Mine Water Tracer Substances for Biogeochemical Processes in Flooded Uranium Mines

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Abstract

Analysis of redox-sensitive ions, dissolved gases, sulphate isotopes, arsenic species, organic compounds and microbial DNA in four flooded uranium mines revealed the presence of highly diverse and metabolizing microbial communities comprising different species of bacterial, archaeal and fungal domains. Microbial sulphate reduction and methanogenesis were evident from both chemical and microbial analysis. A whole suite of other microbial metabolic activities was deduced from the presence of certain pollutant species, organic compounds and established metabolic pathways of the analysed microbial community. Biochemical processes are relevant for the water quality of the investigated mines.

Keywords: mine water microbial community, microbial metabolism, mine water quality

Introduction

After East German uranium industry was decommissioned in 1990, an unprecedented close-out and remediation programme was launched immediately thereafter. The programme is run by Wismut GmbH, a governmentally owned remediation enterprise. In 1990, the operation of five underground uranium mines was stopped. Mine flooding started between 1992 and 2001 and was completed more than one decade ago, except for one mine. Mine water concentrations of uranium, arsenic, 226-radium, metals and salts exceed regulatory discharge limits and require active water treatment. According to current estimates, water treatment is expected to continue beyond 2040. Within the first decade of flooding, dilution of dissolved pollutant inventory has been the determining process for mine water quality. As flooding proceeds, concentration curves of several pollutants deviate from an ideal exponential decline behavior. Site specific, both rapid pollutant concentration declines and permanent stable pollutant concentration levels were observed in different mine waters. Biogeochemical reactions within the flooded uranium mines might be involved in these effects. For example, microbial uranium reduction can cause rapid drops in uranium concentrations. Likewise, stable pollutant concentrations can relate to microbial pollutant mobilization from primary and secondary minerals within the flooded mines. In order to identify the relevance of biogeochemical processes for mine water quality and to deduce consequences for water treatment operations, Wismut's flooded uranium mines were analysed for microbial mine water tracer substances.

Methods

Mine water sampling was performed in several sampling campaigns during 2013 – 2017. Four mines were sampled at least twice. Two mines are characterized by iron rich mine water with acid to slightly acidic pH (Königstein mine and Ronneburg mine), while mine water from the two other mines are poor in iron, rich in arsenic and of neural pH (Pöhla mine and Schlema mine).

Since microbial mine water tracer substances are sensitive to aeration, special effort was made to realize fast on-site measurement and sample conservation procedures. On-site measurements included probe measurements (pH, redox potential) and photometric measurements for ferrous and sulphide ions. For sample conservation, on-site filtration and chemical stabilization, quick-freezing using dry ice, and filling of deaerated sampling containers

Table 1. Parameter selection, sample conservation and analytical methods.

Parameter	Sample conservation	Analytical method
pH, redox potential	Sample conservation Analysis immediate after sampling	pH / redox measuring apparatus multi 9430; pH probe Sentix 980, redox probe Sentix ORP 900 (WTW) Spectrophotometer photoLab 6600 UV-VIS (WTW);
Ferrous iron, Sulphide ions	Analysis immediate after sampling	Spectroquant test kits for ferrous iron (1 – 5 mg/L) and sulphide (0.02 – 1.5 mg/L) analysis (Merck)
Dissolved gases (CO_2, CH_4, H_2, O_2)	Filling of deaerated sampling containers	GC-TCD after gas phase equilibration (GFI Groundwater Consulting Institute Dresden)
^{34}S, ^{18}O sulphate isotopes	Zinc acetate -stabilization	ES/IRMS and TC/EA after barium sulphate precipitation (Helmholtz Centre for Environmental Research, Halle)
Arsenic species (arsenite, arsenate, monomethyl arsonate, dimethyl arsenate, thioarsenates)	Membrane filtration (0.2 µm), EDTA stabilization and quick-freezing	IC- / HPLC-ICP-MS (GFI Groundwater Consulting Institute Dresden; sub-contractors University of Bayreuth and Medical Laboratory Bremen)
Carbohydrates	Quick-freezing	Spectrophotometric analysis after phenol-sulfuric acid reaction (GFI Groundwater Consulting Institute Dresden)
Trace organic compound screening	Filling of air tight sampling containers	GC-MS after ethyl acetate extraction / MSTFA derivatization; NIST data base identification (GFI Groundwater Consulting Institute Dresden)
Microbial DNA	Membrane filtration (sterile) or ethanol stabilization in sterile sampling containers	DNA fragment Next-Gen sequencing after extraction, amplification and purification; BLAST analysis and classification (contract analysis Blue Biolabs GmbH, Berlin)

were used. Table 1 contains the parameters selected to trace microbial processes in mine water with reference to sample conservation, analytical methods, and contracted labs.

Results and discussion

For overall water quality characterization of the four mines, table 2 shows selected average solute concentrations of the respective mine effluents entering the water treatment plants (average over last five years; Wismut database). Mine water microbial tracer contents are given in table 3. They were analysed at the monitoring points used for routine sampling. Isotopic analysis was performed for Ronneburg mine water only. Therefore, another three mine water monitoring points were sampled additionally. Data are shown in table 4. Table 5 contains results of qualitative screening analysis for mine water trace organic compounds. Table 6 summarizes prevailing bacteria, archaea and fungi in the mines identified from DNA analysis of the sampled mine water.

Provided that oxidation of the mined and accessory minerals (e.g. uraninite, sulphides, arsenides) during mining operation generated the primary pollutant source, oxidized geochemical conditions are expected for the polluted mine water. When mine flooding proceeds beyond first flush, both chemical and microbial reactions might consume oxidizing solutes, cause drops in overall redox potentials and change water speciation. Redox potential data in table 2 and 3 indicate oxidized geochemical conditions for Königstein but oxygen depletion for all other mines. Ferrous dominates the iron speciation in all mines. Besides for Königstein, sulphide ions and dissolved methane were detected in all mine waters. Both solutes trace microbial sulphate reduction and methanogenesis, respectively. Consequentially, sulphate reducers (*Desulfurivibrio* spp.) and methanogens (*Methanoregula*, *Methanomassiliicoccaceae*, *Methanobacterium*) were identified in the microbial population of the three mines (see table 6).

Results of isotopic analysis at dissolved sulphate in Ronneburg provide further evidence for microbial sulphate reduction (see table 4). Microbial sulphate reduction enriches both ^{34}S and ^{18}O isotopes in the remaining dissolved sulphate (Knöller 2004). Figure 1 illustrates a proportional increase in heavy S and O isotopes corresponding to a decrease in sulphate concentration of Ronneburg mine water from different monitoring wells.

Table 2. Average solute concentrations (2013 - 2017) of mine effluents.

Mine site	Königstein (well A & B)	Ronneburg (well 2)	Schlema	Pöhla (mine drainage collector)
pH / E_H [mV]	3 / 640	6.4 / 170	7.1 / 120	7.1 / 84
EC [mS/cm]	2.5	4.4	1.7	0.5
HCO_3 [mg/L]	<5	360	580	330
SO_4 [mg/L]	1,100	2,750	500	<2
Ca [mg/L]	270	465	150	48
Mg [mg/L]	23	420	98	16
Fe [mg/L]	75	165	4.3	4.7
U [mg/L]	10	0.75	1.4	0.013
As [µg/L]	38	204	1,450	1,900

Table 3. Microbial tracer substance concentrations in sampled uranium mine effluents (mean values).

Mine site	Königstein	Ronneburg Probe readings	Schlema	Pöhla
pH / EH [mV]	3 / 650	6.6 / 130	7.3 / 185	7.3 / 110
		Photometric on-site measurements		
Fe^{II} [mg/L]	35	170	3.7	5
total Fe [mg/L]	45	184	4.3	4.6
S^{2-} [µg/L]	< 20	25	38	120
		Dissolved gases		
O2 [mg/L]	5	0.4	1.4	0.2
CO2 [mg/L]	55	125	66	26
CH4 [mg/L]	< LD	0.2	0.3	4
H2 [mg/L]	< LD	0.3	< LD	< LD
		Arsenic species		
total As [µg/L] (WISMUT database)	43	150	1,570	1,850
As (III) [µg/L]	22.4	0.4	806	1,132
As (V) [µg/L]	4.9	0.3	21	57
Monomethylarsonic acid MMA, [µg/L]	< LD	0.2	< LD	< LD
Arsenobetain [µg/L]	0.3	0.1	< LD	< LD
Thioarsenates (Mono-, Di-, Trithio-arsenate)	< LD	< LD	1 – 2 % of total As	1 – 2 % of total As
not identified As species [µg/L] (Fe-/ DOM-As-colloids)	15.4	149	727 - 743	637 - 661
		Dissolved organic carbon		
DOC [mg/L]	1	2.2	3	3
Carbohydrates [mg/L]	n.a.	<0.5 – 2.9	6.9	7.7

Results of arsenic species analysis (table 3) also point to microbial processes. While the speciation predominance of arsenite might be explained by arsenolite dissolution, the existence of organoarsenic compounds (Königstein and Ronneburg) and thioarsenates (Schlema and Pöhla) is exclusively related to microbial metabolism. Methylation is a well-known microbial strategy for arsenic detoxication (Bentley 2002) and highly mobile thioarsenates were shown to form under sulphate reducing conditions (Burton 2013). Despite the application of sophisticated analytical methods, a considerable arsenic fraction could not be identified (probably Fe- or DOM-As-colloids). Dissolved organic car-

Table 4. Isotopic signatures of sulphate in Ronneburg mine water.

Monitoring point	Inflow WTP	Mine water monitoring wells		
	Well 2	e-1292	e-1303	e-1328
	Isotopic signature			
$\delta^{34}SO_4$-S [‰ VCDT]	2.0	8.9	10.7	2.4
$\delta^{18}SO_4$-O [‰ VSMOW]	1.6	6.2	9.6	-0.2
	Water quality parameter (date of isotopic analysis)			
pH	6.5	8.6	7.4	6.7
SO_4 [mg/L]	2,750	487	91	741

Table 5. Trace organic compounds in sampled uranium mine effluents.

Mine site	Königstein	Ronneburg	Schlema	Pöhla
	Carboxylic acids (qualitative screening)			
Benzoic acid	detected		detected	detected
Butanoic acid			detected	
Succinic acid	detected	detected	detected	detected
3,4-Dihydroxy-butanoic acid	detected		detected	detected
2-Hydroxyglutaric acid				detected
Lactic acid	detected			
Glyceric acid	detected		detected	detected
Glycolic acid	detected			
	Miscellaneous organics (qualitative screening)			
Isosaccharinic acid lactone			detected	detected
Tetrahydroxyhexanoic acid				detected
lactone				detected
Phenol derivates			detected	detected
Pyridine derivates			detected	
Alkanes		detected	detected	

bon in mine water traces microbial activity insofar as individual substances are microbial substrates and / or metabolites. Table 3 shows surprisingly high concentrations of carbohydrates, which are known to be produced as extracellular polymeric substances (EPS) by e.g. autotrophic iron oxidizing bacteria (Johnson 2014). Fungal metabolites might constitute another source of carbohydrates. Table 6 discloses a great diversity of fungi detected in the mine water, most of them known as soil fungi related to leaf and wood decay (e.g. Cadophora spp., Patinella spp.). The existence and role of fungal communities in waterlogged, oxygen deficient environments was under debate for long. Within the last decade, it has been recognized e.g. in marine habitats, sulfidic hydrothermal spring systems, anaerobic groundwater and acid mine water environments (Brad 2008), (Luo 2005), (Amaral Zettler 2002). The detection of fungal DNA, carbohydrates and further fungal metabolites as well as cellulose degradation products like organic acids, phenol derivates and isosaccharinic acid lactones (see table 5) suggests fungal activity in the flooded mines. By supplying biodegradable organic carbon, fungal mine timber decay might even be the important driver of other biogeochemical processes in flooded mines.

As evident from table 6, the flooded mines contain diverse bacterial and archaeal communities as well. Autotrophic sulphur (*Sulfuricurvum spp*. at Schlema and Pöhla, *Acidithiobacillus spp*. at Königstein) and iron oxidizers (*Galionella spp*., *Sideroxydans spp*. at Ronneburg) are the most abundant bacteria, some of them able of using nitrate for anaerobic oxidation. Nitrifying bacteria (*Nitrospira spp*., *Candidatus Nitrotoga*), an-

Table 6. Mine water microbial biocenosis.

Königstein mine	Ronneburg mine	Schlema mine	Pöhla mine
	Bacterial DNA		
Acidithiobacillus spp.	Galionella sp.	Sulfuricurvum spp.	Sulfuricurvum spp.
Sulfuriferula spp.	Sideroxydans spp.	Sulfurimonas spp.	Sulfurovum spp.
Bacteriovoracaceae	Candidatus Nitrotoga	Thiobacillus spp.	Synthropus spp.
Xanthomonadales	Nitrospira spp.	Desulfurivibrio spp.	Nitrospira spp.
Leptospirillum spp.	Sulfuricurvum spp.	Galionella sp.	Desulfurivibrio spp.
Halanaerobiales	Desulfurivibrio spp.	Nitrospira spp.	Sulfuritalea spp.
	Archaeal DNA		
Candidatus Nitrosotalea	Crenarchaeota MCG [pGrfC26]	Crenarchaeota MCG [pGrfC26] & [B10]	Methanosarcinales [Candidatus Methanoperedens]
Thermoplasma spp.	Parvarchaeota [WCHD3-30]	Parvarchaeota [WCHD3-30]	Candidatus Nitrosopumilus
	Thermoplasmata [BSLdp215]	Thermoplasmata [DHVEG-1]	Methanoregula
	Methanomassiliicoc-caceae	Methanomassiliicoc-caceae	Methanobacterium
	Methanosarcinales [ANME-2d]	Methanosarcinales [ANME-2d]	
	Fungal DNA		
Cladosporium spp.	uncultured soil fungus	Acremonium spp.	Patinella spp.
Cadophora spp.	uncultured Pseudeurotium	Exophiala spp.	Acremonium spp.
Boothiomyces spp.	uncultured Glomeromycota	Patinella spp.	uncultured fungi
Articulospora spp.	Saccharomycetales	uncultured fungi	Exophiala spp.
Rhizophydium spp.		Cladosporium spp.	Cladosporium spp.
		Mycosparella spp.	Mortierella spp.
			Aspergillus spp.

aerobic fermenting bacteria (*Synthropus spp*., *Halanaerobiales*), sulphate and iron reducers (*Desulfurivibrio spp*., *Xanthomonadafaceae*) and a wide diversity of archaea involved in methane and nitrogen cycling (*Methanosarcinales*, *Methanomassiliicoccaceae*, *Methanoregula*, *Methanobacterium*, *Candidatus Nitrosopumilus*) add to the highly diverse microbial community in the flooded mines. In total, around 100 bacterial and half as much fungal and archaeal species were detected in each mine. Complex interactions of the individual microbial species as known for biofilm communities is highly probable. For example, heterotrophic bacteria can metabolize fungal cellulose degradation products and provide substrates like organic acids, alcohols, hydrogen and carbon dioxide for e.g. sulphate reducing bacteria, which can live in syntrophy to anaerobic methanotrophic archaea by extracellular electron transfer too. Likewise, nitrate based sulphur oxidation might relay on nitrification of ammonium which in turn is produced by wood degrading fungi and nitrogen fixing methanogenic archaea. Organic matter, nitrogen and electron cycling are yet unknown but most relevant to understand biogeochemical processes and their effects on pollutant mobility in flooded uranium mines.

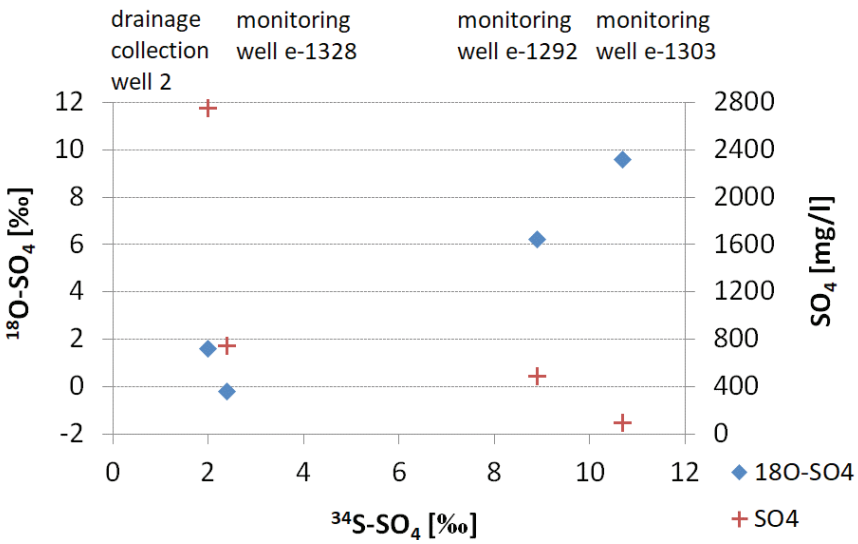

Figure 1 Ronneburg mine water: 34S- and 18O –Isotopes and SO4 concentrations.

Conclusions

Analysis of redox sensitive ions, dissolved gases, sulphate isotopes, arsenic species, organic compounds and microbial DNA in four flooded uranium mines revealed the presence of highly diverse and metabolizing microbial communities comprising species of different genera of bacterial, archaeal and fungal domains. Their interplay in organic matter, nitrogen and electron cycling is of high relevance for mine water speciation and pollutant mobility. For example, the identified iron and sulphate reducers are capable of uranium reduction, which can lead to in-situ uranium immobilization and uranium concentration decline as observed in Pöhla mine. On the other hand, anaerobic microbial oxidation of arsenic containing primary sulphides by e.g. identified sulphur oxidizing microbes and complexation of dissolved arsenic by microbial metabolites might cause constantly high arsenic levels in mine waters as is also observed in Pöhla and Schlema. Further investigation of the metabolic interplay of identified microbial communities and its effects on pollutant speciation can help to reveal mine water quality trends and to develop innovative mine water treatment technologies.

References

Amaral Zettler L.A., Gomez F., Zettler E., Keenen B.G., Amils R., Sogin M.L. (2002) Eukaryotic diverity in Spain's River of Fire, Nature 417:137

Bentley R., Chasteen T. G. (2002) Microbial methylation of metalloids: Arsenic, antimony, and bismuth. Microbiol. Mol. Biol. Rev. 66:250–271

Brad T., Braster M., van Breukelen B.M. (2008) Eukaryotic Diversity in an Anaerobic Aquifer Polluted with Landfill Leachate. Appl. Environ. Microbiol. 74:3959-3968

Burton E.D., Johnston S.G., Planer-Friedrich B. (2013) Coupling of arsenic mobility to sulfur transformations during microbial sulfate reduction in the presence and absence of humic acid. Chem Geol 343:12–24

Johnson D.B., Hallberg K.B., Hedrich S. (2014) Uncovering a Microbial Enigma: Isolation and Characterization of the Streamer-Generating, Iron-Oxidizing, Acidophilic Bacterium "Ferrovum myxofaciens". Appl. Environ. Microbiol. 8:672-680

Knöller K, Fauville A, Mayer B, Strauch G, Friese K, Veizer J. (2004) Sulfur cycling in an acidic mining lake and its vicinity in Lusatia, Germany. Chem Geol 204:303–323

Luo Q., Krumholz L.R., Najar F.Z., Peacock A.D., Roe B.A., White D.C., Elshahedi M.S. (2005) Diversity of the Microeukaryotic Community in Sulfide-Rich Zodletone Spring (Oklahoma). Appl. Environ. Microbiol. 71:6175-6184