

Bioleaching of Low Grade Uranium Ore of Saghand Mine

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ABSTRACT: *This study deals with bioleaching of the low grade uranium ore of Saghand mine in Iran using a mixed culture of mesophilic bacteria (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*). Experiments were carried out in shaking flask examining the effect of temperature, initial ferrous ion concentration, pulp density and particle size. Variations of pH, Eh, ferrous ion concentration, cells growth and uranium extraction were monitored. The highest rates of leaching and extraction were obtained at 35°C, 2.5% (w/v) pulp density, 4 g/l ferrous ion and - 106µm particle size.*

KEY WORDS: *Bioleaching, Mesophilic bacteria, Uranium ore.*

INTRODUCTION

The current process for the uranium extraction employs strong acids as leaching agents, and MnO₂ as oxidant using high temperature. This method often creates an environmental problem, which generates Mn²⁺ in the effluents, which requires costly and time-consuming treatment before their disposal to the tailing ponds [1]. The method also requires large amounts of energy, and involves a complex operational plant [2-4]. While it is not economical to extract uranium from low-grade ores by chemical leaching, the bioleaching process,

which employs microorganisms such as bacteria and fungi as the leaching catalysts, is known to be economical and environmentally acceptable [2-5].

The oxidative leaching of uraninite (UO₂) involves a redox reaction with Fe³⁺ as an oxidant and bacterial reoxidation of Fe²⁺. The redox reaction resulting in uranium oxidation, which is between, oxidized uranium as the electron donor and Fe³⁺ as the electron acceptor. Uranium oxidation is relatively slow with O₂ as the electron acceptor compared with the Fe³⁺ mediated oxidation.

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Table 1: Chemical composition of the ore sample.

Th (ppm)	U (ppm)	S (%)	P ₂ O ₅ (%)	K ₂ O (%)	MgO (%)	Na ₂ O (%)	CaO (%)	Fe ₂ O ₃ (%)	Al ₂ O ₃ (%)	SiO ₂ (%)
27	465	0.75	0.23	0.34	19.03	0.18	1.03	49.67	1.49	24.83

Table 2: Uranium percent at different particle sizes of ore.

Size (μm)	U (%)
-106	0.0465
-212+106	0.0243
-300+212	0.0196
-400+300	0.0245

Dissolved Fe³⁺ and Fe²⁺ usually originate from the bacterial oxidation of Fe-sulfides such as pyrite, pyrrhotite, marcasite and mackinawite. Pyrite is by far the most abundant Fe-sulfide present in the uranium ores. Continuous regeneration of ferric sulfate by bacterial oxidation of Fe²⁺ and Fe-sulfides maintains the high Fe³⁺/Fe²⁺ ratios and redox potential values needed for uranium leaching.

The regeneration of Fe³⁺ containing leach solution by *Acidithiobacillus ferrooxidans* mediated oxidation proposed as a separate process as it could control to maximize oxidation kinetics via nutrient addition, aeration and pH adjustment. Therefore, during the process, microorganisms would not directly attack the uranium ore but they would create the necessary chemical conditions for its dissolution [5-8]. In uranium leaching systems, iron is invariably present in solution and the rate of uranium oxidation to soluble species is a function of the rate of ferric regeneration by iron-oxidizing bacteria. According to the effect of ferric ion on uranium bioleaching, it can be concluded that the acidic ferric sulfate is a strong and effective oxidizing agent, which could replace environmentally undesirable MnO₂ as an oxidant in leaching circuits [1, 4, 7].

In this study, bioleaching of low grade uranium ore of Saghand mine using a mixed culture of mesophilic bacteria was investigated. The effects of temperature, ferrous ion concentration, pulp density and particle size on the uranium bioleaching were evaluated.

EXPERIMENTAL SECTION

Ore sample

A low-grade uranium sample with high iron and pyrite content was prepared from Saghand uranium mine

(Anomaly NO.2) of Yazd in south of Iran. The constitute elements of ore samples obtained by X-Ray Fluorescence (XRF) spectrometry. The chemical compositions of ore sample (-106μm) are shown in Table 1. Table 2 shows U (percentage) at different particle size fractions. According to the X-Ray Diffraction (XRD) analysis of the ore, main minerals are magnetite, pyrite, and quartz, plus uraninite as the main uranium-bearing mineral in the ore. The ore finally ground to particles 80% less than 106μm in size (optimum liberation degree of uranium mineral [9].

Microorganisms and media

Three pure strains used in this study were *Acidithiobacillus ferrooxidans* (DSM 11477), *Acidithiobacillus thiooxidans* (DSM 622) and *Leptospirillum ferrooxidans* (DSM 2705), obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Germany. A basal salt medium containing (NH₄)₂SO₄: 0.4 g/L, MgSO₄.7H₂O: 0.4 g/L and K₂HPO₄: 0.4 g/L [10] used for bioleaching experiments. Energy source: 6.7 g/L Fe²⁺ and 1.0 g/L sulfur powder; mixed cultures were also prepared from the pure strains. All three microorganisms adapted with different pulp densities and different media were used. Experiments were carried out using mixed bacterial cultures and mixture of several microorganisms: *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* (mesophilic and heterotroph). The initial bacterial populations in inoculum were counted about 4×10⁷ cells/mL.

Experimental procedures

Bioleaching experiments were carried out in 250-mL Erlenmeyer flasks containing 95 mL of nutrient medium and 5 mL of inoculums containing mixed bacteria. Flasks were shaken in an orbital shaker at 200 rpm.

Initial pH was adjusted to 1.7 with H₂SO₄ (10N) and during experiments pH was allowed to vary freely. Control experiments were carried out by addition of 5mL 0.5% (v/v) formaline in ethanol to the medium and replacing of the bacterial inoculums by an equal volume of medium. Water losses by evaporation were compensated by the addition of distilled water. Samples

were taken at regular intervals to determine total soluble uranium, ferrous iron concentration and cell number. The pH and Eh were also measured. The experiments were performed in duplicate.

Analysis

The quantity of free bacteria in solution was determined by direct counting, using a Thoma chamber of 0.1 mm depth and 0.0025 mm² area with an optical microscope (×400). Uranium was analyzed by ICP-AES. The ferrous ion concentration in the solution was determined by o-phenanthroline method (using a Varian UV-Vis spectrophotometer, model Cary 50) [11, 12]. The bioleaching residuals were analyzed by XRF and XRD. The Eh (vs. Ag/AgCl) and pH of the supernatant at room temperature was measured with a Metrohm pH meter, model 691.

RESULTS AND DISCUSSION

Effect of temperature

Fig.1a shows the effect of different temperatures 25°C, 30°C, 35°C and 40°C on uranium extraction. According to Fig.1a, while temperature was increased from 25°C to 35°C, uranium extraction increased clearly. Temperature increasing from 30°C to 35°C had not more effect on uranium extraction but the rate of extraction at 35°C was a little more than 30°C before 195 h. At 35°C, uranium extraction was 100% after 195 h while at 30°C it was 97%.

When temperature was increased to 40°C, uranium extraction was reduced to about 63% for 530 hours. The uranium extraction was almost negligible for the control test. These results could be justified by bacterial growth (Fig.1b) and oxidation of ferrous ion (Fig.1c). Bacterial activities as seen in figure 1b have a long lag phase at 40°C and the numbers of cells were less compare to 25°C, 30°C, 35°C. Lower extraction rate at 25°C is due to longer lag phase, as can be seen at Fig.1b, whereas at 35°C and 30°C, the lag phase is short and increasing number of cells indicated successful growth of bacteria due to more adaptation of bacteria with uranium ore. According to Fig.1c, oxidation rate of ferrous ion was high at 35°C, 30°C and 25°C. However, the ferrous ion oxidation at 40 °C is incomplete, (i.e. Fe²⁺ never got zero). For the control test, ferrous ion was chemically oxidized slightly by air. Eh variation with time was shown in Fig.1d. According

to this figure, rapid increase in Eh observed for 195 hours at 35°C, which is due to the bacterial oxidation of ferrous ions to ferric ions. This proves an increasing rate of ferrous oxidation as mentioned for Fig.1c.

Variation of pH with time in the bioleaching experiments is presented in Fig.1e. The pH have been increased as a result of ferrous ion oxidation reaching the maximum value in the first 75 h but the maximum value of pH at 40°C (Fig.1e) was due to bacterial growth (Fig.1b). After this time, the pH decreased so that the rate of decrease at 35°C and 30°C were high compared with 25°C and 40°C. Low pH could be seen at 30°C and 35°C. For control test, pH increased until 195 h and almost remained constant with a little variation afterwards.

When control test is compared to bacterial tests, the effect of bacterial leaching could be understood. The rapid increase of pH could be associated with acid-consuming reactions involving the leaching of carbonate minerals [13]. After this time, pH decreased progressively for which two reasons could be taken into account: 1) a great bacterial growth (Eq. 3), detected by microscopic observations, and 2) a precipitation of jarosites according to [14]:



Where X=K⁺, Na⁺, NH₄⁺ and H₃O⁺.

Moreover, the pH in the leaching experiment would have an important effect on the iron precipitation. The change of pH value for the control test was caused by the progressive ferrous oxidation and the acid dissolution of minerals.

Effect of ferrous ion

The effects of different values of initial ferrous ion concentrations 6.7 g/L, 4.0 g/L, 2.0 g/L and 0 g/L on uranium extraction were tested (Fig.2a). According to this figure, the extraction of uranium is completed by using different initial ferrous ion concentrations. Extraction of uranium was obtained 100% after 195 h, using 6.7 g/L or 4.0 g/L ferrous ion while 220 h needed to reach 100% extraction using 2.0 g/L. There is no clear difference for uranium extraction using initial concentrations of 6.7 and 4.0 g/L ferrous ion, but the rate of extraction by 4.0 g/L ferrous ion is more rapid than that of 6.7g/L ferrous ion before 195 h.

Without initial ferrous ion, the rate of extraction was very low and uranium extraction was obtained about 98%

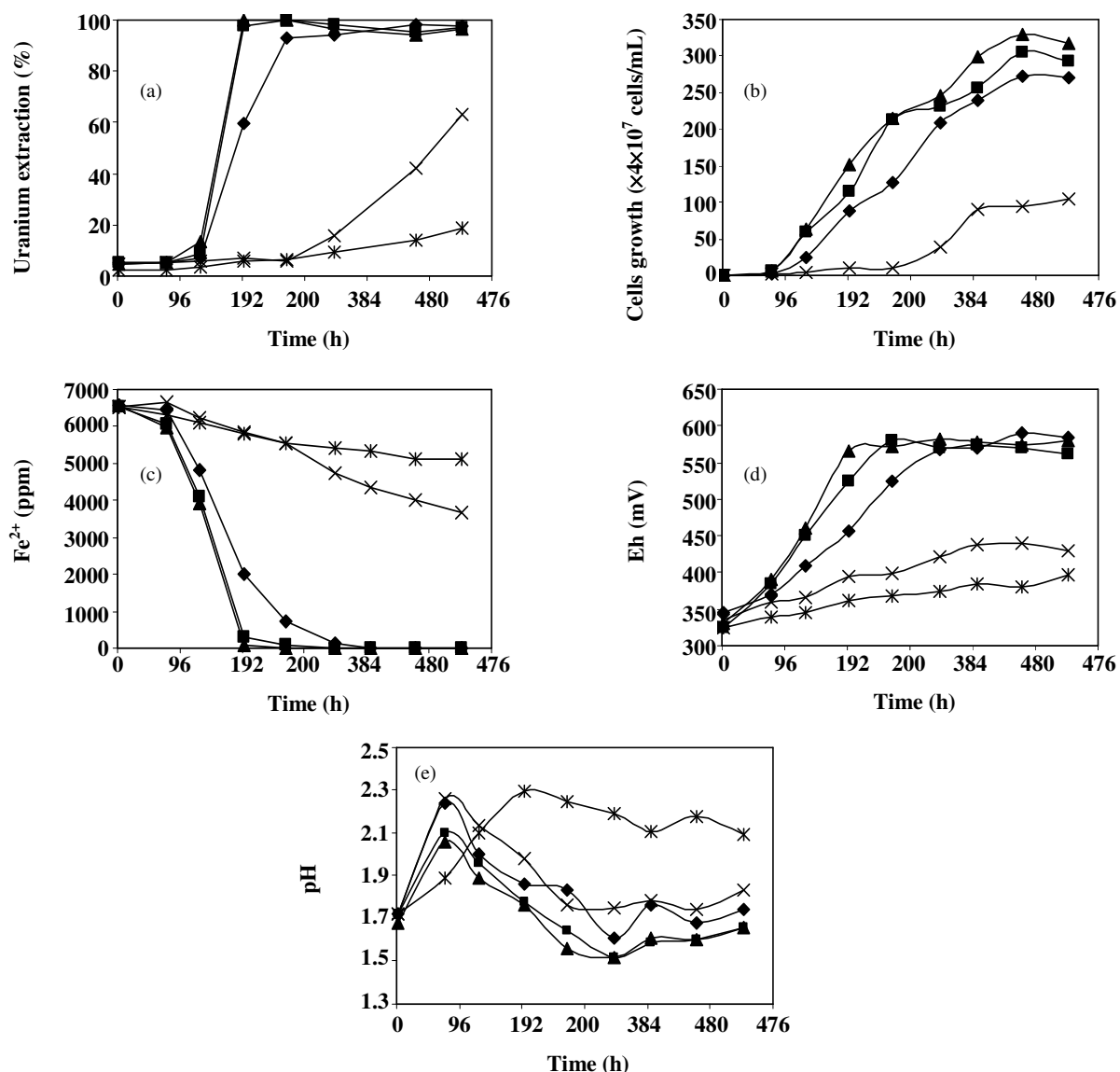


Fig. 1: Effect of different temperatures on the bioleaching of low grade uranium ore: a) Uranium extraction, b) Cells growth, c) Fe^{2+} , d) Eh and e) pH versus time respectively. ♦ 25°C, ■ 30°C, ▲ 35°C, × 40°C, * Control test (35°C).

for longer period (460 h). Control test in the absence of bacteria showed that the extraction of uranium was almost 18% after 530 h (Fig.1a). It was found that the bacteria have an important role in uranium leaching.

The variation of ferrous ion concentration versus time was shown in Fig.2c. This figure illustrates that when different amounts of ferrous ion were added complete oxidation of ferrous ion achieved almost for the same period (after 192 h).

Fig.2d illustrates Eh variation with time for different initial ferrous ion. According to this figure, bacterial

oxidation of Fe^{2+} to Fe^{3+} resulted in Eh increasing. Therefore, in this study Eh variation can be used as a criterion for bacterial oxidation of Fe^{2+} to Fe^{3+} and bacterial activity. There is no clear difference in Eh variations when 6.7 g/L, 4.0 g/L and 2.0 g/L initial ferrous ion were added.

Without initial addition of ferrous ion, Eh decreased until 195 hours and then increased sharply then remained constant. The decrease of Eh was due to lag phase of cells growth (Fig.2b) and chemical leaching, and the increase of Eh was because of ferrous oxidation (Fig.2d).

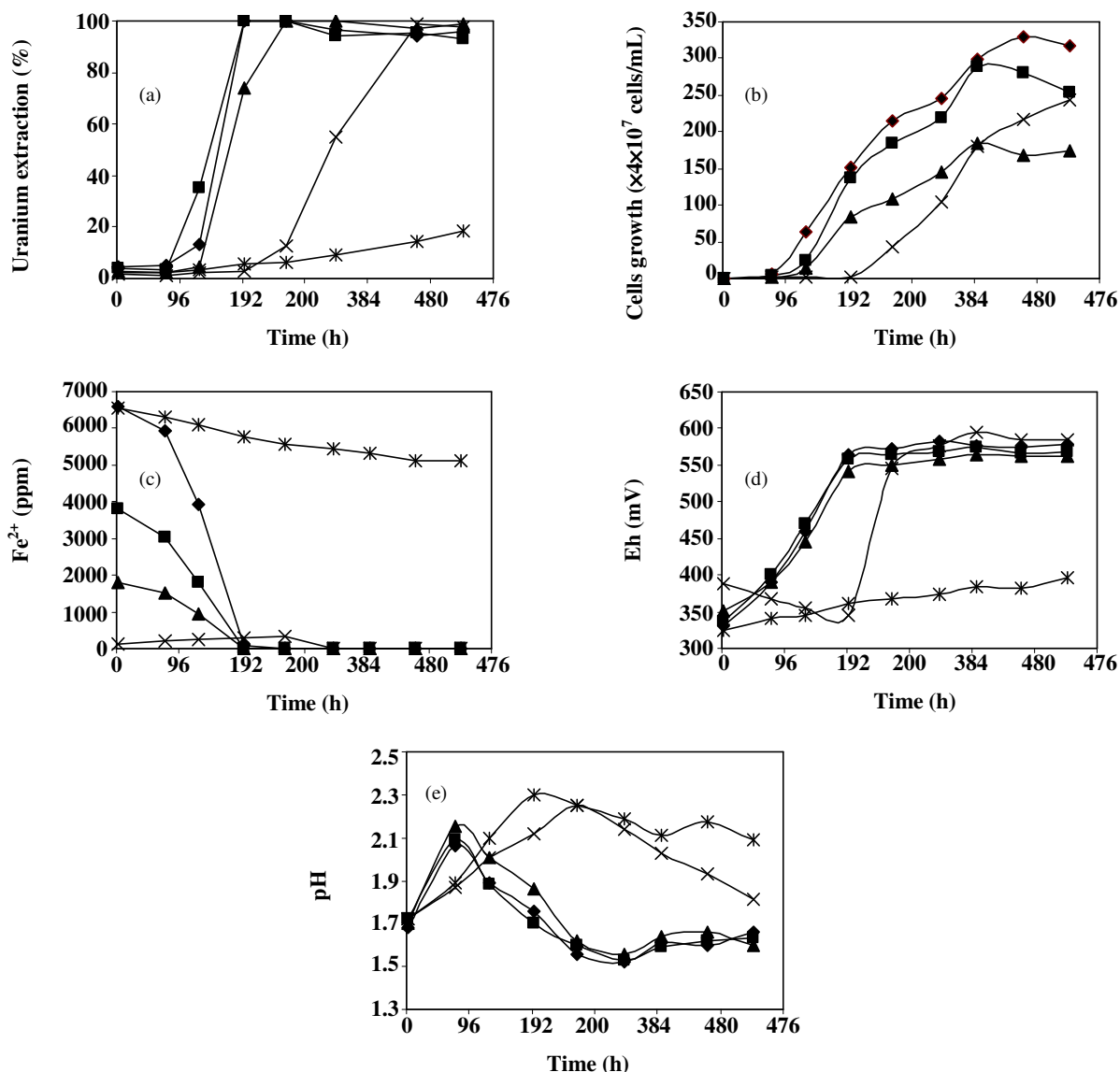


Fig.2: Effect of different initial ferrous ions on the bioleaching of low grade uranium ore: a) Uranium extraction, b) Cells growth, c) Fe²⁺, d) Eh and e) pH versus time respectively. ♦) 6.7 g/L Fe²⁺, ■) 4 g/L Fe²⁺, ▲) 2 g/L Fe²⁺, ×) 0 g/L Fe²⁺, *) Control test (6.7 g/L Fe²⁺).

According to Fig.2b, the number of bacteria increases with increasing the concentration of ferrous ion as providing energy source while the lag phase was long without ferrous ion addition and the increase of cells number was due to the release of energy from the ore.

Fig.2e shows changes of pH with time for different initial ferrous ion concentrations. This figure shows that the test with no ferrous ion addition reaches to its maximum pH value after 260 hours, but the others does not. This is because there weren't any energy source as indicated by long lag phase of cells growth, but after

that time oxidation of ferrous ion of ore content gives rise resulted in cells growth, pH reduction and Eh increasing.

Effect of pulp density

The uranium bioleaching results at different pulp densities as a function of time are shown in Fig.3a. High pulp density (7.5% and 10% (w/v)), had almost similar behaviors. The extraction of uranium for 10% and 7.5% pulp density reached about 98%(maximum value) and 100% respectively after 260 hours whereas at 2.5% and 5% pulp densities, reached 100 % extraction at shorter time.

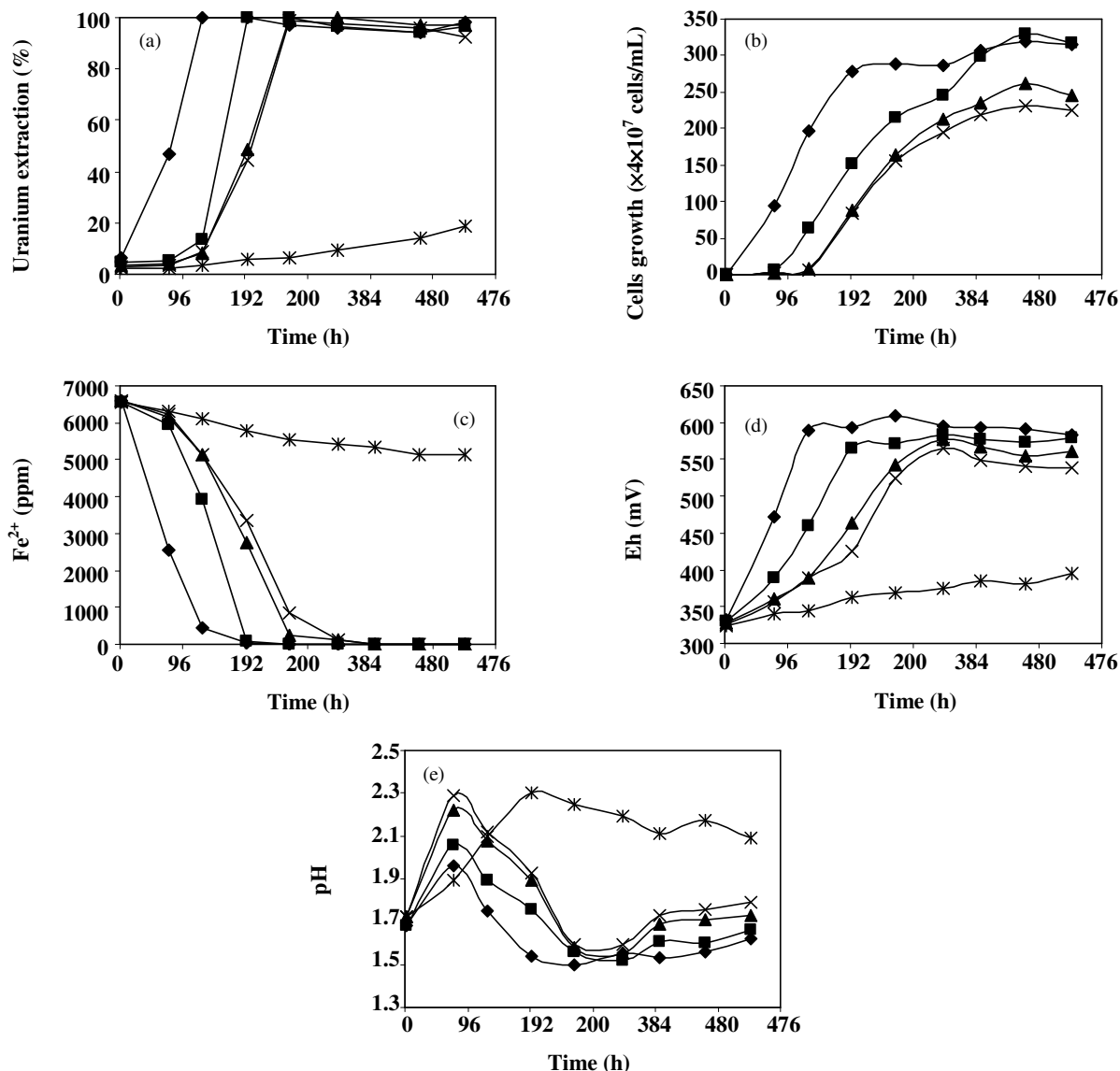


Fig. 3: Effect of different pulp densities on the bioleaching of low grade uranium ore: a) Uranium extraction, b) Cells growth, c) Fe^{2+} , d) Eh and e) pH versus time respectively. ◆) 2.5%, ■) 5%, ▲) 7.5%, ×) 10% (w/v), * Control test (5%).

The main reason for leaching rate decreased, at higher pulp densities, is the more acid consumption of the ore, which led to pH increasing, and precipitation of Fe^{3+} from solution. Fig.3b shows the variation of cells growth with time. According to this figure, the rate of cells growth decreased with increasing pulp density. The bacterial counting was only based on free bacteria in solution with no attempt to determine bacterial densities on mineral surfaces.

The decrease of the growth of bacteria in solution as pulp density is increased (Fig.3b) could be as a result of

the attachment of bacterial cells to minerals and the degradation of cells by solids attrition, although the latter would be expected to occur to a minimal extent due to the relatively low pulp densities.

The ferrous oxidation variations with time are shown in Fig.3c. This figure illustrates the high rate of ferrous oxidation at pulp density of 2.5% is high. As can be seen in Fig.3d, Eh value decreased with increasing pulp density.

Fig.3e shows variation of pH versus time for different pulp densities. During 96 hours, the pH increases with

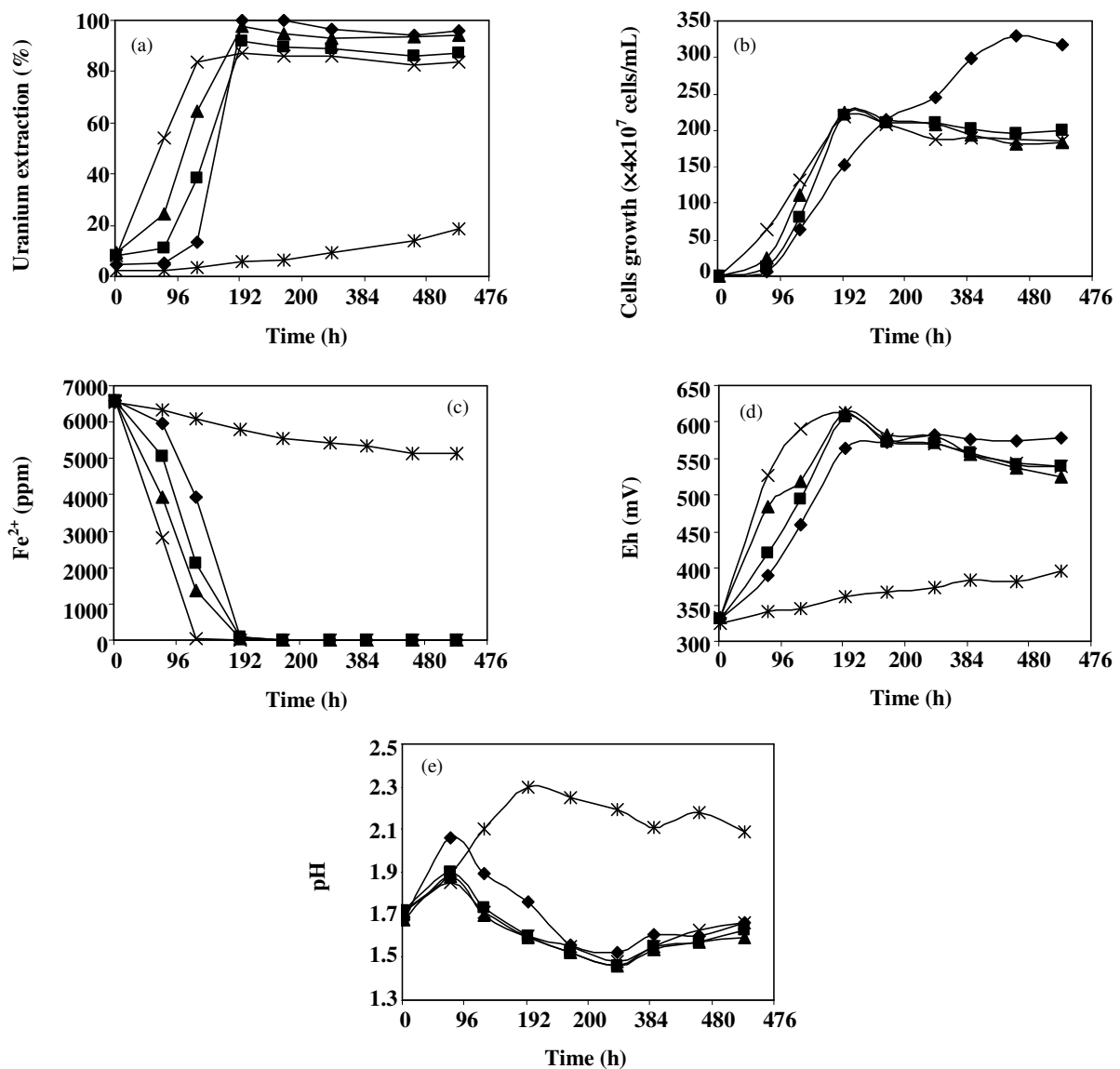


Fig. 4: Effect of different particle sizes on the bioleaching of low grade uranium ore: a) Uranium extraction, b) Cells growth, c) Fe^{2+} , d) Eh and e) pH versus time respectively. \blacklozenge – $106\mu\text{m}$, \blacksquare – $212 + 106\mu\text{m}$, \blacktriangle – $300 + 212\mu\text{m}$, \times – $400 + 300\mu\text{m}$, \ast Control test ($106\mu\text{m}$).

increasing pulp density. After 96 hours, decreasing pH was occurred gradually. This is due to low bacterial activities at high pulp densities.

Effect of particle size

The results of uranium extraction at different particle sizes are shown in Fig.4a. It was found that before 127 h, the rate of uranium extraction increases with increasing particle size. Uranium extraction for 127 hours for particle sizes of $400 + 300\mu\text{m}$, $300 + 212\mu\text{m}$, $212 + 106\mu\text{m}$ and $106\mu\text{m}$ were 84%, 64%, 38% and 14% respectively.

The rate of uranium extractions after 127 h changed for particle size of $106\mu\text{m}$ having maximum rate of extraction. At 195 h, uranium extraction was 100% for $106\mu\text{m}$ particle size, while other particle sizes never could reach to this point. For $400 + 300\mu\text{m}$ after 127 h variation of uranium extraction were negligible. The differences of uranium extraction for various particle sizes before 127 h could be due to difference of bacteria growth (Fig.4b) in various fractions. According to Table 2, while particle size increases, uranium percentage decreases, resulted in an increase in bacterial growth (Fig.4b).

According to Fig.4b, the lag phase at particle sizes of $-400+300\mu\text{m}$ is very low, so well growth of bacteria in this particle sizes cause high uranium extractions.

After 127 hours, the highest extractions for particle sizes of $-106\mu\text{m}$ could be due to higher bacterial growth (Fig.4b) and ferrous complete oxidation (Fig.4c). According to Fig.4b maximum numbers of cells are obtained for $-106\mu\text{m}$ for full period of bioleaching. Nevertheless, for the larger particle sizes bacterial growth was more before 195 h. For the larger sizes cell damage, deactivation, inhibition of the attachment of bacteria to the minerals and detachment of the cells from the mineral surface could occur, resulting in lower extractions [15, 16]. Fig.4c shows ferrous ion variation with time for different particle sizes. The high rate of ferrous oxidation occurred with larger particle sizes ($-400+300\mu\text{m}$). The rate of ferrous oxidation for particle sizes of $-106\mu\text{m}$ was lower than that of larger particles. With comparing Fig.4c to Fig.4a, it could be understood that high rate of extraction and cells growth (Fig.4b) are related to ferrous oxidation, so this is another reason for extraction increasing with the increase of particle sizes before 195 h. The variation of Eh as shown in Fig.4d was a criterion for evaluation of ferrous oxidation. Changes of pH against time are presented in Fig.4e. For smaller particles, the increase in pH at initial stage of bioleaching is higher. It means that small particles consume more acid because of containing carbonate compounds. After initial increasing, pH is decreased. Decreasing of pH for larger particle sizes is higher than smaller particle sizes. Decreasing of pH could be because of iron hydrolysis, the consequent precipitation of ferric compounds and the oxidation of sulfur.

In general, it was concluded that $-106\mu\text{m}$ is the optimum fraction for maximum uranium extraction by bioleaching because it has sufficient surfaces for attachment of bacteria and higher uranium bearing minerals with suitable degrees of liberation.

CONCLUSIONS

The effects of temperature, initial additive ferrous ion, pulp density and particle size on bioleaching of low grade uranium of Saghand mine using mixed culture of mesophilic bacteria (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*) were demonstrated. The results showed that

the optimum temperature for uranium extraction was 35°C . The rate of extraction was low at 25°C compared to 30°C and 35°C . The rate of cells growth was very low at 40°C and the extraction of uranium was not considerable for 530 h.

For different pulp density, the best results obtained at 2.5% (w/v) and uranium extraction declined with increasing pulp density. The addition of initial ferrous ion showed that the optimum initial ferrous ion was 4.0 g/L and initial ferrous ion caused an increase in jarosite precipitation and had restraint effects on uranium extraction during bioleaching process but the values of ferrous ion were considerable. Comparative study on different particle sizes showed that the optimum particle size for maximum uranium extraction was $-106\mu\text{m}$, due to nearly complete minerals liberation degrees.

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