Optimization of Bioleaching Process of High-Fluoride-Bearing Uranium by Response Surface Methodology

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Abstract. In the bioleaching system of high-fluorine uranium, the dissolution of fluorine must be considered, which is also affected by the parameters of the leaching process. In order to match the fluoride-containing system, the strain was domesticated under different fluoride level. In order to match the process parameters in the bioleaching of high-fluoride-bearing uranium ore, response surface methodology was used to predict the effective parameters and their interactions in bioleaching. Four significant variables (\([\text{Fe}^{2+}]_{\text{initial}}\), pH, solid-liquid ratio and inoculation percent) were selected for the optimization studies. The optimal values of the variables for the maximum uranium bioleaching recovery (94.230±0.74)% were as in agreement with the predicted models 94.41%.

1. Introduction

With the rapid development of nuclear power, the demand for uranium resources is increasing. China's uranium resources have the characteristics of small total amount, low grade, complex ore properties, it is imperative to increase the utilization of uranium resources[1]. Many studies have shown that[2-5], sulfuric acid and \(\text{Fe}^{3+}\) oxidants produced by microorganisms during the leaching process can effectively replace the conventional oxidants[6,7] (\(\text{MnO}_2\), \(\text{H}_2\text{O}_2\), \(\text{HClO}_3\)), to reduce the consumption of oxidants and acid during the metallurgical process. Therefore, bioleaching of uranium has attracted wide attention from many researchers due to its low leaching costs and high leaching efficiency.

With the application of bioleaching of uranium, two major engineering problems are gradually presented. First, the tolerance of leaching bacteria, about 60% of uranium resources in China were found in fluoride-bearing uranium deposits, and the concentration of fluorine in heap bioleaching solutions of high-fluorine uranium ore were as high as 2-4 g/L[8], which seriously inhibits the iron oxidation activity of leaching microorganisms. Second, the interactive influence of process parameters. The bioleaching process conditions have interactive effects. It is necessary to match the process conditions to obtain the maximum leaching rate.

Due to the lack of research on the interaction of various influencing factors in the bioleaching of high-fluorine uranium, four main factors: initial \(\text{Fe}^{2+}\) concentration, pH value, inoculation percent, and solid-liquid ratio were selected. In this study, the response surface method Box-Behnken was used to optimize the bioleaching of high-fluorine uranium, the optimal values of different factors were obtained, the interaction among different factors was analyzed, and the regression equation of biological leaching of high-fluorine uranium was established. The optimization results under the optimum conditions were verified.
2. Experimental

2.1. Ore Characterization
The uranium ore used in the experiment was taken from a fluorine-bearing uranium deposit in Jiangxi province, China. The particle size of the sample used in the shake flask test was 90% -0.074 mm. The chemical composition of the ore are shown in Table 1.

**Table 1.** Analysis of uranium ore chemical composition (%)

|   | Si   | Al  | Fe   | Ca | Ti | K  |
|---|------|-----|------|----|----|----|
| 30.7 | 9.38 | 3.434 | 2.1  | 0.375 | 3.34 |
| Na | 0.361 | 0.055 | 0.293 | 0.114 | 0.079 | 0.022 |
| Zr | 0.023 | 0.772 | 0.382 | 0.516 | 0.234 | 0.06 |

The main chemical composition of the ore (%): U_T 0.234%, U(VI) 0.06%, F 0.516%. U(IV) accounts for 74.36% of total uranium, indicating that the uranium ore is reducibility and requires a high oxidizing environment to increase the uranium leaching rate. With high fluorine content, it belongs to high-fluorine uranium ore.

2.2. Fluoride-Resistant Bacteria Domesticated
A mixed microbial strain CJ6-0 (the original strain) was used in the experiment, which was derived from the National Engineering Laboratory of Biohydrometallurgy. The dominant microbes identified by 16SrDNA sequences were Acidithiobacillus sp., Ferrimicrobium sp., Leptospirillum sp., and Acidiphilium sp. In order to adapt the original strain to the fluorine-bearing leaching system, the strain was domesticated under different concentration of fluoride. After the strain was adapting, switch it to a higher concentration of fluoride environment until it reached the limit.

2.3. Response Surface Methodology
A response surface method based Box-Behnken design was used to optimize the bioleaching of fluorine-bearing uranium. As presented in Table 2, four independent variables used in this work were A: pH value; B: solid-liquid ratio; C: initial Fe^{2+}; D: inoculation percent, which were prescribed into three levels coded, (-1, 0, +1). The dependent variable is the uranium leaching rate (%).

**Table 2.** Coded levels for the factors used into response surface analysis

| No. | Factors            | unit | Code level |
|-----|--------------------|------|------------|
| A   | pH value           | -    | -1 0 1     |
| B   | solid-liquid ratio | w/v  | 1.0 2.0 3.0 |
| C   | initial Fe^{2+}    | g/L  | 4.0 6.0 8.0 |
| D   | inoculation percent| v/v %| 10.0 20.0 30.0 |

3. Formatting the Text

Results and Discussion

3.1. Fluoride-Resistant Strain Domesticated
Comparing the fluoride resistant ability with the original stain (CJ6-0) and the domesticated strain (Table 3). The results showed that after domesticated the strains showing a good fluoride tolerance, when the concentration of F was 800 mg/L, the Fe^{2+} oxidation rate was very close to the non-fluoride control group.
Table 3. Domesticated of CJ6 strain with fluoride ions

| F⁻ concentration (mg/L) | Time for microbes to completely oxidize Fe²⁺ in leachate / d |
|------------------------|-------------------------------------------------------------|
|                        | Transfers 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| 0                      | 2           | - | - | - | - | - | - |
| 100                    | 6           | 3 | 2 | - | - | - | - |
| 200                    | 5           | 3 | 2 | - | - | - | - |
| 300                    | 7           | 6 | 3 | 2 | - | - | - |
| 400                    | 8           | 6 | 5 | 3 | 2 | - | - |
| 500                    | 10          | 10| 7 | 4 | 2 | - | - |
| 600                    | -           | 12| 10| 7 | 5 | 4 | 2 |
| 700                    | -           | 18| 13| 9 | 7 | 5 | 3 |
| 800                    | -           | 19| 17| 11| 8 | 5 | 3 |

Analysis of microbial community changes in different domesticated periods. The original strain is defined as CJ6-0, the strain fluoride resistant to 400mg/L was defined as CJ6-1, the strain fluoride resistant to 800mg/L was defined as CJ6-2. The microbial community structures were different on genus level(Fig. 1). The dominant bioleaching microbes in the CJ6-0 were Acidithiobacillus sp., Ferrimicrobium sp., Leptospirillum sp. and Acidiphilium sp., which were commonly used in heap-bioleaching as mixed microbes. These results indicated the original strain was richness relatively. With the domestication, there was no Ferrimicrobium sp. existed in the samples of CJ6-1, furthermore, only Acidithiobacillus sp. existed in the CJ6-2. The poorness abundance of microbial changes should be related with the fluoride stressed.

Figure 1. Structures of the samples dominant genus

3.2. Effect of pH on Bioleaching

Fig. 2 showed that during the pre-acid leaching stage, the lower pH value, the faster the uranium leaching speed. After 7 days pre-acid leaching, the recovery of uranium was about 43%, when the pH value was 1.5. When the initial pH values were 2.0, 2.5, and 3.0, the recovery of uranium were only 30%, 13%, and 8%, respectively. After 5 days pre-acid leaching, the dissolving motivation of uranium was obviously insufficient. It could be explained by the fact that U⁶⁺ has completely dissolved out, however, U⁴⁺ was insoluble in acid. Therefore, there is no significant change of the concentration of uranium ions with increasing leaching time.
3.3. Effect of Solid-Liquid Ratio on Bioleaching

Fig. 3 showed that the solid-liquid ratios have a great influence on the growth of leaching microbes. When the solid-liquid ratio was 0.4 (w/v), the recovery of uranium only slightly increased from 27% to 38% after 7 days of bioleaching.

3.4. Effect of Initial Fe\(^{2+}\) on Bioleaching

Through the analysis of the chemical composition of uranium ore, the iron content in the mineral was as high as 3.4%, mainly in pyrite and biotite. The experiment mainly examines whether the leaching microbes could use the iron contained in the minerals. Fig. 4 showed that when the initial Fe\(^{2+}\) was 2 g/L, the recovery of uranium was lower than the other control groups about 23%.
Therefore, the effect of Fe$^{2+}$ addition during uranium leaching is not obvious. It only needs to provide the initial energy source for the inoculated bacteria. More importantly, Fe$^{3+}$ produced by the oxidation of Fe$^{2+}$ by bacteria can be used as an oxidant to oxidize insoluble U$^{4+}$ to soluble U$^{6+}$. Experiments have shown that the bacteria inoculated during the leaching process cannot directly use the solid iron in the mineral. In the leaching process, Fe$^{2+}$ is still needed to ensure the growth of bacteria and the uranium leaching efficiency.

3.5. Effect of Inoculation Percent on Bioleaching

According to Fig. 5, when the inoculation percent (v/v) were 10% and 20%, the recovery of uranium in the solution have no significant change compared with that in pre-acid leaching stage. When the inoculation percent (v/v) were 30% and 40%, the recovery of uranium ion can be observed increasing significantly. After 7 days of bioleaching, the recovery of uranium can reach about 80%. Results have shown that increasing the inoculation percent helps to shorten the microbial adaptive phase and accelerate the leaching speed.

![Figure 5. Uranium recovery under different inoculation percent](image)

According to Fig. 5, when the inoculation percent (v/v) were 10% and 20%, the recovery of uranium in the solution have no significant change compared with that in pre-acid leaching stage. When the inoculation percent (v/v) were 30% and 40%, the recovery of uranium ion can be observed increasing significantly. After 7 days of bioleaching, the recovery of uranium can reach about 80%. Results have shown that increasing the inoculation percent helps to shorten the microbial adaptive phase and accelerate the leaching speed.

3.6. Response Surface Methodology Optimization Results

Box-behnken design was used to carry out 4 factors and 3 levels of test design. According to the design, 29 sets of test conditions were executed, from which 5 tests are replicates of the central point. Fig. 6 represents the design matrix of the variables together with the experimental results and predicted results.

![Figure 6. Comparison of experimental and predicted values for uranium recovery](image)
By applying a multiple regression analysis to the experimental data, the experimental results of the Box-Behnken were fitted with a quadratic polynomial model (Eq. (1)). The empirical function between uranium recovery and the four variables in coded by using of RSM method are given by the following equation:

\[
R = 83.04 - 22.02A - 14.41B + 1.57C + 7.62D + 3.70AB + 0.22AC - 1.51AD + 0.35BC + 9.55BD + 1.63CD - 41.20A^2 - 6.21B^2 - 2.62C^2 - 5.34D^2
\]  

(1)

The statistical significance of the model equations (Eq. (1)) and the model terms were assessed by the F-test for analysis of variance. The “p-value” value for the model was <0.0001 (p-value<0.01), which indicates that the regression equation was statistically significant with a confidence interval of 99.00%. Besides, the coefficient of the model R^2 that shows the quality of fit of the second-polynomial equations was 0.9755 for uranium recovery, which implies that the model was suitable for sufficient representation of the real relationship among these variables.

Eq. (1) express that two variables in giving ranges (A, B) have the negative linear effect on the modle, in addition, (C, D) have the positive linear effect on the modle. The most effective factor is pH value and after that solid-liquid ratio and inoculation percent respectively, finally, initial Fe2+ is less effective factor. The model also shows the negative effect interaction between variables A (pH value) and D (initial Fe2+) and positive effect interaction between variables (A and B (solid-liquid ratio)), (A and C (inoculation percent)), (B, C), (B, D) and (C, D).

4. Conclusions

According to the analysis of ANOVA, the greatest influence factors on the leaching rate is the pH value, it both affects the activity of microbes in the fluoride-bearing environment and the leaching kinetics of uranium. Secondly, the solid-liquid ratio, increasing the solid-liquid ratio leads to the increase of the F concentration in the leachate, which has a higher requirement for the fluoride-tolerance ability of the microbes. Followed by the inoculation percent, increasing the inoculum size will help shorten the microbial adaptation period. Finally, the effect of initial Fe2+ on the uranium leaching rate is not significant.

5. References

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