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## **BIOLEACHING OF URANIUM MINERALS AND BIOSYNTHESIS OF UO<sub>2</sub> NANOPARTICLES**

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**Abstract:** Uranium mining has resulted in generation of significant amounts of solid wastes. The mechanism and chemical conditions of uranium waste bioleaching were considered. The initial results of bioleaching of solid waste from closed Kowary mine were presented. The process of U(VI) bioreduction by anaerobic reduction microorganisms was described. The factors that contributed to UO<sub>2</sub> nanoparticles fabrication were discussed. The obtained UO<sub>2</sub> particles are promising for development of novel catalysts.

**Keywords:** *bioleaching, U(VI) reduction, Acidithiobacillus ferrooxidans, Shewanella, nanoparticles, UO<sub>2</sub> precipitation*

### **Introduction**

During the last decades the mining activities created significant amounts of uranium solid wastes. For instance, in the south-east part of Germany (Saxony and Thuringia) the intensive uranium mining has resulted in generation of significant amounts of mining waste piles (Pollmann et al., 2006).

Uranium is produced conventionally using a process that employs a strong acid and large amounts of energy. This technology creates environmental problems. Autochthonic microorganisms are able to mobilize metal ions through autotrophic and heterotrophic leaching. The important environmental goal is to prevent uranium transport. One potential method is to use iron-reducing bacteria for multivalent metals and radionuclides transport halt [Sani et al., 2008]. The objective of this paper is to obtain information on the bioleaching of uranium solid wastes and microbial reduction of U(VI) to U(IV) and UO<sub>2</sub> precipitation.

## Bioleaching uranium minerals

Bioleaching of uranium from natural sources was applied to uranium-bearing ores from Daejeon and Okchen districts of South Korea (Choi et al., 2005). The quantity of uranium in these ores is estimated to be over 100 million megagrams. The average content of  $U_3O_8$  in these deposits is below 0.1% by weight.

One of the most widely employed bacteria in the bioleaching process is *Acidithiobacillus ferrooxidans*. This acidophilic bacteria oxidizes ferrous ions ( $Fe^{2+}$ ) to ferric ions ( $Fe^{3+}$ ) which can act as a strong oxidant capable oxidizing uranium minerals. Bioleaching experiments were carried out with 10g of ground and sieved black schist in a 250 cm<sup>3</sup> Erlenmeyer flask and 100 cm<sup>3</sup> of 9 K medium (medium contains 9 g/dm<sup>3</sup>  $FeSO_4$ ). The uranium transfer from the schist into the solution increases as the  $Fe^{2+}$  ions oxidation was increased. The highest uranium leaching efficiency increased from 18% in the absence of bacteria to almost 80% following the introduction of *A. ferrooxidans*.

The effect of *A. ferrooxidans* on uranium leaching from the black shale ore has also been investigated by Lee and coworkers (2005). The black shale ore was taken from Deokpyeong area (Korea). The ore contains 349 mg uranium per kg. Uranium in the ore was associated with carbonates (58%) and organic matter and sulfides (42%). The pH of mineral suspension was systematically decreases to the level of pH = 2.5. The redox potential was mainly due to bacteria oxidation of  $Fe^{2+}$  to  $Fe^{3+}$ . After 250 h of the bioleaching process the redox potential has value of 0.59 V.

Initial bioleaching experiments were carried out using solid waste from the closed Kowary mine. Figure 1 presents bioleaching data using *Acidithiobacillus ferrooxidans*.

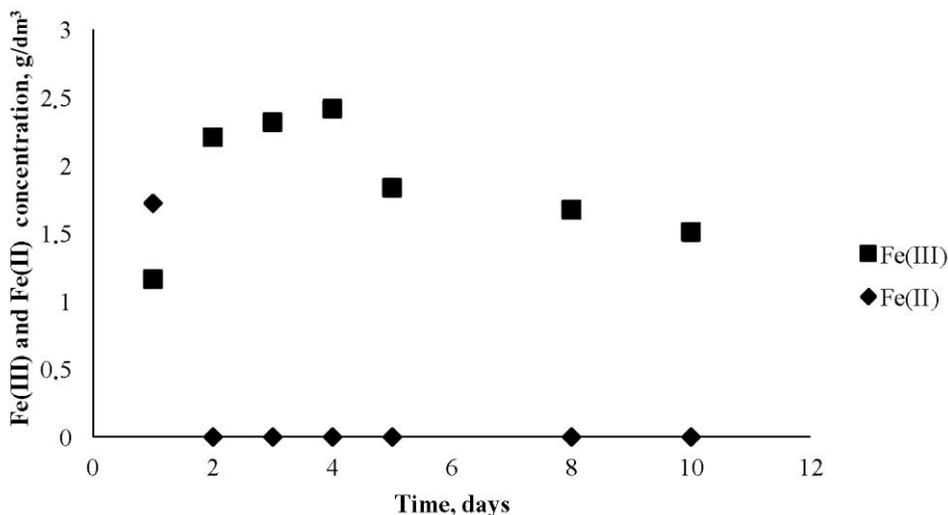


Fig. 1. Bioleaching of solid waste from Kowary by *Acidithiobacillus ferrooxidans*

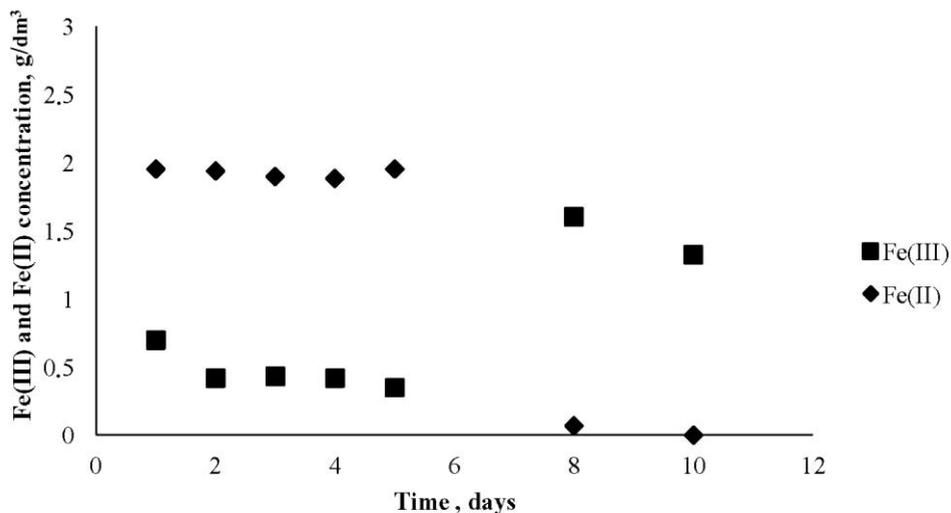


Fig. 2. Bioleaching of solid waste from Kowary using autochthonic bacteria

The bioleaching results using indigenous bacteria are presented in Fig. 2. As can be observed, an intensive oxidation of Fe(II) ions occurred after a 6 days period. Such behavior is in contrast to the literature report of Pal et al., 2010 and it will be investigated.

Since uranium dissolution from the solid wastes requires a strong oxidant therefore the bioleaching experiments are carried out in the presence of ferrous sulfate. The bioleaching path can be composed of three stages within 32 days. In the first stage up to 7 days, the oxidation rate was very fast. From the seventh to the twentieth day the rate of biooxidation was slowly decreased. After the twentieth day the rate of biooxidation was parallel to the rate of biooxidation without iron (Pal et al., 2010).

Leaching of uranium-bearing minerals is accomplished by oxidation of the insoluble  $U^{4+}$  form to the acid soluble  $U^{6+}$  form in an acid environment. The indirect mechanism by using ferric ( $Fe^{3+}$ ) ions as an oxidant is proposed for the uranium bioleach process (Lottering et al., 2008; Abhilash et al., 2011). The uranium bioleaching process has an electrochemical nature. It is confirmed by Eh changes during biooxidation.

Figure 3 presents the Eh changes during uranium waste biooxidation. An increase of Eh corresponds to an increase of Fe(III) ions concentration. From the data present in Fig. 3 the efficiency of Fe(II) biooxidation was better for *Acidithiobacillus ferrooxidans*.

Gold deposits located at Vaal River (South Africa) also contain uranium-bearing minerals. The brannerite-type minerals ( $U_{1-x}Ti_{2+x}O_6$ ) are a major source of uranium in these ores. The insoluble uranium(IV) is oxidized to the water soluble uranium(VI) sulfate as follows:

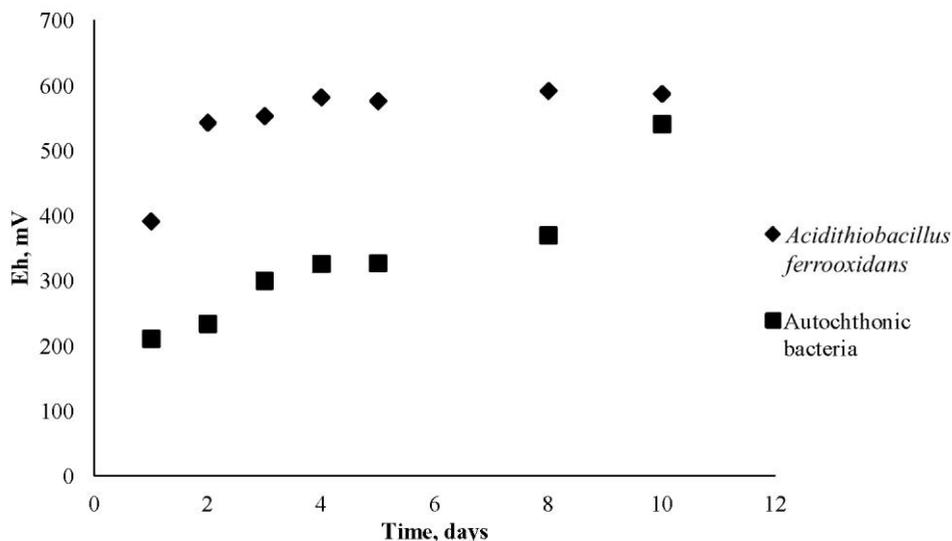


Fig. 3. Changes of Eh during bioleaching of Kowary solid waste by *Acidithiobacillus ferrooxidans* and autochthonic bacteria



A model for uranium minerals bioleaching was developed (Rashidi et al., 2012). The material balances of three reactions provided a correlation between the chemical and biological phenomena.

The efficiency of uranium recovery is depended on the mineralogical characteristics of the ore. In the case of the Vaal River ores 90% uranium dissolution was maximum (Lottering et al., 2008).

A high biosorption of toxic metals such as U, Cu, Pb, Al and Cd accompany to the bioleaching process. For instance, *Acidithiobacillus ferrooxidans* is able to accumulate uranium ions. The ability of *A. ferrooxidans* to bind uranium decreased from 44.62 to 33.81 (mg/g dry weight) when the pH was reduced from 4 to 1.5 (Merroun Selenska-Pobell, 2001).

The Curilo uranium deposit is located in Western Bulgaria. This deposit is a permanent source of acid mine drainage. The generation of drainage water is connected with biooxidation of pyrite and other sulphide minerals. The drainage water was treated by natural and constructed wetlands and alkalized limestone drains (Groudev et al., 2008).

The migration of soluble U(VI) forms can be realized by the reaction of soluble U(VI) to quite insoluble U(VI). Anaerobic microbial activity may lead to U(VI) reduction coupled with oxidation of organic carbon.

Some photosensitive cyanobacteria and algae as *Porphyridium cruentum*, *Spirulina platensis* and *Nostoc linkia* were used for uranium leaching from ores content less than

0.5% of uranium(VI) (Cecal et al., 2000). The bioleaching data showed that the leaching process is more evident in the presence of cyanobacteria (40-90%).

### Removing uranium from pregnant solution

Interest is in removing uranium from the acid mine drainage solution in a cost efficient way. During the last two decades, different groups of microorganisms, such as *Actinomyces* and other bacteria demonstrated the ability to remove uranium from the leaching solution. The capacity to enzymatically reduce U(VI) has been demonstrated for sulphate reducing bacteria (SRB) (Martins et al., 2010). This process can be realized by sulfate and iron reducing bacteria under anaerobic conditions (Gargarello et al., 2010). Microbial reduction of U(VI) to sparingly soluble and immobile U(IV) compounds is a promising method for uranium migration control. However, under anaerobic conditions many iron reducing and sulfate-reducing bacteria can participate in the uranium(VI) reduction process. These bacteria must compete with the bacteria which did not participate in U(VI) reduction, for instance methanogenes bacteria.

*Clastridium spheroids* bacteria are able to reduce Fe(III) to Fe(II), Mn(IV) to Mn(II), U(VI) to U(IV), Pu(IV) to Pu(III) and Tc(VI) to Tc(IV) (Francis, Dodge, 2008). Under anaerobic conditions (pH = 6) uranium complexed with organic ligand (citric acid) was reduced to uranium(IV)-citrate. The reduced uranium remained in the solution as a complex of citric acid. The above results indicate that selection of an appropriate electron donor (organic compound) is important for uranium bioreduction and immobilization.

### Rate of bioreduction

Reduction rates did not vary for the bacteria grown under aerobic and anaerobic conditions. Among the microbial reduction processes that can be determined the environmental behavior of radionuclides, biosorption of uranium and other radionuclides is important for the bioremediation strategic (Kazy et al., 2009). Microbial synthesis of nanoparticles is another very promising aspect of microbial interaction with metal ions. Initial works showed that uranium and thorium biosorption by *Rhizopus* sp. consists of the three processes such as: coordination, adsorption and precipitation. Also, the role of phosphate and carboxylic groups in a complexation phenomenon was considered. The X-ray microanalysis strongly indicated the possibility of uranium and thorium binding with the biomass (*Pseudomonas*) by displacing cellular potassium through ion-exchange mechanism (Kazy et al., 2009). FTIR spectroscopy data showed that after U and Th biosorption, the spectra exhibited changes in peak position further indicating a strong role of carboxyl groups in radionuclide bonding.

Topography analyses using the AFM tapping method, of the cells before and after uranium and thorium sorption revealed an enlargement of bacteria cells and increase in surface roughness. It is interesting that *Acidithiobacillus ferrooxidans* bacteria can

grow under anaerobic condition using iron(III) as the electroacceptor and sulfur as substrate.

### Biosynthesis of $\text{UO}_2$ nanoparticles

The most stable forms of uranium at ambient temperature are  $\text{U}_3\text{O}_8$  and  $\text{UO}_2$ . However,  $\text{UO}_2$  will gradually convert to  $\text{U}_3\text{O}_8$  (Gavrilescu et al., 2009). The bioreduction of U(VI) to U(IV) by abiotic and biotic processes has a significant effect on the uranium ions mobility in the natural environment.  $[\text{UO}_2]^{2+}$  ions can be enzymatically reduced to  $\text{UO}_2$  by sulfate reducing bacteria such as *Shewanella*, *Geobacter* and *Desulfovibrio*.

*Shewanella* species belong to a relatively well-characterized group of U(VI)-reducing bacteria (Burgos et al., 2008). They have a complex and apparently electron transfer system for the reduction of U(VI) ions. For instance, *Shewanella putrefaciens* was grown aerobically on soy broth and harvested by centrifugation. Biomass was resuspended and washed three times in anoxic bicarbonate buffer. The pure cell suspension (approximately  $1.1 \cdot 10^{10}$  cell/dm<sup>3</sup>) was used to bioreduction of U(VI). The medium contains 5 mM of sodium lactate and 1.4 mM uranyl acetate. pH solution was equal to 6.8. Bioreduction was maintained at 23°C in darkness (Senko et al., 2007).

Upon complete bioreduction of U(VI) by *Shewanella putrefaciens*,  $\text{UO}_2$  nanoparticles were located in the periplasm or extracellularly. The localization of  $\text{UO}_2$  particles suggests that U(VI) may be reduced in the periplasm and exported out of the cell via active or passive processes.

The rate of U(VI) reduction is determined by the geochemical conditions under which U(VI) reduction takes place, and the physiological state of the microorganisms used. For example, the presence of  $\text{Ca}^{2+}$  ions may inhibit U(VI) bioreduction (Burgos et al., 2008). The kinetics of U(VI) bioreduction process was observed to be first-order with respect to the concentration of U(VI) ions according to following equation:

$$R_{red} = \frac{d[\text{U(VI)}]}{dt} = -k_{red} [\text{U(VI)}_{solution}]$$

where:  $k_{red}$  is the first-order bioreduction rate constant [ $\text{d}^{-1}$ ].

The rate of U(VI) bioreduction was also depended on the lactase concentration, cell/U(VI) ration, cell/lactase ration and temperature. The obtained results suggested that careful manipulation of addition of electron donor (lactase) could give rise to  $\text{UO}_2$  precipitation (Burgos et al., 2008).

The U(VI) bioreduction rate constants were dependent on the used buffer solution and are presented in Table 1.

Table 1. First-order rate constants for U(VI) bioreduction by *Shewanella oneidensis* (Burgos et al., 2008)

| Buffer solution           | $k_{red}$<br>[d <sup>-1</sup> ] | Cell concentration<br>[cell ml <sup>-1</sup> ] | Temperature<br>[°C] | $r^2$ |
|---------------------------|---------------------------------|--|---------------------|-------|
| NaHCO <sub>3</sub> “fast” | 11                              | 2 10 <sup>8</sup>                              | 37                  | 0.97  |
| NaHCO <sub>3</sub> “slow” | 0.45                            | 1 10 <sup>8</sup>                              | 20                  | 0.87  |
| PBAGW “fast”              | 0.99                            | 2 10 <sup>8</sup>                              | 37                  | 0.98  |
| PBAGW “slow”              | 0.13                            | 1 10 <sup>-8</sup>                             | 20                  | 0.86  |

PBAGW-buffer contains piperazine-NN-bis-2 ethansulfonic acid.

For uranium reduction under iron reducing conditions, the nanoparticles of hematite, as the Fe(III) substrate, and the facultative anaerobic bacterium *Shewanella putrefaciens* were used. The reduction of U(VI) in the bacteria+hematite experiments run was fast (Behrends, van Cappellen 2005). In this case, the competition between abiotic and enzymatic reduction of U(VI) can be explained as a competition between the adsorption onto the hematite surface or the bacterial cell walls.

The redox potential of the U(VI)/U(VIV) couple strong by depends on the chemical and geochemical conditions. Table 2 presents two examples of the uranium couple.

Table 2. Standard stage reduction potential E[V] for uranium (Zhang et al., 2009)

| Redox couple  | E [V] |
|---|-------|
| Uranyl carbonate $UO_2(CO_3)_3^{4-}$<br>$0.5 UO_2(CO_3)_3^{4-} + 1.5 H^+ + e^- \rightarrow 0.5 UO_2 + 1.5 HCO_3^-$                        | 0.687 |
| Ca-U(VI)-CO <sub>3</sub> complex $CaUO_2(CO_3)_3$<br>$0.5CaUO_2(CO_3)_3 + 1.5 H^+ + e^- \rightarrow 0.5 UO_2 + 1.5 HCO_3^- + 0.5 Ca^{2+}$ | 0.424 |

SEM images of biogenic nanoparticles of  $UO_2$  are presented in Fig. 4. As can see the majority of  $UO_2$  particles were located outside of the cells.

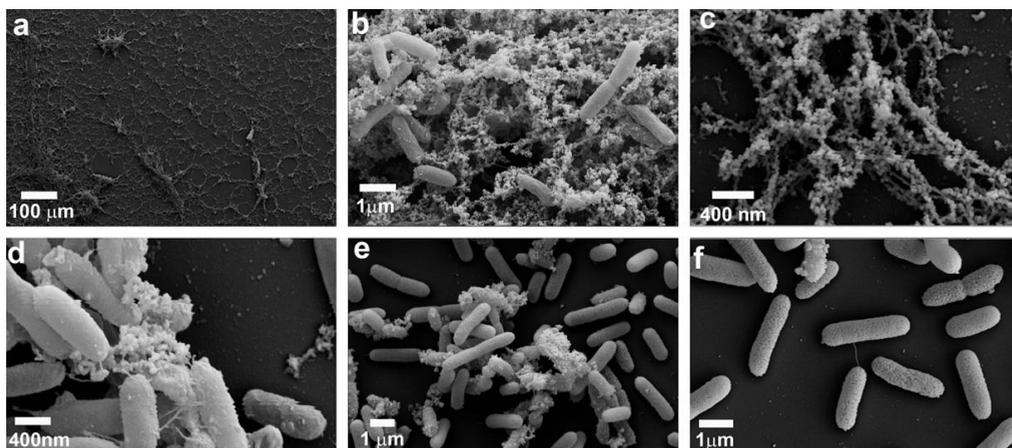


Fig.4. Scanning electron micrographs of  $UO_2$  particles and *Shewanella oneidensis* cells (a–d) NaHCO<sub>3</sub> buffer and (e–f) PBAGW buffer, from (Burgos et al., 2008)

The uranium ions can be adsorbed by carbon nanotubes. The adsorption isotherms of uranium(VI), preferentially the  $\text{UO}_2^{2+}$  and  $\text{UO}_2\text{OH}^+$  ions on the carbon nanotube surfaces were presented by Schierz and Zanker (2009). An increase from 18 mmole/g to 193 mmole/g in uranium ions sorption was observed at pH 5. Such behavior was caused by the increases number of functional groups on the modified surface of carbon nanotubes.

A promising alternative for removing palladium from solution can be use of bacteria for bioreductive deposition of Pd(0) nanoparticles. The formation of Pd(0)-nanoclusters using *Bacillus sphaericus* JG-A12 isolated from the uranium mining pile was investigated (Pollmann et al., 2006). Initial studies showed that the Pd-nanoparticles produced by the cell and deposited onto the cell surfaces are able to catalyze various chemical processes.

## Summary

Biobleaching is a profitable alternative to the conventional chemical process of uranium recovery. The leaching of U from low-grade ores and solid wastes is realized by chemoautotrophic bacteria such as *Acidithiobacillus ferrooxidans*. The initial results demonstrated that biobleaching of uranium solid wastes, located at Kowary, is an effective method for uranium removal. Uranium reducing bacteria, particularly *Shewanella putrefaciens* and *Shewanella oneidensis*, can be used for  $\text{UO}_2$  particles synthesis. The rate of U(VI) bioreduction is first order with respect to the concentration of U(VI) ions. The bioreduction of U(VI) in the presence of hematite particles can be a way to new catalyst fabrication.

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