Journal of Environmental Radioactivity 101 (2010) 969–973

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

journal homepage: www.elsevier.com/locate/jenvrad

Biosorption of uranium by chemically modified *Rhodotorula glutinis*

Jing Bai [a,b,*], Huijun Yao [a], Fangli Fan [a,b], Maosheng Lin [a,b], Lina Zhang [a], Huajie Ding [a], Fuan Lei [a], Xiaolei Wu [a], Xiaofei Li [a,b], Junsheng Guo [a], Zhi Qin [a]

[a] Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, PR China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 4 January 2010
Received in revised form
21 July 2010
Accepted 22 July 2010
Available online 24 August 2010

Keywords:
Biosorption
Chemical modification
Uranium
Functional groups
Rhodotorula glutinis
FTIR analysis

ABSTRACT

The present paper reports the biosorption of uranium onto chemically modified yeast cells, *Rhodotorula glutinis*, in order to study the role played by various functional groups in the cell wall. Esterification of the carboxyl groups and methylation of the amino groups present in the cells were carried out by methanol and formaldehyde treatment, respectively. The uranium sorption capacity increased 31% for the methanol-treated biomass and 11% for the formaldehyde-treated biomass at an initial uranium concentration of 140 mg/L. The enhancement of uranium sorption capacity was investigated by Fourier transform infrared (FTIR) spectroscopy analysis, with amino and carboxyl groups were determined to be the important functional groups involved in uranium binding. The biosorption isotherms of uranium onto the raw and chemically modified biomass were also investigated with varying uranium concentrations. Langmuir and Freundlich models were well able to explain the sorption equilibrium data with satisfactory correlation coefficients higher than 0.9.

1. Introduction

Uranium is a threatening heavy metal because of its high toxicity and radioactivity. Excessive amounts of uranium have found their way into the environment through the activities associated with the nuclear industry (Barkay and Schaefer, 2001; Benedict et al., 1981). Uranium disposed into the environment can reach the top of the food chain and be ingested by humans (Anke et al., 2009), causing kidney or liver damage to humans (Craft et al., 2004; Priest, 2001; Xie et al., 2008). Therefore, it is necessary to treat wastewater containing uranium in order to prevent its contamination of the environment. Moreover, with the development of nuclear industry, uranium will be used more extensively. As a non-renewable resource, the removal and recovery of uranium is meaningful.

Biosorption, a process, has been increasingly considered as a potential alternative way to remove contaminants from industrial effluents (Bhainsa and D'Souza, 2001; Wang, 2002). Compared with the conventional methods, biosorption process offers several advantages, such as low operating cost, high efficiency in detoxifying very dilute effluents and a minimal volume of disposable sludge. Studies have already been performed on uranium biosorption by various microorganisms *viz.* fungi, yeast, algae and unicellular bacteria (Kalin et al., 2005; Kazy et al., 2009; Parab et al., 2005). *Saccharomyces cerevisiae* has been used as a model to assay the heavy metal resistance of the yeast species due to its wide use in the fermentation industry. Nourbakhsh (Nourbakhsh et al., 1994) studied chromium (VI) removal form industrial wastewater by using *S. cerevisiae*. The efficiency of *S. cerevisiae* for uranium sorption was also tested in pure uranium solution (Kedari et al., 2001) and wastewater from uranium mill (Tykva et al., 2009). However, other yeasts, including *Rhodotorula*, which have been used in lead and cadmium biosoption (Cho and Kim, 2003; Salinas et al., 2000), have not been studied for uranium sorption. In order to make a better use of the microorganisms and improve their efficiency in uranium biosorption, intensive research is needed, including determination of the cell functional groups involved in uranium binding.

Metal ion uptake by biomass is believed to occur through interactions with functional groups native to proteins, lipids, and carbohydrates that make up the cell wall (Chen et al., 2007). To maximize the efficiency of the biomass, it is important to identify the important functional groups responsible for metal binding. The information obtained from these determinations will be useful for future attempts at chemically or biosynthetically altering the biomass to enhance its sorption capacity or selectivity for the specific metal ions. The identity of the functional groups would also

* Corresponding author. Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, PR China. Tel.: +86 931 4969692; fax: +86 931 4969693.
E-mail address: baijing@impcas.ac.cn (J. Bai).

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doi:10.1016/j.jenvrad.2010.07.003

be helpful for determining the mechanisms responsible for the binding of targeted metal ions.

Chemical modification of functional groups is a useful technique in characterizing the functional groups responsible for metal binding. For instance, Gardeatorresdey et al. (1990) studied Cu(II) binding after esterification of carboxyl groups on an algal species and observed the decreased sorption capacity of Cu(II). This suggested that carboxyl groups are responsible for Cu(II) binding. Similarly, in order to investigate the significance of amino groups in lead and cadmium sorption, Holan and Volesky (1995) employed formaldehyde modified *Ascophyllum nodosum* biomass, since the formaldehyde treatment can result in the methylation of amino groups (Loudon, 1984). Bai and Abraham (2002) reported the role of amino and hydroxyl, as well as carboxyl groups in the sorption of Cr (VI) by chemically modified *Rhizopus nigricans* biomass. However, chemical modification has not been used to date to study the role played by functional groups in uranium biosorption.

The objective of this study was to investigate the removal of uranium from aqueous solution by chemically modified *R. glutinis*. A combination of chemical modifications, metal-binding experiments, and infrared spectroscopy was performed to gain insight into the role played by functional groups in uranium binding.

2. Materials and methods

2.1. Biosorbent

The *R. glutinis* used in this study was obtained from the Institute of Microbiology, Chinese Academy of Sciences. The culture medium contained glucose 20 g/L (Factory of chemical experiments, Laiyang China), peptone 10 g/L (Hangzhou microbial regent Co. Ltd, China), yeast extract powder 10 g/L (Baorui microbial technology Co. Ltd, China), NaCl 2.5 g/L (Sinopharm chemical reagent Co., Ltd, shanghai, China), KH_2PO_4 1.0 g/L (Laiyang shuangshuang chemical Co. Ltd, China) and $MgSO_4{\cdot}7H_2O$ 0.5 g/L (Laiyang shuangshuang chemical Co. Ltd, China). After cultivating the cell in the sterilized medium mention above at 30 °C for 24 h, 5 mL of the cell suspension were added to the same fresh culture medium of 100 mL for further incubation. Cells at the stationary growth phase were killed by heating them in an oven at 60 °C for 1 day. The dead cells were harvested by centrifugation at 5000 rpm for 5 min and washed several times with distilled water until the biomass looks whitish. The biomass obtained was referred as raw biomass in this paper.

2.2. Chemicals

The stock solution of uranium (1.0 g/L) was prepared by using uranyl nitrate ($UO_2(NO_3)_2$) purchased from Sigma–Aldrich and diluted to appropriate concentration with distilled water. The initial pH of the uranium solutions were adjusted with 0.1 M HCl or 0.1 M NaOH and not monitored during the whole experimental process. 0.05% Arsenazo III solution was prepared by dissolving 0.5 g of the reagent in 1000 mL of distilled water. HCl, NaOH and Arsenazo III were purchased from sinopharm chemical reagent Co., Ltd, shanghai, China. All other reagents used were of analytical reagent grade unless otherwise stated.

2.3. Chemical modifications of biomass

Portions of raw biomass were chemically modified in different ways to understand the role of functional groups in uranium biosorption. The chemical modifications used to treat the biomass were as follows.

2.3.1. Methanol treatment

The method described by Kapoor and Viraraghavan (1997) was followed. Briefly, 80 mg of the well washed raw biomass was suspended in 4.8 mL of anhydrous methanol (CH_3OH, Tianjin guangfu fine chemical research institute, China), and 480 μL of concentrated hydrochloric acid was added to the suspension. The mixture was agitated on a rotary shaker at 125 rpm for 6 h. The reaction occurs as follows:

$$RCOOH + CH_3OH \xrightarrow{H^+} RCOOCH_3 + H_2O \quad (1)$$

The treated cell suspension was then centrifuged and sequentially washed with distilled water, 0.2 M sodium carbonate, and finally distilled water. The obtained biomass was re-suspended in 8 mL distilled water and immediately used in biosorption experiment.

2.3.2. Formaldehyde treatment

According to Kapoor and Viraraghavan's (1997), 80 mg of biomass was contacted with 1.6 mL of formaldehyde (HCHO, Shanghai experimental reagent Ltd, China) and 3.2 mL of formic acid (HCOOH, Tianjin guangfu fine chemical research institute, China). The reaction mixture was shaken on an agitator at 125 rpm for 6 h. This treatment was expected to result in the methylation of amines (Loudon, 1984). The general reaction occurs as follows:

$$RCH_2NH_2 \xrightarrow{HCOH,HCOOH} RCH_2N(CH_3)_2 + CO_2 + H_2O \quad (2)$$

The biomass obtained was washed and re-suspended as the method used in methanol treatment. To conveniently measure the same amount of each biomass used in uranium sorption, 80 mg of raw biomass was also re-suspended in 8 mL distilled water.

2.4. Biosorption studies

Sorption experiments were carried out using raw and chemically modified biomasses to investigate the effect of chemical modifications on biosorption capability. 0.4 mL (80mg/8 mL ° 0.4 mL = 4 mg) of raw and chemically treated cell suspensions were sampled, added to polypropylene centrifuge tubes, and centrifuged at 5000 rpm for 5 min to separate the biomass from water. Then 4 mL uranium solutions of known concentration were mixed with the biomass and shaken on a rotary shaker at 150 rpm for 30 min (Bai et al., 2009) at room temperature (25 °C). The mixtures were centrifuged and the supernatant was assayed spectrophotometrically for uranium with Arsenazo III (Khan et al., 2006; Genç et al., 2003). The amount of adsorbed uranium per unit yeast biomass (mg metal ions/g dry yeast biomass) was calculated using the following expression,

$$q = \frac{(C_0 - C_e)V}{m} \quad (3)$$

where q is the amount of uranium adsorbed onto the unit amount of the biomass (mg/g), $C_0$is the initial uranium concentration (mg/L), $C_e$is the equilibrium or final uranium concentration (mg/L), V is the volume of the aqueous phase (L) and m is the amount of the biomass (g).

Previous study revealed that initial pH of 6.0 is the most favorable condition for uranium sorption by *R. glutinis* and the precipitation of uranyl hydroxides can be avoided at this pH (Bai et al., 2009). Therefore, all the sorption experiments were done at initial pH 6.0. Control experiments without biomass were carried out to determine the degree of uranium removal by plastic tube.

Duplicate experiments were performed and mean values were used in the analysis of data.

2.5. Analytical methods

Uranium concentration was determined on UV-1801 spectrophotometer (Beijing rayleigh analytical instrument Co., Ltd, China). 100 μL uranium solution sample, 500 μL 0.3 M HCl and 300 μL 0.05% Arsenazo III aqueous solution were added to a glass flask, the final volume of solution was filled up to 10 mL by distilled water, then the absorbance of the mixed solution was analysis at 650 nm. Mixed solution prepared in the same way but without uranium was used as reference. Uranium concentration was calculated from the calibration curve. The detection limits and sensitivity of this method were 7.02 mg/L and 1.01 mg/L, respectively.

The infrared (IR) spectra of raw, chemically modified and uranium bounded *R. glutinis* cells were obtained using Fourier transform infrared spectrometer (PelkinElmer Spectrum GX FTIR, USA). Sample dicks were made by mixing 5 mg of dry biomass with 150 mg of KBr (Sinopharm chemical reagent Co., Ltd, shanghai, China) and pressed them into tablet form. All infrared spectra were recorded over 4000–400 cm^{-1} region with a resolution of 0.2 cm^{-1}.

3. Results and discussion

Fig. 1 shows the FTIR spectra of raw and chemically modified cells before uranium sorption. As shown in Fig. 1, the cellular IR spectra were very complex and obvious adsorptions were observed covering the total range of the wave number. Trough assignment was made basing on the references (Drake et al., 1996; Kiefer et al., 1997; Mantsch and Chapman, 1995) and listed in Table 1.

The effects of chemical treatment on the functional groups could be evaluated from the difference among the spectrums. The curve b in Fig. 1 shows the IR spectrum of *R. glutinis* treated with methanol. By comparing curve b with curve a, a new shoulder at wave number of 1732 cm^{-1}, which was due to the stretching vibration of ester carbonyl, was observed. And the peak at wave number of 1068 cm^{-1} in raw biomass (Fig. 1, curve a) was assigned to the C–O