Journal of Environmental Management 187 (2017) 490–496

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

# Tolerance and bioaccumulation of U(VI) by *Bacillus mojavensis* and its solid phase preconcentration by *Bacillus mojavensis* immobilized multiwalled carbon nanotube

Sadin Özdemir [a, *], M. Kadir Oduncu [a], Ersin Kilinc [b], Mustafa Soylak [c]

[a] Department of Biology, Faculty of Art and Science, Siirt University, 56100 Siirt, Turkey
[b] Health Services Vocational High School, Medical Marketing and Promotion Programme, Mardin Artuklu University, 47200 Mardin, Turkey
[c] Department of Chemistry, Faculty of Sciences, Erciyes University, TR-38039 Kayseri, Turkey

ARTICLE INFO

*Article history:*
Received 26 April 2016
Received in revised form
5 July 2016
Accepted 2 November 2016
Available online 14 November 2016

*Keywords:*
*Bacillus mojavensis*
Resistance and bioaccumulation
Solid phase extraction preconcentration
U(VI)

ABSTRACT

In this study, uranium(VI) tolerance and bioaccumulation were investigated by using thermo -tolerant *Bacillus mojavensis*. The level of U(VI) was measured by UV–VIS spectrophotometry. The minimum inhibition concentration (MIC) value of U(VI) was experimented. Bacterial growth was not affected in the presence of 1.0 and 2.5 mg/L U(VI) at 36 h and the growth was partially affected in the presence of 5 mg/L U(VI) at 24 h. What was obtained from this study is that there was diversity in the various periods of the growth phases of metal bioaccumulation capacity, which was shown by *B. mojavensis*. The maximum bioaccumulation capacities were found to be 12.8, 22.7, and 48.2 mg/g dried bacteria, at 24th hours at concentration of 1.0, 2.5 and 5 mg/L U(VI), respectively. In addition to these, U(VI) has been preconcentrated on *B. mojavensis* immobilized MWCNT. Several factors such as pH, flow rate of solution, amount of biosorbent and support materials, eluent type, concentration and volume, the matrix interference effect on retention have been studied, and extraction conditions were optimized. Preconcentration factor was achieved as 60. Under the optimized conditions, the limit of detection (LOD) and quantification (LOQ) were calculated as 0.74 and 2.47 μg/L. The biosorption capacity of immobilized *B. mojavensis* was calculated for U(VI) as 25.8 mg/g. The results demonstrated that the immobilized biosorbent column could be reused at least 30 cycles of biosorption and desorption with the higher than 95% recovery. FT-IR and SEM analysis were performed to understand the surface properties of *B. mojavensis*.



## 1. Introduction

There has been a significant interest about the removal of pollutant such as radionuclides, heavy metals, drugs, dyes, and pesticides from environmental polluted areas and samples in recent years (Aksu, 2005). The biological processes of biosorption and bioaccumulation have been indicated to have well potential to replace traditional processes for the recovery of various pollutants. Some confusion has prevailed in the literature about the use of the terms "biosorption" and "bioaccumulation" based on the state of the biosorbent. The bioaccumulation is described as the phenomenon of living biosorbents, while biosorption mechanisms are based upon the use of non-living biosorbent (Vijayaraghavan and Yun, 2008). Biosorption has been described as the adsorption features of the biological substances including non-living organism to bind the pollutants by various mechanisms such as complexation, electrostatic attraction, covalent binding, ion-exchange, adsorption, and Van der Waal's forces (Breierova et al., 2002; Mack et al., 2007). Heavy metal recoveries by living cells usually take place rapid initial surface binding followed by a second, slower stage of transport across the cell membrane into the cell. This method is named 'bioaccumulation' which is employed to demonstrate the concomitance of biosorptive and metabolism dependent mechanisms, in contrast to 'biosorption', which does not require metabolic contribution and can be influenced also by dead biosorbent (Vecchio et al., 1998). The using of the biosorbent for the removal of the pollutants ensures low-cost, eco-friendly, speed, and effective process. For this purpose, plant, yeast, fungus, and bacteria have

* Corresponding author.
*E-mail address:* sadin77@hotmail.com (S. Özdemir).

http://dx.doi.org/10.1016/j.jenvman.2016.11.004
0301-4797/

been used as biosorbent (Vijayaraghavan and Yun, 2008).

Uranium is not only a source in nuclear energy applications (Dolatyari et al., 2016), but also a potential environmental contaminant with longtime toxic effects primarily caused by its chemical and/or biological toxicity (Song et al., 2012). Uranium, due to the most significant nuclear energy sources, its separation, preconcentration and retention from nuclear industrial waste water, seawater, ore water and other aqueous mediums have major importance in either efficient application of resources or environmental safety (Li et al., 2015).

For its importance, the determination of uranium in real samples is required by an effective process. Recently a great deal of study has been devoted to solid phase extraction (SPE) as an enrichment method (Saeed et al., 2011). Recently SPE is used to enhance the selectivity and sensibility of the process because it delivers for selective binding of metal ions to a solid support where it will be accumulated and thereafter desorbed with a few volume of solvent. Solid phase extraction (SPE) based on the utilization of various biosorbent such as alga, fungus, bacteria, is the preferred process for the enrichments of analytes at very low concentrations by investigators. Nanoparticles, Amberlite XAD resins, silica etc. have been widely used for this purpose as support materials (Ozdemir et al., 2016). The primary superiorities of bacteria based solid phase extraction processes such as selective and sensitive process, decrease the consumption of analysis period and quantity of chemicals, low LOD, and request to use of reusable sorbents for the enrichments of analytes were evaluated in recent review with details (Ozdemir et al., 2013). On the other hand, different analytical devices such as spectrophotometry, liquid scintillation detection, inductively coupled plasma atomic emission spectrometry, fluorometry, inductively coupled plasma-mass spectrometry (ICP-MS), gamma-ray spectrometry, ion chromatography, adsorptive stripping voltammetry (ASV) and capillary zone electrophores is used for determination of trace concentration uranium (Dutta et al., 2008; Saeed et al., 2013). Among these devices spectrophotometry is cheaper than others.

Biosorption of metal ions by using various biosorbents have been widely investigated. However, there was not enough study on solid phase extraction of U(VI) by using bacteria immobilized sorbent according to in our literature survey. On the other hand, most of the investigations focused on the bioaccumulation and resistance mechanisms in mesophilic bacteria. But, a number of investigations have been examined about U(VI) resistance and bioaccumulation on thermo-tolerant bacteria. The aims of this study were to investigate the U(VI) resistance and bioaccumulation and develop a preconcentration methods for U(VI) before its detection by UV–VIS spectrophotometry.

## 2. Materials and method

### 2.1. Cultured of Bacillus mojavensis

*Bacillus mojavensis* were grown in 1000 mL glass bottle containing 250 mL Nutrient Broth (NB) media. The pH of fermentation medium were adjusted with 0.1 mol/L HCl or NaOH and then autoclaved. After autoclaved, each glass bottle was inoculated with 2.5 mL of $3.6 \times 10^7$ cell suspension and cultured on shaker at 35 °C and 120 rpm.

### 2.2. Determination of minimum inhibitory concentration (MIC) of U(IV)

The uranium-tolerance of thermo-tolerant *B. mojavensis* was experimented by the minimum inhibitory concentration (MIC) process. *B. mojavensis* were inoculated to NB and Nutrient Agar which contains various concentrations of U(VI) (prepared from its chloride salt in sterile distilled water). After inoculation, the agar plates were incubated for 24 h at 35 °C. The lowest concentration of the uranium, which inhibited *B. mojavensis* growth, was determined as the MIC of the uranium against the bacteria studied.

### 2.3. Influence of U(VI) concentration on B. mojavensis growth and bioaccumulation

To investigate the effect of U(VI) concentrations on bacterial growth, *B. mojavensis* was inoculated into 250 mL of culture media containing U(VI) at various concentrations. The bacteria were incubated for 72 h. Growth of *B. mojavensis* was measured periodically (12, 24, 36, 48 and 72 h) by UV–VIS at 540 nm.

*B. mojavensis* were cultured in 250 mL of medium containing different concentration of uranium in 1000 mL erlenmeyer flasks on a shaker at 35 °C and 120 rpm. Samples of mediums were collected at different time (12, 24, 36, 48, 72 and 96 h) and centrifuged at 8 min at 10,000 rpm. Pellets and upper solutions were dried 12 h at 80 °C and pellets were then weighed. Pellets and upper solutions were digested by concentrated $HNO_3$ and were separately used to detect the bioaccumulated U(VI) concentration by UV-VIS. Bioaccumulation amounts were calculated as the difference between the initial U(VI) concentration and the one in the sample. All experiments were tested at least twice.

### 2.4. Preparation and packing of solid phase extraction (SPE) column

A 150-mg amount of dried and dead *B. mojavensis* was mixed with 100 mg multi-walled carbon nanotubes (MWCNT), respectively. The SPE columns were then prepared by a method from our previous investigation (Ozdemir and Kilinc, 2012). One hundred and 50 mg of MWCNT immobilized with *B. mojavensis* were separately wetted with 4 mL of distilled water for packing of (SPE) column. The mixtures were separately transferred polyethylene columns. Before use, HCl solution (1 mol/L) and distilled water were passed through the columns in order to condition and wash them. Then, the columns were preconditioned by passing buffer solution.

### 2.5. General biosorption studies

A 50 mL mixtures of U(VI), Th (IV), La (III) and Ce (IV) at 1.0 mg/L were taken and the pHs were adjusted with HCl and $NH_3$ to 6.0. Then, they were passed through the column with peristaltic pump. The retained metal ions were then eluated from the solid phase with a 5.0 mL of 1.0 mol/L HCl. The concentrations of the U(VI), Th (IV), La (III) and Ce (IV) in the eluate were determined by ICP-OES (Inductively coupled plasma-optical emission spectrometry). The highest preconcentration was achieved for U(VI) as 94.5% (67.5% for Th (IV), 49.0% for La (III) and 59.3% for Ce (IV)). So, further studies focused on optimization of experimental conditions for U(VI) preconcentration. Perkin-Elmer Spectrum 400 Fourier Transform Infra-Red spectrometer (Waltham, MA, USA) was used for FT-IR records. Scanning electron microscope (SEM) images were obtained on a LEO 440 SEM with an accelerating voltage of 20 kV.

### 2.6. UV–VIS spectrophotometric analysis of U(VI)

After the desorption of the biosorbed U(VI), the desorption solution was diluted to desire times. A 0.5 mL of diluted U(VI) solution was mixed with 0.5 mL HCl (2 mol/L), 0.125 mL Arsenazo III in a test tube. And then the final volume was completed to 2.5 mL with distilled water. It was centrifuged to provide the complete mixture of the ingredient at 1000 rpm and 30 min. Then, the absorbance of