

Uranium Reduction in Mine Water via Glycerol Biostimulation: Spectroscopic Evidence of U(IV) Formation

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Abstract

Uranium (U) contamination in mine water remains a challenge despite conventional remediation efforts. This study explores the biostimulation of the indigenous microbial community in Schlema-Alberoda mine water using glycerol, vanillic acid, and gluconic acid as electron donors to promote microbial U(VI) reduction. Glycerol demonstrated the highest efficiency, leading to a near-complete decrease in U concentration in the supernatant. Cryo-TRLFS and UV/Vis analyses confirmed the reduction of U(VI) to U(IV) through glycerol. The results highlight the potential of biostimulation for U remediation and its viability as an efficient and sustainable strategy for low-level U contaminated mine water.

Keywords: Uranium, mine water, bioremediation, spectroscopy, tetravalent uranium

Introduction

Anthropogenic activities have left radionuclide-contaminated environments worldwide and uranium (U) contamination from mining activities poses serious environmental and human health risks. The Schlema-Alberoda mine (WISMUT GmbH, Saxony, Germany) was a major U mining site until the 1990s. After mining ceased, conventional remediation efforts were applied to mitigate U contamination in mine water (Newman-Portela et al. 2024). However, U remains in the mine water at 1 mg/L. In aqueous environments U exists mainly as soluble U(VI) and less soluble U(IV). Under oxidizing conditions, U(VI) forms uranyl (UO_{2}^{2+}) complexes, enhancing mobility, whereas under reducing conditions, U(IV) precipitates as uraninite (UO_2) , limiting its transport.

Over the past three decades, bioremediation has emerged as a cost-effective and efficient strategy for addressing U conta-

mination (Banala et al. 2021). Its success relies on biogeochemical processes mediated by microorganisms, which can alter U solubility through different biochemical interactions. The primary approaches for U bioremediation involve biomineralization in oxic environments and enzymatic reduction under anoxic conditions (Banala et al. 2021). Additionally, microbial enzymatic U reduction can be enhanced by the addition of electron donors, accelerating environmental detoxification and improving the overall bioremediation process (Newsome et al. 2014). Therefore, bioremediation presents itself as a viable, sustainable, and scalable approach, offering an effective microbiological technology to complement conventional remediation techniques.

Schlema-Alberoda mine water has a circumneutral pH and redox potential that favor U mobility. It contains high sulfate and iron concentrations, with a diverse microbial



community linked to carbon, sulfur, iron, and nitrogen cycles, which may play a role in U cycling. In this study, we investigate the potential of the indigenous microbial community in Schlema-Alberoda to reduce U in mine water contaminated with low U concentrations (1 mg/L) by stimulating its activity with different electron donors (gluconic acid, vanillic acid, and glycerol) in various experiments. Additionally, we assess the effectiveness of glycerol as an electron donor for biostimulation using a spectroscopic approach. The results demonstrate that glycerol is highly effective in stimulating the native microbial community, leading to the near-complete removal of soluble U(VI) and the formation of U(IV).

Methods

The chemical composition of the mine water from a former U mine in Saxony (Germany) was determined by inductively coupled plasma-mass spectrometry (ICP-MS) and high-performance ionic chromatography (HPIC). Microcosms (1 L serum bottles) were prepared with fresh mine water (1 mg/L U) and amended separately with 10 mM glycerol, 10 mM gluconic acid, or 10 mM vanillic acid as electron donors. Additionally, abiotic controls were prepared using sterile mine water from Schlema-Alberoda amended with 10 mM glycerol, vanillic acid, and gluconic acid to evaluate whether these electron donors influenced mine water chemistry. The microcosms were incubated at 28 ± 2 °C for four months in darkness. Redox potential (E_b) and pH were measured, and aliquots were taken to determine U, Fe, As, and SO_4^{2-} concentrations using ICP-MS and HPIC. At the end of the experiment, a black precipitate was observed at the bottom of the glycerolamended microcosm.

To further investigate the black precipitate using a spectroscopic approach, a new set of biostimulated anaerobic microcosms was established. These microcosms contained Schlema-Alberoda mine water (1 mg/L U) supplemented with 10 mM glycerol. They were incubated at 28 ± 2 °C for four months in darkness. U concentrations were monitored at the end of the experiment using ICP-MS. A microcosm was terminated when the soluble U concentration decreased by 20%, 60%, or 90%. U(VI) in the aqueous phase of the microcosm was investigated using cryo-timeresolved laser fluorescence spectroscopy (cryo-TRLFS). Samples for cryo-TRLFS were collected in single-use 2 mL plastic cuvettes, and measurements were performed under cryogenic conditions using a Nd:YAG pulsed laser system, and luminescence spectra in the wavelength range of 350 to 650 nm were recorded with an iHR550 spectrograph and an amplified CCD-camera system. Ultraviolet-visible (UV/Vis) spectroscopy was used to detect the formation of U(IV) in the black precipitate formed at the bottom of the microcosms during the experiment. Samples were prepared by dissolving the black precipitate in 5 M HCl, and the spectral range between 500 and 750 nm was captured by the Cary 5G UV/Vis-NIR spectrophotometer. An aquo ion U(IV) solution of 100 µM in 5 M HCl was used as the reference spectrum.

Results and discussion

The biostimulation experiments showed a notable decrease of soluble U(VI) in Schlema-Alberoda mine water. The pH values ranged from neutral to slightly basic values (7.00 to 8.01). However, adding glycerol, the E_h of the mine water showed a sharp drop, from 445 mV to -246 mV. Among the tested electron donors, glycerol showed the highest efficiency, achieving nearly complete U(VI) removal (99%), whereas vanillic acid led to a reduction of approximately 90% (Tab. 1). However, in the gluconic acid microcosm, no decrease in U(VI) concentration was observed. The electron donor may promote the growth of bacteria involved in glycerol fermentation, leading to the production of organic compounds that can be utilized by sulfate-reducing bacteria (SRB) or ironreducing bacteria (IRB), which play a key role in U reduction. For example, IRB such as Geobacter can utilize acetate as an electron donor for U(VI) reduction (Anderson et al. 2003). Similarly, bacteria such as Desulfovibrio, an SRB also capable of iron reduction, can oxidize glycerol while coupling it to sulfate reduction (Santos et al. 2018; Lovley et al. 1993). Indirectly, the sulfide produced in this process could facilitate the indirect reduction

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of U(VI) to U(IV) (Boonchayaanant *et al.* 2010). However, further studies on the microbial community are needed to identify the bacterial genera involved in this process.

On the other hand, no notable changes were observed in the abiotic controls. This is because vanillic acid and gluconic acid do not form stable complexes with U(VI) under the given physicochemical conditions, as thermodynamic speciation calculations reported by Newman-Portela *et al.* (2024) predict no coordination between these compounds and U. In the case of glycerol, there were no thermodynamic data for complexation with U(VI). However, no formation of U(VI)-glycerol complexes was expected since glycerol exhibits only three hydroxyl groups that do not deprotonate in aqueous solutions (Newman-Portela *et al.* 2024).

Cryo-TRLFS analysis (Fig. 1) reveals distinct fluorescence bands at 480, 501, 519, 542, and 566 nm, matching the spectral features previously identified for $Ca_2UO_2(CO_3)_3(aq)$ (Newman-Portela *et al.* 2024). The UV/Vis spectra (Fig. 2) show a well-defined peak at 650 nm, which aligns with the characteristic signal of U(IV) when compared to the reference. Additionally, a peak at 560 nm is observed, consistent with prior reports of U(IV) formation (Gao and Francis 2008). A separate peak between 675 nm and 700 nm suggests potential U(IV) interaction with chloride, likely influenced by the elevated HCl concentration used during sample preparation (Tutschku et al. 2003).

Overall, the UV/Vis spectra provide clear evidence of U(IV) formation, with its bands becoming more pronounced as soluble U(VI) decreases in the supernatant (Fig. 2). In contrast, the cryo-TRLFS spectra exhibit a reduction in fluorescence intensity as soluble U(VI) decreases (Fig. 1). These spectral changes clearly indicate a progressive transformation of U(VI) to U(IV) under biostimulated conditions using glycerol as electron donor, supporting the effectiveness of the selected electron donors in promoting microbial U(VI) reduction to U(IV). The observed shifts in spectral features are consistent with previous studies on U speciation, reinforcing the role of microbial activity in controlling U mobility. Further investigations using advanced spectroscopic and microscopic techniques, along with microbial community analyses, could offer a more comprehensive understanding of the mechanisms governing U bioreduction in mine water environments.

Conclusions

This study demonstrates the effectiveness of biostimulation in promoting U(VI) reduction in Schlema-Alberoda mine water. Among the tested electron donors, glycerol was the most efficient, leading to a near-complete removal of soluble U(VI) and the formation of U(IV). The sharp decrease in redox potential upon glycerol addition suggests the stimulation of anaerobic microbial activity, likely involving sulfate- and iron-reducing bacteria. The UV/ Vis and cryo-TRLFS analyses confirm the

| electron don | stron donor biostimulation (glycerol, vanillic acid and gluconic acid). Data presented in mg/L and E_h in m | | | | | | |
|--------------|---|----------|---------------|---------------|--|--|--|
| | Schlema-Alberoda mine water | Glycerol | Vanillic Acid | Gluconic Acid | | | |
| | 7.22 | 7.00 | 7.00 | 0.01 | | | |

| Table 1 Geochemical parameters observed in the | original mine water and | d at the end of the expe | eriment after the |
|---|-------------------------|--------------------------|-------------------------|
| electron donor biostimulation (glycerol, vanillic a | cid and gluconic acid). | Data presented in mg | L and E_h in mV . |

| рН | 7.32 | 7.99 | 7.00 | 8.01 |
|------------------|------|------|------|------|
| E _h | 445 | -246 | 218 | -248 |
| Fe | 0.99 | 0.05 | 0.32 | 0.35 |
| As | 0.92 | 0.46 | 0.40 | 0.17 |
| U | 1.05 | 0.01 | 0.10 | 1.37 |
| SO4 ² | 335 | 141 | 338 | 144 |



Figure 1 Cryo-TRLFS spectra of the aqueous phase (supernatant) at different stages of uranium(VI) reduction in glycerol-amended microcosms. The sample with no U decrease in the supernatant is shown in black, while samples with 20%, 60%, and 90% uranium decrease are represented in red, blue, and green, respectively.



Figure 2 UV/Vis absorption spectra of the black precipitate samples with different levels of uranium decrease at the supernatant in glycerol-amended microcosms. The U(IV) reference is shown in black, while samples with 20%, 60%, and 90% uranium decrease are represented in green, red, and blue, respectively.

reduction of U(VI) to U(IV), highlighting the role of microbial processes in controlling U mobility. The findings emphasize the potential of bioremediation as a complementary strategy to conventional remediation techniques for low-level U-contaminated environments. However, further studies on the microbial community composition and metabolic pathways are necessary to refine biostimulation strategies and optimize U immobilization. Additionally, the long-term stability of reduced U(IV) should be evaluated to ensure its environmental persistence and effectiveness as a remediation approach.

Acknowledgements

WISMUT GmbH (Germany) is gratefully acknowledged by the authors for providing the case study, sharing relevant remediation site information, and technical support during the sampling campaigns. Furthermore, the authors express their appreciation to the staff at Helmholtz-Zentrum Dresden-Rossendorf (Germany), especially Sabrina Beutner and Sylvia Schöne for analytical measurements. This research was funded by the RadoNorm project under the Euratom research and training programme 2019-2020 (grant agreement No 900009). Additional support was provided by the mobility grants programme of the European Radioecology Alliance (ALLIANCE).

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