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Bioleaching of cobalt from Cu/Co-rich sulfidic mine tailings from the polymetallic Rammelsberg mine, Germany

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ABSTRACT

A microbial consortium of mesophilic and acidophilic bacteria and archaea was applied in shake flasks as well as in 2 L stirred tank reactors (STR) to bioleach cobalt, copper, and other valuable metals from sulfidic mine tailings (Rammelsberg polymetallic massive sulfide deposit, Harz Mountains, Germany). After succession from low to high pulp density, the microbial consortium was well adapted to 10% pulp density and showed high bioleaching efficiency. Microbial activity and abundance were measured by microcalorimetry, microscopy, and quantitative, real-time PCR. The adapted mesophilic microbial consortium consisted mainly of *Acidithiobacillus* (*At.*) *ferrooxidans* and *At. thiooxidans* which achieved 91% cobalt and 57% copper extraction from the bulk tailings (Co 0.02%; Cu 0.12%) after 13 days in STR. Bioleaching tests with a tailings flotation concentrate (Co 0.06%; Cu 0.57%) showed a recovery of 66% cobalt and 33% copper. In addition, mineralogical analysis showed that cobalt occurred on the surface of framboidal pyrite and was mainly leached by microbial attack. Attached cells were microscopically observed on the surface of solid particles of the bulk tailings and tailings flotation concentrate. The amount of sulfides (mainly pyrite) in the tailings was sufficient to sustain microbial growth and thus no additional substrate was required for tailings bioprocessing. Bioleaching is considered to be an important processing step in the concept for reprocessing of the Rammelsberg mine tailings, and for many sulfidic mine tailings worldwide.

1. Introduction

Billions of tons of mining wastes and residues have been stockpiled or dumped as tailings worldwide. Mine tailings are among the largest mining wastes on Earth and can occupy large areas. Mine tailings result from the flotation process of sulfide mineral ores. Exposed to water and oxygen these very likely produce acid rock drainage (ARD), causing ground and surface water contamination (Dold, 2008; Dold, 2014; Kalin et al., 2018). From another point of view, mine tailings may contain significant amounts of valuable metals including indium, magnesium, cobalt, platinum group elements (PGE), or rare earth elements (REE), which are of high economic interest (Alcalde et al., 2018; Ulusoy, 2019). Resources from mining residues are an option for diversifying critical mineral supply, as the need for metals and raw materials is rapidly increasing due to the global economic growth. The recovery of valuable metals from mine wastes and tailings is becoming increasingly important (Kaksonen et al., 2018; Schippers and Hedrich, 2018).

Bioleaching refers to biological solubilisation of metals from minerals and wastes. Biomining uses microorganisms to enhance the extraction and recovery of metals from minerals and wastes (i.e. both leaching and recovery). Engineering applications for bioleaching (and hence also biomining which also includes bioleaching) include e.g. bioreactors, vats, heaps and in situ leaching (Kaksonen et al., 2018). Bioleaching of ore concentrates, mine tailings, and secondary resources such as electronic waste has been demonstrated (Schippers et al., 2014). Acidophilic microorganisms (Hedrich and Schippers, 2021) facilitate dissolution of insoluble metal sulfides via indirect bioleaching mechanisms by generating ferric iron and/or protons, either in contact with the mineral surface or as planktonic cells in the liquid phase (Sand et al., 1995; Vera et al., 2013). Several laboratory studies have already demonstrated efficient metal extraction from sulfidic mine tailings using bioleaching. Bioleaching has shown to be more efficient than acid leaching with Cu extraction between 68% and 98% after five weeks from mine tailings (covellite, chalcopyrite, enargite and chalcocite as most abundant Cu minerals) with high pyrite content of 22% (Stanković et al., 2015). Bioleaching of sulfidic tailings (chalcopyrite as chief Cu mineral) in an Iranian mine using moderate thermophiles reached 55% Cu and 59.5% Co extraction after 30 days at 5% pulp density (Ahmadi

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et al., 2015). The Cu and Co bioleaching from tailings (pyrite, chalcopyrite and valleriite as main ore minerals) of a Chinese mine resulted in 84% and 86% extraction, respectively, at 10% pulp density for 12 days in shake flasks (Wen and Ruan, 2002). A combination of oxidative and reductive bioleaching carried out at extremely low pH values (~1) and at 45 °C was shown to be highly effective in extracting copper from mineral tailings (mainly quartz, pyrite and gypsum, small amount of chalcopyrite) in two mines currently operating in Spain and Serbia (Falagán et al., 2017). Bioleaching of low grade flotation tailings (pyrrhotite, pyrite, chalcopyrite, pentlandite, and sphalerite as main ore minerals) at 5% pulp density achieved 60% of Co extraction in reactor experiments at 32 °C for 15 days (Altinkaya et al., 2018). A commercial application of stirred-tank reactor (STR) bioleaching for Co recovery has even been demonstrated for a cobalt-containing pyrite concentrate stockpiled in the former copper mine in Kasese, Uganda (Morin and d'Hugues, 2007).

The abandoned Rammelsberg mine is located in the historic Harz Mountains mining district in central Germany. The Rammelsberg ore deposit was mined until 1988 (Römer et al., 2018) and flotation tailings from the mining activities were deposited in the Bollrich tailings pond (Supplementary Fig. S1). Recovery of valuable metals and minerals from the tailings pond has been proposed (Kuhn and Meima, 2019). A recent research project (REWITA) funded by the German Federal Ministry of Education and Research (BMBF) investigated the presence of Cu, Co, Zn, Ag, Ga, In and other valuable metals in minerals (barite, sulfides, silicate, etc.) in the mine tailings in detail (Schirmer et al., 2017). A process scheme for their recovery and complete reprocessing of the Bollrich tailings material was developed and tested at laboratory scale (Römer and Goldmann, 2019). One important process step in this scheme might be bioleaching. A preliminary study by the German company G.E.O.S. Ingenieurgesellschaft mbH demonstrated the suitability of bioleaching for metal extraction. Economic (bio-)hydrometallurgical processing of mine tailings requires a sufficiently high metal concentration in the leaching solution for downstream treatment. In general, an increase in pulp density in stirred tank reactor bioleaching results in decreased cobalt and copper recovery, due to inhibition of microbial activity at high pulp density (Bampole and Mulaba-Bafubiandi, 2019; Liu et al., 2007). Thus, for the bioleaching process a compromise between an economically relevant pulp density and microbial activity and growth is required to achieve a high metal leaching efficiency. Studies on how microbial cells interact with low grade ores, especially mining tailings, are still scarce.

In this study, we tested a mesophilic and acidophilic microbial consortium to bioleach copper, cobalt and other valuable metals from the bulk Rammelsberg mine tailings and a sulfidic tailings flotation concentrate. Results of the metal dissolution and microbiological analysis during bioleaching are presented. Microbial community composition in bioreactors under different operational conditions was evaluated. In addition, the interaction of microorganisms with the tailings particles was investigated.

2. Materials and methods

2.1. Tailings and tailings concentrate samples

Tailings samples from Rammelsberg mine (Goslar, Harz Mountains, Germany) were obtained from various tailings depths after coring (BMBF project r4 REWITA). A mixed sample from 15 to 26 m tailings depths was assigned as the bulk tailings. Tailings concentrate samples were obtained after flotation processing of the bulk tailings (Römer et al., 2018) and washed with water in order to remove floatation reagents. Both, bulk tailings and tailings concentrate, were milled to a grain size of < 500 μm and were stored under nitrogen in gas-tight bottles.

2.2. Material characterization

The main elemental composition of the two tailings samples and bioleaching residues was determined by peroxide fusion ICP-MS (FUS-MS-Na_2O_2, Actlabs), X-ray fluorescence analysis (XRF), and chemical analysis. For the latter, the samples were digested by aqua regia and then analyzed for their metal ion concentration by ICP-OES. For the digestion, 0.5 g of an air-dried sample were digested with 12 mL concentrated HNO_3-HCl (1:3 v/v) mixture. After heating at 110 °C for 3 h, the dried residues were diluted with 20 mL 2% HNO_3. After filtration with Whatman no. 42 paper, the suspension was transferred to 100 mL volumetric flask and then filtered through a 0.2 μm membrane filter. The filtrate was used for ICP-OES analysis.

Total sulfur and organic carbon contents were analyzed using a LECO CS 230 carbon-sulfur analyzer. For the latter, the samples were first treated with 10% HCl at 80 °C to remove the carbonaceous carbon (inorganic carbon). Treated ssamples were then combusted in a high frequency oven at about 2000 °C with an oxygen stream. The resulting carbon dioxide was analyzed by infrared detection, carbon monoxide was catalytically oxidized to carbon dioxide before detection. The sulfide content of the samples was determined by using chromium-reducible sulfur distillation as previously described (Gröger et al., 2009). The specific surface area was determined by N_2 adsorption with a Micromeritics Gemini III 2375 surface area analyzer (Kaufhold et al., 2010). The mineralogical composition was determined via X-ray diffraction analysis (XRD). Surface analyses of solids was done by electron probe microanalysis (EPMA-SEM).

2.3. Microbial consortium

A microbial consortium of acidophilic bacteria and archaea of the BGR (Bundesanstalt für Geowissenschaften und Rohstoffe) strain collection (Schippers et al., 2010) was selected according to previous studies in BGR (Hedrich et al., 2016; Marrero et al., 2015). It consisted of *At. thiooxidans* Ram 8 T, *At. ferrooxidans* Ram 6F, *Leptospirillum (L.) ferrooxidans*^{T}, *Ferroplasma (Fp.) acidiphilum* BRGM 4. The mixed culture was pre-grown on 50 mM ferrous iron and 10 g/L S^0 at an initial pH of 2.0 at 30 °C in basal salt medium (Wakeman et al., 2008).

2.4. Bioleaching tests

Different assays were performed to comparatively study the efficiency of metal bioleaching to select the optimal bioleaching conditions. These included 1) inoculated assay: addition of the microbial consortium mentioned above, 2) not inoculated assay: no microorganisms were added (only indigenous bacteria from the mine tailings were present), and 3) abiotic control: 0.5 mM formic acid was added to the assay to inhibit bioleaching bacteria (Ballerstedt et al., 2017) under no inoculation conditions representing chemical leaching only. The initial pH was set to 1.6, 1.8 or 2.0 and pulp density was 2%, 5% or 10%. The leaching experiments were first conducted in shake flasks at 2% pulp density and then at 5% and 10% pulp density in both, shake flasks and stirred-tank reactors (STR). The pre-cultivation strategy of Hedrich et al. (2016) for increasing the pulp density was applied. The experimental conditions for tests in 2-L STR and shake flasks were as follows: Temperature at 30 °C and pH 2.0; leaching time of ~two weeks; two or three inoculated parallel assays and one abiotic control. Once the experiments started, the pH in the abiotic reactors was set according to the pH in the bioleaching reactors. The parameters temperature, pH and agitation were controlled by the STR operation system (Fermac 200 modules, Electrolab, UK) equipped with the agitation module Fermac 231. Temperature control was achieved using an industry standard Pt100 sensor. The pH was controlled by a pH controller through pumping 2 M or 5 M sulfuric acid. Aeration (0.5 L/min) was achieved by pumping air through a syringe filter (pore size 0.2 μm, Sartorius, Germany). Water evaporation was compensated at regular intervals by addition of diluted sulfuric acid (pH 2) during the experiments, and samples were taken at different time intervals.

2.5. Chemical analytical and microbiological/molecular biological methods

The pH values and oxidation/reduction potentials (ORP) were intermittently measured with digital pH − /redox meters (Mettler Toledo and WTW) with semi-micro pH electrodes (Blue Line pH 16, SI Analytics), and an ORP electrode (Ag/AgCl reference, PCE Instruments), respectively. The amount of acid consumed during leaching was recorded for comparison. Liquid samples were withdrawn each day or each second day till the leaching experiments were finished. Cells were counted directly by using a light microscope and after SYBR Green staining by using a fluorescence microscope and/or quantified by quantitative real-time PCR (qPCR) using copy numbers of bacterial 16S rRNA genes (Hedrich et al., 2016). Dissolved ferrous iron and total iron concentrations were measured by the ferrozine assay (Lovley and Phillips, 1987). ICP-OES was used to determine the concentration of metal ions. Terminal restriction fragment length polymorphism (T-RFLP) was used to analyze the composition of microbial communities (Hedrich et al., 2016). Microcalorimetric activity measurements were done according to previous reports (Hedrich et al., 2016; Schippers and Bosecker, 2005). The measured heat output in μW was given per gram tailings.

2.6. Confocal laser scanning microscopy (CLSM)

Cells were visualized by using nucleic acid stain Syto 9 or protein stain Sypro orange. Samples were neutralized with filter-sterilized tap water and incubated with fluorescently labelled stains for 20 min at room temperature. Afterwards, stained samples were washed three times with filter-sterilized tap water in order to remove the unbound stains. Samples were directly observed using confocal laser scanning microscopy (CLSM) without any further treatment. Stained samples were examined using a Leica TCS SP5X instrument, controlled by the LASAF 2.4.1 build 6384 (Leica, Heidelberg, Germany). The system was equipped with an upright microscope and a super continuum light source (470–670 nm) as well as a 405 nm diode laser. Images were collected with a 63× water immersion lens with a numerical aperture (NA) of 1.2 and a 63× water-immersible lens with an NA of 0.9.

Fluorescence image analysis and surface coverage calculation were done by using an extended version of the software ImageJ (v1.50i). Maximum intensity projection (MIP) was produced with the software IMARIS version 8.1.2 (Bitplane AG, Zurich, Switzerland).

3. Results and discussion

3.1. Characterization of the bulk tailings and tailings concentrate

Results of elemental analyses of the tailings samples are shown in Table 1. Apart from quartz, pyrite was identified as one of the main mineral phases in these tailings samples (Supplementary Table 1). This enables the possibility and feasibility to use bioleaching for tailings processing, since pyrite serves as energy substrate for bioleaching microorganisms. Both samples contained considerable amounts of cobalt (0.02–0.06%) and copper (0.12–0.57%). The total resources in the whole tailings were estimated to be 1221 t Co and 12,593 t Cu (Römer et al., 2018). Most interesting metals including Cu were concentrated within the sulfide fraction, and Co was primarily related to pyrite (Supplementary Fig. S2), followed by chalcopyrite and sphalerite (Schirmer et al., 2017). Specific surface analysis of the tailings showed that the bulk tailings and tailings concentrate had surface areas of 8.08 m^2/g and 2.30 m^2/g, respectively (Supplementary Table 1).

Table 1
Elemental composition of the bulk tailings and tailings concentrate (%).

Tailings	C_{org}	Fe	TS	S^{2-}	S^0	Co	Cu	Zn	Pb	As
Bulk	0.16	10.2	9.28	7.52	0.08	0.02	0.12	1.49	1.35	0.08
Concentr.	1.44	27.9	34.25	30.7	0.10	0.06	0.57	4.85	4.09	0.25

TS: total sulfur content; C_{org}: organic carbon.

3.2. Bioleaching tests of bulk tailings in shake flasks

Bioleaching was tested at 2%, 5% and 10% pulp density at different initial pH values after 16 days. Results from bioleaching of bulk mine tailings at different initial pH values and SL in shake flasks are presented in Fig. 1. For iron dissolution, bioleaching was more efficient than the abiotic control for chemical leaching; when the SL increased, dissolved iron concentration in bioleaching assays increased while the iron bioleaching efficiency decreased. T-RFLP analysis showed that L. *ferrooxidans* accounted for more than half of the community composition at 2% and 5% SL. In contrast, *At. ferrooxidans* and *At. thiooxidans* dominated the microbial community at 10% SL (data not shown). For abiotic controls, lower pH resulted in higher iron leaching. For bioleaching tests, iron leaching depends on both initial pH and SL (Fig. 1). Since no sulfuric acid was added in the experiments, the final pH value of the abiotic controls was higher compared to the initial one regardless of the pulp density. In contrast, the final pH value of the bioleaching tests was lower (not shown), due to sulfuric acid production by sulfide and sulfur biooxidation. An initial pH of 2 was chosen for bioleaching tests with 10% SL.

3.3. Bioleaching of bulk tailings and flotation concentrate in 2-L STR

After adaptation of the mixed microbial consortium at 2% and 5% SL, a robust growth of the mixed culture at 10% SL in the STR was obtained. An improved iron leaching of the bulk tailings from 58% to 85% was measured after succession on SL from 2% to 10% SL (not shown). The mixed consortium containing mainly *At. ferrooxidans* and *At. thiooxidans* remained stable throughout the experiment. The data of the bioleaching of bulk tailings are shown in Fig. 2.

In the bioleaching reactors, redox potential increased rapidly from 389 mV to 573 mV within 4 days. This corresponded to a rapid decrease of the iron(II) concentration from 53 mM to 1.6 mM. After 4 days, the redox potential remained around 573 mV and iron(II) concentration at 2–3 mM. The pH values kept decreasing from 2.0 to 1.5 and total iron concentration increased to 182 mM (~10.2 g/L) after 13 days. This indicates that due to bacterial activity, the iron(II) was constantly converted to iron(III), causing metal sulfide dissolution. The redox potential was kept between 550 and 600 mV. The observed microbial activities in the bioreactor with flotation concentrate (Supplementary Fig. S3) indicate that potentially remaining flotation reagents had no obvious influence on the bacterial activities (Jafari et al., 2019).

In the abiotic control reactor, no obvious iron oxidation was detected, indicated by only slight increase of redox potential (up to 426 mV) and almost constant iron(II) concentration (Fig. 2). In case of leaching tests of tailings concentrate (Supplementary Fig. S3), the iron (II) concentration increased over the 13 days of incubation. All these showed that formic acid with a concentration of 0.5 mM was sufficient to inhibit microbial activity of acidophiles, as described for sodium azide applied during leaching tests (Martin et al., 2015). Previously, formic acid was proposed for decommissioning of deep in situ biomining operations by eliminating microbial activities (Ballerstedt et al., 2017).

As shown in Fig. 3, the adapted mixed culture showed good performance for leaching of the bulk tailings and tailings concentrate at 10% pulp density in 2-L STR. The microbial consortium achieved 91% cobalt and 57% copper extraction from bulk tailings after 13 days in STR. For the flotation concentrates, bioleaching tests showed 66% cobalt and 33% copper recovery after 13 days. A moderately thermophilic

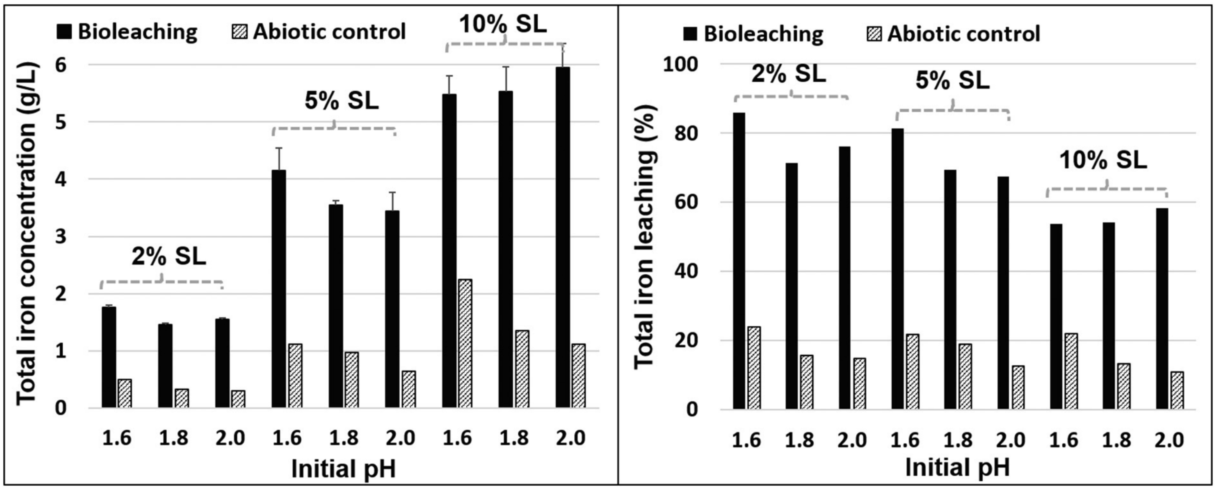

Fig. 1. Bioleaching of bulk tailings at different initial pH and pulp density in shake flasks. Experiments in triplicates were done at 30 °C and at 150 rpm for 16 days. Bars show the concentration of total dissolved iron (left) and total iron recovery (right). Error bars show standard deviation.

consortium BRGM-KCC achieved comparable Co recovery from flotation tailings in 2-L STR reactor at 40 °C within 8 days (Guezennec et al., 2015). Operation at higher temperature is usually associated with higher costs. In the current tests, the metal recovery was lower for the tailings concentrate than for bulk tailings, however further optimizing of the operating conditions should allow for higher recovery.

As and Co bioleaching (Fig. 3) correlated with iron leaching. This is most likely due to the association of As and Co with pyrite (Supplementary Fig. S2). The removal of As together with the recovery of valuable metals reduces the process costs for detoxification of the tailings waste (Park et al., 2014). The leaching dynamics of Cu from the tailings concentrate seem to reach a dissolution plateau earlier than Co (Fig. 3). For the bulk tailings, Co and Cu leaching dynamics showed similar trends (data not shown). Thus, Cu in the tailings concentrate may be more refractory than Co for dissolution (Schirmer et al., 2017). The large content of the silicate minerals was neither altered by microbial nor by ferric iron attack as revealed by the XRD analysis of leaching residues (not shown). Leaching of other metals in the tailings like Mg was most likely related to an acid dissolution process, independent of tailings material.

The comparison of STR bioleaching of bulk tailings and tailings concentrate revealed an increase of Co extraction from 17 mg/L to 36.5 mg/L after 13 days, and a Cu concentration increase from 68 mg/L to 189 mg/L (Fig. 4a). The microcalorimetrically measured heat output related to microbial pyrite oxidation activity for bulk tailings was about 40 μW/g (Fig. 4b), which was somewhat higher than microbial activities in a mine waste dump of Ilba Mine in Romania (Jozsa et al., 1999; Sand et al., 2007). Microbial communities grown on tailings

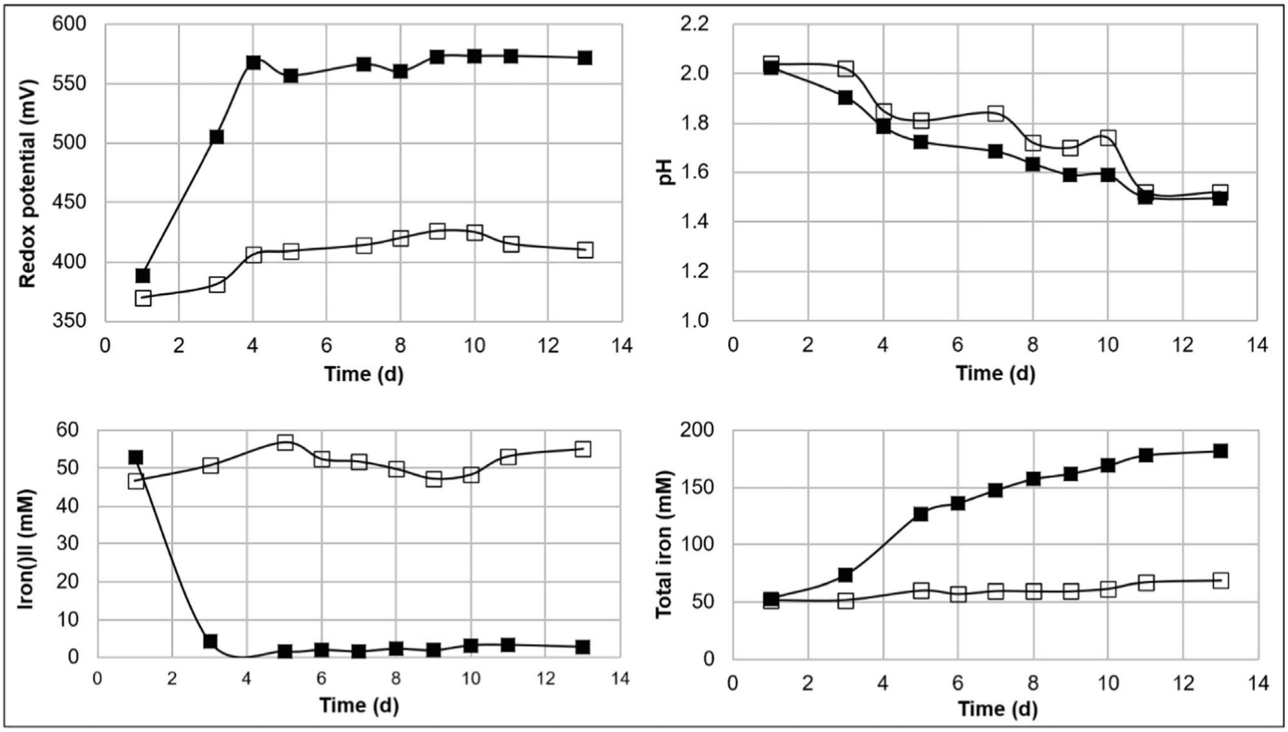

Fig. 2. Bioleaching of the bulk tailings by the mixed culture in 2-L STR at an initial pH of 2.0 and at 30 °C. Changes in redox potential, pH, iron(II) and total iron concentration. ■: bioleaching, □: abiotic control. The pH in control reactors was adjusted according to the bioleaching runs by adding 5 M sulfuric acid. Values represent mean of two parallel bioleaching reactor runs.

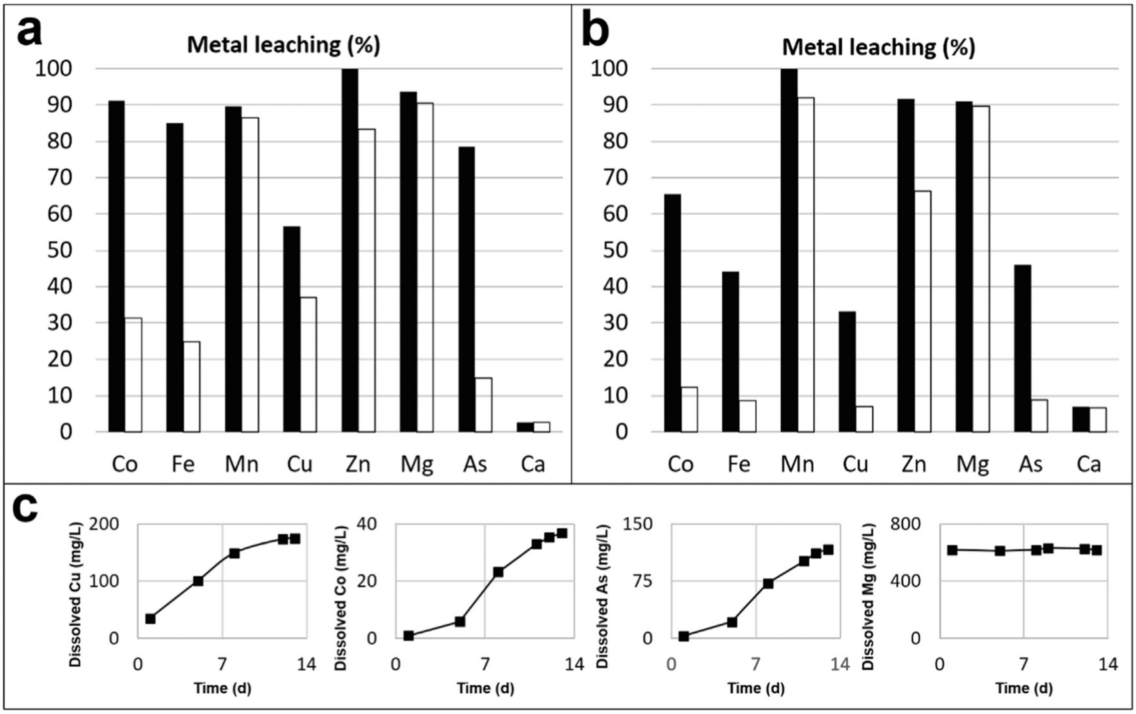

Fig. 3. Bioleaching of the bulk tailings (a) and tailings concentrate (b, c) in 2-L STR by the adapted culture at 30 °C and 10% SL after 13 d. ■ bioleaching □ abiotic control. Values represent mean of two parallel bioleaching reactor runs.

concentrate produced a heat output of 109 μW/g (Fig. 4b), which was 2.7 times higher than for bulk tailings, but lower than values obtained for copper concentrate bioleaching at higher temperature (Hedrich et al., 2018). The higher sulfide content related to pyrite in the tailings concentrate than in the bulk tailings (Table 1) provided more energy for microbial growth and activity. This can be further supported by the fact that cell numbers on tailings concentrate were higher than those on the bulk tailings (Fig. 5a, Supplementary Fig. S4). A slight discrepancy between SYBR counting and qPCR quantification was noticed, which was not observed for the quantification of a moderate thermophilic consortium grown on copper concentrate (Hedrich et al., 2016). Microbial heat output mostly depends on the substrate availability as revealed by microcalorimetry tests, proving that microcalorimetry is suitable for the assessment of bioleaching activity of tailings containing sulfide or reduced sulfur compounds (Kock and Schippers, 2006; Schippers et al., 2007).

Active pyrite dissolution occurs at a redox potential > 645 mV (Liu et al., 2008), whereas fast chalcopyrite dissolution is favored at lower redox potentials (420–450 mV) (Christel et al., 2018; Córdoba et al., 2008; Gericke et al., 2010; Hedrich et al., 2018). The redox potential around 560 mV seems to be sufficient to dissolve pyrite contained in the bulk tailings. Thus, Co was efficiently extracted (Figs. 2 and 3). For tailings concentrate bioleaching, an increased redox potential may be needed for higher Co extraction (Fig. 3 and Supplementary Fig. S3).

Attached cells were microscopically observed on surfaces of solid particles of both, the bulk tailings and tailings concentrate (Fig. 4c and d). No obvious difference of the number of attached cells was observed by CLSM.

Microbial community analysis by T-RFLP indicated that the mixed consortium contained mainly *At. ferrooxidans* and *At. thiooxidans* (Fig. 5b, Supplementary Fig. S4). These two organisms are the most frequently used bacteria for bioleaching of tailings and flotation tailings at mesophilic conditions (Muñoz et al., 2009; Park et al., 2014; Sand et al., 2007; Wang et al., 2018). Some indigenous bacteria (~19%) were detected at the end of the leaching tests in STR (Fig. 5b). These were identified as gram-positive bacteria related to *Sulfobacillus* and *Alicyclobacillus* (not shown). Among these a likely new species of *Sulfobacillus* as indicated by the T-RFLP analysis (data not shown) was isolated for further characterization. Acidophilic microorganisms related to *Sulfobacillus* sp. were also detected in other mine tailings (Chen et al., 2013; Kock and Schippers, 2006; Kock and Schippers, 2008; Korehi et al., 2013).

Fig. 6 presents the EPMA analysis of tailings concentrate and the leaching residues. Mineral phases like pyrite, barite, and galena were detected. The identified pyrite framboids (Wilkin and Barnes, 1997) seem to be the main feature of the tailings concentrate and cobalt is primarily associated with pyrite (Supplementary Fig. S2). The existing form of cobalt in the Rammelsberg tailings differs from cobalt tailings in Luanshya Mine (Zambia), which mostly occurs as carrollite ($CoCu_2S_4$) (Chen et al., 2016). By comparison of the leaching residues, it seems that Co was preferentially leached from the framboidal structures by microbial/ferric iron attack (Fig. 6; Supplementary Fig. S2). Co dissolution was much lower in the abiotic control compared to the bioleaching tests (Fig. 3). The bioreactor results suggested that the bioleaching process could be scaled up, however further process optimization is required.

Additional sulfur or iron substrates are recommended for bioleaching of tailings materials, since these materials often contain only low amounts of sulfides or sulfur (Liu et al., 2007; Srichandan et al., 2019). This makes bioprocessing of tailings more costly. However, the sulfide content in the Rammelsberg mine tailings is sufficient to allow bioleaching without substrate addition, and therefore economically more feasible. By using CLSM combined with fluorescent staining, monolayer biofilms were observed on both tailings samples (Fig. 4c and d). The direct observation of attached cells supports the microbial dissolution of minerals, microorganisms attached to the mineral surface facilitate the mineral attack through formation of biofilms composed of extracellular polymeric substances (Vera et al., 2013).

4. Conclusions

This study determined and discussed the dissolution dynamics of

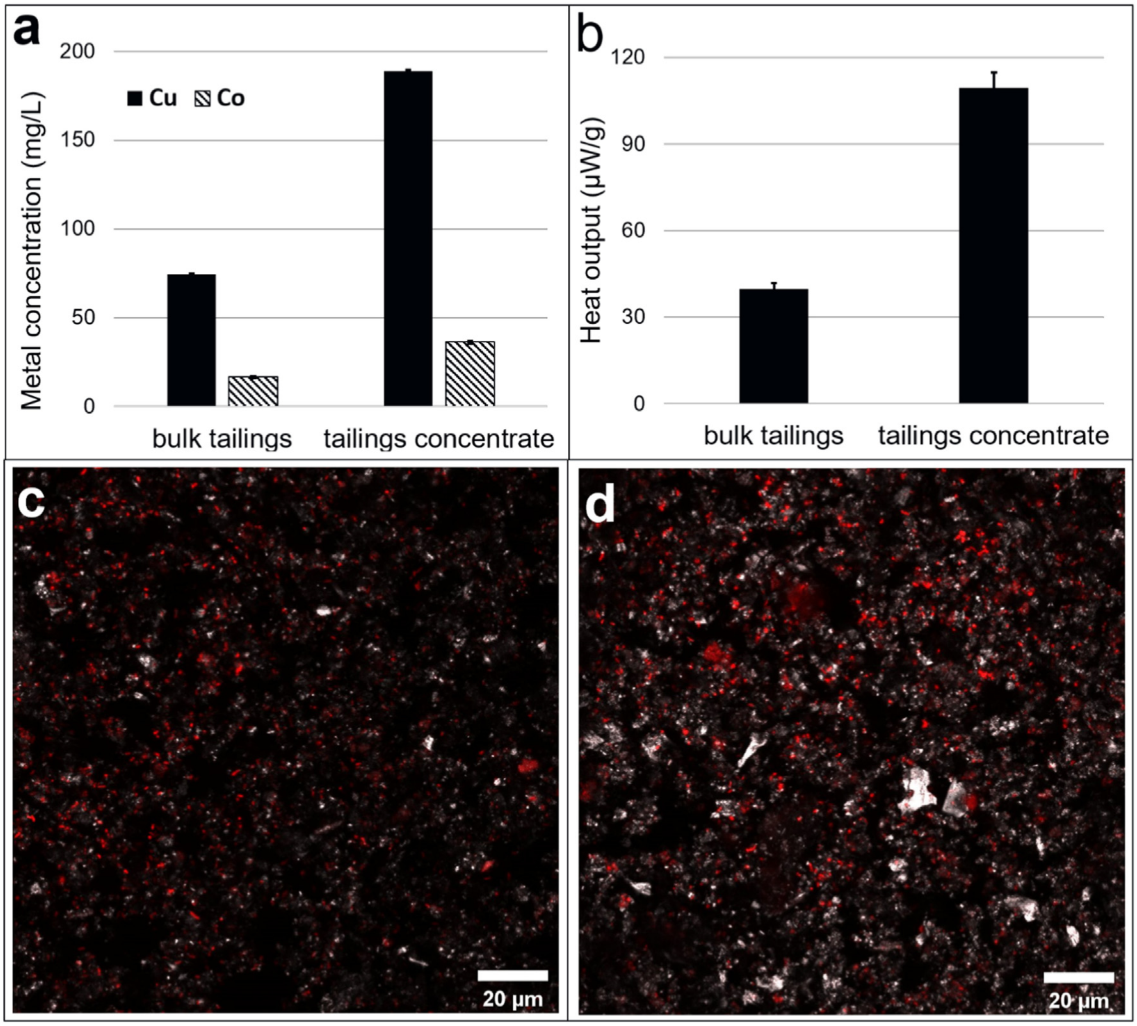

Fig. 4. Comparison of cobalt and copper bioleaching from the bulk tailings and tailings concentrate by the mixed culture (a), microbial activity on the bulk tailings and tailings concentrate determined by microcalorimetry (b), and monolayer biofilms on bulk tailings (c) and tailings concentrate (d). Biofilm (attached) cells on tailings surface were visualized by confocal laser scanning microscopy (CLSM) after staining with SyproOrange (protein specific stain). Cells = red, tailings surface = dark grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

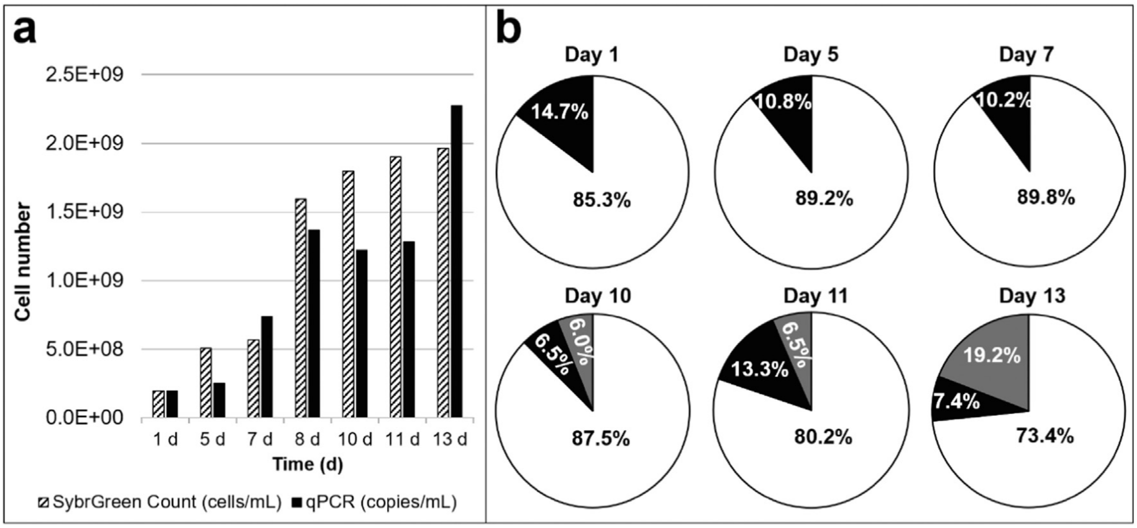

Fig. 5. Changes in cell number and microbial community composition during bioleaching of the bulk tailings by the mixed culture in 2-L STR at an initial pH of 2.0 and 30 °C. (a) changes of cell numbers analyzed via counting of SybrGreen stained cells and 16S rRNA gene copy numbers via qPCR; (b) changes of microbial community over time analyzed via T-RFLP, white: *At. ferrooxidans*, black: *At. thiooxidans*, grey: indigenous bacteria.

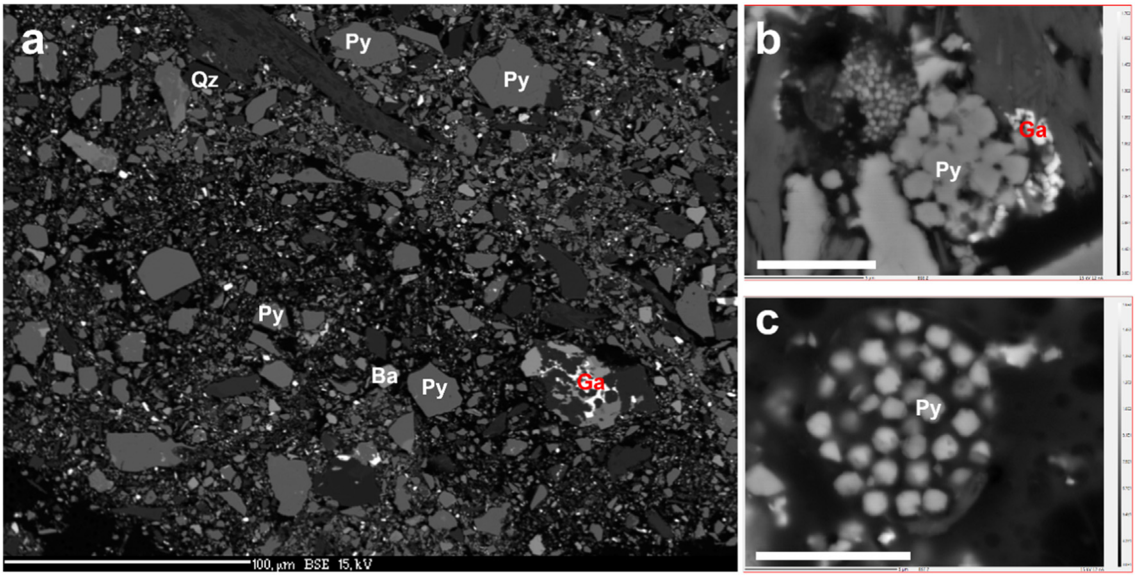

Fig. 6. EPMA-SEM of tailings concentrate (a) and tailings concentrate after bioleaching (b). Qz: quartz, Py: pyrite, Ba: barite, Ga: galena. Bar in b and c represents 5 µm.

metals from tailings and tailings concentrate in laboratory scale stirred tank reactor bioleaching experiments. STR bioleaching using a mesophilic and acidophilic consortium mainly consisting of *At. ferrooxidans* and *At. thiooxidans* has been established for efficient Co extraction from Rammelsberg bulk mine tailings and tailings flotation concentrate. Further process optimization (residence time, bioleaching rate) and upscaling are required for full process development. Bioleaching is an important processing step in the concept for reprocessing of the Rammelsberg mine tailings, and for many sulfidic mine tailings worldwide.

Author statement

Conceptualization: R.Z., D.G. and A.S.; Data curation: R.Z., S.H. and A.S.; Formal analysis: R.Z., S.H. and A.S.; Funding acquisition: D.G. and A.S.; Investigation: R.Z., S.H. and F.R.; Methodology: S.H., F.R. and A.S.; Project administration: A.S.; Resources: D.G. and A.S.; Supervision: D.G. and A.S.; Validation: D.G. and A.S.; Visualization: R.Z. and F.R.; Writing - original draft: R.Z.; Writing – review & editing: R.Z., S.H. and A.S. All authors read and confirmed the revised manuscript.

Declaration of Competing Interest

None to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.hydromet.2020.105443.

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