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Immobilization of chlorine dioxide modified cells for uranium absorption

Shengbin He [a], Binbiao Ruan [b], Yueping Zheng [a], Xiaobin Zhou [a], Xiaoping Xu [a, *]

[a] Research Institute of Photocatalysis, College of Chemistry, Fuzhou University, Fuzhou 350108, China
[b] China Nuclear Power Engineering co.,LTD (FuQing), China

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ABSTRACT

There has been a trend towards the use of microorganisms to recover metals from industrial wastewater, for which various methods have been reported to be used to improve microorganism adsorption characteristics such as absorption capacity, tolerance and reusability. In present study, chlorine dioxide(ClO_2), a high-efficiency, low toxicity and environment-benign disinfectant, was first reported to be used for microorganism surface modification. The chlorine dioxide modified cells demonstrated a 10.1% higher uranium adsorption capacity than control ones. FTIR analysis indicated that several cell surface groups are involved in the uranium adsorption and cell surface modification. The modified cells were further immobilized on a carboxymethylcellulose(CMC) matrix to improve their reusability. The cell-immobilized adsorbent could be employed either in a high concentration system to move vast UO_2^{2+} ions or in a low concentration system to purify UO_2^{2+} contaminated water thoroughly, and could be repeatedly used in multiple adsorption-desorption cycles with about 90% adsorption capacity maintained after seven cycles.

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1. Introduction

Heavy metal pollution is one of the most important environmental problems today. Various industries produce and discharge wastes containing different heavy metals into the environment, such as mining and smelting of metalliferous ores, energy and fuel production, aerospace and atomic energy etc (Wang and Chen, 2009). Thus, metal as a kind of resource is becoming short and also brings about serious environmental pollution, threatening human health and the ecosystem.

Among the heavy metals, uranium, which exist commonly in UO_2^{2+} form in 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, and abnormal and slow embryonic development (Kalin et al., 2004; Domingo, 2001). Therefore, seeking 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 et al., 2009). Biosorption, as a biological method, has become a favorable method of choice due to its good performance, low cost and large available quantities (Vijayaraghavan and Yun, 2008; Farooq et al., 2010).

Many living microorganisms have been reported to have the capability of accumulating metallic elements. However, further research has revealed that pretreated inactive/dead microbial biomass could improve their absorption characteristics by surface group modification (Vijavaraghavan and Yun, 2008). A number of methods have been employed for cell wall modification of microbial cells in order to enhance the metal binding capacity of biomass and to elucidate the mechanism of biosorption. These methods include physical treatments like heating, freezing and lyophilization, and chemical treatments like detergents, alkali and methanol (Suh and Kim, 2000; Sar et al., 1999; Rincon et al., 2005; Göksungur et al., 2005). And more advanced applications are the immobilization of whole cells in a suitable support matrix to improve its features, such as increasing reusability and utilization efficiency, decreasing inhibitions (Choudhary et al., 2011; Nakajima et al., 1982; Genc et al., 2003). In the present study, chlorine dioxide, a high-efficacy disinfectant, was first reported to have a positive effect on UO_2^{2+} biosorption by *Bacillus*.sp.B26 cells. An effective immobilization method for whole cells of *Bacillus*.sp.B26 was determined, thus laying the foundations for its further application.

* Corresponding author. Tel.: +86 13067237982.
E-mail addresses: comhsb@163.com (S. He), xu@fzu.edu.cn (X. Xu).

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2. Materials and method

2.1. Microorganism and culture media

Bacillus sp.FB12 was isolated from the vicinity of a power plant previously (Xu et al., 2013).

Cell growth medium and condition are as described by Xu et al, (2013), with medium(g/L) compose of maltose 10, casein peptone 10, beef extract 6, $NH_4H_2PO_4$ 4, K_2HPO_4 1.2, KH_2PO_4 2.5, $MgSO_4 \cdot 7H_2O$ 0.15, $MnSO_4$ 0.012, $ZnSO_4$ 0.008, pH 7.2, and growth at 32 °C with an agitation speed of 180 r/min in a rotating shaker.

2.2. Pretreatment

The cells were harvested at a suitable time by centrifugation at 10,000 ×*g* for 10 min at 4 °C and washed three times with distilled water. The cell sediments of four groups were treated by NaOH(0.1M/L), methanol, chlorine dioxide(ClO_2,150 mg/L) and heat(96 °C) respectively for 10min, and then washed with distilled water.

2.3. FTIR analysis

Infrared spectra of the pure/uranium-beating and ClO_2 treated biomass were recorded in the range 400–4000 cm^{-1} on a Nicolet FTIR spectrophotometer using KBr pallets.

2.4. Absorption experiment

The wet biomass (0.01 g or equal cell-immobilized matrix) were added to 30 ml solutions containing UO_2^{2+}(300 mg/L). The suspensions were incubated at 25 °C, ph 6.0 on a shaker at 180 rpm and sampled periodically. The samples were centrifuged at 10,000 ×*g* for 10 min to obtain the supernatant which was then filtered through a 0.45 micron cellulose membrane and the residual uranium was detected by micro-uranium analyzer (MUA) with detection range of 3.0×10^{-11} ~ 2.0×10^{-5} g/mL and relative standard deviation (RSD) of less than or equal to 8%. The MUA was brought from Beijing feather nylon technology co., LTD and is possessed by China Nuclear Power Engineering co., LTD (FuQing). The MUA is known to work something like this. In a solution, uranyl ion (UO_2^{2+}) can react specially with fluorescence enhancer (J-22) and form stable complex, which can easily emit strong fluorescence (at 500, 522,546 nm) under ultraviolet pulse irradiation. The fluorescence intensity is proportional with the content of uranyl ion. All the absorption experiments were performed under condition of 25 °C and ph 6.0 due to its economical and similar to natural environment.

2.5. Preparation of immobilized whole cells

Wet biomass (0.05 g) was added to 10 ml distilled water and preserved at 4 °C in the refrigerator for subsequent experiments.

Agar immobilization. The agar(1 g) was dissolved in 40 ml distilled water at 96 °C, and then cooled to 40 °C and added 10 ml above bacterium suspension. The mixtures were dropped into a layer of vegetable oil (Mamo and Gessesse, 2000). Hard beads were washed with distilled water and dried in drying closet.

Sodium alginate immobilization. 10 ml bacterium suspension was mixed homogenously with 40 ml distilled water containing 1 g alginate. The mixture was added into 2% calcium solution by peristaltic pump and left to harden for 2 h (Cabrita et al., 2013). The alginate beads were washed with distilled water and dried in a drying closet before use.

Acrylamide immobilization. To a sterile acrylamide monomer solution(40 ml distilled water containing 7.12 g acrylamide and 0.38 g bis-acrylamide) in 0.05 M phosphate buffer(pH 6.5), 10 mg of ammonium persulphate was added. Ten microliters of tetramethyl ethylene diamine was then added followed by the addition of 10 ml cell suspension and the solution was mixed and poured into sterile plates with about 2 mm in thickness (Hemachander et al., 2001). The contents were allowed to polymerise and cut into blocks (3 mm × 3 mm × 2 mm), and then washed and dried.

Carboxymethylcellulose(CMC) immobilization. Na-CMC soution was prepared in distilled water (40 ml water containing 0.5 g Na-CMC) and then mixed with 10 ml cell suspension. The mixture was introduced into a solution containing 0.2 M $FeCl_3$ through a nozzle using a peristaltic pump, and the solution was stirred to prevent aggregation of the CMC beads (Bayramoğlu et al., 2003). The cell-immobilized beads (about 2 mm in diameter) were cured in this solution for 60min and then washed with distilled water and dried.

2.6. Desorption and reusability experiments

The *Bacillus* sp.UV32 cells with adsorbed UO_2^{2+} in them were harvested by centrifugation and incubated with 30 ml solution containing 1 mol/L Na_2CO_3 or 0.1 mol/L HCl at 25 °C with an agitation speed of 180 r min for 150 min. Two groups were desorbed by Na_2CO_3 and HCl(or HCl and Na_2CO_3) orderly. The supernatant obtained by centrifugation was subjected to determination of UO_2^{2+} content to calculate the desorption rates of various desorption reagents by the following equation: desorption rate (%) = (desorption content/sorption content) * 100%. In order to determine the reusability of the immobilized and dried cell preparations, consecutive adsorption-desorption cycles were repeated seven times by using the same biosorbent.

3. Result and discussion

3.1. Effect of various treatment methods on adsorption time course

Time course for adsorption of UO_2^{2+} by ClO_2 pretreated *Bacillus*.sp.B26 cells and untreated ones are shown in Fig. 1. In all cases, a large amount of UO_2^{2+} was absorbed rapidly by cells within 30min. The absorption quantities reach to maximum at about 150min with absorption quantity of about 386 mg/L for control group, heated and alkali treated groups, 370 mg/L for methanol treated group, and 425 mg/L for ClO_2 treated group. Therefore the ClO_2 modified *Bacillus*.sp.B26 cells demonstrated a 10.1% higher UO_2^{2+} ions adsorption capacity than the control group.

As the biosorption process involves mainly in cell surface sequestration, the modification of cell wall can greatly alter the

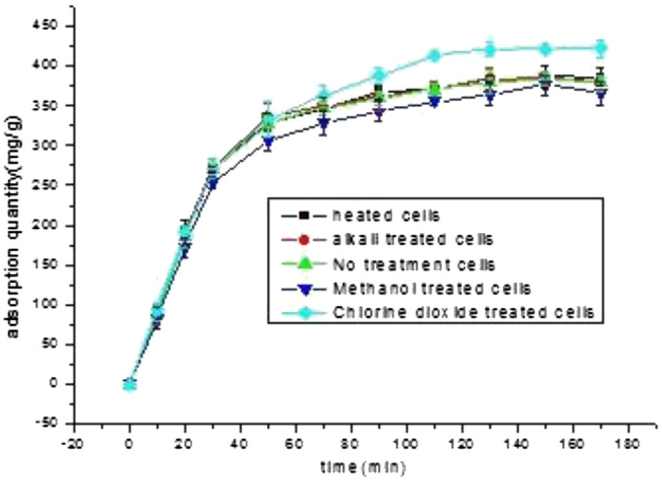

Fig. 1. Effect of different reagents treatment on UO_2^{2+} adsorption time courses.

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