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Enhanced chalcopyrite dissolution in stirred tank reactors by temperature increase during bioleaching

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ABSTRACT

Primary copper sulfides, such as chalcopyrite, represent the most important copper resource and are currently mainly exploited by pyrometallurgy. Bioleaching is considered as environmental-friendly and economic alternative, and the technical feasibility of stirred tank reactor (STR) bioleaching of copper ore concentrates using acidophilic, iron- /sulfur-oxidizing microorganisms was proven. In case of copper concentrates from Kupferschiefer-type ore the copper recovery at temperatures suitable for moderately thermophilic acidophiles was, however, incomplete due to inefficient chalcopyrite dissolution.

The presented study therefore aimed to enhance chalcopyrite dissolution by decoupling of growth and bioleaching activity of the acidophiles. This was achieved by a controlled temperature increase in laboratory bioreactor experiments with copper concentrate from Kupferschiefer-type ore. Total cell number and microbial activity monitoring allowed to define three stages of the bioleaching process: (i) growth phase, (ii) bioleaching phase and (iii) resting phase. The total residence time was close to seven days, according to copper recovery and iron oxidation activity. Experiments at constant temperatures (42 °C, 48 °C, 50 °C) and a controlled temperature increase at the end of the growth phase of 42/46 °C, 44/48 °C and 46/50 °C, showed that metal recovery was enhanced by incremental temperature increase and resulted in a lower redox potential. Copper recovery was improved from originally 86% to 97% by only 4–8 °C temperature increase. Mineralogical analysis confirmed an almost complete absence of chalcopyrite in the residues after the 46/50 °C bioleaching experiments. These results indicate a distinct temperature effect on copper recovery. The study defines parameters to make STR bioleaching of chalcopyrite-rich ores more efficient and economically feasible and ensures an optimal control of the process for upscaling.

1. Introduction

Stirred tank reactors (STR) are mainly used at industrial scale for biooxidation of refractory gold ores, however, also commercial base metal bioleaching has been described. Bioleaching of copper mainly takes place as heap or dump bioleaching and significantly contributes to the world copper production (Schippers et al., 2014; Brierley, 2016). One drawback with the latter technology is, besides the lower leaching kinetics compared to STR, that the primary copper sulfides such as chalcopyrite ($CuFeS_2$) are scarcely leached in the heaps and that copper production only relies on the dissolution of secondary copper sulfides (Watling, 2006). However, incomplete copper recovery due to insufficient dissolution of chalcopyrite has also been observed in European research projects addressing the development of a STR bioleaching process for copper concentrates from Kupferschiefer-type ores (d'Hugues et al., 2008; Spolaore et al., 2009, 2011; Kamradt et al., 2018).

The main copper-bearing sulfide minerals in black shale are bornite (Cu_5FeS_4), chalcocite (Cu_2S), covellite (CuS) and chalcopyrite. As observed by Spolaore et al. (2009, 2011) during bioleaching, chalcocite is oxidized by ferric ions in two steps, with covellite as intermediate product (Dixon, 2000; Leahy et al., 2007):

$$Cu_2S + 2Fe^{3+} \rightarrow Cu^{2+} + CuS + 2Fe^{2+} \quad (1)$$

$$CuS + 2Fe^{3+} \rightarrow Cu^{2+} + S^0 + 2Fe^{2+} \quad (2)$$

The elemental sulfur produced in Eq. (2) is then enzymatically oxidized to sulfate by microorganisms. The intrinsic rate of Eq. (1) is so

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high, that the reaction rate is limited by diffusion of ferric ions to the mineral surface (Dixon, 2000), but since Eq. (2) is very slow, the overall copper recovery is driven by covellite dissolution.

Chalcocite and bornite were almost completely dissolved in the first bioreactor of a three-stage STR bioleaching experiment, whereas the amount of covellite increased in the residue, but was completely dissolved in the second bioreactor. The final residue in the third bioreactor was dominated by the presence of chalcopyrite, supporting the hypothesis of its recalcitrance in bioleaching (Spolaore et al., 2011).

Chalcopyrite only dissolves in acidic medium (below pH 4) at redox potentials above 400 mV (vs. Ag/AgCl electrode), whereas the dissolution kinetics depend on ferric ion concentration and pH value. The observed decreasing chalcopyrite dissolution over time during bioleaching is often described as the result of passivation layer formation on the mineral surface, hindering the further oxidation process (Cordoba et al., 2008). Evidence of elemental sulfur, sulfides (like covellite) (Barriga Mateos et al., 1987) and ferric iron-bearing precipitates on the surface of the mineral were confirmed by mineralogical analysis (Sandstrom et al., 2005; Stott et al., 2000).

Strategies to resolve incomplete chalcopyrite dissolution in bioleaching processes comprise (i) the application of thermophilic archaea (Gericke et al., 2001; d'Hugues et al., 2002), (ii) operating at controlled redox levels by adjusting the oxygen supply (Third et al., 2002; van der Merwe et al., 1998) or by applying electrochemical methods (Lotfalian et al., 2015; Ahmadi et al., 2011), (iii) the addition of catalysts such as silver (Cancho et al., 2007) as well as (iv) fine grinding of the concentrate (Rhodes et al., 1998).

Chalcopyrite bioleaching with moderately thermophilic iron- and/or sulfur-oxidizing acidophiles has received increased attention, since their optimal growth temperatures of about 45–52 °C represent a kinetic advantage over those for meso-acidophiles, and the operation costs are lower than for operations at higher temperatures for thermo-acidophiles (Brierley, 2008). In a study by Norris et al. (2016) efficient copper extraction from chalcopyrite was achieved with moderately thermophilic bacteria at about 50 °C and using thermophilic archaea at up to 80 °C (Norris 2007). The high-temperature bioleaching with thermophiles has, however, also disadvantages, such as corrosion of the bioreactors, the formation of secondary minerals hindering further bioleaching and causing valuable leached elements to re-precipitate (Batty and Rorke, 2006). Moderately thermophiles are also capable of growth at higher pulp densities than thermophiles and allow bioleaching in STR without significant corrosion (Nemati and Harrison, 2000). Recently an industrial scale STR process with a planned annual production of 50,000 t copper cathode per year using moderate thermophiles has been developed in Iran (Naghibzadeh et al., 2017).

The objective of this recent study was to improve copper recovery from copper concentrate derived from Kupferschiefer-type ore and to optimize the STR bioleaching process. A detailed study of metal bioleaching and the microbial community composition changes during the course of the experiment was undertaken. The idea for improved copper recovery was to slightly increase the operating temperature to achieve better chalcopyrite dissolution, but to avoid enhanced precipitation of secondary minerals and passivation layer formation. This approach was tested by either operating at constant temperature or an incremental, two-step temperature increase combined with microbial community monitoring. Analysis of the bioleaching kinetics, metal recovery and mineralogy of the bioleach residues should indicate the favored operating temperature for efficient chalcopyrite dissolution.

2. Materials and methods

2.1. Copper concentrate from Kupferschiefer-type ore

The European Kupferschiefer-type deposits in central Europe belong to the sediment-hosted base metal deposits, which are widespread and mined worldwide (Borg et al., 2012).

The material used for bioleaching was a copper concentrate retrieved from black shale ore deposits (KGHM, Poland). The concentrate had a particle size of < 90 µm and had the following main characteristics: 13.3 wt% Cu, 9.4 wt% Fe, 16.5 wt% S, 0.064 wt% Ag, 1.1 wt% total inorganic carbon (TIC), 9.1% total organic carbon (TOC) and an approximate particle size of < 63 µm (cumulative passing 89%, Table S1). The abundance of sulfide minerals was determined by SEM-based automated mineralogy (mineral liberation analysis–SEM/MLA-GXMAP).

2.2. Microbial culture and growth conditions

The acidophilic, moderately thermophilic bioleaching consortium used was the BRGM-KCC consortium maintained for many years on copper concentrate (d'Hugues et al., 2003; Morin and d'Hugues, 2007) comprising *L. ferriphilum*, *At. caldus* and *Sulfobacillus* spp. (Spolaore et al., 2011). The consortium was maintained on 3% Cu concentrate in basal salt medium pH 1.8 (Wakeman et al., 2008) at 42 °C, in which it was sub-cultured several times and adapted to 10% (w/v) solids load before serving as inoculum for bioleaching tests.

2.3. Bioleaching set up

Bioleaching experiments were carried out in 2 L pH- and temperature-controlled, stirred tank bioreactors (Electrolab, UK). The reactors were fully baffled and agitated by a dual impeller system consisting of a standard 6-blade Rushton turbine in combination with a 6-blade 45° axial flow impeller with speed set to 400 rpm. After initial acid treatment of the sulfidic material with H_2SO_4 (acid consumption of 182.3 g H_2SO_4/kg) to about pH 1.8 in order to dissolve carbonates and avoid inhibition of the cells by inappropriate pH values, the experiment was inoculated with 150 mL culture adapted to the mineral according to the protocol described by Hedrich et al. (2016). The bioreactors were operated at 10% (w/v) solids load and sparged with air at 120 L/h.

2.4. Determination of experimental parameters

A preliminary test to study the relation between bacterial activity (via microcalorimetry), cell numbers (SYBR Green staining) and metal dissolution was carried out (Hedrich et al., 2016). Cell numbers, microbial activity, pH, redox and dissolved metals were followed daily in order to determine key parameters during the experiment to better understand and enhance the bioleaching process. Previous experiments with the same material, microbial consortium and using similar conditions showed that a reaction time of about seven days for the bioleaching was suitable; we therefore allowed ten days for the complete monitoring of the experiments.

2.5. Temperature experiments

Data from previous experiments in continuous cascade bioreactors at BRGM indicated that sulfide dissolution and metal recovery was enhanced when temperature raised by just a few degrees from one tank to the other due to exothermic reactions during metal sulfide leaching. This observation led to the proposed two-step temperature set up, where according to the above findings, the temperature was kept close to the optimum of the bioleaching consortium for about three days to allow proper growth and colonization of the ore, and was increased by 4 °C afterwards to enhance metal bioleaching. Constant temperatures (42 °C, 48 °C, 50 °C) and two-step temperature profiles (42/46 °C, 44/48 °C, 46/50 °C) were tested.

Each experiment at constant temperatures of 42 °C, 48 °C, 50 °C followed the previously described adaptation protocol (Hedrich et al., 2016), whereas, for the two-step temperature experiments at 42/46 °C, 44/48 °C and 46/50 °C, cultures were adapted to the starting temperature. Three parallel bioreactors were run for each experiment and

abiotic set ups served as chemical control experiments. The pH in control experiments was adjusted with sulfuric acid according to the final pH determined in bioleaching experiments to provide similar conditions. Copper mineral dissolution and kinetics during bioleaching were followed and the experiments terminated when the copper mineral dissolution reached a plateau. Regular monitoring of pH, redox potential, dissolved metal concentration, microbial activity and community structure was performed as described above.

2.6. Analytical methods

2.6.1. Chemical analysis

Regular offline pH (BlueLine 18 pH electrode, Schott, Germany) and redox potential (BlueLine 31 Rx electrode, Schott, Germany) measurements were performed directly in the bioreactor pulp. Measured redox values were corrected to the standard hydrogen electrode (SHE) and reported as E_h. Ferrous and ferric iron concentrations were measured in 0.45 µm-filtered samples using the Ferrozine assay (Lovley and Phillips, 1987). Dissolved metals were regularly determined in filtered, acidified samples using ICP-OES (Varian SpectrAA-300). At the end of the experiment, the residue was harvested, washed with acidified water and dried before performing digestion with nitric acid following analysis of the metals by ICP-OES.

2.6.2. Mineralogical analysis

Scanning electron microscope (SEM) investigations on carbon-coated polished sections of the concentrate and the residues were carried out using a MLA650F (Quanta 650 FEG system; FEI Company) equipped with two energy-dispersive X-ray detectors (XFlash Detector 5030, Silicon Drift Detector; Bruker Nano) for semi-quantitative element analysis without standardization. Dried and homogenized samples were stored under nitrogen until further analysis. The mineralogical composition of the rocks was quantitatively determined using SEM/mineral liberation analysis techniques (SEM/MLA-GXMAP; Gu, 2003; Fandrich et al., 2007). Duplicate representative sections were measured for the concentrate and each residue sample. In each section, the mineralogy was identified using energy-dispersive X-ray spectrometry (EDS) combined with backscattered electron (BSE) imaging. The intense intergrowth/accumulation of very fine-grained minerals in larger particles (commonly non-ore minerals, e.g. silicates) required application of a high spectrum-matching threshold of 80% for proper identification of minerals, however, it also resulted in an increased percentage of grains treated as unknown minerals by the system.

2.6.3. Microbiological analysis

Total cell numbers were determined in slurry samples by microscopic counting after SYBR Green staining according to Lunau et al. (2005), following homogenization of the samples by ultrasonic treatment (20 s, 20 cycles, 20% intensity). After appropriate dilution, 1 mL staining solution (20 µL 1 M ascorbic acid, 280 µL Mowiol, 20 µL SYBR Green I) was added to 50 µL of the sample and incubated for at least 10 min in the dark. The mixture was then applied onto a membrane filter (Whatman Nucleopore, d = 25 mm, 0.2 µm pore size), rinsed with 1 mL 0.1% Tween20/PBS solution followed by rinsing with 30 mL TE buffer to enhance the visibility of the cells and to avoid interactions with particles. Afterwards, the filter was put onto a microscopic slide and covered with 25 µL anti-fading solution (7% Mowiol, 1% ascorbic acid) before counting cells under the microscope. Cell numbers were determined for each sample by counting across the whole filter area and at least 50 fields of view.

Microcalorimetric measurements were performed at the start and end of each experiment in order to determine the bioleaching activity of the cultures (Rohwerder et al., 1998; Makaula et al., 2017). Therefore 1 mL of the bioreactor pulp was put into a 4 mL glass ampoule and the supernatant was removed after 5 min of settling. The ampoule was sealed and the heat output (µW per g material) measured in a TAM III microcalorimeter (TA Instruments). Samples were measured in triplicate for about 12 h. The weight of the residue before the experiment and the dry weight afterwards were determined in order to calculate the heat output per g of solids. Chemical control experiments were conducted with the same set up.

The microbial community structure and abundance were monitored in slurry samples after DNA extraction by terminal restriction fragment length polymorphism (T-RFLP) and quantitative real-time PCR (qPCR) as described previously (Hedrich et al., 2016). Mean values of the relative abundance determined by qPCR and T-RFLP were compared and plotted.

3. Results

3.1. Determination of bioleaching stages

The performance of copper concentrate bioleaching in a STR inoculated with the acidophilic and moderately-thermophilic BRGM-KCC consortium was followed at 42 °C. Daily monitoring of total cells via SYBR Green staining clearly showed that cell numbers increased within the first three days of the experiments and stayed constant afterwards at about $5*10^9$ cells/mL with a slight decrease at days 9 and 10 (Fig. 1). Microcalorimetric data supported the initial increase of cell numbers with a raise in microbial activity to about 680 µW/g and constant activity for the next days. The activity decreased between days 7 and 8 and dropped within the last two days to about 140 µW/g (Fig. 1). Three stages can be defined from this experiment: (i) growth stage (days 1–3), (ii) bioleaching stage (days 3–7) and (iii) resting stage (days 8–10) (Fig. 1). Correspondingly, iron and sulfide oxidation activity in terms of E_h and pH changes (Fig. 2) and bioleaching kinetics as copper released from the mineral (Fig. 3) correlated with the detected bacterial activity and the cell number development. An increase in copper bioleaching and iron oxidation occurred after three days.

3.2. Temperature experiments

First experiments were conducted in STR at three constant temperatures (42 °C, 48 °C and 50 °C). Copper bioleaching kinetics and metal recovery were improved by increasing the constant temperature from 42 °C to 48 °C, but decreased and stopped at 50 °C (Table 1). The resulting Cu recovery reached 94% at 48 °C (compared to 86% at 42 °C) but decreased to 46% at 50 °C (Table 1).

The highest copper recovery of 97% was achieved when starting the growth phase at 46 °C and increasing the temperature to 50 °C at day 3 (Table 1). Copper recovery and dissolution rate were similar at 48 °C and 42/46 °C (94%, Table 1) and improved even more when increasing the starting temperature of the two-step runs from 42 °C to 44 °C and

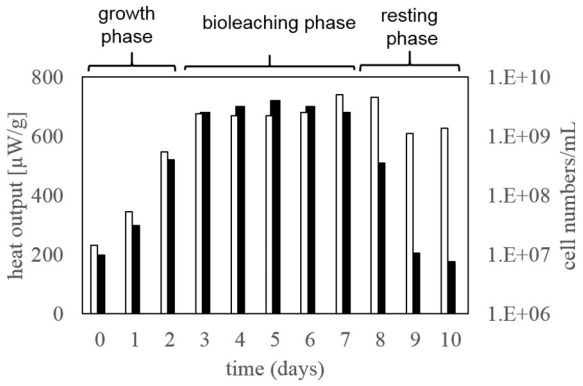

Fig. 1. Total cell numbers determined via SYBR Green staining (white bars) and microbial activity measured by microcalorimetry (black bars) during STR copper bioleaching at 42 °C.

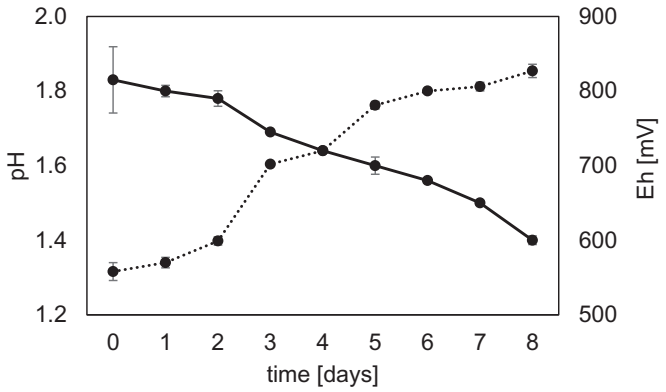

Fig. 2. Evolution of pH (solid line) and E_h (dotted line) during STR copper bioleaching at 42 °C. Data are mean values from triplicate bioreactor runs, error bars show standard deviation.

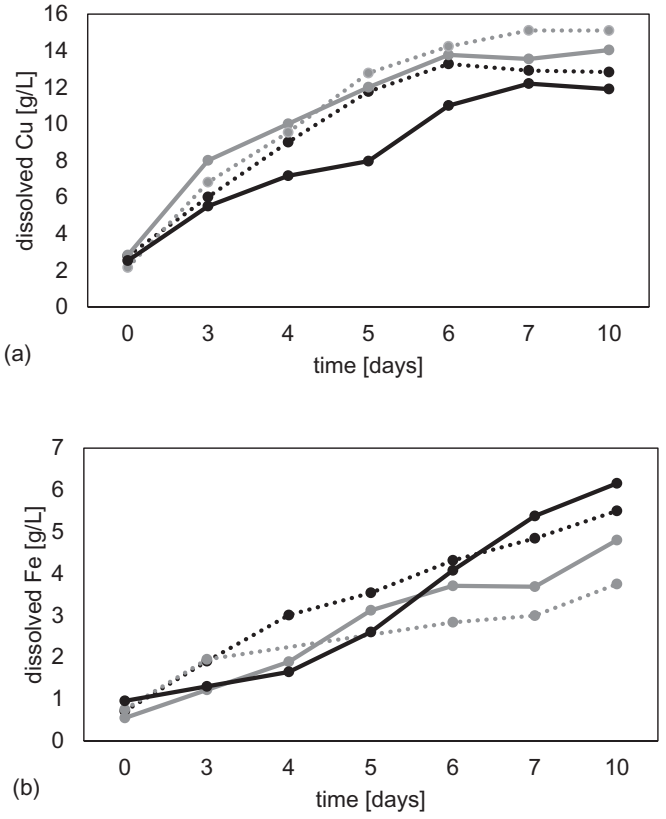

Fig. 3. Comparison of (a) copper and (b) iron dissolution kinetics in the STR at 42 °C (black solid line), 42/46 °C (black dotted line), 44/48 °C (grey solid line) and 46/50 °C (grey dotted line).

46 °C, indicating the advantage of the two-step temperature condition. There was only a slight difference in copper recovery between the 44/48 °C and 46/50 °C profiles.

In addition, the bioleaching kinetics of copper improved with increased temperature. Fig. 3 shows the kinetics at standard 42 °C compared to the temperature profiles (42/46 °C, 44/48 °C, 46/50 °C) where maximum copper recovery (97%) was achieved at 46/50 °C. Metal recovery data showed decreased iron bioleaching from the mineral at higher temperatures (Fig. 3b).

Table 2
Comparison of metal recovery [in %] from copper concentrate in STR with acidophilic microorganisms at various temperature profiles.

	Co	Cu	Fe	Mn	Ni	Zn
42 °C	83	86	62	95	25	55
42/46 °C	83	94	64	95	33	63
44/48 °C	85	96	57	93	40	70
46/50 °C	86	97	44	94	42	74

Further analysis of the residue proved that not only the recovery of copper was enhanced at higher temperatures but also the recovery of nickel and zinc (Table 2).

The pH developed similarly in almost all experiments independently from the temperature and was between 1.3 and 1.5 after 8 days (Fig. S1). Only the pH of the 50 °C experiment remained almost constant at about 1.8 during the complete course of the experiment. The final redox potential (E_h) of higher temperature experiments was significantly lower (up to 100 mV less) than for the experiments at 42 °C and 42/46 °C (~850 mV; Fig. 4). The E_h of the 50 °C run was also low (614 mV) due to bioleaching inhibition throughout the experiment. The E_h of control experiments was always below 400 mV and ferric iron concentration < 1 g/L (data not shown).

3.3. Microbial community

The microbial community showed similar activity (533–596 µW/g) during (day 3) and at the end (day 8) of the experiment for most of the different temperature profiles, apart from the 50 °C run. In the latter, activity declined to 225 µW/g at the end of the bioreactor run (Fig. 5).

Total bacterial 16S rRNA gene copy numbers determined by quantitative *real-time* PCR showed no significant difference in all, but the 50 °C experiments. They increased at day 3 of the bioleaching followed by a slight decrease at day 8 (Fig. S2). Bacterial gene copy numbers of the 50 °C experiment were similar to the others at the beginning, only slightly decreased at day 3 and dropped to $6.8*10^3$ gene copy numbers/mL at the end of the run.

Detailed bacterial species abundance determined by T-RFLP and species-specific qPCR (Fig. S3) revealed that the microbial community at 48 °C was dominated by *Sb. thermosulfidooxidans*, but still contained about 20% of *At. caldus*. No *L. ferriphilum* was found at the end of the bioleaching phase. The 50 °C experiment was dominated by *Sb. thermosulfidooxidans* at all stages, whereas *L. ferriphilum* was absent throughout the experiment. The latter observation is consistent with the loss of iron oxidation activity in the bioleaching experiments (Fig. 6).

As in the samples from the 48 °C bioleaching run, *At. caldus* was dominant at day 3 in all two-step temperature experiments (Fig. S3). *Sb. thermosulfidooxidans* dominated over the other two taxa in all but the 42/46 °C temperature experiment. *At. caldus* was the key player at the end of the 42/46 °C bioleaching similarly to the 42 °C experiment. *L. ferriphilum* was only detected in low numbers (1–3%, Fig. S3) in the 42/46 °C experiment, and was not found at higher temperatures (Figs. 6 and S3).

Table 1
Copper recovery [%] determined by residue digestion analysis and dissolution rate [mg $L^{-1}H^{-1}$] during bioleaching at different temperature profiles. Mean values are given for triplicate bioreactor runs including standard deviation.

Temperature [°C]	42	48	50	42/46	44/48	46/50 chemical control
Cu recovery	86 ± 2	94 ± 1	46 ± 3	94 ± 1	96 ± 1	97 ± 1 48 ± 2
Cu dissolution rate	163 ± 9	182 ± 4	66 ± 12	173 ± 7	201 ± 5	263 ± 3 93 ± 2

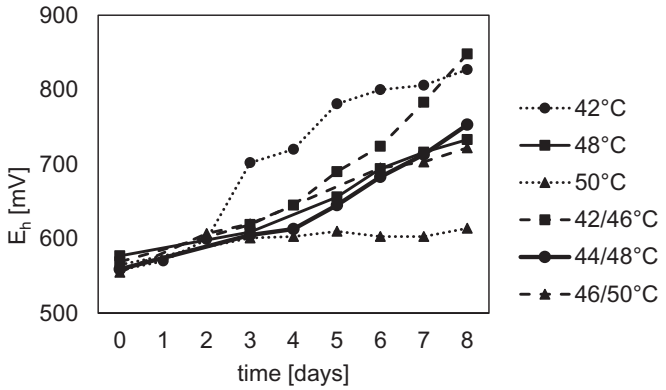

Fig. 4. Development of E_h in the batch STR during bioleaching of the copper concentrate at various temperature profiles. Data are mean values of triplicate bioreactor runs.

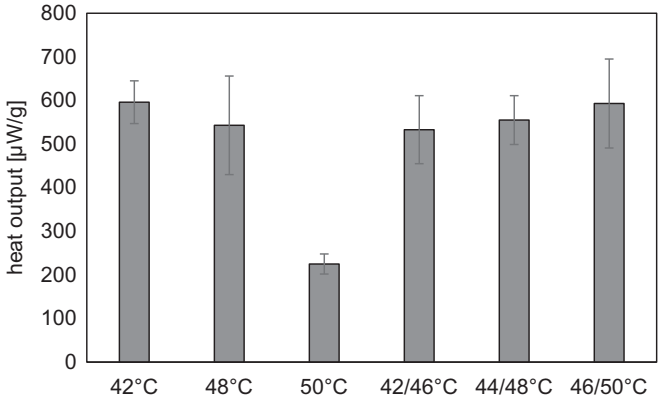

Fig. 5. Microbial activity determined by microcalorimetry of slurry samples from bioleaching experiments of copper concentrate in the STR at various temperature profiles. Samples were taken at the end (day 8) of the experiment. Data are mean values of triplicate bioreactor runs and triplicate microcalorimetric measurements. Error bars show standard deviation.

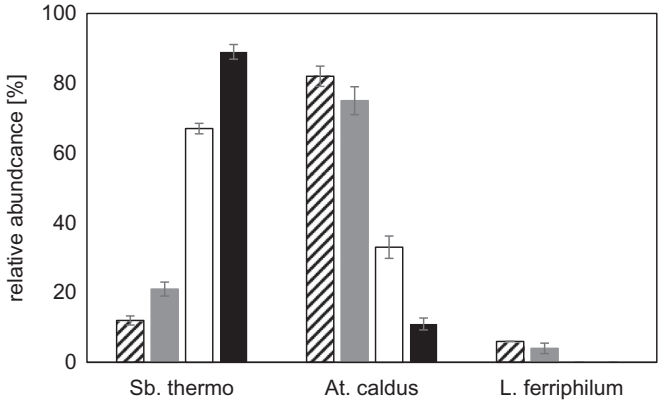

Fig. 6. Relative abundance of acidophilic species in batch bioleaching STR at the end of the experiment at different temperatures (42 °C – hatched columns, 42/46 °C – grey columns, 44/48 °C – white columns and 46/50 °C – black columns). Data are mean values, error bars indicate standard deviation.

3.4. Mineralogical analysis

SEM/MLA-GXMAP data reconstructed the mineralogical composition of the copper concentrate and the bioleaching residues. The copper concentrate contained 12.3 wt% chalcopyrite, 9.6 wt% bornite (+ idaite), 1.8 wt% chalcocite + covellite, 5.6 wt% pyrite, as well as other

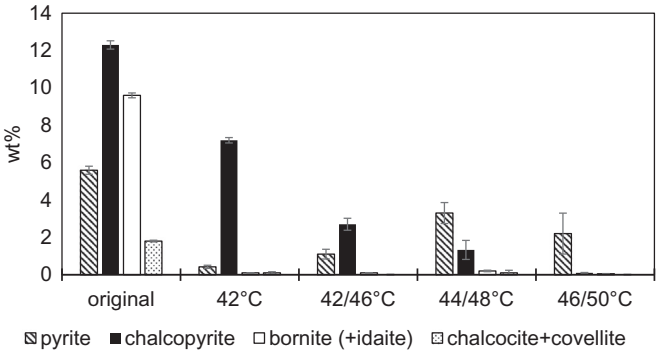

Fig. 7. Mineral distribution in the original copper concentrate and the bioleaching residues from various temperature profile experiments as determined by SEM/MLA-GXMAP. Data are mean values of duplicate samples. Error bars show standard deviation.

minor sulfidic minerals (mean values of two measured sub-samples). Fig. 7 illustrates the almost complete dissolution of bornite and chalcocite/covellite at 42 °C, as well as some remaining pyrite and significant amounts of chalcopyrite. The amount of chalcopyrite in the bioleaching residue decreased dramatically with increasing temperatures and the mineral was almost completely dissolved at 46/50 °C. In contrast, pyrite concentrations are at a rather high value at higher temperatures. The SEM/MLA-GXMAP analysis of the 46/50 °C residue proved difficult as > 50% of the particles were < 6.3 µm in size (Table S1) and no proper copper mineral grains were present anymore to perform reliable analysis. The absence of chalcopyrite particles, however, provided evidence for its dissolution. While X-ray diffraction analysis could detect about 3 wt% of chalcopyrite in the 42 °C residue, its concentration in the higher temperature residues was below the detection limit of this method.

4. Discussion

Bioleaching experiments in batch bioreactors at 42 °C housing the acidophilic and moderately thermophilic BRGM-KCC consortium were carried out at various temperatures with the aim to study the effect of temperature on the bioleaching of a copper concentrate retrieved from Kupferschiefer-type ore and to improve metal recovery.

Preliminary experiments to determine the kinetics and microbial growth behavior during the bioleaching run served as basis for further tests. Daily total cell number and microbial activity monitoring allowed to determine three stages during the bioleaching experiment: (i) adaptation/growth stage, (ii) bioleaching stage, where microorganisms are active and the major part of the metal leaching occurs but cell numbers and microbial activity remained constant, and finally, (iii) resting stage, where the substrate (i.e. the metal sulfide) is depleted and bacteria are not active anymore, but cell numbers do not decrease immediately. These data clearly show that even so cells are prominent during the whole experiment, not all of them are actively contributing to metal bioleaching at all times. From these findings, it is assumed that the parameters within the first three days of the bioleaching experiment are essential for the microbial growth and should be adjusted according to the needs of the target organisms, e.g. by operating at optimum pH and temperature. In the next stage, when the desired microbial consortium has well established, the focus can be put on efficient metal extraction and the parameters adjusted accordingly.

In the STR experiments, maximum bioleaching of metals was achieved after about seven days when the microbial activity reached a plateau; it decreased afterwards, indicating a termination of the bioleaching process due to the fading of substrate (metal sulfides and ferrous iron). Metal recovery and kinetics of copper and iron bioleaching confirmed results of previous experiments (Spolaore et al.,

2009, 2011; Hedrich et al., 2016). The data of the bioleaching at 42 °C in STR therefore served as base case for further experiments with the aim to improve copper recovery and bioleaching kinetics.

In a previous experiment, an automated temperature increase was observed in continuous STR bioleaching of cobaltiferrous pyrite due to the exothermic mineral dissolution (unpublished data). Since this temperature increase was concurrent with enhanced metal recovery, two-step temperature profiles involving a temperature increase at the end of the growth stage were tested in the current study. These temperature experiments showed that the copper recovery was enhanced with increasing temperature and reached its maximum in the 44/48 °C and 46/50 °C experiments. In contrast, experiments at constantly high temperatures of 48 °C and 50 °C did not achieve the high copper recoveries of the two-step temperature runs. Data clearly show that a three day growth phase for the microorganisms, before increasing the temperature by just 4 °C, had a positive effect on copper recovery and chalcopyrite dissolution. Furthermore, the microbial community was inhibited in experiments at constant 50 °C, indicated by low microbial activity, iron oxidation rate and metal recovery, but provided most positive results when allowing three days of adaptation at 46 °C before increasing to 50 °C for enhancing the metal bioleaching. The two-step incremental temperature procedure was clearly more efficient than running the experiment at a constant temperature level, as it allows the microorganisms to grow and to adapt first before their actual bioleaching stage.

The microbial community was dominated by three key players of the BRGM-KCC consortium, which are typically found in bioleaching operations at moderate temperatures (d'Hugues et al., 2008; Gericke et al., 2010; Spolaore et al., 2009, 2011). The autotrophic iron-oxidizer *L. ferriphilium*, which has a distinct tolerance to low pH and high ferric iron concentrations, the autotrophic sulfur-oxidizer *At. caldus* and the mixotrophic iron-/sulfur-oxidizer *Sb. thermosulfidooxidans,* which is capable of growth at higher temperatures than the other two.

As temperatures increased, the microbial community became dominated by the moderately thermophilic bacteria (*Sb. thermosulfidooxidans*) of the consortium according to their optimum growth temperatures (50–55 °C). After initial dominance at day 3 of the experiments, the abundance of *At. caldus* decreased towards the end, but the organism was still present at 50 °C, as it can grow up to 52 °C but has its optimum at 40–45 °C. *L. ferriphilum* was only present in low numbers at 42–46 °C, but was not detected at higher temperatures, as it only grows up to 45 °C with an optimum at 37 °C. One other reason for the low abundance of the chemolithoautotrophic *L. ferriphilum* and the late development of *Sb. thermosulfidooxidans* in the system, apart from the temperature range, could be the lack of CO_2. Contrary to former studies, where *L. ferriphilum* was found (Spolaore et al., 2009, 2011; Norris et al., 2016), no additional CO_2 was applied. Since *L. ferriphilum* is the key iron-oxidizer in the system until *Sb. thermosulfidooxidans* takes over, the redox potential and therefore iron oxidation rate remained low in higher temperature experiments. However, as proposed before by Spolaore et al. (2011), a larger proportion of sulfur-oxidizers in the bioleaching process actually seems to enhance chalcopyrite dissolution, e.g. by counteracting the formation of elemental sulfur as passivation layer on the chalcopyrite surface and keeping a lower redox potential as less iron is oxidized.

Mixed communities are therefore very important when running these kind of experiments in order to assure stable process development under varying conditions.

Mineralogical studies confirmed that bornite and chalcocite were immediately dissolved even at 42 °C as they are the easiest to bioleach copper sulfides with the lowest rest potential. The reported covellite formation, following chalcocite and chalcopyrite dissolution (Dixon, 2000; Leahy et al., 2007), could not be confirmed in residues of the higher temperature experiments as the chalcocite + covellite value was below 0.1% in all residues, suggesting an immediate dissolution of the potentially formed covellite. Enhanced chalcopyrite dissolution occurred with increasing temperature and the mineral was almost completely dissolved at 46/50 °C, proving that chalcopyrite dissolution is favored at higher temperatures and lower redox potentials. The critical redox potential for chalcopyrite dissolution is reported to be about 450 mV (Ag/AgCl electrode, Cordoba et al., 2008). The redox potential of the 42 °C bioleaching experiment raised very fast and already reached the critical potential for ferric iron precipitates after three days (498 mV, converted to Ag/AgCl), whereas the redox value in the other experiments increased slowly and only got to 490 mV (Ag/AgCl) after five days and stayed below 420 mV (Ag/AgCl) until day 4. As reported by Gericke et al. (2010) copper bioleaching rates of chalcopyrite-rich ore at 45 °C are favored at redox potentials around 420 mV (Ag/AgCl) compared to 600 mV (Ag/AgCl). They also reported the decreased pyrite oxidation at these low redox potentials combined with higher final pH values due to the lack of acid production from pyrite oxidation. Although more pyrite remained in the higher temperature residues, pH values were almost as low as at 42 °C. Only the pH of the 44/48 °C experiment was higher (1.53 compared to 1.41), which was also supported by a higher pyrite abundance in the residue.

5. Conclusion

The presented study gives insights into the microbial effect on efficient metal bioleaching and how this process can be controlled by simply understanding the concurrence of the bacterial species, the mineralogy of the ore and the physico-chemical conditions. After detailed understanding of the microbial performance during bioleaching of the copper concentrate, a novel temperature-driven concept based on decoupling of growth and bioleaching phase, was applied to enhance metal bioleaching. The two-step temperature procedure proved to be superior to experiments at constant high temperature in terms of microbial community composition, chalcopyrite dissolution and copper recovery. A slight operating temperature increase, applied just at the end of the bacterial growth stage, lead to improved bioleaching kinetics and overall metal recovery in combination with near-complete chalcopyrite dissolution at lower redox potential. This study demonstrates efficient bioleaching of chalcopyrite-rich concentrate at moderate temperatures with the advantage of low operation costs due to lower temperatures for complete chalcopyrite dissolution. By operating the STR at moderate temperatures under the reported conditions, formation of passivation layers, precipitation of secondary iron minerals and damage to the tanks can be reduced relative to higher temperature operations. Furthermore, the enhanced recovery of various other metals from the concentrate raises the overall value of the material.

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Appendix A. Supplementary data

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