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Indirect *in situ* bioleaching is an emerging tool for accessing deeply buried metal reserves, but can the process be managed? – A case study of copper leaching at 1 km depth

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ABSTRACT

Copper is a strategic raw material widely needed for electrification. One possibility to diversify the supply to answer the market demand is to produce copper with in situ technology. In this study, feasibility of in situ bioleaching of copper was tested in a deep subsurface deposit. During in situ bioleaching of copper, copper is leached using a biologically produced ferric iron solution, which is recycled back to the in situ reactor after valuable metals are recovered, after which the solution is re-oxidized by iron-oxidizing microorganisms (IOB). A rock reactor was constructed in the Rudna Mine at ca 1 km depth and the microbiology and hydrogeochemistry of the water circulated through the reactor after blasting for fracturing the rock was monitored over time. The test site was rich in carbonates requiring large quantities of acid to remove the buffering capacity. The bacterial, archaeal and fungal communities in the rock reactor were monitored and characterized by quantitative polymerase chain reaction (qPCR) and amplicon sequencing, and acidophilic, iron oxidizing activity of the microbial communities during operation and pre- and post-operation phases was tested by cultivation. No acidophilic iron oxidizers were detected in the water samples during construction of the pilot reactor. Acidic leaching solution originating from the underground ferric iron generating bioreactor (FIGB) contained acidophilic IOB, which were also viable after the leach liquor was returned from the rock reactor. In the post-operation phase, when the rock reactor was neutralized with $CaCO_3$/$Ca(HCO_3)_2$ solution, to inhibit the acidophilic IOB, iron oxidizing microorganisms were still present in the effluent solution one week after termination of the leaching and start of neutralization. Therefore, the post-operation phase needs further attention to completely stop the activity of added microorganisms. Copper was abundantly leached during the acid wash and leaching phases, proving the concept of deep in situ bioleaching.

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1. Introduction

The need to access deeply buried, lower grade ores is growing as more readily accessible high-grade ores are dwindling at the same time as the demand for metals is growing. *In situ* biomining, i.e. using microorganisms to facilitate leaching of metals on site, could be a possible solution (Wadden and Gallant, 1985), as this technique may be both cost and energy efficient for extracting metals from ores that would not otherwise be economical to mine (Johnson et al., 2013; Johnson, 2015). In addition, in situ biomining may be more discrete on the landscape and the environment compared to conventional mining as there is no need for an excavation pit, no heaps for overburden rock materials nor tailings. Nevertheless, fracking used instead of mining may have impacts on groundwater quality due to e.g. production of acid mine drainage and dissolution of heavy metals (Meng, 2017; Burton et al., 2014; Li et al., 2017). For in situ biomining to be an economic option the regeneration of the lixiviant must be ensured, i.e., reoxidation of ferrous to ferric iron in bioreactors in order to reduce waste and save raw material (Johnson et al., 2013). The principle, as described by Johnson (2015) is based on using highly acidic, ferric iron rich solution, for ferric iron-catalyzed oxidative dissolution of copper from the deeply buried ore, and recovery of the copper containing reduced leaching fluid, which is regenerated for reuse in a ferric iron generating bioreactor (FIGB). At the end of operations, the in situ leaching process needs to be properly terminated when the ore deposit has been exhausted, to avoid uncontrolled production of acid mine drainage (AMD), which is formed due to the accelerated oxidation of sulfidic ores when exposed to water and oxygen or another oxidant, such as ferric iron (e.g. Johnson and Hallberg, 2005). When the ferric iron is oxidized to ferrous in this process, remaining IOB in the leached fractured rock could potentially re-oxidize the ferrous to ferric iron, thus keeping the process going, which could have detrimental effects on the environment in the form of increase in heavy metal contamination and acidification of water and aquatic ecosystems in the vicinity of the mine site (e.g. Seredkin et al., 2016; Luís et al., 2009; RoyChowdhury et al., 2015). It is also important to ensure that no leakage of acidic and metal rich lixiviant solution from the leaching site into the environment occurs by carefully inactivating the process at the end of operations, as tested previously in simulation studies (Ballerstedt et al., 2017; Bomberg et al., 2018).

Acidophilic iron oxidizing microorganisms derive energy from the oxidation of soluble ferrous iron and exist naturally in acidic environments, such as acidic mine waters (e.g. Wendt-Potthoff and Koschorreck, 2002; García-Moyano et al., 2007; Bomberg et al., 2019). Consortia or isolated species of such acidophilic iron oxidizing microorganisms are used in bioleaching applications, where metals are extracted from ore using biological means (e.g. Rawlings and Johnson, 2007). Ferric iron solution oxidizes the ore and becomes reduced. Acidophilic iron oxidizers re-oxidize ferrous iron to ferric iron, which can then oxidize more ore. Typical microbial species used in bioleaching applications are for example *Leptospirillum* (*L.*) *ferriphilum*, *L. ferrooxidans*, *Acidithiobacillus* (*At.*) *caldus*, *At. ferrooxidans*, *Acidimicrobium* (*A.*) *ferrooxidans*, and *Sulfobacillus* (*S.*) *benefaciens*, either in single-species applications or as mixtures (Hong et al., 2019; Falco et al., 2003; Wang et al., 2018; Giaveno et al., 2007; Johnson et al., 2008; Watling et al., 2014; Christel et al., 2018; Khaleque et al., 2017; Pakostova et al., 2018).

In situ leaching has been commercially used mainly for uranium and copper, but in situ bioleaching is still in the developmental phase because it yet needs to be proven both economically feasible, efficient and environmentally safe (Roberto and Schippers, 2022; Watling et al., 2014; Richter et al., 2018). In in situ leaching applications, deeply buried ores are usually reached by drilling wells from the ground surface (e.g. Johnson, 2014). The BIOMOre project (https://cordis.europa.eu/project/id/642456, Grant Agreement #642456) constructed the first bioleaching rock reactor pilot (Fig. 1 AB) in combination with hydrofracking at approximately 1 km depth in the Rudna copper mine in Poland. The unique hydrofracking-bioleaching in situ pilot plant applied indirect leaching of copper with ferric iron lixiviant, i.e., the acidophilic microorganisms were contained in a Ferric Iron Generating Bioreactor (FIGB) on site at 1 km depth, into which the reduced iron solution after Cu recovery was directed for reoxidation by an iron oxidizing microbial community and further reused in the rock reactor.

The motivation of this study was that indirect in situ bioleaching is a possible future metal recovery technology (Johnson, 2015), but it may be challenging to control in the deep subsurface. Despite being attached in biofilms on the granulated activated carbon carrier material of the on-site FIGB, it is expected that some microorganisms will follow the regenerated lixiviant from the FIGB into the subsurface, where they may present risks by remaining active and continue uncontrolled leaching of the rock after the end of the leaching operations. Although deeply buried ores characteristically contain high hydrostatic pressure, which is likely to reduce the

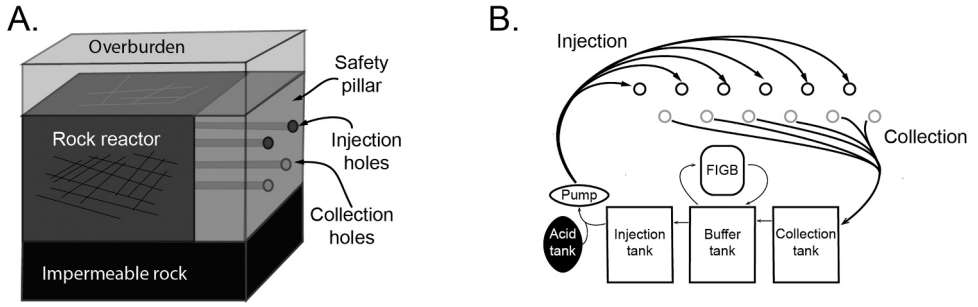

Fig. 1. A schematic presentation of the A) rock reactor showing the fracked block between the overburden and impermeable rock, safety pillar and inflow and outflow collection holes, and B) the pilot plant with collection basin for the outflow of the rock reactor, the buffering basin and FIGB, injection tank with in-line acid tank for pH adjustments, pump and inlet injection holes to the rock reactor.

activity of non-piezophilic/-piezotolerant microorganisms (Johnson, 2015), it has recently been shown that meso- and thermophilic bioleaching microorganisms may retain their metabolic activity at pressures up to at least 100 bar (Zhang et al., 2018). The lack of oxygen in the deep subsurface may inactivate obligately aerobic bioleaching microorganisms, but facultatively anaerobic microorganisms may grow and metabolize under anaerobic conditions. Post operation procedures require that the biological processes employed for the bioleaching operation cease also in the subsurface. It is thus of great importance that escaped bioleaching microorganisms are irreversibly inactivated, in order to reduce the risks of causing uncontrolled leaching similar to that occurring naturally in e.g. the long-abandoned Mynydd Parys mine in Wales or the drainage into the Rio Tinto in Spain (Coupland and Johnson, 2004; España et al., 2005).

One way to inactivate the added bioleaching microorganisms is to use elevated chloride ion concentrations, as chloride is present in some mining sites and high concentrations have been shown to be detrimental to acidophilic microorganisms (Shiers et al., 2005; Bomberg et al., 2018; Gahan et al., 2009; Kinnunen and Puhakka, 2004). Only some extremely halotolerant acidophilic iron oxidizing bacteria, such as *Acidihalobacter* (*Ah.*) *prosperus*, have been shown to tolerate chloride concentrations of up to 35 g L^{-1} (Nicolle et al., 2009). Previously, tests to inactivate acidophilic iron oxidizing bacteria were performed with Rudna mine Kupferschiefer sandstone and black shale ore in laboratory simulation studies (Ballerstedt et al., 2017; Bomberg et al., 2018). Different compound combinations, including formate, nitrate, alcohols sodium dodecyl sulfate (SDS), were shown to inactivate most iron oxidizers in the experiment (Ballerstedt et al., 2017), but the naturally prevailing chloride rich water in the mine was likewise effective (Bomberg et al., 2018).

The present objectives were to upscale previous laboratory studies (Pakostova et al., 2018; Ballerstedt at al, 2017; Bomberg et al., 2018) to a field study at the Rudna Mine to 1) monitor the development of iron oxidizing potential of the microbial communities circulating in the pilot in situ rock reactor over the time of construction, preparation, operation and neutralization, 2) examine whether any intrinsic acidophilic, iron-oxidizing microorganisms were enriched in situ during the construction of the rock reactor, which may cause undesired leaching after operations have stopped, and 3) to investigate how successful the decommission procedures were for inactivating the introduced acidophilic consortia in the in situ rock reactor. We used DNA based qPCR and amplicon sequencing techniques together with cultivation-based techniques and sampling throughout the construction, operation, and decommissioning of the rock reactor pilot.

2. Materials and methods

2.1. Site description

The Rudna Mine is situated in Western Poland in the central part of the copper ore deposit in the pre-Sudeten monocline and represents one of the largest copper reserves in Poland (Gregor et al., 2012). The deposit consists of three lithological zones, the Rotliegendes sandstone, lower Zechstein black shale and dolomites. The copper deposits are situated in the Kupferschiefer, which consists of layers of sandstone and black shale and has been described in detail by Gregor et al. (2012). The copper is mainly present in chalcocite, bornite and chalcopyrite (Vaughan et al., 1989). The Kupferschiefer generally has a carbonate content of 10–40 %, which needs to be removed for the acidic leaching of the copper to be successful (Pakostova et al., 2018). It contains high amounts of calcareous minerals, such as calcite and dolomite, as well as chloride rich minerals, such as mainly halite, but also in the Cu containing atacamite ($Cu_2Cl(OH)_3$) (Pakostova et al., 2018). The average content of elements in the Kupferschiefer has been reported by Ineich et al. (2018) and the Cu content was 1.52 mass-% (m%), Fe 0.71 m%, total S 0.88 m% and Cl 0.41 m%.

Table 1
Operational phases and durations during the *in situ* test work.

Operational phase	Duration (weeks)	Reason
Blasting and drilling		Preparing the rock reactor
Pre-water washing	15	First rinsing to remove blasting residue and fine-grained rock material, total volume of tap water 1.6 m^3, repeated 6 h injection, 6 h passive drainage
Pause	12	
Pre-water washing	8	Second rinsing to remove blasting residue and fine-grained rock material, pumping with a custom designed Atlas Copco pump
Water washing	19	Continuous pumping between rock reactor inlet and outlet via a fluid treatment plant ro remove remaining fine grained material and blasting residues from the washing water
Acid washing	9	Rinsing with 95 % sulfuric acid solution to remove excess carbonate to condition the rock reactor for leaching and maintain the solution below pH 3
Pause	18	Drilling of new injection holes
Acid washing	11	Rinsing with 95 % sulfuric acid solution to remove excess carbonate to condition the rock reactor for leaching and maintain the solution below pH 3
Ferric leaching and biological generation	5	Leaching of copper
Post operational inactivation	1	Rinsing with concentrated $CaCO_3$ solution to increase pH and inactivate IOB

2.2. Description of the in situ rock reactor

2.2.1. Description of the in situ rock-reactor development

The construction and monitoring of the rock reactor have previously been reported (Anacki et al., 2018; Anacki and Kirej, 2017; Ineich et al., 2018). Briefly, a rock reactor comprizing approximately 100 m^3 (10 m x 5 m x 2 m, length x width x hight) of copper deposit surrounded by sandstone was produced by fracking inside the Rudna Mine, Poland, behind a 4 m thick safety pillar at a depth of 1000 m below ground level (Fig. 1A). A total of 11 fluid injection inlets and 13 collection outlets were installed. Fluids were circulated between the rock reactor and a pilot plant containing a collection basin for outflow from the rock reactor, a buffering tank, the FIGB and a basin for injection fluids that were pH adjusted with sulfuric acid from an acid tank and pumped into the injection holes into the rock (Fig. 1B). The test was divided into three main operational phases: water washing for removal of blasting residues and fine grained rock material, sulfuric acid washing for removal of carbonates in the rock, and bioleaching with ferric iron lixiviant for extraction of Cu, followed by decommissioning of the rock reactor with alkaline solution in order to inhibit iron oxidizing microorganisms (Table 1).

Blasting residue and fine-grained rock material was initially removed by a pre-water washing with a total of 1.6 m^3 tap water during a repeated 6 h injection phase followed by passive drainage over 6 h (Table 1). A second pre-water wash with circulation was performed after a pause of 12 weeks using a specially custom designed Atlas Copco pump. A continuous water wash using 72 m^3 water followed where the water was pumped between the inlet and outlet to the fluid treatment plant containing a series of basins flowing over to the next and allowing for sedimentation of particles before it was returned via the inlets to the rock reactor. The rinsing water was replaced every two weeks with fresh tap water.

The rock reactor was prepared for leaching by performing an acid wash with concentrated sulfuric acid solution (ca 95 %) in order to remove excess carbonate from the rock and ensure that the pH of the solution in the rock reactor remained below pH 3. Due to leakage around the injection holes of the reactor the acid wash had to be paused for 18 weeks during which time the old injection holes were plugged and new injection holes were drilled. The acid wash was thereafter continued with a total of approximately 550 L of concentrated sulfuric acid used.

After the acid wash the leaching using biologically oxidized ferric iron solution (pH < 2.0) started. During the leaching phase, the reduced, copper containing iron solution was reoxidized biologically by leading it into a 600 L polypropylene fluidized bed ferric iron

Table 2
The physicochemical composition of the in situ rock reactor fluids. All measurements are reported from the outflow, except where explicitly marked with "in" and cursive font. Sampling times when the microbiology was also measured are indicated in bold.

Sample	Sampling date	pH	ORP vs Ag/AgCl mV	Cl^- g L^{-1}	Cu mg L^{-1}	Na g L^{-1}	SO_4^{2-} g L^{-1}	Ca g L^{-1}	Fe mg L^{-1}	Mg mg L^{-1}	K mg L^{-1}
Pre-water wash - in	12.12.2016	7.6		0.05	<0.002	0.02	0.09	0.08	0.06	12.9	1.74
Pre-water wash	13.12.2016	6.2		35.3	1.03	21.4	0.12	2.01	<0.05	335	288
Pre-water wash 1	18.1.2017			2.4	1.2	1.1	0.7	1.0		150	
Pre-water wash 2	5.5.2017	5.9		91.1	0.14	58.8	2.3	5.2		790	
*Water wash – in**	6.7.2021 – 24.10.2017	7.4	300 -> 200 * *	42 -> 20 * *							
Water wash 1	4.7.2017	7.2	310	1.0	0.4	1.8	0.2	1.3	0.03	230	180
Water wash 2	19.7.2017	7.2	300	1.5	0.4		0.8	1.0	0.13		
Water wash	25.7.2017	7.5	250	17.6			0.83		0.41		
Water wash	19.8.2017	7.7	196	8.99			0.43		0.038		
Water wash	12.9.2017	7.5	230	17.4			0.66		0.017		
Water wash 3	24.10.2017	7.7	400	23.2	0.3	1.3	0.72	1.3	0.18	200	140
Acid wash - in	24.10.2017–13.6.2018	0.3	420 -> 400 * *	50 -> 10 * *							
Acid wash	10.11.2017	<1.0	654	38.9	643	18.5	56.3	0.92	1180	546	171
Acid wash	23.11.2017	0.4	199	15.3	89.4	8.4	40.7	0.89	1330	295	169
Acid wash 1	5.12.2017	1.6	360	35.79	13.6	23.30	5.10	2.06	0.5	390	295
Acid wash	12.12.2017	<1.0	644	14.6	17.1	10.7	29.5	0.89	648	261	78.7
Acid wash	2.5.2017	1.4		14.23	17.3	8.21	5.16	1.13		121	
Acid wash 2	10.5.2018			10.0	<30	5.0	7.0	0.75	600	110	80
On-site FIGB	18.6.2018										
Leaching - in	13.6.2018 – 13.7.2018	1.5	400–420	4–14							
Leaching 1	18.6.2018	1.9	420	6.0	270	11.0			6640	470	nd
Leaching	21.6.2018			18	275	11.0	20			460	
Leaching	12.7.2018	1.7	421	41.4	443	10.0	22.8	0.63	12,500	525	12.8
Leaching 2	13.7.2018										
Neutralization - in											
Neutralization 1	18.7.2018	5.9		16.6	0.36	13.4	10.2	0.86		415	
Neutralization 2	30.7.2018										

*injection between 6.7.2017 and 24.10.2017, * * -> = decrease over time.

generating bioreactor (FIGB) located on site, that contained activited carbon granules as carrier material for iron oxidizing biomass. The biomass was maintained by adjusting the pH of the solution with sulfuric acid, addition of nutrients and continuous oxygen flow as described in Supplement A.

After the leaching process was finalized, the rock reactor was drained of leaching solution. Thereafter, a concentrated solution of $CaCO_3$ mixed in tap water was pumped into the rock reactor in order to increase the pH and stop the acidic leaching. Neutralization solution was pumped into the reactor for the duration of one week.

The pH and oxidation-reduction potential (ORP) were measured over the whole study in order to monitor the conditions of the rock reactor during the conditioning towards suitably acidic and oxidative conditions for successful leaching as well as to follow the conditions (increase in pH) in the neutralization phase. The concentration of copper was measured to determine the success of the leaching process.As the rock material was dissolved during the acid wash and leaching phases, Fe, Cl^-, Na, sulfate, Ca, Mg and K were measured as they were major constituents of the rock. In addition, the IOB are sensitive to Cl-, which was thus especially important to monitor. The physicochemical measurements are presented in Table 2.

2.2.2. Chemistry of the rock reactor fluids

Water chemistry was tested at every stage of the construction and operation of the rock reactor, i.e., pre-water wash, water wash, acid wash and leaching. The analyses and frequencies of the physicochemical measurements are presented in Table S1 and were adjusted to properly control the conditions in the pilot rock reactor during construction in order for adjustments to be made to the construction process according to the results.

The laboratory chemical analyses were performed using the methods listed in Table S2. The detection limits of these methods depended on the characteristics of the samples, such as salinity and pH, and could vary greatly between samples, as shown in Table S2. Water samples were analyzed in two separate laboratories in Germany (UIT Dresden) and Poland (Centrum Badań Jakości, Lubin).

2.3. On-site FIGB

Acidophilic iron oxidizing biomass on carrier material was produced in laboratory-scale reactors at Tampere University, Finland, followed by a semi-pilot reactor at the Geological Survey of Finland, Outokumpu, and transported to the Rudna mine site, Poland, as described in Supplement A. Briefly, the pre-produced carrier-bound iron oxidizing biomass was decanted to a 600 L polypropylene reactor on site, i.e., the on-site FIGB. The on-site FIGB was operated in semi-continuous mode for 7 months before the leaching phase began. During this phase the pH was maintained at between 1.3 and 1.9 with continuous aeration of 2–5 L of air min^{-1}. Twice a week, 100 L of the solution was replaced with tap water, nutrients, and ferrous sulphate as described in Supplement A to maintain the biomass. The oxidation reduction potential (ORP) mostly stayed between 570 and 600 mV (measured vs Ag-AgCl electrode), but occasionally decreased to as low as 500 mV.

2.4. Sampling for microbiology

Water samples from the injection lines and collection lines from the different phases of the in situ rock reactor and on-site FIGB were collected on site into sterile 1 L (Nalgene) plastic bottles. Microbial biomass was concentrated on syringe filters (Sterivex™) 0.22 -μm pore-size, and frozen at − 20 °C within hours of the sampling. In addition, injection and collection line samples as well as on-site FIGB samples were collected in sterile 1 L plastic bottles or 50 mL screw capped test tubes (Falcon) for cultivation tests. The number of replicate samples, sample volumes and sampling dates are presented in Table S3.

2.5. Testing for activity of iron oxidizing bacteria

The aptitude for aerobic biological iron oxidation by the microbial community in the solutions used in the different phases of the rock reactor and leaching processes was tested by cultivation. A total of 4 different media were used in 5 mL batch cultures inoculated with 0.5 mL sample. All cultures were done in 2 or 3 replicates. Iron oxidation was tested at pH 1.4 in a culture medium by Ahoranta et al. (2020) as described in Bomberg et al. (2018) (Medium Fe-1), containing $(NH_4)_2SO_4$ (3.75 gL^{-1}), Na_2SO_4 •10 H_2O (1.875 gL^{-1}), $MgSO_4$ •7 H_2O (0.625 gL^{-1}), KCl (0.125 gL^{-1}), K_2HPO_4 (0.0625 gL^{-1}), and $Ca(NO_3)_2$• 4 H_2O (0.0175 gL^{-1}), supplemented with 1 % (v/v) trace element solution containing (gL−1) $FeCl_3$ •6 H_2O (1.375 gL^{-1}), $MnSO_4$ •4 H_2O (0.319 gL^{-1}), H_3BO_3 (0.25 gL^{-1}), Na_2SeO_4 (0.1125 gL^{-1}), $ZnSO_4$ •7 H_2O (0.1125 gL^{-1}), Na_2MoO_4 •2 H_2O (0.1 gL^{-1}), $CoCl_2$ •6 H_2O (0.075 gL^{-1}), and $CuSO_4$ •5 H_2O (0.0625 gL^{-1}), and ferrous iron as $FeSO_4$ •7 H_2O (5.6 gL^{-1}). Based on the dominance of *Leptospirillum* sp. in the iron oxidizing community of the laboratory-scale (Bomberg et al., 2018; Hajdu-Rahkama et al., 2019), Medium 882 (https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium882.pdf) with higher ferrous iron content (20 g L^{-1}) designed for the cultivation of *Leptospirillum* was also used. In order to detect heterotrophic iron oxidizers, such as *Sulfobacillus* sp., DSM medium 271 (https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium271.pdf) containing 8.0 g L^{-1} ferrous sulphate was supplemented with yeast extract 0.25% w/v, and the presence of halophilic iron oxidizers was tested with medium 477 (https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium477.pdf). All media were prepared and sterilized according to the instructions for each medium.

One or two sampling points were tested for each stage of the pilot as shown in Table S3. The cultures were incubated at 30 °C in aerobic conditions, in a horizontal mixer set to 140 rpm. The cultures were checked visually weekly. Positive iron oxidation activity was indicated by colour change of the medium from uncoloured to rust colour indicating activity of acidophilic iron oxidizing bacteria via iron oxide precipitates. Non-inoculated medium was used as a negative control.

2.6. Molecular biological methods

The methods used for microbial DNA extraction, qPCR, amplicon library preparation and sequence analysis are described in Supplement B. Briefly, the abundance of bacteria, archaea and fungi in the water samples from the different phases of the pilot rock reactor and the on-site FIGB effluent was examined with qPCR targeting the bacterial and archaeal 16 S rRNA genes and the fungal 5.8 S rRNA gene. The microbial DNA was extracted using the NucleoSpin Soil DNA extraction kit (Macherey-Nagel) and the DNA extract was further purified with the NucleoSpin gDNA Clean-up (Macherey-Nagel) to remove remaining impurities and inhibitors. The microbial communities (bacteria, archaea, fungi) were characterized using Iontorrent high throughput amplicon sequencing. The sequences were deposited in the European Nucleotide Archive (www.ebi.ac.uk/ena/) under Study Number PRJEB56924.

3. Results

3.1. Leaching

The concentration of Cu in the outflow of the rock reactor during the pre-water wash and water wash phases was low, at most 4 mg L^{-1} and 0.4 mg L^{-1}, respectively, although at the time of microbiological sampling the Cu concentration in the pre-water wash phase was lower (Table 1). The concentration of total iron was at most 0.24 mg L^{-1}, and generally lower in both pre-water wash and water wash samples.

At the beginning of the acid wash phase a peak in Cu concentration was observed reaching approximately 650 mg L^{-1} Cu, and another peak reaching approximately 280 mg L^{-1} Cu in the outflow, which were likely caused by the rapid dissolution of acid-soluble Cu bearing minerals, such as atacamite, which is present within the ore (Pakostova et al., 2018), and addition of fresh sulfuric acid to the system, respectively. Otherwise, the concentration of Cu in the rock reactor outflow was steady at around 30 mg L^{-1} and the total amount of dissolved Cu during the acid wash phase was 30 kg, which was mostly dissolved during the first two months of acid wash (Fig. 2A).

The concentration of total iron in the rock reactor outflow during the acid wash phase increased over time from below detection limit to up to 3000 mg L^{-1} during the first 2 months of acid wash, but decreased to approximately 700 mg L^{-1} after this (Fig. 2B).

During the indirect bioleaching phase, the concentration of leached Cu in the outflow of the rock reactor increased from 30 mg L^{-1}

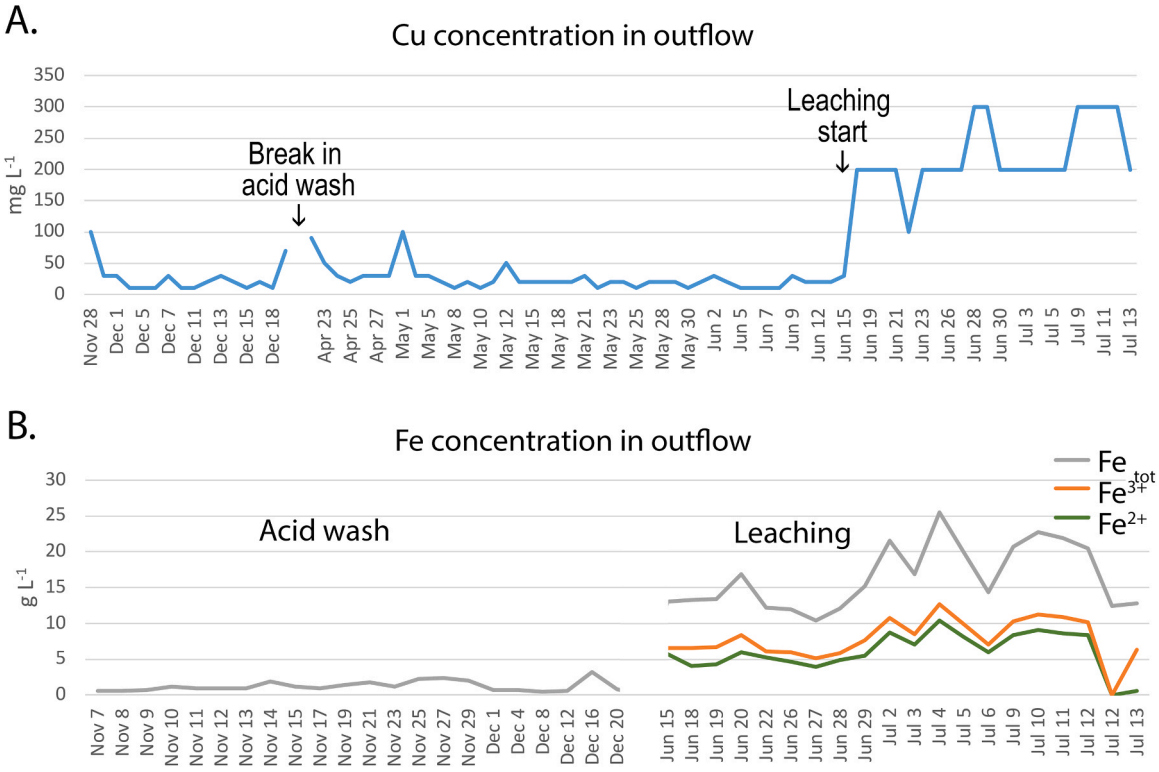

Fig. 2. The concentration of A) Cu and B) iron over time in the outflow of the rock reactor measured during the acid wash and indirect bioleching phase. The Cu concentrations were measured with Cu indicator strips (Tektrak, Twyning, UK). The break in measurements in A) was due to leakage around the injection holes and the acid wash was discontinued for 18 weeks. The break in measurements in B) was also due to the pause in the acid wash phase and measurements were continued when the leaching phase started.

to a maximum of 450 mg L^{-1} at the end of the leaching phase. The concentration was followed frequently on site by the use of Cu test strips, which showed that the Cu concentration increased to 200 mg L^{-1} within the first 5 days and remained on this level with periodical peaks reaching more than 300 mg L^{-1} concentrations (Fig. 2A).

The concentration of total and ferrous iron tended to increase over the leaching period from 6500 mg L^{-1} and 5900 mg L^{-1}, respectively to up to 13,000 mg L^{-1} and 9000 mg L^{-1}, respectively, towards the end. The concentration of ferric iron was lower, varying between 500 mg L^{-1} and 2200 mg L^{-1} over the whole leaching phase (Fig. 2B). The total amount of Cu leached during the bioleaching phase was 37 kg. However, the leaching tests were terminated before all Cu was leached.

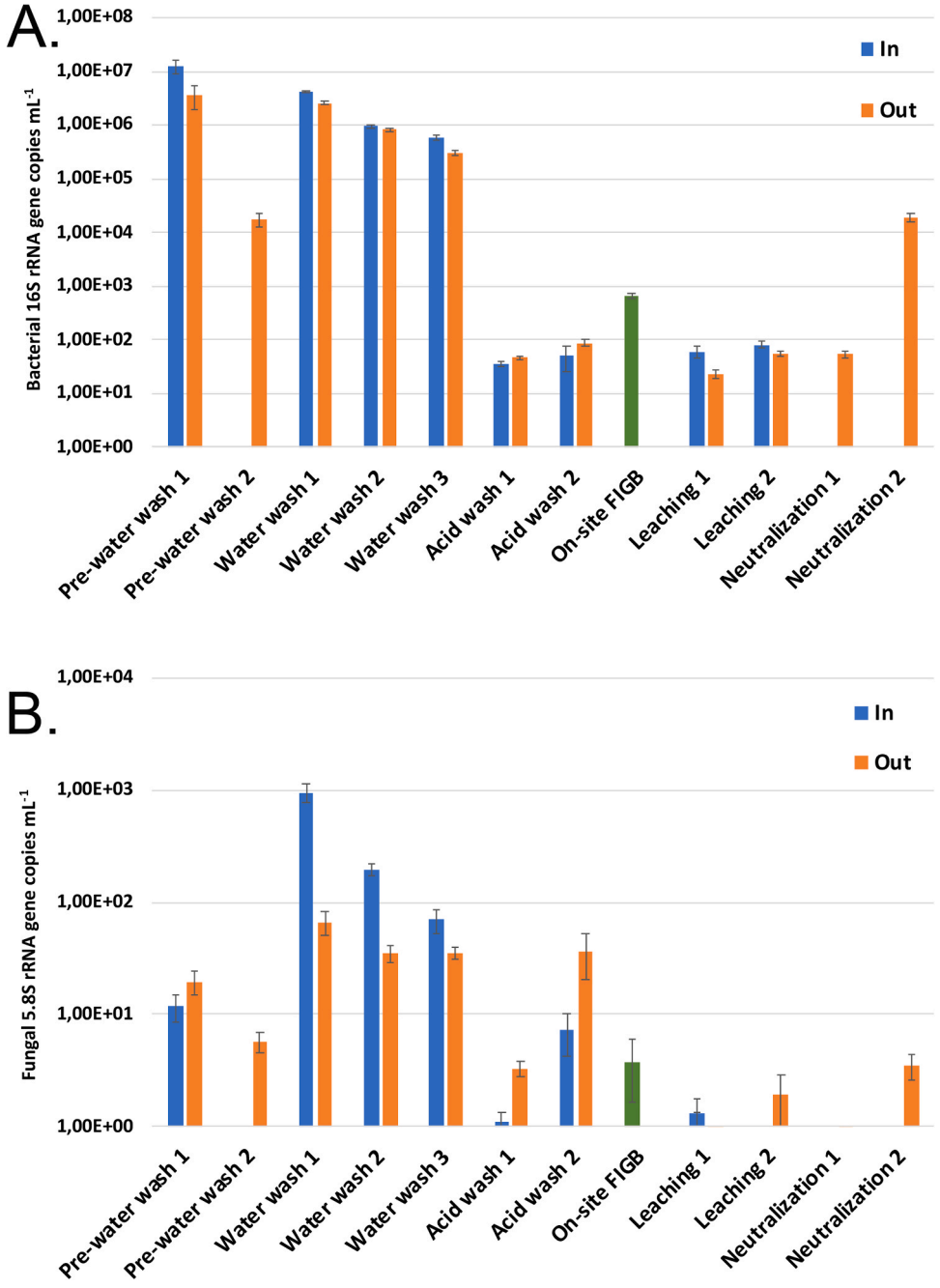

Fig. 3. The number of A) bacterial 16 S rRNA gene copies mL^{-1} sample fluid and B) fungal 5.8 S rRNA genes mL^{-1} sample fluid estimated by qPCR. The inflow samples are shown in blue and outflow samples in orange. The sample from the outflow of the on-site FIGB is shown in green. Each bar represents the average value of three parallel reactions from 2 or 3 replicate samples. The error bars represent standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Estimation of microbial community sizes

The number of bacterial 16 S rRNA genes in the input and output water during the pre-water wash and water wash of the rock reactor was between 3.0×10^5 and 1.2×10^7 16 S rRNA gene copies mL^{-1} except for the second pre-water wash, which had a lower number of bacterial 16 S rRNA genes (Fig. 3). In general, the number of detected bacterial 16 S rRNA genes was slightly lower in the output water compared to the input water. In the acid wash, leaching and beginning of the neutralization phase the number of bacterial 16 S rRNA genes detected by qPCR was below 10^2 genes mL^{-1}, but increased to 1.9×10^4 gene copies mL^{-1} in the second neutralization sample. The number of bacterial 16 S rRNA gene copies detected in the leaching solution going into the rock reactor was 6.7×10^2 mL^{-1}. The number of bacterial 16 S rRNA genes in the FIGB solution was 6.7×10^2 copies mL^{-1}.

Fungi were detected by qPCR mainly in the water wash phase injected water at a maximum of 9.6×10^2 5.8 S rRNA gene copies mL^{-1} and in all other samples the fungal 5.8 S rRNA gene copy number was below 1.9×10^2 copies mL^{-1}. Archaea were not detected by qPCR from these samples.

3.3. Amplicon sequening

The bacterial and fungal communities of the on-site FIGB and different phases (see Section 2.2) of the construction and operation of the rock pilot reactor were characterized by amplicon sequencing. The number of bacterial sequence reads from the effluent of the first and influent of the second acid wash samples were low, less than 500 reads/sample, but all other samples generated generally thousands of high-quality bacterial sequence reads (Table S4).

The number of fungal sequences varied between the sample types, with the lowest numbers obtained from the pre-water wash samples, the second and third water wash, the first acid wash and the second influent acid wash samples (Table S5). From all other sample types, generally thousands of quality-screened fungal ITS1 reads were obtained.

No archaeal sequence reads were obtained.

In the PCoA plot, a clear difference in the bacterial community composition in the samples collected from different phases of the in situ reactor could be seen (Fig. 4A). The pre-water wash and water wash samples fell along the right side of the plot showing changes in

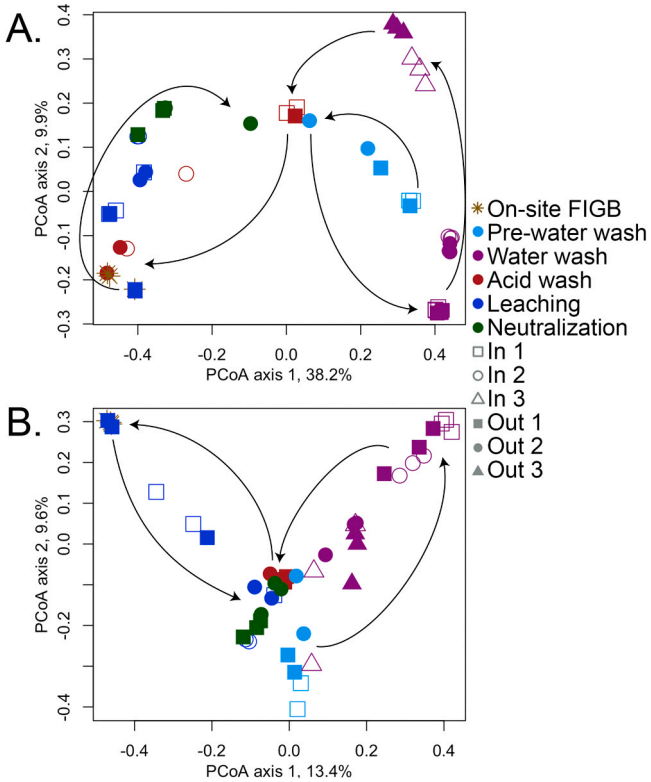

Fig. 4. Principal Coordinates Analysis of the A) bacterial and B) fungal communities in the different phases of the rock reactor based on the Bray-Curtis dissimilarity model and relative abundance of OTUs. Each replicate sample is represented by a symbol. The pre-water wash and water wash samples are light blue and purple, respectively, the acid wash samples are red, leaching blue and neutralization green. Inflow samples are indicated with open symbols and output samples with filled symbols. First, second and third (when relevant) sampling times are distinguished by squares, circles, and triangles, respectively. The on-site FIGB samples represented by brown stars. Only samples with more than 100 sequence reads were included in the analysis. The axis legends indicate percent of variation between the samples.

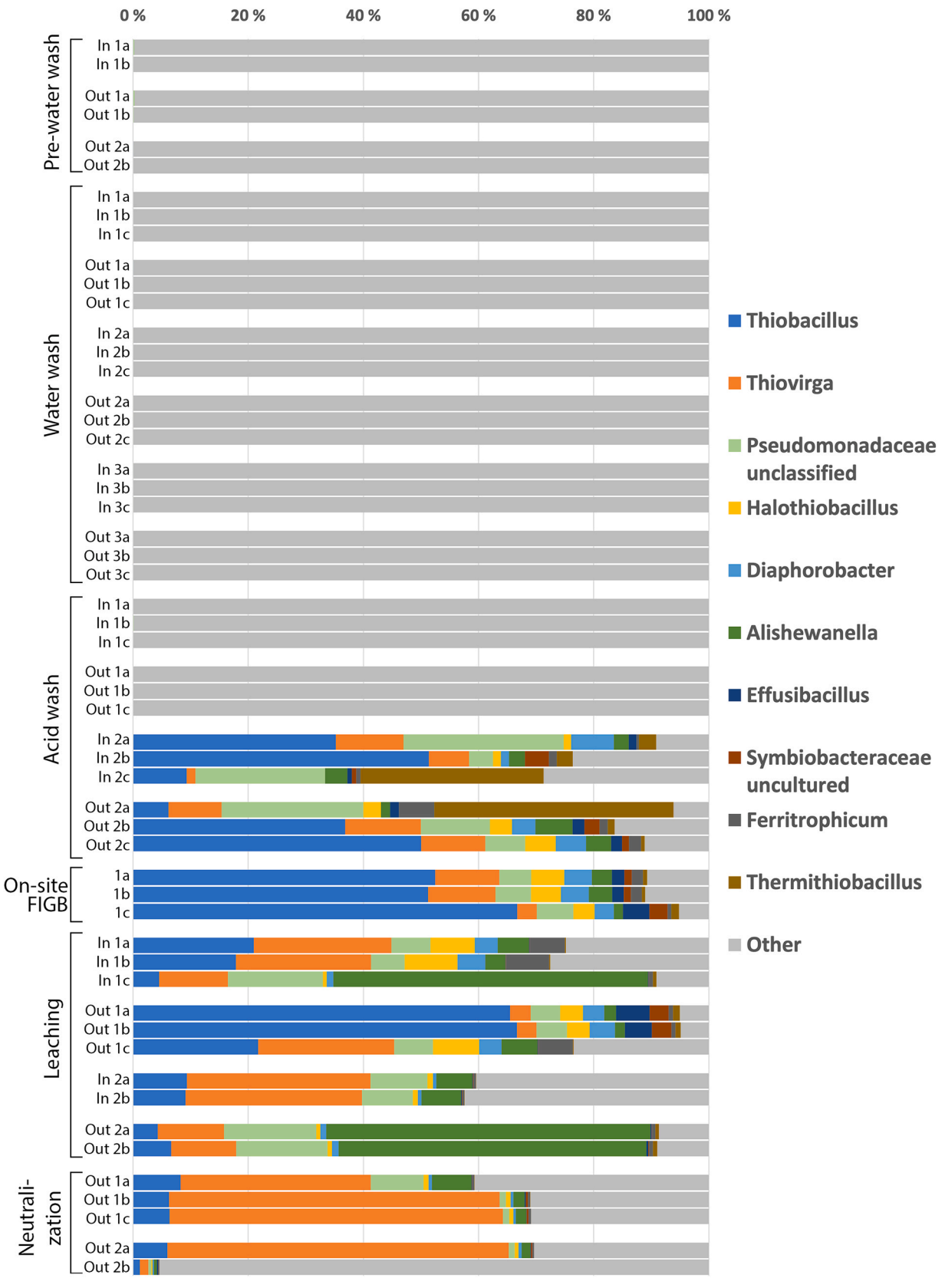

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Fig. 5. The relative abundance of the 10 most prominent bacterial genera of the on-site FIGB community and their distribution throughout the pilot rock reactor samples. The grey bars show the combined relative abundance of bacterial genera that were not among these 10.

the bacterial communities over time. The first acid wash influent and effluent samples fell to the center part of the plot whereas the second acid wash samples fell to the lower left of the plot together with the leaching and on-site FIGB samples. With the exception of one of the second replicate neutralization samples, all neutralization samples fell close to the acid wash and leaching samples in the left of the plot. In general, the pre-water wash and water wash communities were clearly separated from the communities in the acidic acid wash and leaching samples indicating highly different community composition between these water types.

A total of 22,337 bacterial OTUs belonging to 819 different bacterial genera were detected from the whole sample set. With the exception of the acid wash samples, most of the bacteria belonged to the classes Alpha- and Gammaproteobacteria (Fig. 5, Figs. S4AB). Sequence reads identified as Alphaproteobacteria were sorted into several groups based on sequence similarity, but the groups could not be assigned to specific alphaproteobacterial genera. The Gammaproteobacteria belonged to Marinobacter in the pre-water wash and water wash samples (Figs. S4AB).

No bacterial genera found in the on-site FIGB community were detected in the influent or effluent samples of the pre-water wash or water wash phases, despite the high number of bacterial 16 S rRNA gene counts in these samples (Fig. 5). This was also the case for the first acid wash samples, but in the second acid wash Thiobacillus represented up to 51 % of the bacterial community in both influent and effluent samples and Thiovirga up to 11 %. In the FIGB effluent used as lixiviant in the rock reactor, Thiobacillus and Thiovirga contributed with up to 66.7 % and 11.7 %, respectively, and unclassified Pseudomonadaceae, Halothiobacillus, Diaphorobacter, Alishewanella, Effusibacillus, unclassified Symbiobacillus, Ferrotrophicum and Thermithiobacillus contributed with lower relative abundances. The similarity between the second acid wash sample community and the on-site FIGB community is likely due to the fact that the leaching phase and thus the circulation of lixiviant from the in situ rock reactor to the on-site FIGB had been on-going for one week at the time of sampling from the on-site FIGB. The first leaching influent solution had lesser relative abundance of Thiobacillus (up to 21.0 %) and higher relative abundance of Thiovirga (up to 23.4 %) compared to the on-site FIGB effluent, but in the leaching effluent the relative abundance of Thiobacillus had increased to up to 66.7 % and Thiovirga had decreased to at most 23.6 %, with great variation between the replicate samples. The next leaching sample showed that Thiobacillus had markedly decreased both in the in situ rock reactor influent and effluent samples to at most 9.4 %, whereas the proportion of Thiovirga was almost 32 % in the influent to the rock reactor samples. However, in the effluent from the rock reactor, the proportion of Thiovirga was only approximately 11.5 %, whereas the proportion of Alishewanella was up to 66.3 % and that of unclassified Pseudomonadaceae was approximately 16 %. In the first neutralization effluent samples the proportion of Thiovirga was 33.0 – 57.9 % of the bacterial community and Thiobacillus contributed with at most 8.3 %, unclassified Pseudomonadaceae with 1.1 – 9.2 %, Alishewanella with 1.8 – 6.9 % and the other on-site FIGB genera with less than 1 % each. Of the second neutralization sample, after one week of flushing with Cl^- rich water, one of the replicate samples contained high relative abundance of Thiovirga (59.3 %) with low contributions of Thiobacillus (6.0 %) and other on-site FIGB taxa at less than 1 % relative abundance each, whereas the bacterial community of the other replicate sample consisted to 95.4 % of bacteria not found in the on-site FIGB community.

The PCoA of the fungal communities clustered the pre-water wash, water wash and first leaching phase samples into defined clusters placed in opposite parts of the plot (Fig. 4B) indicating distinct fungal communities in these water types. The acid wash, second leaching phase and the neutralization phase samples clustered together close to the middle of the plot indicating high similarity between in the fungal communities between these sample types. The on-site FIGB samples fell together with the first sampling point of the leaching phase outlet samples.

A total of 1797 fungal OTUs belonging to 237 fungal genera were detected from all samples combined. The majority of the fungal community in the on-site FIGB effluent consisted of unclassified Ascomycota with the closest match in the The National Center for Biotechnology (ncbi) nucleotide database to a clone S2_31796 (accession number KU000393), which contributed with 26.2–72.2 % of the fungal sequence reads in the on-site FIGB community, and a likewise unclassified ascomycotal clone LMRF_48 (accession number EU826922) 15.7 – 52.3 % of the community (Fig. 6, Figs. S5AB). Other fungal groups were present at less than 5 % relative abundance. The only fungal genus present in the water wash samples that could also be found in the FIGB community had Rhodothorula-like sequences. This fungus was found in the first and second influent and effluent water wash samples at maximum relative abundance of 11.3 %, but was present in the FIGB community at less than 1 %. The first leaching influent sample community did not contain S2_31796 sequences, but had high relative abundance of Fusarium-like fungal sequences, up to 81.7 %, and up to 20.7 % of LMRF_48 sequences in two out of three replicate samples. The effluent samples contained up to 28.2 % S2_31796 and 57.2 % LMRF_48 sequences in two out of three replicate samples and 89.7 % Fusarium-like sequences in the third replicate sample. The second leaching influent sample had a completely different fungal flora consisting of up to 65.0 % of uncultured fungi with the closest matching ncbi database sequence being 87_NA11_P31_H7 (KC965607). The second leaching effluent sample consisted of 99.3 % of fungal types not present in the FIGB community. Of the three replicates of the first neutralization effluent samples, one contained 65.2 % 87_NA11_P31_H7 like sequences, while the other community identified from the other two replicates consisted to more than 90 % of fungal types not present in the on-site FIGB. The second neutralization sample consisted of 98.2–99.8 % of fungi not present in the on-site FIGB.

3.4. Testing iron oxidation capacity by cultivation

The iron oxidation capacity of the microbial community during the different stages of the rock reactor construction, preparation, leaching, and neutralization was tested by cultivation on four different acidic, ferrous iron containing media. Samples from the water

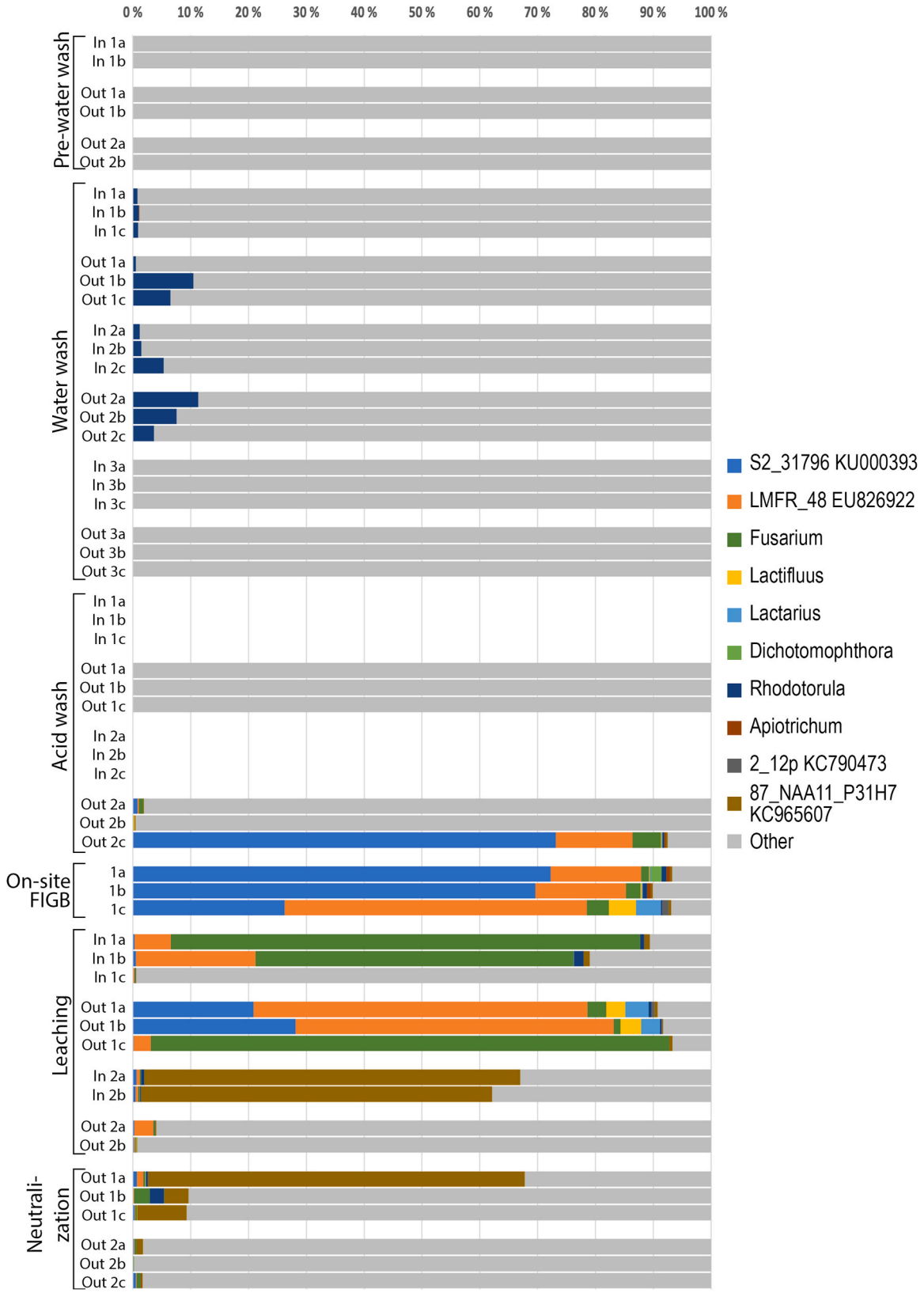

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Fig. 6. The relative abundance of the 10 most prominent fungal genera of the on-site FIGB community and their distribution throughout the samples. The grey bars show the combined relative abundance of fungal genera that were not among these 10. The sequence data for both acid wash influent samples were omitted due to too low sequence counts.

wash and acid wash stages contained only low or no iron oxidizing capacity, i.e., acidophilic iron oxidizers, as seen by no or only slight color change in any of the media after 1 week of incubation (Table 3, Fig. S6). During the leaching phase, noticeable iron oxidation capacity was seen in both In and Out samples in all culture media, with the exception that in the first leaching samples the iron oxidation capacity appeared less prominent in the DSM 477 medium containing NaCl. However, the iron oxidation capacity was noticeable in the second leaching sample as determined by clear color change in the media. In the neutralization phase, the iron oxidation capacity remained noticeable in Medium Fe-1 and DSM 477 at both sampling times, whereas the color change was only slightly different from the negative control in the DSM 882 and DSM 271 + YE media.

4. Discussion

In situ bioleaching of metals is considered a more environmentally friendly, inexpensive, and less invasive mining approach than conventional open pit mining and excavations, but may still pose some environmental hazards, such as uncontrolled AMD and pollution of surrounding surface and groundwater (Johnson, 2015, 2018; Vargas et al., 2020; Seredkin et al., 2016). In the BIOMOre project issues like the spontaneous acidification of the rock reactor site due to indigenous acidity producing microorganisms and inactivation of bioleaching microorganisms flowing from the on-site FIGB to the rock reactor were investigated. To our knowledge, this is the first study examining the possibility to inactivate introduced iron oxidizing acidophiles in a deep in situ pilot bioleaching rock reactor complex and is of high importance for the future development of in situ bioleaching operations globally.

The outflow of the rock reactor before acidification contained only low amounts of acidophilic iron oxidizing microorganisms as they were not detected by sequencing and only slight color change was visible in the iron oxidation cultivations from the water wash or first acid wash samples (Fig. S6). This was in accordance with a previous study on saline water in Rudna Mine that showed that the indigenous microbial community did not contain acidophilic microorganisms or acidification of the water (Bomberg et al., 2022). Obligate acidophilic iron oxidizers require constant low pH and may be sensitive to great increases in pH. These microorganisms maintain a near neutral cytoplasmic pH in extremely acidic environments by different active strategies, such as a highly controlled proton motive force (PMF) and by changing their cytoplasmic membrane fluidity (Michels and Bakker, 1985; Mykytczuk et al., 2010). It is likely that a community of these microorganisms requires some time to become established, which would explain the occurrence of acidophilic bacterial genera during the later stages of the acid washing phase, as this phase stretched over approximately 6 months and was discontinued for part of this time, thus allowing for a low biomass, indigenous acidophilic community to develop (Figs. 4 and 5). In addition, it is highly likely that the IOB attach to the surfaces of the rock reactor where they can form biofilms with suitable acidic microenvironments even in circumneutral environments, as has been shown for in waste rock and bioleaching heaps (Dockrey et al., 2014; Henne et al., 2019; Mielke et al., 2003), from where the IOB can detach into the surrounding fluids.

The FIGB microbial community did not contain typical acidophilic iron oxidizing bacterial lineages, such as *Leptospirillum* and *Acidithiobacillus*. Nevertheless, the consortium managed to maintain low pH and high iron oxidizing activity and in addition showed unusually high microbial diversity. It is likely that the typical IOB were attached to the carrier material and thus were present at levels below the detection limit of the assay in the fluids. It has been shown that IOB in biofilm on carrier material are not washed out with the outflow from bioreactors (Giaveno et al., 2008) and that non-attached cells are much more likely to die in the solution if conditions become unfavourable (Pace et al., 2005). Bacteria belonging to the *Thiobacillus* genus dominated in the effluent of the on-site FIGB at the start of the leaching phase. However, their relative abundance decreased with an increase of the relative abundance of *Thiovirga* and unclassified Pseudomonadaceae bacteria in the leaching solution running from the on-site FIGB into the rock reactor, and in addition to these, variable amounts of *Alishewanella*. The pH of the leaching solution increased slightly during the time in the rock reactor and the concentration of Cu and Cl^- increased, which may affect the microbial community structure. However, the Cu was removed before the leaching solution was returned to the on-site FIGB and should not have affected the bioreactor community.

Table 3
Visual determination of iron oxidation activity in the culture tubes compared to a negative control (non-inoculated growth media) after one week of incubation at 30 °C. Nd indicates 'Not determined', - no change compared to negative control, + colour change compared to negative control indicating iron oxidation, + + strong colour change compared to negative control, indicating strong iron oxidation activity.

Sample	Sampling point (In/Out)	Medium Fe-1	DSM 882 (*Leptospirillum*)	DSM 271 with Yeast extract	DSM 477 (*Halothiobacillus*)
Water wash 3	In	+	Nd	+	-
Water wash 3	Out	+	Nd	+	-
Acid wash 2	In	+	-	+	-
Acid wash 2	Out	+	-	+	-
Leaching 1	In	++	++	++	+
Leaching 1	Out	++	++	++	+
Leaching 2	In	++	++	++	++
Leaching 2	Out	++	++	++	++
Neutralization 1	Out	++	+	++	++
Neutralization 2	Out	++	+	+	++

Nevertheless, the Cl^- concentration increased in the on-site FIGB during operations and varied between 4 and 14 g L^{-1} and rose to as much as 41.4 g L^{-1} in the outflow from the rock reactor by the end of the leaching phase, which may have affected the on-site FIGB community. Acidophilic microorganisms are often sensitive to high chloride concentrations (Shiers et al., 2005; Gahan et al., 2009; Ballerstedt et al., 2017) because of their positive internal membrane potential. Chloride rich conditions cause an influx of chloride ions that leads to a negative gradient that allows for an influx of protons and acidification of the cytoplasm and death of the acidophiles (Alexander et al., 1987). In addition, a higher salt concentration outside than inside the cell also leads to osmotic stress, which may either dehydrate or lyse the cell. Nevertheless, some acidophiles, such as *At. ferrooxidans* and *At. caldus*, have been suggested to react to osmotic stress by adaptation of their cell membranes to higher osmotic stress, accumulation of amino acids as osmoprotectants in the cytoplasm, and expression of specific proteins (YceI family) that are involved in acid and osmotic stress related actions (Zammit et al., 2011, 2012). Some extremely halotolerant bacteria, such as *Ah. prosperus*, have been shown to tolerate chloride concentrations of up to 35 g L^{-1} (Nicolle et al., 2009). It should be noted, though, that rock reactor inflow and outflow leaching solution contained a very low bacterial load, although there was clear iron oxidation activity detected in the cultivation tests.

The neutralization phase beginning with flushing the rock reactor with alkaline $CaCO_3$ solution increased the pH in the rock reactor with the intention to irreversibly inhibit acidophilic iron- and sulfur-oxidizing microorganisms and end the leaching process. Several different inhibition solutions have previously been tested in laboratory scale experiments showing that the locally found chloride-rich water as well as mixtures of organic acids, primary alcohols, nitrate, and chloride with simultaneous increase in pH could inhibit the bioleaching acidophilic community, whereas tap-water did not (Bomberg et al., 2017; Ballerstedt et al., 2017). Nevertheless, during the short neutralization period in this pilot experiment, the number of bacterial 16 S rRNA gene copies mL^{-1} increased indicating microbial growth in general and after one week of neutralization, the microbial iron oxidation activity in the solution outflowing from the rock reactor was still visible in the enrichment cultures. The outflowing neutralization solution also contained a high relative abundance of *Thiovirga* in the bacterial community. *Thiovirga* have been found at neutral pH in mine water overflow and minerals processing in several studies (Kadnikov et al., 2019; Miettinen et al., 2021). This type of species has pH and temperature optimum at 7.5 and 30 °C, respectively (Ito et al., 2005), which are close to that of the rock reactor. However, the known *Thiovirga* species require at least microaerobic conditions, and do not tolerate high salinity, with more than 50 g NaCl L^{-1} in the outflow of the rock reactor at the end of the leaching phase and between 40 g and 105 g L^{-1} in the local mine water ponds (Bomberg et al., 2022). The pilot rock reactor has been disassembled and a longer neutralization time with longer follow-up was not possible due to time restrictions and operations in the mine. However, it is likely that after operations cease and this type of rock reactor would be flooded, the pH and Cl^- concentration of water in a rock reactor in Kupferschiefer would dramatically increase and turn anoxic, which would decrease the activity of the *Thiovirga*.

The naturally occurring fungal consortia detected during the construction of the rock reactor differed from the community in the FIGB and in the rock reactor during leaching. Fungi are known to weather rock by secreting organic acids and thus release nutrients for bacterial and archaeal communities (Jongmans et al., 1997) and have been shown to leach copper from carbonate copper ore (Kiel and Schwartz, 1980). Fungal numbers were generally low, but highest during the water wash phase and low in the FIGB and during the leaching phase, and hardly any fungi were detected in the neutralization phase. The fungi in the FIGB and leaching phase were not detected in the neutralization phase. Fungi have been found in acidic environments (e.g., reviewed by Gross and Robbins, 2000), also in metal-rich and acidic mine environments (Bomberg et al., 2018) and mineral processing plants (Bomberg et al., 2020; Miettinen et al., 2021). In this study, fungi were most abundant in the water during the construction of the rock reactor, i.e. during the pre-water wash and water wash phases when tap water was used to wash the rock reactor, and may have originated from the tap water rather than from the ore. Archaea were not detected in any of the samples and likely did not have a role in this environment.

Copper was abundantly leached both during the acid wash and leaching phases, but different Cu fractions, i.e. from different types of Cu-bearing minerals (chalcocite, bornite, chalcopyrite, atacamite), were likely dissolved during the different phases. Acid washing was used to condition the rock reactor for the actual leaching process by removing carbonates with acid. This also led to the dissolution of Cu bearing minerals, assumedly e.g. atacamine, that were easily dissolved in acid, but not the metal-sulfide minerals, as shown previously (Pakostova et al., 2018). However, based on Pakostova et al. (2018) and the more than 400 % increase in copper in the outflow of the rock reactor when the leaching phase started (Fig. 1), the metal-sulfide minerals were likely leached with the acidic ferric iron solution from the on-site FIGB, indicating that indirect deep in situ bioleaching of copper from sulfidic ores by regeneration of ferric iron lixiviant in an on-site FIGB is possible, not taking into account costs, efficiency or even suitability of the site. It should also be considered that the rock of the test site was rich in carbonates, which makes the site less suitable for this process as large quantities of acid are needed to remove the buffering capacity of the carbonates to make the rock reactor environment suitable for leaching with acidic lixiviant and obligately acidophilic FIGB microorganisms. Acid treatment of carbonaceous rocks also releases great amounts of CO_2, an estimated 74 m^3 CO_2 per ton rock in the rock reactor (Szubert, 2018) and the estimated size of the rock reactor pilot of this study was 250 tons, which is something to be considered when choosing in situ bioleaching sites.

5. Conclusions

The concept of in situ biomining is an attractive alternative compared to conventional excavation intense mining operations. However, this technique may still face some environmental challenges, such as contamination of groundwater by leaching agents and uncontrolled leaching and iron oxidation caused by residual IOB originating from the on-site FIGB (e.g. Seredkin et al., 2016; Johnson, 2015, 2018). Thus, the iron oxidizing microorganisms need to be inactivated at the end of the operation. In this study, we examined the change in microbial community structure and acidophilic iron oxidation capacity over time during the construction, operation and post operation phase of the hydrofracking-bioleachingpilot rock in situ reactor in the Rudna mine. There was only slight acidophilic,

iron oxidation activity detected in the pre-operational water washing steps of the rock reactor and no known IOB were found in the sequence data, but after acid treatment, during leaching and at the beginning of the decommissioning phase, iron oxidation was evident. Iron oxidizing bacterial taxa were also detected in samples from the late acid wash phase and throughout the leaching and neutralization phase by amplicon based high throughput sequencing. These taxa were similar to those detected in the on-site FIGB and from the leaching phase onwards. The reason for this may be the timing of the sampling as it occurred at the transition between the acid wash and leaching stages and the leaching solution was injected directly after the last acid solution. The fungal community in the on-site FIGB, leaching and first neutralization phase samples consisted of similar groups, although the relative abundances of the detected groups differed. In the second neutralization sample the fungal community was clearly different from that of the acidic samples.

The results show that some of the iron-oxidizing microorganisms from the separate iron oxidation reactor were transported to the rock reactor from the on-site FIGB when the solution was returned to the circulation in the rock reactor. This may improve the leaching in the rock reactor during the operation but cause undesired leaching after the operations have been stopped. Therefore, the inactivation of introduced acidophilic consortia in the in situ rock reactor needs to be properly managed after the operations by raising the pH of the rock reactor to circumneutral by rinsing with $CaCO_3$ solution (this study), solutions of formate, nitrate, alcohols and/or SDS (Ballerstedt et al., 2017) or highly saline chloride-rich water naturally present in the mine (Bomberg et al., 2018) and testing for active IOB before ending the decommissioning operations. However, in this particular site the rock type contains high amounts of carbonate and chloride, which will hinder the development of undesired leaching by raising the pH and increasing the chloride concentration in the water rendering the environment unfavorable for obligately acidophilic IOB.

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CRediT authorship contribution statement

Malin Bomberg: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – original draft, Visualization. **Hanna Miettinen:** Conceptualization, Methodology, Validation, Writing – review & editing, Visualization. **Réka Hajdu-Rahkama:** Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Visualization. **Aino-Maija Lakaniemi:** Conceptualization, Methodology, Validation, Writing – review & editing, Visualization, Supervision. **Wojciech Anacki:** Conceptualization, Methodology, Validation, Writing – review & editing. **Kajetan Witecki:** Conceptualization, Methodology, Validation, Writing – review & editing. **Jaakko A. Puhakka:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Funding acquisition. **Théodore Ineich:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Wickus Slabbert:** Conceptualization, Methodology, Writing – review & editing. **Päivi Kinnunen:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

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Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2023.103375.

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