Investigation of Microbial In-Situ Remediation of Uranium Mine Site Pollutants in the Flooded Mine Königstein

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ABSTRACT

Mining activities in the former uranium mine Königstein left acidic, highly oxidized drainage waters with pollutant concentrations (e.g. uranium) of several milligrams per litre. In the current process of mine flooding, pump and treat prevents pollution spreading. Since acid mine drainage will be generated over long periods of time, possibilities of in-situ pollutant retention within the pollutant plume are under investigation as cost saving alternative to decades of pump and treat.

A novel reactive zone technology for retention of inorganic mine site pollutants based on the stimulation of autochthonous sulphate reducing bacteria and a sequence of mineral forming processes was developed. The key process is the microbial mediated precipitation of iron sulphides, which bind pollutants and act as redox buffers under gradual transformation of their surface bound iron oxy(hydr)oxides when exposed to oxygen containing groundwater.

The stimulation of autochthonous sulphate reducing bacteria, whose existence in the mine sediments was previously shown, was tested in column experiments using a novel reactive material. It consists of silicate foam glass and zero-valent iron powder. In contact with AMD, the reactive material emits molecular hydrogen and serves as pH-buffer. Microbial colonization, precipitation of pyrite and other mineral species and pollutant accumulation to several weight percent (e.g. 1 to 3 wt.% for uranium, arsenic and zinc) were analysed at the surface of the reactive material after contact with mine waters and sediments for about one year. At redox transition to oxic conditions, iron oxyhydroxide crystallization was observed as coatings around reduced mineral formations ensuring a long term stable pollutant immobilization. Effluent pollutant concentrations during the column experiments remained beneath 150 μg/l.

Keywords: bioremediation, microbial sulphate reduction, biofilm, pollutant bonds

INTRODUCTION

From 1946 to 1990, SDAG WISMUT in former East Germany was the major foreign uranium supplier to the Soviet Union. During that period, around 215,000 tonnes of uranium were produced from more than 20 deposits. The Königstein deposit provided around 10 % of the total amount. Around 6,000 tonnes of U from the Königstein deposit were mined by acidic in-situ leaching. Mining activities caused primary uranium ores as well as sulphidic co-mineralisation to oxidize and shift to water soluble forms. The mine's inventory of mobile uranium prior to flooding was estimated 2,000 tonnes.

The Königstein mine is situated in the deepest of four sandstone aquifers. Groundwater would seep through the mine at natural flow conditions. At mine flooding, pump-and-treat prevents pollutant spreading into adjacent aquifers. Within the first fifteen years of flooding, 25 – 50 tonnes of uranium were discharged from the mine every year. Presuming constant mass flow rates, removal of the estimated amount of mobile uranium would last 40 to 80 years. Declining pollutant concentration with time will even prolong the period of potential pollution of adjacent aquifers.

In order to find cost saving alternatives to decades of pump and treat, the in-situ retention of mine site pollutants within a reactive zone was investigated. The aimed key process within the reactive zones is reductive precipitation of pollutants and thus their transfer back to water insoluble forms.

METHODOLOGY

For initiation of reductive pollutant precipitation, reducing geochemical conditions and a surplus of sulphide ions is required. Therefore, microbial sulphate reduction by autochthonous SRB is to be stimulated within the reactive zone. Microbial screening of mine drainage water, sandstone and iron hydroxide sludge samples was carried out to assess the presence of autochthonous SRB. DNA extractions were performed and 16S rDNA clone libraries were constructed [Seifert et al, 2008].

Batch tests were used to stimulate and quantify microbial sulphate reduction and to identify pollutant fixation at sulphate reducing conditions. Mine drainage water (1.07 l) was added to a mixture of milled sandstone (50 g) and milled iron hydroxide sludge (17.5 g) in gas tight glass bottles. Stimulation of SRB was performed by addition of ZVI (3 g cast iron powder) releasing H_2 as substrate for autotrophic microbes and iron ions to stimulate the formation of reduced pollutant precipitates. The headspace of the test bottles was first flushed with N_2 and later with a mixture of H_2/CO_2 after each water sampling. Tests were conducted for 240 d. Sulphate reduction rates were obtained from $^{32/34}S$ isotope analysis at dissolved sulphate ions (Helmholtz Centre for Environmental Research). Pollutant fixation was investigated by ICP-OES analysis of mine water solutes and XPS (X-ray photoelectron spectroscopy) analysis of pollutant coordination within the sediment (SGS Institute Fresenius).

To induce autotrophic sulphate reduction and pollutant precipitation within the flooded mine, a reactive material was developed. It consists of silica covered foam glass. The silica cover contains cast iron powder. The granules (1-3 cm diameter) float at mine typical pressures. They provide substrates that favour microbial growth and mineral precipitation. The reaction sequence of microbial sulphate reduction and mineral precipitation induced by the reactive material was investigated in column experiments. 25 kg of sandstone (< 3 mm), 4.5 kg of iron hydroxide sludge and 2.4 kg of reactive material were place in horizontal glass columns equipped with water in- and outlets as well as sample ports for water, gas and solids. The columns were filled with 8 l of mine water and exposed to continuous mine water flow-through at ~250 ml / d for 250 days. Pollutant fixation was investigated by

ICP-OES analysis of mine water solutes and REM-EDX analysis (TU Bergakademie Freiberg) of reactive material surfaces. For identification of pollutant bonds, sequential extractions were used [Graupner et al., 2007 for method description]. Microbial colonization was investigated by DNA extraction and classification using the clone bank constructed. Furthermore, $^{32/34}$S isotope analysis of dissolved sulphate ions (Helmholtz Centre for Environmental Research) and GC-analysis of dissolved permanent gases were performed.

RESULTS AND DISCUSSION

Microbial screening

The mine drainage water was dominated by acidophilic iron oxidizing *Betaproteobacteria* (abundance of sequences related to *Ferribacter polymyxa*; second major sequence group with similarities to *Acidithiobacillus ferroxidans*), and heterotrophic acidophiles in minor parts.

A dominance of the sulfate-reducing bacteria *Desulfovibrio* (ca. 70%) in association with other members of the *Deltaproteobacteria*, members of the *Alpha-*, *Beta-* and *Gammaproteobacteria*, *Actinobacteria,* and *Firmicutes* was obtained for the analysed iron hydroxide sludge sample. The sandstone sample revealed a high bacterial diversity. About 20% of the analysed clones could be classified as sulphate-reducing bacteria, others were similar to aerobic and anaerobic bacteria with various physiological properties.

Fig. 1 depicts the frequency of the bacterial classes obtained by sequence analyses of clone libraries constructed with DNA of the sludge and the sandstone sample. The phylogenetic tree (Fig. 2) shows the distribution of sequenced clones belonging to the classes of *Firmicutes* and *Deltaproteobacteria* from the two clone libraries. Both harbor genera described as sulphate, sulphite, thiosulphate, and sulphur reducing bacteria. The obtained clones were used to create a fragment library for T-RFLP. This database was used to investigate further samples from the mine site, in which sulfate-reducing bacteria, like *Desulfovibrio* and *Desulfosporosinus* were detected [Seifert et al., 2008].

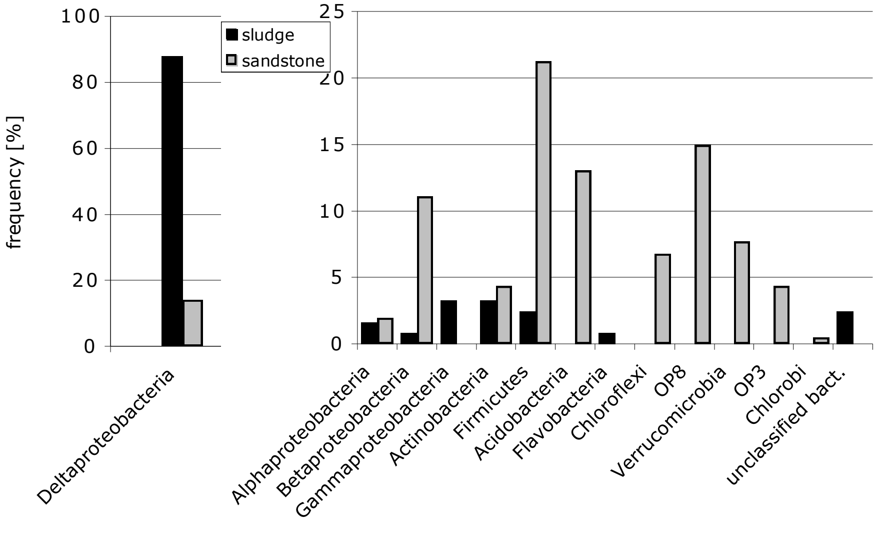

Figure 1 Frequency of bacterial classes obtained from DNA extracted from mine sandstone and mine iron hydroxide sludge [Seifert et al, 2008]

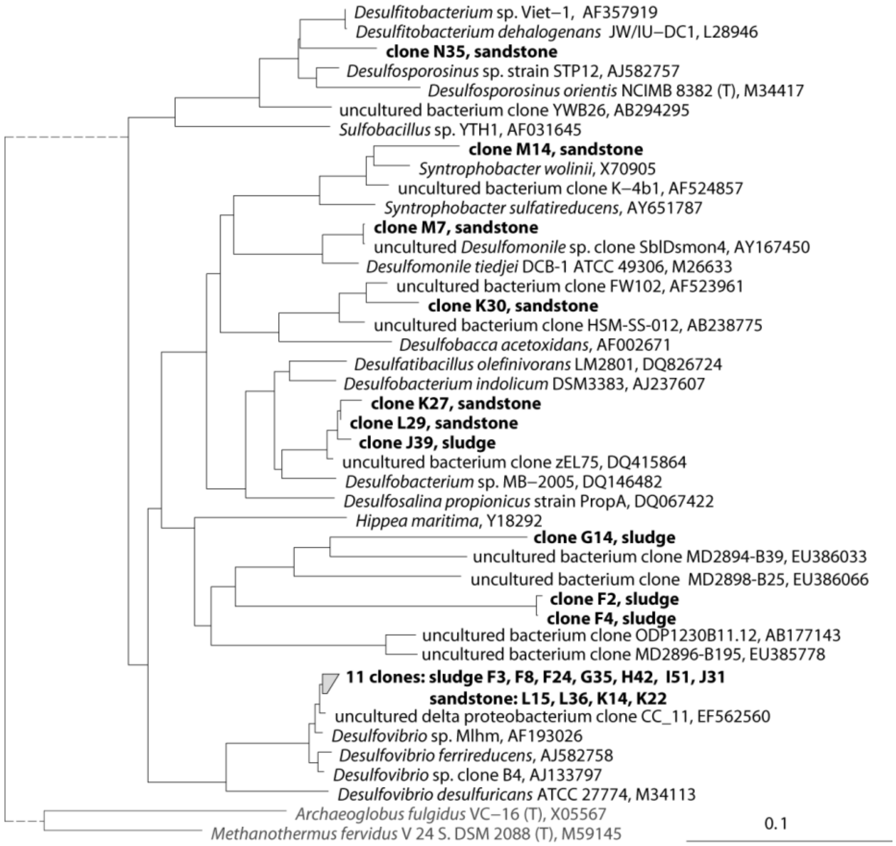

Figure 2 Phylogenetic tree of 16S rDNA sequences of *Deltaproteobacteria* and *Firmicutes* from the sludge and the sandstone sample (the sequence group within the *Desulfovibrionales* branch consists of 11 sequences from both samples; Seifert et al, 2008)

Batch tests

Tab. 1 contains the average mine water composition used for pollutant removal batch tests.

Table 1 Selected chemical parameter of mine water

pH	E_H [mV]	EC [mS/cm]	SO_4 [mg/l]	Fe [mg/l]	Ca [mg/l]	U [mg/l]	Zn [mg/l]	Ni [mg/l]	Pb [mg/l]	Cd [mg/l]	As [mg/l]
2.8	640	3	1,500	120	300	15	15	0.5	0.5	0.1	0.05

Resulting from anaerobic iron corrosion (eq. 1), redox potential decreased to -35 mV after 7 days while H_2 was detected in the bottles headspace and the concentration of iron ions increased by a factor of 3.5. After 35 days, redox potential further dropped to – 120 mV causing pollutant concentrations to fall below 200 µg/l. At the same time, pH increased to > 6.5 (Fig. 3 - upper graph).

$$\mathrm{Fe}^0 + 2\mathrm{H}_2\mathrm{O} \rightarrow \mathrm{Fe}^{2+} + \mathrm{H}_2 + 2\mathrm{OH}^- \tag{1}$$

Sulphate reduction was observed after a lag-phase of about 40 days in all batch tests. At first it was verifiable by a complete blackening of the bottles, which indicates iron sulphide precipitation. Because of excess of ferrous iron, no dissolved sulphide was detectable. Simultaneous decrease of iron and sulphate concentrations was measured from day 40 onwards until the end of the tests (Fig. 3 - lower graph). With decreasing SO_4 concentration in solution, the heavy isotope ^{34}S enriched within the remaining dissolved sulphate (Fig. 4). This serves as indicator of microbial sulphate reduction, since the light isotope ^{32}S is favoured in metabolism of sulphate reducing bacteria. T-RFLP investigations of batch tests aqueous phase (day 130) showed, that the microbial community was dominated by *Desulfovibrio sp.* and *Desulfosporosinus sp.*. Autochthonous sulphate reducing bacteria were stimulated successfully. The microbial sulphate reduction rate was 1-5 $\mathrm{mg} / \mathrm{l} \cdot \mathrm{d}$.

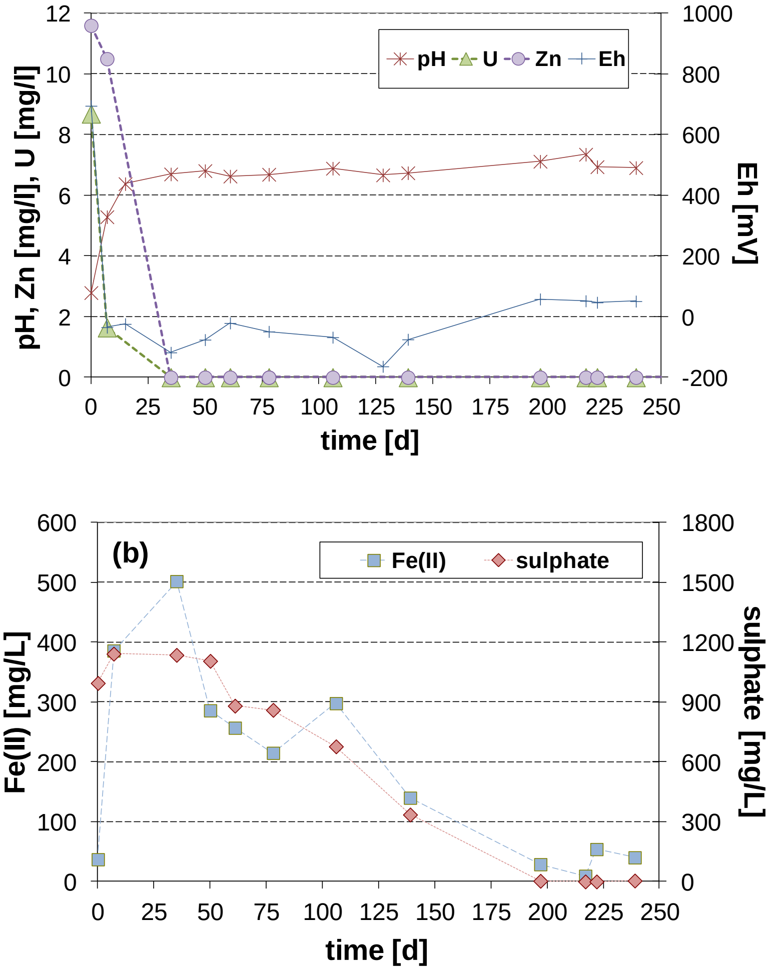

Figure 3 Selected solution parameter (above: pH, E_H, U, Zn; below: Fe, SO_4) during batch tests

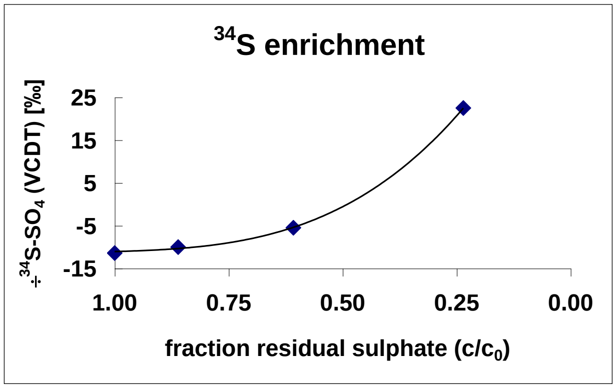

Figure 4 ^{34}S isotope enrichment in the residual dissolved sulphate of the batch test aqueous phases

XPS analysis of the solid phase (day 240) identified sulphur to occur completely reduced in monosulphide (50.9%) and disulphide phases (49.1%). This implies complete sulphate reduction and partly alteration of the initially precipitated iron monosulphide within the time period of the test. Iron was shown to be bond to sulphides (19.9%) and oxides (80.1%). The high percentage of iron oxides is related to the iron hydroxide sludge used in the test systems. Moreover, XPS analyses revealed uranium to be nearly complete immobilized in reduced mineral phases. It occurs predominantly tetravalent (80.1% UO_2) and partially in polysulphide phases (9.1% US_x (x>1)). 10.8% of the sediment bonded uranium remained hexavalent, most probably adsorbed to the iron hydroxide phase. Zn was found in ZnO coordination, which may result from $Zn(OH)_2$ precipitation with subsequent phase transformation. The test results indicate pollutant precipitation and iron sulphide formation as independent processes. Pollutant precipitation occurs prior to iron sulphide formation and is related to changes in pH and pe resulting from anaerobic iron corrosion. Microbial sulphate reduction is stimulated by H_2 evolving at anaerobic iron corrosion. Sulphide precipitates with ferrous iron to iron monosulphide, which in turn transformed partly to iron disulphide.

Column tests

The initial composition of the column feed is summarized in Tab. 2. Selected chemical parameter of the column effluents are depicted as function of test duration in Fig. 5 (above: U and Zn; below: pH, redox potential, dissolved H_2, H_2SiO_3). The pollutant concentrations[1] of the column effluent remained beneath 150 µg/l over the entire test period. Column passage caused a rise in pH from pH=3 to pH>8 and a drop in redox potential from Eh=770 mV to Eh≤250 mV due to immediate silica dissolution at the surface of the reactive material.

Table 2 Initial concentrations of column feed

pH	E_H [mV]	EC [mS/cm]	SO_4 [mg/l]	Fe [mg/l]	Ca [mg/l]	U [mg/l]	Zn [mg/l]	Pb [mg/l]	As [mg/l]	H_2SiO_3 [mg/l]	TIC [mg/l]
2.9	770	1.5	625	65	110	8.9	6.7	0.5	0.03	25	3

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[1] also for Pb, As, Cd and Ni (not depicted in Fig. 5)

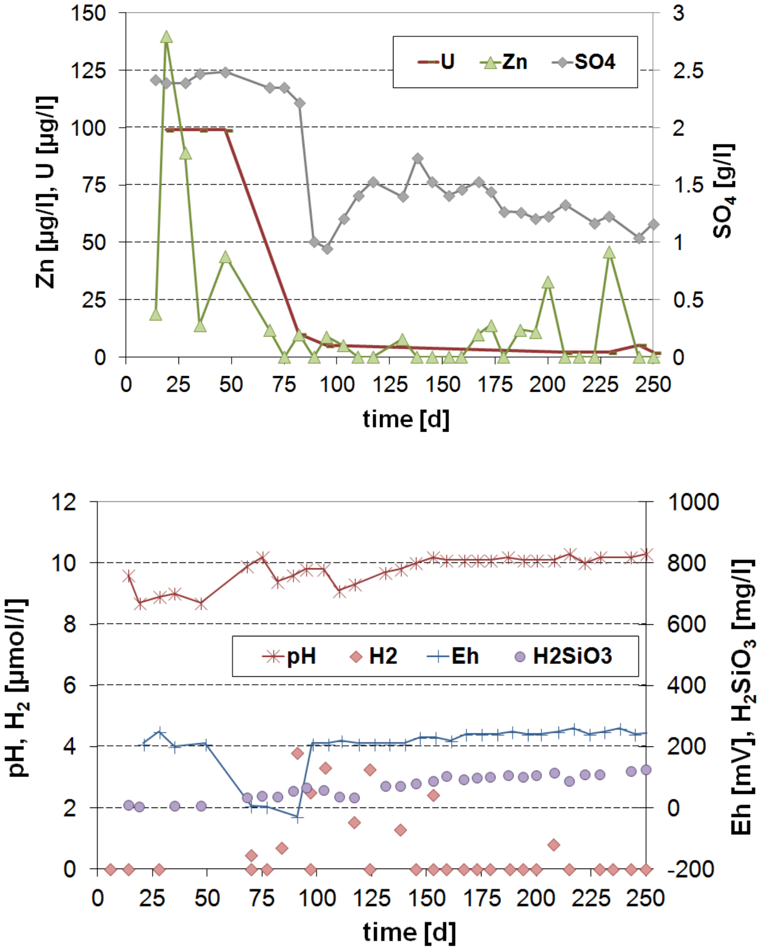

Figure 5 Selected parameter of column effluent (above: U, Zn, SO$_4$; below: pH, E$_H$, H$_2$, H$_2$SiO$_3$)

Around 70 days after the experiment started, anaerobic oxidation of the silica embedded ZVI set in as indicated by H$_2$ formation and redox potential decrease. At the same time, dissolved silica concentrations of the column effluent increased (Fig. 5 - lower graph). Dissolution of reactive material silica coatings led to pH buffering and exposure of the embedded ZVI. H$_2$ was detectable in water and gas samples sporadically until the end of the experiments (gas phase data not shown). Due to high pH, mine water iron precipitated within the column immediately and the effluent did not contain any dissolved iron. At the beginning of the experiments, sulphate concentrations multiplied by factor 4 during column passage because of sulphate leaching from the rock material (Fig. 5 - upper graph). At day 90, effluent SO$_4$ concentrations declined sharply, while anaerobic iron oxidation and H$_2$ supply was ongoing already. Because SO$_4$ feed concentrations stayed constant (data not shown) and microbial sulphate reduction counts for a maximum concentration decrease of 160 mg/l only (32 days residence time within the column; 5 mg/ l · d maximum SO$_4$ reduction rate), sulphate leaching rates and mineral reactions affect the effluent sulphate concentrations significantly. This prevents clear evidence for microbial sulphate reduction from concentration decrease and ^{34}S enrichment in the residual sulphate. ^{34}S isotope data of the effluent sulphate show only a slight enrichment of the heavy isotope (Fig. 6).

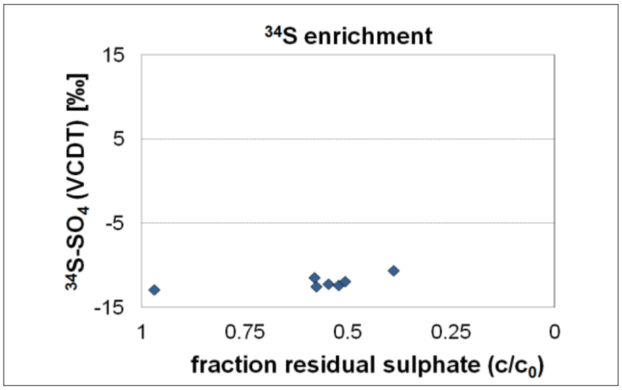

Figure 6 ^{34}S isotope enrichment in the residual dissolved sulphate of the column test effluent

The presence of sulphate reducing bacteria in the column effluent at the end of the experiment was confirmed by T-RFLP investigations. 35 % of the identified microbial DNA was assigned to sulphate reducers. Iron and sulphur oxidizing bacteria and nitrogen fixing bacteria were also identified. Next to species which were detected before in mine water and sandstone samples, the column effluent also contained typical soil bacteria (*Bosea thiooxidans sp.*, *Solirubrobacter soli sp.*).

After the experiment, the reactive material was sampled from the column for investigation of pollutant content and mineralization. Fig. 7 shows REM photographs of the reactive material surfaces, which were found to be extensively colonized by microbes (A). Within the biofilm, pollutant enrichments were identified by EDX-analysis. The bright particles (B - strong backscattering in BSE mode of heavy elements) contain U, Pb, Fe, S and C and are interpreted as sulphidic and reduced mineralization. The smaller spheres are iron (hydr)oxides (C). Their EDX spectra contain U, As, Fe, C and lack S. The coexistence of reduced and oxidized minerals is possible due to their embedding in the biofilm matrix which allows for redox niches.

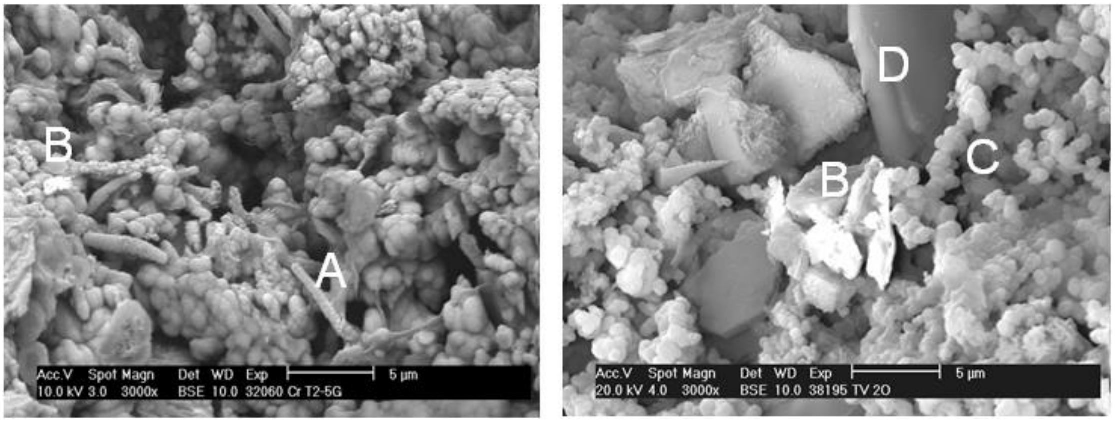

Figure 7 REM photographs of reactive material surfaces after the experiment (left: biofilm remnants and microbial structures with cell wall mineralisation (A) and heavy element accumulations (light coloured particle B); right: heavy element accumulation (light coloured particles B), iron (hyr)oxides spheres (C) and silica sheets (D)

The coexistence of oxidized and reduced minerals also occurred in a larger scale. The left photograph in Fig. 8 shows a glass column segment after the experiment with blackening of iron hydroxide sludge (orange) and sandstone dust (grey) in contact with reactive material. The right photograph shows the alteration of the reactive material (originally grey granules), on which mineral coatings precipitated (orange and blackish-red granules). Sequential extractions of the blackish-red coating revealed the highest pollutant content within the reduced mineral fraction, followed by the fraction content of amorphous iron hydroxides and biofilm organic substances (Fig. 8 – upper bar chart). In contrast, most iron is bond to amorphous iron hydroxides, followed by biofilm organic substances (Fig. 8 - lower bar chart). The reduced phases contain ~1 wt% iron, which equals 1.6 wt% iron monosulphide or 2.15 wt% iron disulphide, respectively.

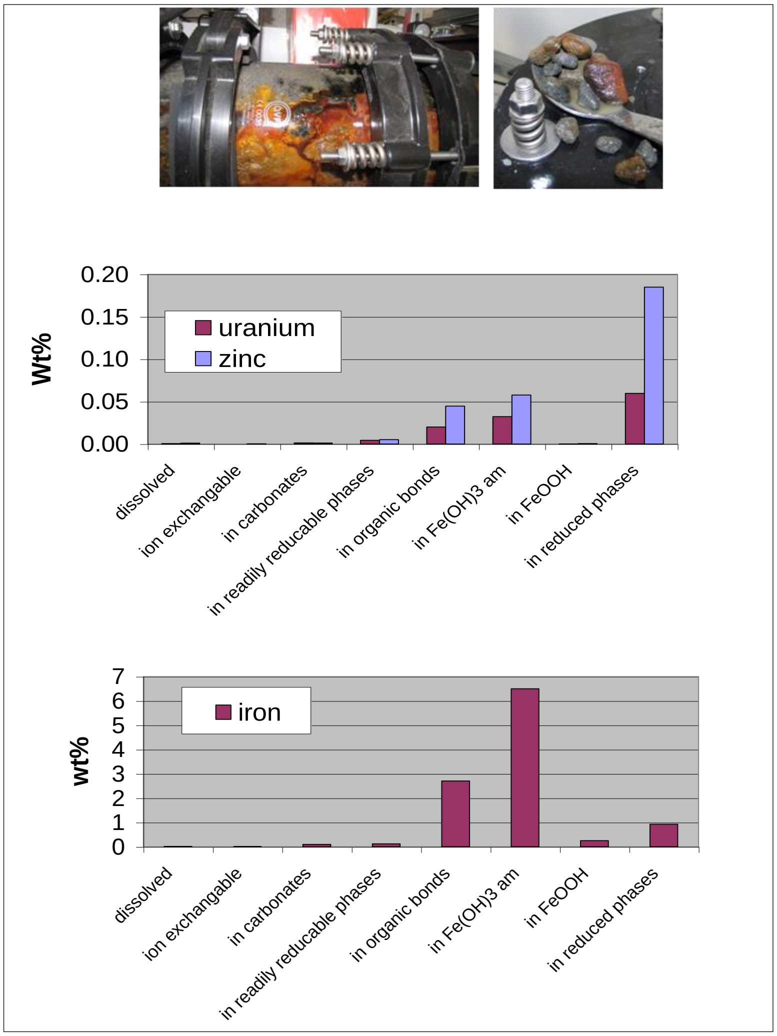

Figure 8 Photographs of glass column segment and reactive material after the experiment and bar charts on uranium, zinc (upper graph) and iron contents (lower graph) in different binding forms as extracted from the blackish-red reactive material coating after the experiment (sequential extraction)

CONCLUSION

The flooded mine Königstein hosts a diverse autochthonous microbial biocenosis. Its autotrophic growth can be stimulated by anaerobic iron corrosion products (OH^-, H_2). At anaerobic oxidation of cast iron powder in mine water, autochthonous sulphate reducing bacteria form sulphide ions at rates of 1 – 5 mg / l · d. The presence of sulphide facilitates pollutant immobilization by direct precipitation (e.g. lead, zinc, uranium) and by iron monosulphide formation, which in turn transforms to iron disulphide. Iron disulphide surface reactions reduce oxidized solutes and cause pollutant reduction comparable to ZVI (e.g. for uranium). Pollutant immobilization from acid mine water in contact with ZVI depends on sorption and reduction at ZVI surfaces, sulphidic precipitation, sorption and reduction at iron sulphides and its ferrous iron hydroxide surface layers as well as on redox buffering. The latter is based on the oxidant scavenger effect of elemental and ferrous iron oxidation with subsequent ferric iron hydroxide formation. If ferric hydroxide formation occurs in close vicinity to the reduced minerals, it may form coatings that protect them from re-oxidation. That is of special importance for preventing future pollutant remobilization in contact with dissolved oxygen upon completion of microbial activity stimulation.

A reactive material designed to carry ZVI, microbes and precipitates by floating in mine water is suited for in-situ-application of ZVI induced pollutant retention. Comparable to cast iron powder, the reactive material is able to emit H_2, to stimulate autochthonous sulphate reducers, to buffer pH and to induce pollutant and iron sulphide precipitation. Moreover, the material provides dissolved silica and serves as microbial growth support and biofilm carrier. This facilitates sustainable pollutant retention considerably. Biomolecules promote mineral formation by serving as precipitation templates. Biofilm matrices provide niches that allow for different redox conditions and thus for coexistence of reduced and oxidized minerals in very close spatial vicinity. Incorporation of silica in mineral precipitates decreases their water solubility. If exposed to mine water flow, mineral coatings form on the reactive material. They contain both reduced and oxidized pollutant bonds. Their iron hydroxide coatings encapsulate mine water pollutants also under oxidizing conditions.

Based on the investigation of underlying processes, microbial in-situ remediation seems feasible to inhibit the discharge of uranium mine site pollutants from the flooded mine Königstein.

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