			$T = 298$ K	$T = 303$ K	$T = 313$ K	$T = 323$ K
5	13.34	82.43				
10	13.19	80.9				
20	13.85	82.42				
50	11.61	75.02	-10.22	-10.59	-11.33	-12.07
100	15.17	86.19				
150	3.93	37.53				

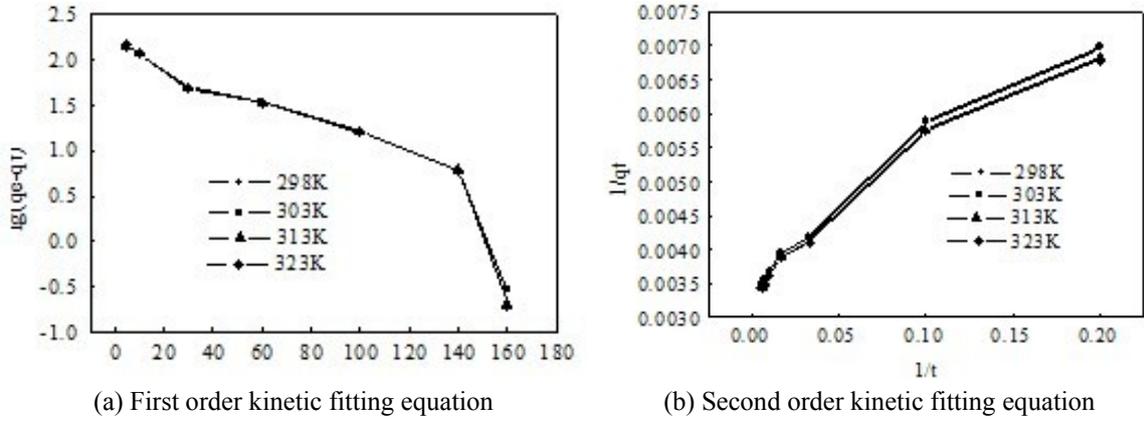


Fig. 12 Fitting equations

Table 5 Fitting equation and constants of the kinetic models

T(K)	First order fitting equation	k_1 (pm)	R^2	Second order fitting equation	$k_2 \times 10^{-3}$ pm	q_e (mg/g)	R^2
298	$Y = -0.0145x + 2.2968$	0.033	0.844	$Y = 0.0184x + 0.0036$	0.704	277.8	0.966
303	$Y = -0.0138x + 2.2684$	0.032	0.868	$Y = 0.0183x + 0.0035$	0.669	285.7	0.966
313	$Y = -0.0144x + 2.2923$	0.033	0.842	$Y = 0.0178x + 0.0035$	0.688	285.7	0.967
323	$Y = -0.0155x + 2.329$	0.036	0.803	$Y = 0.0177x + 0.0035$	0.692	285.7	0.966

3.6 Adsorption kinetics

An adsorption rate expression is necessary in order to design a fast and effective process. The Langergren first-order rate and Ritchie second-order rate equations are models that generally used to study the absorption dynamic models (Yakup *et al.* 2004). The Langergren first-order rate equation is expressed by

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad \text{or} \quad \lg(q_e - q_t) = -\frac{k_1 t}{2.303} + \lg q_e \quad (8)$$

While Ritchie second-order rate equation is expressed by

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad \text{or} \quad \frac{1}{q_t} = \frac{1}{k_2 q_e^2 t} + \frac{1}{q_e} \quad (9)$$

where t is adsorption time; q_t is the amount of adsorbed metal ion at t point of time; q_e is the equilibrium amount of metal ions biosorbed onto biosorbent; k_1 is adsorption rate constant of first order and k_2 is adsorption rate constant of second order.

The Langmuir pseudo-first-order kinetic equation was obtained by the plot (see in Fig. 12(a)) of t against $\lg(q_e - q_t)$ according to result data (not showed) and Freundlich pseudo-second-order kinetic equation was described by the plot(see in Fig. 12(b)) of $1/t$ versus $1/q_t$.

The compared kinetic equation relevant parameters are showed in Table 5. It was determined that the biosorption fitted the pseudo-second order kinetic model with a R^2 value of 0.97.

4. Conclusions

FB12 strain isolated from the vicinity of a power plant exhibited intrinsic abilities to absorb uranium at an absorption rate of 82.4% and was developed to a absorption rate of 95.9% by UV irradiation and UV-LiCl cooperative mutagenesis. The growth responses of developed strain named *Bacillus* sp.UV32 indicated that the *Bacillus* sp.UV32 performed an excellent tolerance in culture containing U concentration range from 20 to 40 mg/L.

The most important parameters that influence the absorption rate and content were pH and co-existing ions. It was also determined that the temperature did not have much effect on the adsorption rate and content. The ideal absorption conditions were determined as 25°C temperature, 6.0 pH and 180 min contact time. In ideal absorption conditions, the initial uranium ion concentration was chosen as 100 mg/L, and an absorption content of 393.4 mg/g was obtained with the application of 0.083 g/L biomass. The strain UV32, therefore, shows a high potential capability in removing UO^{2+} from solutions and may be considered as one of the candidate strains in the application of UO^{2+} scavenging.

The absorption is characterized as multilayered, hetero-geneous, and physical. The experimental data exhibited a good agreement with the isotherm models of Langmuir. Standard free Gibbs energy (ΔG) and ΔH parameters were taken into account, and bioabsorption is believed to have endothermic character and to be natural. The kinetics of absorption was seen to fit a pseudo-second order reaction model.

Acknowledgments

The authors gratefully acknowledge support from the Doctoral Fund of Ministry of education (No. 20103514110002) and the Major Plan Project of Science and Technology of Fujian Province (No. 2009N0046).

References

- Abdelouas, A., Lu, Y., Lutze, W. and Nuttall, H E. (1998), "Reduction of U (VI) to U (IV) by indigenous bacteria in contaminated ground water", *J. Contam. Hydrol.*, **35**(1), 217-233.
- Arica, M.Y., Bektas, S. and Denizli, A. (2003), "Uranium recovery by immobilized and dried powdered biomass: characterization and comparison", *Int. J. Miner. Process.*, 2003, **68**(1-4), 93-107.
- Chen, B., Xu, X., Hou, Z., Li, Z. and Ruan, W. (2011), "Identification and Mutagenesis of a New Isolated Strain *Bacillus* sp.B26 for Producing (R)- α -Hydroxyphenylacetic Acid", *Chin. J. Chem. Eng.*, **19**(4), 636-643.
- Choudhary, S. and Sar, P. (2011), "Uranium biomineralization by a metal resistant *Pseudomonas aeruginosa* strain isolated from contaminated mine waste", *J. Hazard. Mater.*, **186**(1), 336-343.
- Dabbagh, R., Ghafourian, H., Baghvand, A., Nabi, G.R., Riahi, H. and Ahmadi Faghih, M.A. (2007), "Bioaccumulation and biosorption of stable strontium and 90 Sr by *Oscillatoria homogenea* cyanobacterium", *J. Radioanal. Nucl. Chem.*, **272**(1), 53-59.
- Das, N. (2012), "Remediation of Radionuclide Pollutants through Biosorption – an Overview", *CLEAN–Soil*,

- Air, Water*, **40**(1), 16-23.
- Dhami, P.S., Gopalakrishnan, V., Kannan, R., Ramanujam, A., Salvi, N. and Udupa, S. (1998), "Biosorption of radionuclides by *Rhizopus arrhizus*", *Biotechnol. Lett.*, **20**(3), 225-228.
- Domingo, J.L. (2001), "Reproductive and developmental toxicity of natural and depleted uranium: A review", *Reproductive Toxicol.*, **15**(6), 603-609.
- Drancourt, M., Bollet, C. and Carlioz, R. (2000), "16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates", *J. Clin. Microbiol.*, **38**(10), 3623-3630.
- Eroglu, H., Yapici, S., Nuhoglu, C. and Varoglu, E. (2009), "Biosorption of Ga-67 radionuclides from aqueous solutions onto waste pomace of an olive oil factory", *J. Hazard. Mater.*, **172**(2/3), 729-738.
- Farooq, U., Kozinski, J.A., Khan, M.A. and Athar, M. (2010), "Biosorption of heavy metal ions using wheat based biosorbents – A review of the recent literature", *Bioresour. Technol.*, **101**(14), 5043-5053.
- Fourest, E., Canal, C. and Roux, J.C. (1994), "Improvement of heavy metal biosorption by mycelia dead biomass (*Rhizopus arrhizus*, *mucor miechei* and *pencillium chrysogenum*): pH control and cationic activation", *FEMS Microbiol. Rev.*, **14**(4), 325-332.
- Gavrilescu, M., Pavel, L.V. and Cretescu, I. (2009), "Characterization and remediation of soils contaminated with uranium", *J. Hazard. Mater.*, **163**(2), 475-510.
- Genç, Ö., Yalcinkaya, Y., Büyüktuncel, E., Denizli, A., Arica, M.Y. and Bektaş, S. (2003), "Uranium recovery by immobilized and dried powdered biomass: characterization and comparison", *Int. J. Miner. Process.*, **68**(1), 93-107.
- Gok, C., Gerstmann, U. and Aytas, S. (2013), "Biosorption of radiostrontium by alginate beads: application of isotherm models and thermodynamic studies", *J. Radioanal. Nucl. Chem.*, **295**(1), 777-788.
- Goyal, N., Jain, S.C. and Banerjee, U.C. (2003), "Comparative studies on the microbial adsorption of heavy metals", *Adv. Environ. Res.*, **7**(2), 311-319.
- Kalin, M., Wheeler, W.N. and Meinrath, G. (2004), "The removal of uranium from mining waste water using algal/microbial biomass", *J. Environ. Radioact.*, **78**(2), 151-177.
- Kazy, S.K., D'Souza, S.F. and Sar, P. (2009), "Uranium and thorium sequestration by a *Pseudomonas* sp.: Mechanism and chemical characterization", *J. Hazard. Mater.*, **163**(1), 65-72.
- Kedari, C.S., Das, S.K. and Ghosh, S. (2001), "Biosorption of long lived radionuclides using immobilized cells of *Saccharomyces cerevisiae*", *World J. Microbiol. Biotechnol.*, **17**(8), 789-793.
- Kryvoruchko, A.P. and Atamanenko, I.D. (2007), "The effect of dispersed materials on baromembrane treatment of uranium-containing waters", *Desalination*, **204**(1), 307-315.
- Langmuir, I. (1918), "The adsorption of gases on plane surfaces of glass, mica and platinum", *J. Am. Chem. Soc.*, **40**(9), 1361-1403.
- Li, J. and Zhang, Y. (2012), "Remediation technology for the uranium contaminated environment: A review", *Procedia Environmental Sciences*, **13**, 1609-1615
- Li, Q., Liu, Y., Cao, X., Pang, C., Wang, Y., Zhang, Z. and Hua, M. (2012), "Biosorption characteristics of uranium (VI) from aqueous solution by pummelo peel", *J. Radioanal. Nucl. Chem.*, **293**(1), 67-73.
- Liu, N., Liao, J., Yang, Y., Luo, S., Luo, Q., An, Z. and Zhao, P. (2008), "Biosorption of ²⁴¹Am by *Saccharomyces cerevisiae*: Preliminary investigation on mechanism", *J. Radioanal. Nucl. Chem.*, **275**(1), 173-180.
- Mahmood, Q., Hu, B.L., Cai, J., Zheng, P. and Azim, M.R. (2009), "Isolation of *Ochrobactrum* sp. QZ2 from sulfide and nitrite treatment system", *J. Hazard. Mater.*, **165**(1-3), 558-565.
- Morrison, S.J., Spangler, R.R. and Tripathi, V.S. (1995) "Adsorption of uranium (VI) on amorphous ferric oxyhydroxide at high concentrations of dissolved carbon (IV) and sulfur (VI)", *J. Contam. Hydrol.*, **17**(4), 333-346.
- Pollmann, K., Raff, J., Merroun, M., Fahmy, K. and Selenska-Pobell, S. (2006), "Metal binding by bacteria from uranium mining waste piles and its technological applications", *Biotechnol. Adv.*, **24**(1), 58-68.
- Sanke Gowda, H. and Shakunthala, R. (1978), "Naphthidines as redox indicators in titrations with vanadate", *Anal. Chim. Acta*, **97**(2), 385-389.
- Sar, P., Kazy, S.K. and Souza, S.F.D. (2004), "Radionuclide remediation using a bacterial biosorbent", *Int.*

- Biodeterior. Biodegrad.*, **54**(4), 193-202.
- Silva, J.R., De Melo Ferreira, A.C. and Da Costa, A.C.A. (2009), "Uranium biosorption under dynamic conditions: preliminary tests with *Sargassum filipendula* in real radioactive wastewater containing Ba, Cr, Fe, Mn, Pb, Ca and Mg", *J. Radioanal. Nucl. Chem.*, **279**(3), 909-914.
- Vijayaraghavan, K. and Yun, Y.S. (2008), "Bacterial biosorbents and biosorption", *Biotechnol. Adv.*, **26**(3), 266-291.
- Xie, J.Z., Chang, H.L. and Kilbane II, J.J. (1996), "Removal and recovery of metal ions from wastewater using biosorbents and chemically modified biosorbents", *Bioresour. Technol.*, **57**(2), 127-136.
- Yakup Arıca, M., Bayramoğlu, G., Yılmaz, M., Bektaş, S. and Genç, Ö. (2004), "Biosorption of Hg^{2+} , Cd^{2+} , and Zn^{2+} by Ca-alginate and immobilized wood-rotting fungus *Funalia troglitii*", *J. Hazard. Mater.*, **109**(1), 191-199.

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