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Liquid Scintillation Counting Applied for Studying the Influence of Microorganisms on the U(IV) to U(VI) Oxidation Process

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Abstract: The aim of the present study is to investigate the ability of *Escherichia coli* to induce the oxidation of U(IV) to U(VI). This process facilitates the dissolution of uranium from ores to its high oxidation state U(VI) in aqueous solutions, in the frame of establishing new methods for cleaning up radioactive/ nuclear wastes. In this respect we measured the uranium content in aqueous samples collected from *E. coli* cultures grown in the presence of uranium ore. Measurements were done using a liquid scintillation counter, as a direct method to detect radioactive components. Results show moderate ability of U(IV) to U(VI) oxidation which depends on the *E. coli* strain.

Keywords: *Escherichia coli*; Uranium oxidation; Liquid scintillation counting; Gross α activity

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Introduction

Escherichia coli (commonly abbreviated *E. coli*, and named after its discoverer), is a gram negative bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Its microbiological activity was extensively used in biotechnologies (protein expression - insulin production) and bioprocessing. For instance, the biosorption capacity of *E. coli* was used to remove Fe(III), Cd(II), Ni(II) and Cr(VI),[1] as well as inactive or radioactive Tl(I) from diluted aqueous solutions.[2-4]

The respiratory activity of the microorganisms might be also used for increasing the oxidation state of some cations. Thus, *Thiobacillus ferrooxidans* allows metabolizing ferrous ions as the result of its oxidation to ferric one.[5]

Based on those results, the aim of the present work was to oxidize the insoluble tetravalent U(IV) uranium found in ores (such ascoffinite, pitchblende, and uraninite) to the soluble hexavalent U(VI) state by using the respiratory process of several *E. coli* cultures.

For data evaluation, ultra low-level α/β discrimination liquid scintillation counting (LSC) was employed due to high detection efficiencies (up to 100 %) and comparable background count rates, a useful tool for the determination of gross α activities.[6] Pulse shape analysis (PSA) allows identification of the particle which caused it and enables simultaneous recording of pure α and β spectra and counting of very small α activity in the presence of high β activity.

Experimental

Three strains of *E. coli* (further called C1= C3010, C3= C3016, and C4= C2566) used for protein expression (T7 Express Sampler, New England, BioLabs) were used in our experiments.

A rich medium culture, prepared by dissolving 20 g LB powder (Roth) in 1 L distilled water. The medium was sterilized for 30 min in an autoclave.

Precultures of *E. coli* were incubated at 37 °C for 9 h with continuous stirring (100 rpm), in liquid LB medium. Six samples of 1 mL of cell preculture (OD 580= 0.5) were inoculated into 50 mL test tubes containg each 20 mL of medium and 0.5 g uranium ore, as described in,[7] then incubated for another 24 h with continuous stirring (100 rpm). This time allows the cells to attain a stationary state (in 16-20h), after which their density is decreasing. The cells were pelleted by centrifugation at 5000 rpm for 10 min and the supernatant was used for further measurements.

In order to assess the content of U(VI) we preferred using the Liquid Scintillation Counting technique, in order to avoid some disadvantages of photometric methods, like colour quenching and the heavy metals content of the solution.[8] Activity measurements were performed with a Wallac Perkin-Elmer low background liquid scintillation counting (LSC) spectrometer (Quantulus 1220), which combine low-level LSC with pulse shape analysis (PSA). The samples are prepared in 20 mL polyethylene vials. The volume is optimized at 12 mL UltimaGold LLM cocktail (Perkin Elmer) and 8 mL active sample, by determining the minimum background count rate of α particles.

An aqueous solution of uranyl nitrate (Merck) was used as a standard for the α measurements. The amount of uranium dissoluted by the buffer

itself, reffered as the control sample, was subtracted from the obtained values. The substraction results represents the amount of uranium which was dissoluted by *E. coli* oxidative activity.

As a first step, the PSA level has to be optimized. The optimum PSA level is a numeric parameter that is represented by a line, the angle of which is user controlled, divides the dual plane into short (β) and long (α) pulse categories. When dividing line (PSA setting) is correctly set, counts from these two categories are stored as two separate spectra. Measurements at different values of PSA, in a range from 40 to 160, were done for two samples containing an α and a β emitter. The α/ β interference was calculated as the spillover levels of α and β counting and is represented in Figure 1 versus the PS level. The optimized PSA level, which determines the best α/ β separation of the pulses, is located at the interesection of the spill over levels.[9] We found out that the optimized value is PSA= 120.

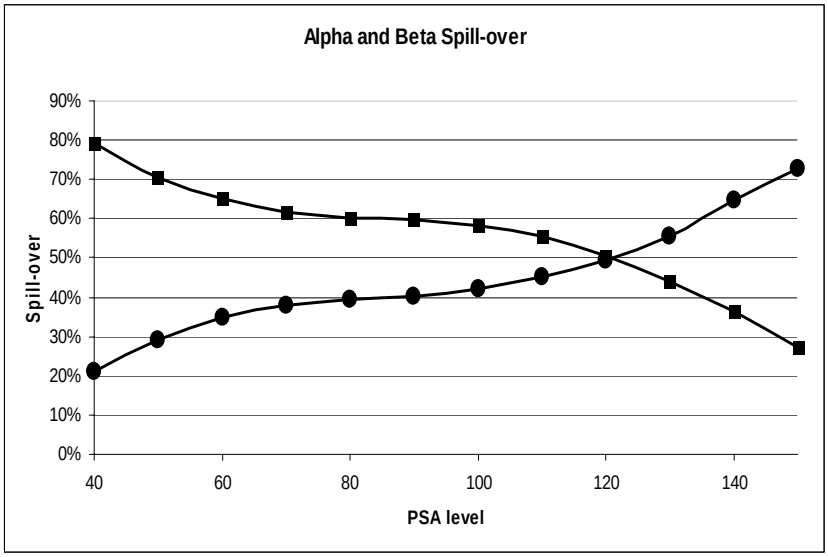

Figure 1. α and β Spill-over for different values of the Pulse Shape Analysis (PSA) parameter; the optimized value corresponds to a 50 % spill-over

Results and discussions

Ten sets of measurements, 30 min each, are registered for every sample, in order to statistically analyze the results. The registered spectra (Figure 2) are analyzed using an EasyView software, which allows determining the activity of each sample.

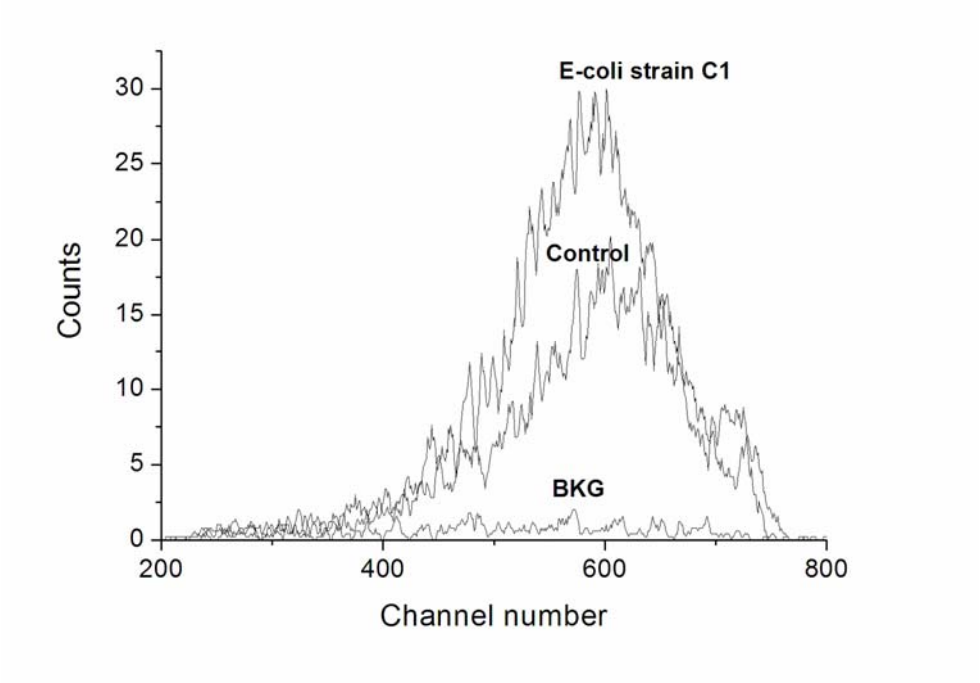

Figure 2. Example of spectrum for strain C1, control and background samples.

Then, the amount of uranium oxide, expressed as number of oxidized uranium nuclei for every set of samples, is calculated using the measured values of activity (Table 1). Results show moderate ability of U(IV) to U(VI) oxidation which depends on the nature of the *E. coli* strain.

Table 1. Experimental results for strains activity, U(IV) nuclei number and oxidation ratio U(VI)/ U(IV).

Results	Strain C1	Strain C3	Strain C4
Activity, Bq	0.04225	0.0337	0.04203
U(VI), nuclei	$2.25 \cdot 10^{16}$	$1.79 \cdot 10^{16}$	$2.24 \cdot 10^{16}$
Oxidation ratio U(VI)/ U(IV), 1‰	0.91	0.74	0.93

The discrimination of α and β pulses is based on the well-known difference between the delayed components of their fluorescence quenching. Quantulus provides superior α/ β discrimination counting, especially for samples containing mixed α/ β/ γ emitters, based on the amplitude independent classification of pulses by their length. The spectrometer incorporates two dual programmable Multi Channel Analyzers which enables simultaneous measurement of four spectra. Setting the PSA at an appropriate level, it is possible to route α events into one half of the MCA (SP12) and β events into other half (SP11). The method becomes attractive for the measurement of α-emitters such as radium, uranium [10], or both.

Conclusions

The present study has shown the moderate ability of *E. coli* in dissolution/ oxidation of U(IV) from a uranium ore to U(VI). Because of the very low activity of the measured samples, as well as the interferences of the uranium daughters in the radiometric system, a Quantulus 1220 α/ β ultra-low level spectrometer was successfully used. The proposed method is must cheaper than the usual instrumental (mass spectrometry) and faster

than the chemical (complexation with arsenazo III and further spectroscopic analysis) ones.

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