The Quarterly Scientific Journal of Applied Biology

Vol.36, No.2, Sering. 76, Summer 2024, p. 1-4.

Journal homepage: https://jab.alzahra.ac.ir

10.22051/jab.2022.40942.1497

Optimization of uranium biosorption process by autoclaved Micrococcus Luteus biomass using response surface methodology

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Accepted:2023.06.12 **Received**:2023.03.10

Introduction

Toxic heavy metal contamination of industrial water is a significant universal problem. They accumulate in living tissues throughout the food chain which has humans at its top. These toxic metals can cause accumulative poisoning, cancer and brain damage. Uranium is one of the most seriously heavy metals because of its high toxicity and radioactivity. Excessive amounts of uranium have found their ways into the environment through the activities associated with the nuclear industry. Conventional methods for removing uranium from wastewaters include; precipitation, evaporation, ion exchange, membrane processing and adsorption. Nevertheless, these methods have several disadvantages, such as high installation and operating costs, requirement of preliminary treatment steps, difficulty of treating the subsequently generated solid waste, and low efficiency at low metal concentration (o 100 mg/L). Owing to increase in environmental awareness, there has been an emphasis on the development of new environmental friendly ways to decontaminate waters using low-cost methods and materials. In this endeavor, microbial biomass has emerged as a complementary, economic and eco-friendly device for controlling the mobility and bioavailability of metal ions. The present work evaluates the performance of the *Micrococcus luteus* biomass to remove uranium ions from aqueous solutions. The effect of pH, temperature, initial concentration, and sorbent dose on biosorption capacity is studied. The results showed that the factor of initial uranium concentration, sorbent dose and pH statistically (p-value‹ 0.05) affect the uranium biosorption process. In contrast, temperature factor (p-value› 0.05) statistically have no effect on uranium removal by M. luteus.

Materials & Methods

Materials: *Micrococcus luteus* bacteria used in this research with PTCC No. 1408 was purchased from the Scientific and Industrial Research Organization of Iran. Uranyl nitrate salt ($UO_2(NO_3)_2.6H_2O$) was obtained from the Research Institute of Nuclear Sciences and Technologies. Nutrient agar culture medium, sulfuric acid and sodium hydroxide and other materials used in this research were supplied from Merck Company.

Preparation of uranium solutions and biomass: A stock solution containing 1000 mg L-1 of U(VI) was prepared of UO2(NO3)4.6H2O. The working solutions were prepared daily from stock solutions.
In this study, the biomass of *Micrococcus luteus* bacteria was heat treated in an autoclave at a temperature of $121^{O}C$ for 15 minutes at a pressure of 1.5 atmospheres. Then the parameters of temperature, pH, initial concentration of uranium and amount of biosorbent were optimized using the response surface method in Design Expert software.

Experimental design and batch biosorption studies: The design of the experiment was done using the response surface method by Design Expert software. Four variables, including initial uranium concentration (10-100 mg/l), temperature (25-40 C°), pH (2-5) and biosorbent dose (5-25 g/l), in five levels α-, -1, 0, +1, α+, 1 were used to design the experiment. Therefore, 27 experiments were presented using a central composite design. Uranium biosorption experiments were performed by adding specified amounts of bacterial biomass in 20 ml Erlenmeyer flasks containing uranium solution with the concentration and pH corresponding to each experiment, with the specified temperature in the Shaker. After 90 minutes, each sample was centrifuged at 4500 rpm for 15 minutes at 4°C. Then, the remaining uranium in the solution was measured by ICP (Perkin Elmer/Optima 7300DV). The percentage of uranium removal (R) was calculated by equation 1:

$$R(\%) = \frac{(C0-Cf)}{C0} * 100 \tag{1}$$

Where C_0 and C_f are the initial and the final concentrations of the metal ion solutions (mg/l), respectively.

Results & discussion

By using the RSM-CCD method, the optimization of the biosorption process was carried out. The experimental results based on each point of the experimental design. Then, using analysis of variance (ANOVA), the obtained results were evaluated.
The equation obtained for the biosorption efficiency of uranium by *Micrococcus luteus* is expressed as follows:

Removal= +381.00267-15.47727* C(ppm)-45.12825 * pH+0.62243* T (^{0}C)-7.08198* M (g/l) +4.10347 *C (ppm) * pH+7.56296E-003* C (ppm)* T (^{0}C) +0.18786* C (ppm)* M (g/l)-0.10822* pH * T (^{0}C) +2.14135* pH * M (g/l)-0.036100* T (^{0}C) * M (g/l) +0.10988* C (ppm)^2-8.84880* pH^2-0.047385* C (ppm) * pH * M (g/l)-0.030184* C (ppm)^2* pH

The F-value and p-value of the proposed model are equal to 12.19 and 0.0001, respectively, reflecting the accuracy of the proposed model. This model with R^2 equal to 0.93 shows that the proposed model can well predict the experimental values.
The results showed that the factor of initial uranium concentration, sorbent dose and pH statistically (p-value‹ 0.05) affect the uranium biosorption process. In contrast, temperature factor (p-value› 0.05) statistically have no effect on uranium removal by *Micrococcus luteus*. With increasing uranium concentration from 10 mg/l to 100 mg/l, the removal decreases from %100 to %99/6. The increase in

absorption efficiency at low uranium concentrations indicates that the bacterial biosorbent used in dilute metal solutions is efficient. On the other, one of the most important effective parameters in biosorption is the pH of the solution. with increasing the pH from 2 to 4/25, the removal increased from %49/18 to %100. Because the number of biosorbent binding sites decreases at low pH due to the protonation of functional groups. But with increasing the pH from 4/25 to 5, the removal decreased to %99/65 due to the formation of uranyl complexes. Also, with increasing the of biosorbent dose due to the increase in the area of the biosorbent surface, which enhances the number of grafting sites, the absorption percentage can be increased. Therefore, the results showed that the removal of uranium increases from %89/82 to %98/83 by increasing the amount of biosorbent from 5 g/l to 25 g/l.

Conclusion

In this research, he results indicated that the pre-treated biomass under the conditions suggested by Design Expert software (19.75 g/liter of biomass, temperature 32.14 ^{O}C and pH 3.33) is able to remove approximately 99.98 percent of uranium from the contaminated area is 26.11 mg/liter of uranium, which shows its valuable potential in bioremediation applications of uranium from acidic wastewaters contaminated with low concentrations of uranium.

Keywords: Biosorbent, Design–Expert, Radionuclide, Bioremediation

Acknowledgement:

This research was done with the support of Nuclear Science and Technology Research Institute. Therefore, the authors sincerely appreciate and thank that center for their financial and spiritual support.

Declaration of conflict of interest:

The authors declare that they do not have any conflict of interest.

Statement on ethics:

The authors declare that this work has not been published elsewhere and has not been submitted to another publication at the same time.