



Research article

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

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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*.

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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

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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, pre-concentration 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 electrophoresis 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 3.6×10^7 cell suspension and cultured on shaker at 35 °C and 120 rpm.

2.2. Determination of minimum inhibitory concentration (MIC) of U(VI)

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 HNO₃ 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 NH₃ 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

the U-Arsenazo complex was measured at 651 nm. The blank without U(VI) was prepared and analyzed with same process pointed out above. The equation between the concentration of the U(VI) as mg/L and absorbance of the complex was $y = 0.0808x + 0.00136$ with the correlation coefficient as $r^2 = 0.9986$ (Ozdemir and Kilinc, 2012).

2.7. Sample preparation

NCS ZC-73014 (tea sample), NWTM-15 (fortified water) and CASS-4 (nearshore sea water) as certified reference samples were applied the developed SPE method to validation. The sample was digested in analytical microwave oven by a procedure from our previous studies (Ozdemir and Kilinc, 2012). 100.0 mL of certified water samples were directly applied to SPE procedure after pH adjustments. Tap waters from Mardin, Siirt and Diyarbakir were sampled in 5.0 L bottles after flushing 5.0 min. 10 L of Van Lake water was filtered through a cellulose membrane filter of 0.45 μm pore size (Millipore). After filtering, a 100 mL of sample was adjusted to the optimum pH then, the sample was passed through the column.

3. Results and discussion

3.1. Minimum inhibitory concentration (MIC)

The results of minimum inhibitory concentration (MIC) rates were acquired after 48 h incubation in both liquid and solid mediums. After 48 h incubation, it was determined that *B. mojavensis* was more sensitive in the liquid culture than in solid culture.

The MIC values of U(VI) were found to be 12.5 mg/L and 85 mg/L for liquid culture and solid culture, respectively. According to these results, *B. mojavensis* was almost 7 times more resistance in solid culture than liquid culture. Similar results reported by Vodnik et al. (1998), Hassen et al. (1998) and Ozdemir et al. (2012). This situation arises the thought that the diffusion rates of metals in solid and liquid culture can be different and the metals solubility in liquid culture is higher than in solid culture (Ozdemir et al., 2012).

3.2. Influence of U(IV) concentration on *B. mojavensis* growth and bioaccumulation

Fig. 1a demonstrates that the influence of various U(VI) concentrations on growth of *B. mojavensis*. It can obviously be shown that growth was not influenced at concentration of 1 mg/L U(VI) during 72 h. *B. mojavensis* growth was partially affected at 2.5 mg/L U(VI) concentration at 12th hours. In presence of 2.5 mg/L U(IV), *B. mojavensis* growth was inhibited by 6.2%, 7.5%, 12.4% and 9.6% at the 24th, 36th, 48th and 72th hours, respectively. When *B. mojavensis* were cultured in media containing 5 mg/L U(VI) concentration, growth was partially affected during 72 h. In the presence of 5 mg/L and 2.5 mg/L U(VI), the growth of *B. mojavensis* was inhibited 21.5%, 17.3%, 22.7%, 27.1% and 24.2% at 12, 24, 36, 48 and 72th, respectively, when compared with the control.

The effect of different U(VI) concentrations on bioaccumulation capacity of *B. mojavensis* are represented in Fig. 1b. Bioaccumulation capacity of *B. mojavensis* raised at 1, 2.5 and 5 mg/L U(VI) from the growth period of 12th to 24th. When bacteria was grown in culture containing 2.5 mg/L U(VI) at 36 and 72 h, bioaccumulation capacity was found to be 19.1 and 21.1 mg/g dried bacteria, respectively. As depicted from Fig. 1b, the highest bioaccumulation capacity amounts performed during 96 h incubation by *B. mojavensis* for U(IV) were determined as 12.8, 22.7 and 48.2 mg/g dried bacteria, at 24th hours at concentration of 1, 2.5 and 5 mg/L U(VI), respectively. In this investigation, bioaccumulation capacity of U(VI)

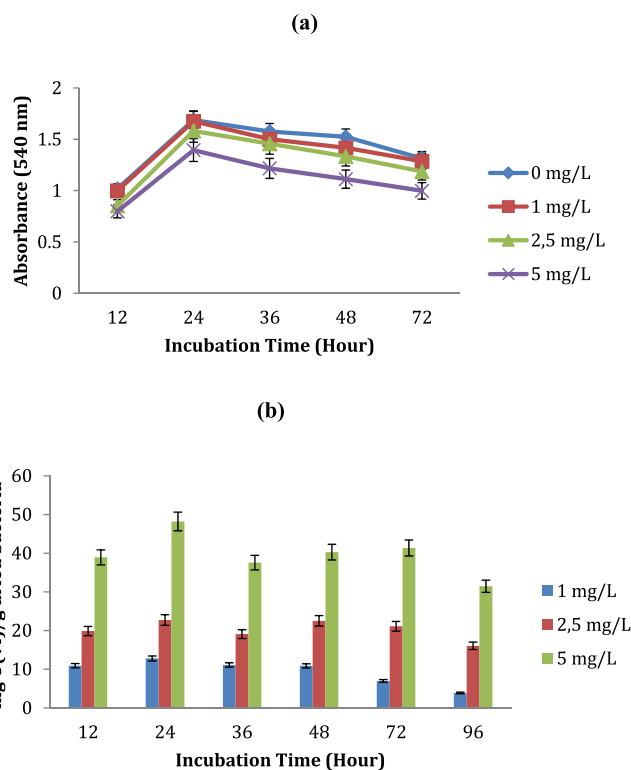


Fig. 1. Influence of U(VI) concentration on *B. mojavensis* growth (a) and bioaccumulation (b).

exhibited difference in various periods of *B. mojavensis* growth. Some studies such as Macaskie and Dean (1984) and Volesky et al. (1993) similarly indicated that bacterial cells displayed difference in various cycles of growth of their metal bioaccumulation capacity. The difference of bioaccumulation capacity of metal ions by bacteria can be controlled with active mechanism and because the bacteria have a live and active metabolism, resistance and bio-sorption mechanisms may perform an act together in metal bioaccumulation (Ozdemir et al., 2012). In addition, the bioaccumulation levels of uranium showed varieties at different incubation time. The uranium active efflux system can be played role in this study as reported by Theodorakopoulos et al. (2015).

3.3. Solid phase extraction of U(IV)

B. mojavensis loaded MWCNT was employed as solid phase sorbent for solid phase extraction of U(VI) before its determination by UV–VIS. Surface functional groups of *B. mojavensis* loaded MWCNT with and without U(VI) were investigated by comparing their FT-IR spectra (Fig. 2). They were recorded on diamond ATR by absorbance mode. Peaks at approximately 3000 cm^{-1} and 1250 cm^{-1} in Fig. 2a were attributed to CH_x groups and D bands of MWCNT (Kouklin et al., 2004). The other peaks arised from surface functionalities of *B. mojavensis*. There was no differentiation after U(VI) biosorbed on its surface (Fig. 2b). However, shifting on peaks was observed as approximately 10 cm^{-1} after it was interacted with U(VI). The $\nu_{\text{as}}(\text{U}=\text{O})$ band of uranyl absorbs in the infrared at circa 900 cm^{-1} , and its frequency depends on the nature of the uranyl complex. Surface macro structure of *B. mojavensis* loaded MWCNT was examined by SEM. The results were presented in Fig. 3. It was easy to see the dead bacterial structures on MWCNT in Fig. 3 a, b, c, f, g and h. From Fig. 3 d, e, i and, j it could be concluded that

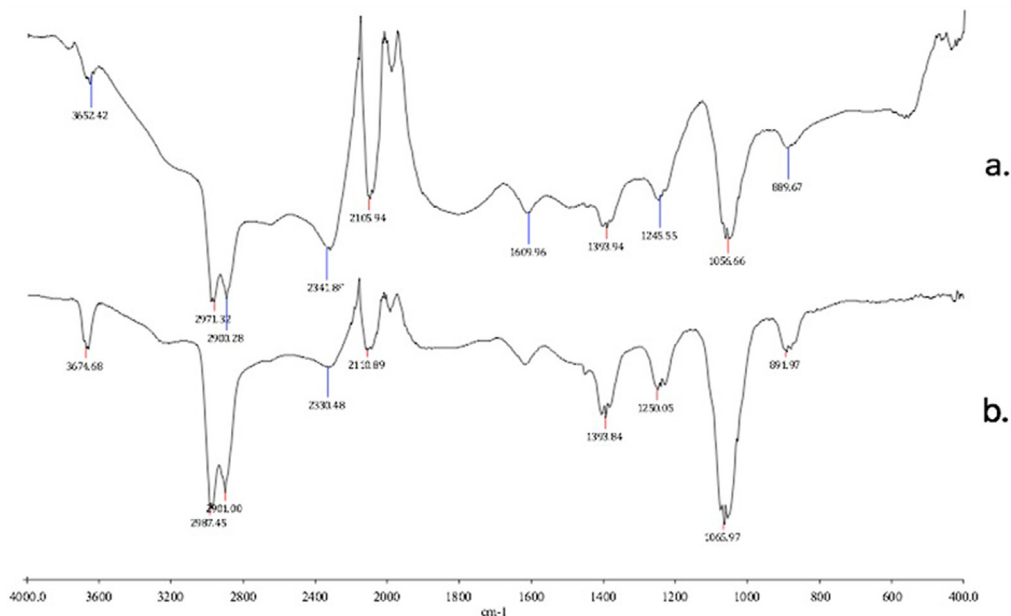


Fig. 2. FT-IR spectral comparison of *B. mojavensis* loaded MWCNT without (a) and with (b) U(VI).

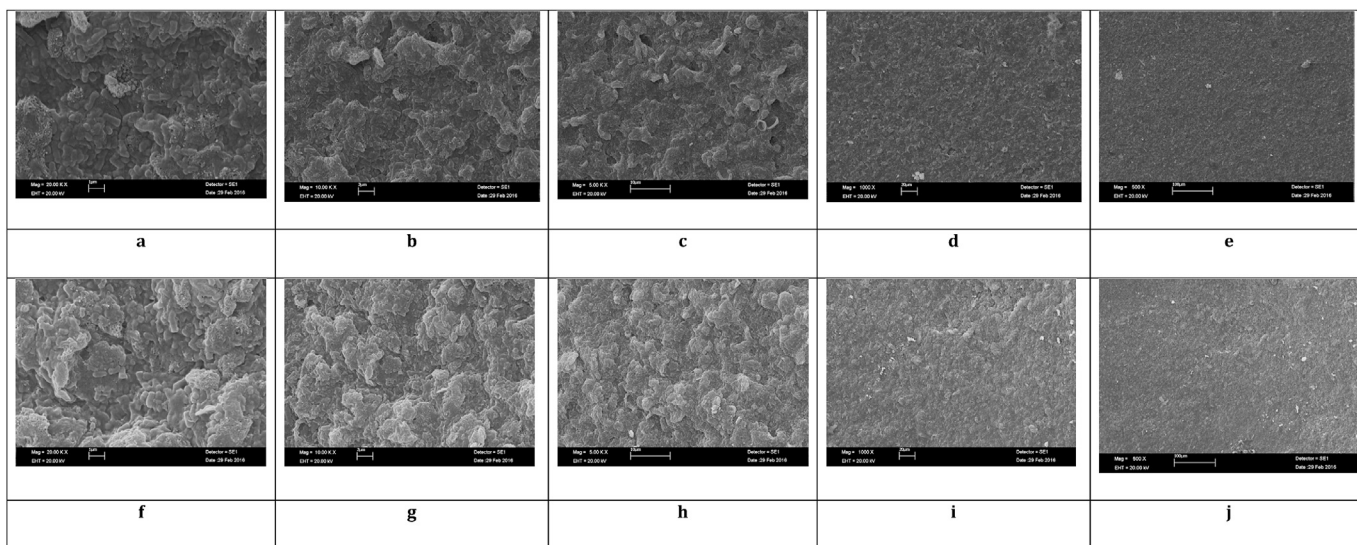


Fig. 3. Investigation of surface macrostructure of *B. mojavensis* loaded MWCNT with (a, b, c, d, and e) and without (e, f, g, h, and i) U(VI) by SEM at different resolutions (1, 2, 10, 20 and 100 μm respectively).

homogenous surface structure was achieved for *B. mojavensis* loaded MWCNT.

3.4. Influence of solution pH on solid phase extraction of U(IV)

The solution pH is one of the primary significant parameters for quantitative retention of analytes in solid phase extraction processes. (Ozdemir et al., 2010). Therefore, the influence of solution pH on the biosorption of U(VI) on the immobilized *B. mojavensis* was examined in the pH range of 2.0–9.0. The solution pH was adjusted using HCl and HNO₃, and then the general SPE method was applied. The influence of solution pH on the recoveries of U(VI) is depicted in Fig. 4. If the solution pH was higher than 3.0, U(VI) was quantitatively retained with high precision. The experimental results of pH indicated that quantitative biosorption (>95%) of U(VI)

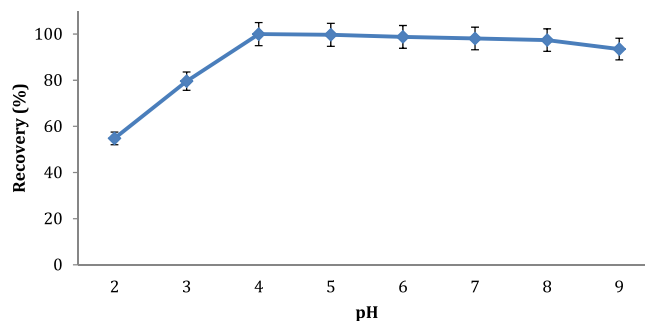


Fig. 4. Influence of pH on solid phase extraction of U(VI).

was obtained with the pH variety 4.0–7.0. Therefore, for following studies, pH 4.0 was selected as solution pH.

3.5. Influence of flow rate on solid phase extraction of U(VI)

The influence of the solution flow rate during the SPE process was investigated in the range of 1–6 mL/min. As seen in Fig. 5, the recovery of U(VI) onto *B. mojavensis* loaded MWCNT was not significantly reduced up to flow rate of 2 mL/min. When the solution flow rate increased from 3 mL/min to 5 mL/min, recovery of U(VI) onto SPE column was decreased from 94.3% to 75.9%. After 2 mL/min, the recovery was not quantitative because of insufficient contact between U(VI) and *B. mojavensis* loaded MWCNT (Anthemidis et al., 2001). The solution flow rate of 2 mL/min was chosen as sample and eluent flow rate for further studies.

3.6. Influence of quantity of *B. mojavensis* and MWCNT

The influence of quantity of *B. mojavensis* at constant rate of MWCNT on the biosorption of U(VI) was tested in the range of 50–300 mg (MWCNT as 100 mg). As demonstrated in Fig. 6a, the biosorption was quantitative with rising quantities of *B. mojavensis* up to 150 mg, and the extra addition lead to reduces on the U(VI) recoveries from the SPE column, presumably because of interaction of binding sites on the immobilized biosorbent. For this result, 150 mg of *B. mojavensis* was selected for following studies. The influences of the MWCNT quantity on the recovery of U(VI) on *B. mojavensis* was also studied. The results are shown Fig. 6b. The recoveries of U(VI) were quantitative (>99%), until the addition of 100 mg MWCNT. After that, the recovery rates did not changed, probably because of the saturation of *B. mojavensis*. In following studies, 100 mg MWCNT was used as support matrix.

3.7. The influence of HNO₃ and HCl volume and concentration on recovery of U(VI)

Selecting suitable elution solution is too significant because of selectivity, performance and compatibility. The influences of various concentration and volume of nitric and hydrochloric acid were studied to determine suitable eluent for desorption of U(VI) from immobilized *B. mojavensis* onto MWCNT. According to Table 1, when using 0.5 mol/L 5 mL HNO₃ and HCl, the recoveries of U(VI) was found as $92.8 \pm 0.7\%$ and $94.2 \pm 0.8\%$, respectively. The maximum recovery extent was observed for 5 mL of eluent volume with 1 mol/L HCl. So, 5 mL of 1 mol/L HCl was selected as proper eluent for next studies.

3.8. Matrix effect

Analytical enrichment processes for trace elements in the high

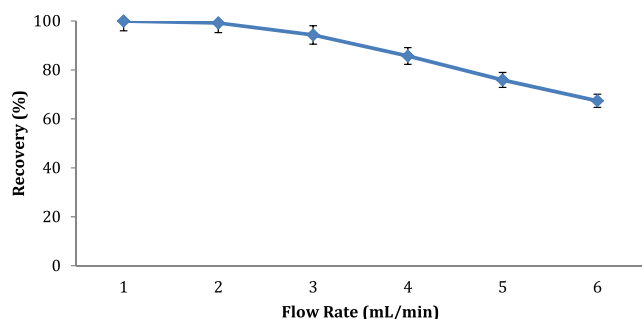


Fig. 5. Influence of flow rate on solid phase extraction of U(VI).

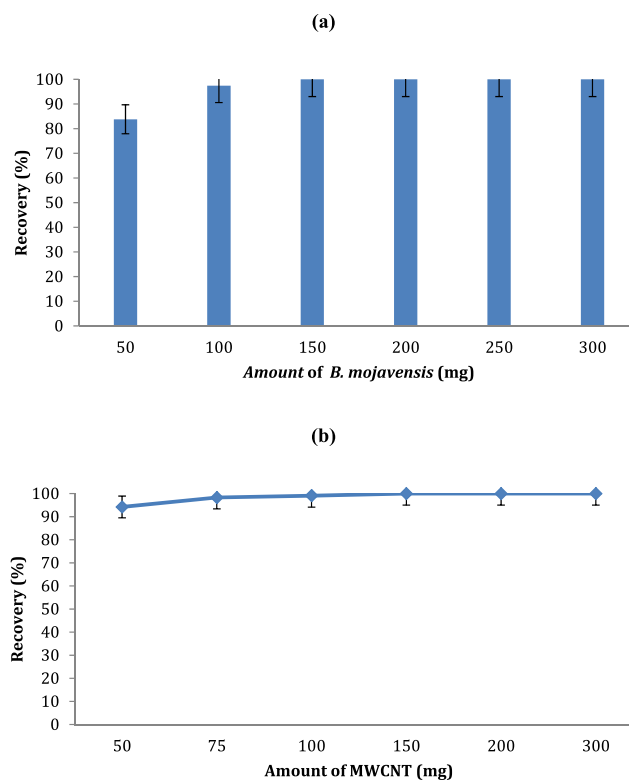


Fig. 6. Influence of amounts of *B. mojavensis* (a) and MWCNT (b).

Table 1
Influence of volumes and concentrations of HNO₃ and HCl on recovery of U(VI).

Eluent type	Volume (mL)	Concentration (mol L ⁻¹)	Recovery (%)
			MWCNT
HCl	3	0.5	88.9 ± 1.1
	5	0.5	94.2 ± 0.8
	3	1	93.9 ± 1.2
	5	1	100 ± 0.9
HNO ₃	3	0.5	85.9 ± 0.6
	5	0.5	92.8 ± 0.7
	3	1	93.2 ± 0.9
	5	1	96.1 ± 1.3

salt concentration can be extremely influenced by the matrix constituents of the sample. This is described as matrix effect (Saracoglu et al., 2002). The effects of some foreign ions on the solid

Table 2
Interference studies.

Interferic ion	Interferic ion to U(VI) ratio	Recovery ^a (%)
		U(VI)
Na ⁺	5000	97.9 ± 0.5
K ⁺	5000	98.1 ± 0.7
Ca ⁺²	200	99.4 ± 1.3
Mg ⁺²	100	98.2 ± 0.8
Fe ⁺²	100	96.4 ± 0.5
Al ⁺³	25	97.2 ± 1.2
Cd ⁺²	5	98.6 ± 1.3
Co ⁺²	5	98.3 ± 0.7
Cu ⁺²	5	97.8 ± 0.6
Ni ⁺²	5	98.8 ± 0.9
Zn ⁺²	5	98.4 ± 1.1

^a Concentrations of the interferic ions are 100 µg L⁻¹.

phase extraction of U(VI) using *B. mojavensis* loaded onto MWCNT were also examined. The results are represented in Table 2. Recoveries ratio were higher than 95% for all tested foreign ions. It can be concluded that studied foreign ions did not interfere with the detection. The described procedure is therefore extremely selective can be used to natural samples containing these ions.

3.9. Sample volume

The maximum convenient sample volume must be detected in solid phase extraction processes, because environmental samples contain very low levels of trace metal ions (Saracoglu and Elçi, 2002; Ozdemir et al., 2016). So, the influence of the sample volume was studied on the recoveries of U(VI). As seen in Fig. 7, the recovery of U(VI) was not influenced till 300 mL of sample volume. When the sample volume was higher than 300 mL, recovery percentages <95% for U(VI) was acquired. The preconcentration factor of U(VI) was determined as 60, because elution volume was 5.0 mL.

3.10. Reusability of *B. mojavensis* loaded SPE column and its biosorption capacity

In SPE methods, reusability of the biosorbent loaded SPE column is an economically significant factor (Okumus et al., 2015). To investigate the reusability of the biosorbent SPE column, a sample volume of 50 mL, which contained 1 mg/L U(VI) was passed through the SPE under the optimum conditions. As seen in Fig. 8, *B. mojavensis* loaded SPE column can be used up to 30 times (>95%). The capacity of the biosorbent is a significant parameter that determines how much biosorbent is necessary to recover a certain quantity of analytes from the sample solutions quantitatively. The biosorption capacity of solid phase extractor was experimented using batch process. A 20 mg of *B. mojavensis* loaded onto MWCNT was added to 100 mL solutions containing 10 mg/L of U(VI). After shaking the solutions for 120 min, the suspension was centrifuged at 7000 rpm for 10 min. The residual quantity of U(VI) metal ions in supernatant were determined by UV–VIS spectrometry. The biosorption capacity was determined as 25.8 mg/g.

3.11. Analytical characteristics and application to real samples

Under the optimized conditions, the limit of detection (LOD) and quantification (LOQ) were calculated as 0.74 and 2.47 µg/L. Linear calibration curve was achieved in the concentration range of 3.0–80 µg/L of U(VI) with a correlation coefficient as 0.9979. By considering the 300 mL initial and 5.0 mL of final volume, 60 fold preconcentration factor was achieved by developed SPE method. Thus, further improvement in sensitivity for U(VI) measurement by UV–VIS successfully reached by applying developed SPE method. By considering the UV–VIS spectrophotometric methods for U(VI)

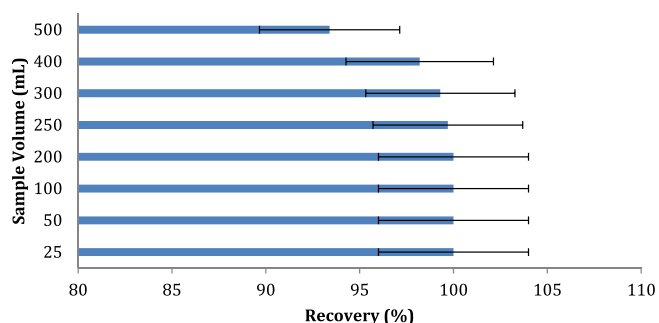


Fig. 7. Effect of sample volume on the recovery of U(VI).

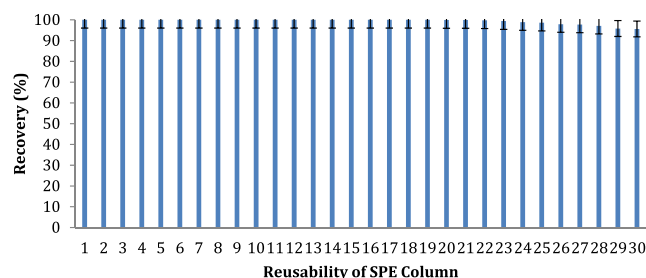


Fig. 8. The reuse of *B. mojavensis* loaded SPE column after applying 5.0 mL of 1.0 mol/L HCl to remove uranyl between two consecutive experiments.

determination, it could be concluded that *B. mojavensis* loaded onto MWCNT is powerful analytical method as an alternative to atomic spectrometric methods (Ozdemir and Kilinc, 2012). During the experiments, we also tested the efficiency of the same column in case of lower uranyl concentrations at 0.01 and 0.025 ng/mL of uranyl. We observed that column efficiency was not affected.

The developed method applied to tap water samples (Siirt, Mardin and Diyarbakır) and Van Lake water (Table 3). U(VI) concentrations in tap waters were found as lower than LOD. They were spiked with 10.0 ng/mL of U(VI). It was determined with high recovery. U(VI) concentration in Van Lake samples (two different location) were found as 66.2 ± 4.3 and 70.5 ± 2.9 ng/mL. The found U(VI) concentrations were in good agreement with literature value as 37–110 ng/mL (Yaman et al., 2011). They were also spiked with known amount of U(VI). Spiked amount of U(VI) was determined with high recovery, also.

4. Conclusion

Tolerance, bioaccumulation and solid phase extraction of U(VI) were investigated in details. It should be highlighted that *B. mojavensis* was almost 7 times more resistance in solid culture than liquid culture. It was possible to conclude from results that bioaccumulation capacity of U(VI) exhibited difference in various periods of *B. mojavensis* growth. Solid phase extraction by using *B. mojavensis* loaded onto MWCNT offers an easy preconcentration procedure of U(VI) in real environmental and food sample. The procedure has been optimized and successfully applied for the preconcentration of U(VI) before its determination by UV–VIS. The preconcentration factor was found as 60. The developed method had high tolerance to other possible metal cations in sample matrix.

Table 3
U(IV) determination in samples.

Sample	Certified ng/mL	Found ng/mL
NCSZC 73014 tea sample ^a	10 ± 2	10 ± 0.6
CASS-4 seawater	3.0	2.9 ± 0.2
NWTM-15 fortified water	14.5	14.1 ± 1.1
Tap water (Siirt)	<LOD	–
Tap water (Siirt) ^b	–	9.7 ± 0.7
Tap water (Mardin)	<LOD	–
Tap water (Mardin) ^b	–	9.9 ± 0.6
Tap water (Diyarbakır)	<LOD	–
Tap water (Diyarbakır) ^b	–	9.9 ± 1.0
Van Lake water (Van)	–	66.2 ± 4.3
Van Lake water (Location 1) ^b	–	75.6 ± 4.0
Van Lake water (Van)	–	70.5 ± 2.9
Van Lake water (Location 2) ^b	–	80.6 ± 3.3

^a ng/g.

^b Spiked with 10.0 ng/mL of U(VI).

References

- Aksu, Z., 2005. Application of biosorption for the removal of organic pollutants: a review. *Process Biochem.* 40, 997–1026.
- Anthemidis, A.N., Zachariadis, G.A., Stratis, J.A., 2001. On-line solid phase extraction system using PTFE packed column for the flame atomic absorption spectrometric determination of copper in water samples. *Talanta* 54, 935–942.
- Breierova, E., Vajczikova, I., Sasinkova, V., 2002. Biosorption of cadmium ions by different yeast species. *Z. Naturforsch.* 57, 634–639.
- Dolatyari, L., Yaftian, M.R., Rostamnia, S., 2016. Removal of uranium(VI) ions from aqueous solutions using Schiff base functionalized SBA-15 mesoporous silica materials. *J. Environ. Manag.* 169, 8–17.
- Dutta, S., Mohapatra, P.K., Dhekane, G.D., Das, A.K., Manchanda, V.K., 2008. Solid phase extraction of europium and uranium using Tulsion CH-90 resin. *Desalination* 232, 216–224.
- Hassen, A., Saidi, N., Cherif, M., Boudabous, A., 1998. Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. *Bioresour. Technol.* 65, 73–82.
- Kouklin, N., Tzolov, M., Straus, D., Yin, A., Xu, J.M., 2004. Infrared absorption properties of carbon nanotubes synthesized by chemical vapor deposition. *Appl. Phys. Lett.* 85, 4463–4465.
- Li, J., Yang, X., Bai, C., Tian, Y., Li, B., Zhang, S., Yang, X., Ding, S., Xia, C., Tan, X., Ma, L., Li, S., 2015. A novel benzimidazole-functionalized 2-D COF material: synthesis and application as a selective solid-phase extractant for separation of uranium. *J. Colloid Interface Sci.* 437, 211–218.
- Macaskie, E.L., Dean, A.C.R., 1984. Cadmium accumulation by a *Citrobacter* sp. *J. Gen. Microbiol.* 130, 53–62.
- Mack, C., Wilhelm, B., Duncan, J.R., Burgess, J.E., 2007. Biosorption of precious metals. *Biotechnol. Adv.* 25, 264–271.
- Okumus, V., Ozdemir, S., Kilinc, E., Dundar, A., Yuksel, U., Baysal, Z., 2015. Preconcentration with *Bacillus subtilis*-immobilized Amberlite XAD-16: determinations of Cu²⁺ and Ni²⁺ in river, soil, and vegetable samples. *Biorem. J.* 19, 47–55.
- Ozdemir, S., Kilinc, E., 2012. *Geobacillus thermoleovorans* immobilized on Amberlite XAD-4 resin as a sorbent for solid phase extraction of uranium(VI) prior to its spectrophotometric determination. *Microchim. Acta* 178, 389–397.
- Ozdemir, S., Kilinc, E., Erdogan, S., 2010. *Bacillus* sp. immobilized on Amberlite XAD-4 resin as a biosorbent for solid phase extraction of thorium prior to UV–VIS spectrometry determination. *Microchim. Acta* 171, 275–281.
- Ozdemir, S., Kilinc, E., Poli, A., Nicolaus, B., Guven, K., 2012. Cd, Cu, Ni, Mn and Zn resistance and bioaccumulation by thermophilic bacteria, *Geobacillus toebii* sub sp. *decanicus* and *Geobacillus thermoleovorans* sub sp. *stromboliensis*. *World J. Microbiol. Biotechnol.* 28, 155–163.
- Ozdemir, S., Okumus, V., Dundar, A., Kilinc, E., 2013. A review on preconcentration of metal ions by bacteria. *Microchim. Acta* 180, 719–739.
- Ozdemir, S., Kilinc, E., Okumus, V., Poli, A., Nicolaus, B., Romano, I., 2016. Thermophilic *Geobacillus galactosidasius* sp. nov. loaded γ -Fe₂O₃ magnetic nanoparticle for the preconcentrations of Pb and Cd. *Bioresour. Technol.* 201, 269–275.
- Saeed, S., Davarani, H., Shejjooni-Fumani, N., Najarian, A.M., Tabatabaei, M., Vahidi, S., 2011. Preconcentration of lead in sugar samples by solid phase extraction and its determination by flame atomic absorption spectrometry. *Am. J. Anal. Chem.* 2, 626–631.
- Saeed, S., Davarani, H., Moazami, H.R., Keshtkar, A.R., Banitaba, M.H., Saeed, N., 2013. A selective electromembrane extraction of uranium (VI) prior to its fluorometric determination in water. *Anal. Chim. Acta* 783, 74–79.
- Saracoglu, S., Elçi, L., 2002. Column solid-phase extraction with Chromosorb-102 resin and determination of trace elements in water and sediment samples by flame atomic absorption spectrometry. *Anal. Chim. Acta* 452, 77–83.
- Saracoglu, S., Divrikli, U., Soylak, M., Elci, L., 2002. Determination of copper, iron, lead, cadmium, cobalt and nickel by atomic absorption spectrometry in baking powder and baking soda samples after preconcentration and separation. *J. Food Drug Anal.* 3, 188–194.
- Song, Q., Ma, L., Liu, J., Bai, C., Geng, J., Wang, H., Li, B., Wang, L., Li, S., 2012. Preparation and adsorption performance of 5-azacytosine-functionalized hydrothermal carbon for selective solid-phase extraction of uranium. *J. Colloid Interface Sci.* 386, 291–299.
- Theodorakopoulos, N., Chapon, V., Coppin, F., Floriani, M., Vercouter, T., Sergeant, C., Camilleri, V., Berthomieu, C., Fèvrier, L., 2015. Use of combined microscopic and spectroscopic techniques to reveal interactions between uranium and *Microbacterium* sp. A9, a strain isolated from the Chernobyl exclusion zone. *J. Hazard Mater.* 285, 285–293.
- Vecchio, A., Finoli, C., Simine, D.D., Andreoni, V., 1998. Heavy metal biosorption by bacterial cells. *Fresenius J. Anal. Chem.* 361, 338–342.
- Vijayaraghavan, K., Yun, Y.S., 2008. Bacterial biosorbents and biosorption. *Biotechnol. Adv.* 26, 266–291.
- Vodnik, D., Byrne, A.R., Gogala, N., 1998. The uptake and transport of lead in some ectomycorrhizal fungi in culture. *Mycol. Res.* 102, 953–958.
- Volesky, B., May, H., Holan, Z.R., 1993. Cadmium biosorption by *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* 41, 826–829.
- Yaman, M., Ince, M., Erel, E., Cengiz, E., Bal, T., Er, Ç.C., Kilicel, F., 2011. Distribution study of U, V, Mo, and Zr in different sites of lakes Van and Hazar, river and seawater samples by ICP-MS. *Clean Soil Air Water* 39, 530–536.