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Original citation:

Pakostova, E, Grail, BM & Johnson, DB 2018, 'Bio-processing of a saline, calcareous copper sulfide ore by sequential leaching', *Hydrometallurgy*, vol. 179, pp. 36-43.

<https://dx.doi.org/10.1016/j.hydromet.2018.05.004>

DOI 10.1016/j.hydromet.2018.05.004

ISSN 0304-386X

Publisher: Elsevier

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Bio-processing of a saline, calcareous copper sulfide ore by sequential leaching

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ARTICLE INFO

Keywords:

Acid leaching
Bioreactor
Copper ore
Indirect bioleaching
Kupferschiefer
Salt

ABSTRACT

Deep *in situ* biomining (DISB) offers the potential for exploiting metal-rich ore bodies that are currently difficult or non-economic to access, with far less environmental impact than current deep or opencast mining practices. DISB is being developed within the EU Horizon 2020 project “BIOMore”, which targets a calcareous sedimentary copper ore, *kupferschiefer*, which is a widespread subterranean rock stratum in central and north-western Europe. Laboratory tests on the two main copper-bearing fractions of *kupferschiefer*, sandstone and black shale, showed that, besides requiring basic minerals to be removed ahead of indirect bioleaching (to avoid the formation of ferric carbonates), both contained significant amounts of chloride minerals (chiefly halite) which could have a major negative impact on DISB. A sequential three-phased protocol was developed, involving (i) leaching with water, which removed most of the chloride, (ii) leaching with sulfuric acid, which removed most of the residual chloride and carbonate minerals, and (iii) indirect bioleaching with an acidic ferric iron lixiviant. The latter was produced in a laboratory-scale ferric iron-generating bioreactor dominated by the iron-oxidizing acidophilic prokaryotes *Leptospirillum ferriphilum*, *Sulfobacillus thermosulfidooxidans* and *Ferroplasma acidophilum*. The iron oxidizing capacity of this bioreactor was not impaired by the concentrations of chloride present during the leaching phase in which it was used. Sequential leaching was applied successfully using a “composite” (sandstone plus black shale) ore column, which mimicked the flow circuit in a DISB, and included a bleed stream where soluble copper was removed from the pregnant leach solutions generated by sulfide precipitation. Water leaching was shown to cause partial disintegration and lowering of the resistance of the sandstone fraction to crushing.

1. Introduction

Biomining, the use of microorganisms to recover metals by oxidative dissolution of metal sulfide minerals in primary ores and wastes (tailings, rock dumps etc.) is now well established as a global technology (Johnson, 2014; Harrison, 2016). Conventionally, biomining uses irrigated systems (rock dumps and bioheaps; principally for recovering base metals) or stirred tanks, which are mostly used for recovering gold from refractory ores. In both of these, the microbial catalysts of mineral oxidation, chiefly acidophilic chemolithotrophic bacteria and archaea, occur in juxtaposition to the metal sulfides, often growing as biofilms on the minerals themselves where they mediate contact leaching (Bellenberg et al., 2014). The primary oxidant involved in bioleaching and bio-oxidation of sulfide minerals in low pH liquors is ferric iron which, in oxygen-containing lixiviant, is continuously regenerated from ferrous iron by the many species of

acidophiles that are now known to use ferrous iron as an electron donor to support their growth (Dopson, 2016). Biogenic ferric iron can also effectively oxidize sulfide minerals in the absence of molecular oxygen, the process known as “non-contact” mineral bioleaching/bio-oxidation. The fact that abiotic mineral oxidation and microbial regeneration of ferric iron can be physically disconnected underpins an alternative engineering design for biomining, referred to as “indirect bioleaching”. This concept has been demonstrated with various mineral ores, such as sphalerite (Carranza et al., 1993) and has the advantage that conditions, such as temperature, may be optimized for both processes independent of each other.

Deep *in situ* bioleaching (DISB) is a variant of the indirect bioleaching concept, the main difference being that the abiotic mineral leaching and microbial regeneration of an oxidized lixiviant may be separated by considerable distances (Johnson, 2015). With DISB, ore bodies are mined *in situ* rather than hauled to the land surface as in deep

Abbreviations: DISB, deep *in situ* bioleaching; FIGB, ferric iron-generating bioreactor; PLS, pregnant leach solutions

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mining or made accessible by opencast excavations. Acidic, ferric iron lixivants are generated in bioreactors (FIGBs; ferric iron-generating bioreactors) located on the land surface and pumped underground through drilled channels to where the ore body is located. Non-porous sulfide ore bodies (which constitute the majority) need to be fractured to open up fissures through which the lixiviant can flow. As it does, ferric iron mediates mineral dissolution and solubilizes target metals such as copper but, in contrast to conventional mining, the limited dissolved oxygen in the lixiviant precludes its re-oxidation, so that the pregnant leach solutions (PLS) generated would be reduced rather than oxidized. These are pumped back to the surface via collector channels, the target metal extracted and recovered using solvent extraction/electrowinning (or an alternative approach), and the ferrous iron-rich raffinate returned to the FIGB to regenerate ferric iron and the oxidized lixiviant returned to the flow circuit.

The concept of DISB as an alternative approach to extract and recover base metals has been around for a number of years, and trialed in at least one location (Arizona, USA). Likewise, the use of bioreactors to generate acidic ferric iron solutions was described over forty years ago by Livesey-Goldblatt et al. (1977) and modified and improved FIGBs have been developed since that time (e.g. Kinnunen and Puhakka, 2004). There are many potential advantages in extracting metals from primary ores using DISB, which include: (i) avoiding haulage and comminution of buried rocks, greatly reducing energy costs and carbon-footprints of mining; (ii) material of no value (~99% of a typical ore body) remains buried underground, resulting in a vast reduction in waste material generated and dumped on the land surface; (iii) the land surface area required by a DISB operation (its “footprint”) would be a fraction of that required by a conventional metal mine. Perceived disadvantages include potential contamination of ground-waters, uncontrolled migration of PLS in the deep subsurface and poorer rates of metal extraction than could be obtained by conventional mineral processing involving comminution and froth flotation.

“BIOMore” is a research and development project sponsored by the European Union as part of its Horizon2020 programme (www.BIOMore.info), and combines DISB and indirect bioleaching technologies. The project covers multiple aspects of the proposed technology, including intensive modelling and environmental issues. A pilot-scale test has been set up ~1 km underground within an operating copper mine (the Rudna mine in Poland, operated by Europe's largest primary copper producer KGHM). Rather than drill directly from the land surface, a purpose-built FIGB has been sited underground next to an ore block, in order to test the feasibility of using indirect bioleaching of an *in situ* ore body. The ore block, which has been fractured by lateral drilling and controlled blasting to open-up fissures to allow percolation of liquors, is *kupferschiefer*, a copper-rich deposit that occurs widely in central and north-eastern Europe. *Kupferschiefer* comprises adjacent layers of sandstone and black shale, with copper deposited chiefly in chalcocite, bornite and chalcopyrite (Vaughan et al., 1989), and is also rich in calcareous minerals (calcite and dolomite) and contains significant amounts of chloride, chiefly as halite (NaCl) but also present in the copper-containing mineral atacamite (Cu₂Cl(OH)₃). The presence of large amounts of carbonates and chlorides is not conducive to bioleaching technologies, which are normally carried out at pH < 2 (i.e. extensive acid consumption would be required to dissolve the carbonate minerals) by prokaryotic microorganisms that are mostly intolerant of relatively small concentrations (< 100–200 mM) of chloride. Ahead of the pilot-scale test within the Rudna mine, tests were carried out on ore packed into laboratory-scale columns to ascertain how these perceived obstacles in using DISB to extract copper from *kupferschiefer* could be eliminated or minimized.

Table 1

Metal contents of *kupferschiefer* sandstone and black shale samples (source: Sylvain Guignot, BRGM).

Metal(loid) (%)	Ag	Co	Cr	Cu	Fe	Ni	Zn
Sandstone	0.012	0.007	0.043	2.98	0.99	0.006	0.002
Black shale	0.024	0.014	0.028	3.61	2.88	0.027	0.10

2. Materials and methods

2.1. *Kupferschiefer* ore samples

Samples taken from the sandstone and black shale strata from the *kupferschiefer* ore at the Rudna mine were sent to the Federal Institute for Geosciences and Natural Resources (*Bundesanstalt für Geowissenschaften und Rohstoffe*; BGR, Hanover, Germany) where they were milled and graded. Gravel-sized samples between 2 and 8 mm diameter were sent to the laboratories at Bangor University and used in the column experiments described below. Elemental analysis data of the sandstone and black shale samples used in experimental work (Table 1) was provided by the *Bureau de Recherches Géologiques et Minières* (BRGM, Orleans, France). Total leachable chloride in the sandstone and black shale were determined by summing that removed from the gravel samples by leaching sequentially with sulfuric acid and acidic ferric sulfate lixiviant, as described in Section 2.3.

2.2. Construction and operation of a laboratory-scale ferric iron-generating bioreactor (FIGB)

A 2 L (working volume) bioreactor vessel (Electrolab, U.K.) was packed with 8 mm diameter sterile porous glass beads (Poraver, Germany), a liquid medium containing 50 mM ferrous sulfate, basal salts and trace elements (Nancucheo et al., 2016) adjusted to pH 1.5 (with sulfuric acid) and inoculated with a mixed culture of acidophilic prokaryotes sourced from the *Acidophile Culture Collection* maintained at Bangor University: *Leptospirillum ferriphilum* strain MT63, *L. ferrooxidans*^T, *Acidithiobacillus* (*At.*) *ferrooxidans*^T, *At. ferridurans*^T, *At. ferrivorans* strain CF27, *At. ferriphilus*^T, *At. caldus*^T, *At. thiooxidans*^T, *Sulfobacillus thermosulfidooxidans*^T, *Acidibacillus* (*Ab.*) *ferrooxidans*^T, *Ab. sulfuroxidans*^T, *Acidiphilium cryptum* strain SJH, *Ferroplasma acidophilum* strain BRGM4 and *Acidiplasma* sp. strain FV. The bioreactor was connected to two 5 L vessels, one of which acted as the feed supply to the FIGB and contained the same liquid medium as above, while the other acted as a drain vessel. The bioreactor was aerated at 1.5 L sterile air min⁻¹ and maintained at 35 °C. When all of the ferrous iron in the liquid medium had been oxidized, fresh medium was pumped into (and oxidized medium out of) the vessel at increasing rates as the bioreactor became increasingly colonized with immobilized iron-oxidizing acidophiles. After ~3 months of operating the FIGB in this way, the ferrous iron in the influent liquor was increased to 180 mM and the pH lowered to 1.3, in order to generate extremely acidic lixiviant solutions with higher concentrations of ferric iron to be used for indirectly bioleaching ore samples.

The effect of dissolved salt on the performance of the FIGB was tested by adding increasing amounts of sodium chloride (equivalent to 0.5, 1, 2, 3, 10 and 20 g/L of Cl⁻) to feed liquor containing 180 mM ferrous iron, pH 1.3, and determining rates of iron oxidation. The microbial composition of the ferric iron-rich lixiviant generated by the FIGB was determined at regular intervals using a combination of terminal restriction enzyme fragment length polymorphism (T-RFLP) analysis and by plating on solid media, as described below.

Table 2

Durations of individual phases during sequential leaching of separate *kupferschiefer* ore fractions (sandstone and black shale): Phase I, acid leaching; Phase II, indirect bioleaching. Each sub-phase (A to H) involved the use of a fresh, fully oxidized lixiviant solution.

Sandstone leaching phases and sub-phases	I	IIA	IIB	IIC	IID
Duration (days)	9.8	3.9	4.0	4.0	10.0

Shale leaching phases and sub-phases	I	IIA	IIB	IIC	IID	IIE	IIF	IIG	IIH
Duration (days)	4.9	1.3	1.8	2.0	4.0	8.1	10.9	9.1	6.9

2.3. Sequential leaching of *kupferschiefer* sandstone and black shale samples

The first column leaching experiment set up involved using separate non-aerated columns, 32 cm high and 9.4 cm wide and maintained at 35 °C, for sandstone (2.3 kg) or black shale (2.0 kg) ore. These were leached sequentially, first with sulfuric acid and afterwards with acidic ferric iron-rich lixiviant generated by the FIGB. Acid leaching, referred to as Phase I in Table 2, involved recirculating 2 L of 100 mM sulfuric acid through the columns for either 10 days (sandstone) or 5 days (black shale). Next, the acid leachates were removed and replaced with 2 L of FIGB lixiviant containing 180 mM ferric iron, pH 1.7 (Phase II). This was again continuously recirculated through the columns until almost all of the ferric iron present had been reduced to ferrous, at which point it was replaced with 1.5 L of fresh, fully oxidized lixiviant. This cycle was repeated four times for sandstone and nine times for the black shale, each new batch of lixiviant being referred to as a “sub-phase” (IIA, IIB etc.; Table 2). Addition of sulfuric acid was required during indirect bioleaching of both sandstone and shale to avoid the pH of the PLS produced increasing to the point that ferric iron precipitated (~pH 2.3). Indirect bioleaching continued for 23 days for the sandstone sample, and 44 days for the black shale.

2.4. Sequential leaching of composite *kupferschiefer* ore

A third column leaching experiment was set up using both components of *kupferschiefer* ore (a 0.3 kg layer of black shale sandwiched between two 1.0 kg layers of sandstone, mimicking the layered structure of the *in situ* ore body and referred to as “composite” *kupferschiefer* ore). Since leaching the separate ore fractions had shown that both contained significant concentrations of acid-leachable chloride, a preliminary 19 day leaching step using reverse osmosis (RO)-grade water (referred to as Phase 0) was included in this experiment. This was followed by a 3 day acid leaching period (using 2 L of 0.9 M sulfuric acid; Phase I) and a 40 day period in which the composite ore was indirectly bioleached (Phase II). The set up used in Phase II of this experiment differed significantly from that used in the first column leaching experiment. Firstly, the FIGB was connected directly to the ore column, rather than just providing single-use lixiviant solutions, and a flow circuit established between them. Secondly, bleed streams (100–200 mL) were withdrawn on seven occasions during Phase II, soluble copper removed from these solutions by sulfide precipitation (using biogenic H₂S generated in a low pH sulfate-reducing bioreactor (Santos and Johnson, 2017) and the copper-free raffinate solutions returned to the flow stream. Thirdly, an additional vessel was included in the flow circuit wherein sulfuric acid was added to compensate for that consumed by reacting with residual carbonate minerals in the composite ore. Fig. 1 shows an image of the column and a schematic of the set up used in each of the three phases of this experiment.

2.5. Assessment of the partial disintegration of *kupferschiefer* sandstone caused by water washing

It was noted that sand grains accumulated in the bottom of the ore column during water washing of the composite *kupferschiefer* ore. To attempt to quantify the extent to which the sandstone gravel samples disintegrated, and assess whether their physical strength had been weakened by water leaching, an empirical experiment was set up.

Four jars, each containing 100 g *kupferschiefer* sandstone (2–8 mm diameter) and 100 mL of RO-grade water were incubated at 30 °C, two of which were agitated by being continuously aerated (“active” treatment) while the other two were not (“passive” treatment). After 14 days, the sandstone from each jar was removed and separated (by sieving) into three fractions: (i) gravel (> 2 mm diameter), (ii) medium and coarse sand (0.2 to 2 mm), and (iii) fine sand (63 to 200 µm), each of which were dried and weighed. Concentrations of chloride, sodium, magnesium and calcium in the aqueous solutions were determined at the end of this experiment, and those of chloride during the first two days of water leaching in a separate experiment. Ninety grams of the gravel fraction samples (and 90 g of unwashed sandstone) were then subjected to incremental crushing using a vibrating mill (TI-100, Heiko Seisakusho, Japan) for 1 h. Sand fractions (< 2 mm) produced were removed by sieving and weighed on 10 occasions during this test.

2.6. Chemical analyses

The pH values of FIGB effluents and column leachates were measured using a pHase combination glass electrode, and redox potentials (adjusted to be relative to a standard hydrogen electrode, E_H values) using a combination platinum silver/silver chloride electrode (VWR International, UK); both electrodes were coupled to an Accumet pH/redox meter 50.

Concentrations of total copper were determined by reducing the copper (II) present with hydroxylamine solution, followed by measuring copper (I) using the bicinchoninic acid colorimetric assay (Anwar et al., 2000). Concentrations of ferrous iron were determined using the Ferrozine colorimetric assay (Stookey, 1970) and total iron using the same assay after reducing soluble ferric iron to ferrous with an excess of ascorbic acid; concentrations of ferric iron were determined from differences between those of total and ferrous iron. Concentrations of Ag, Ca, Co, Cr, Mg, Na, Ni, and Zn in ore leachates were measured by atomic absorption spectrophotometry, using a Varian SpectraAA 220FS AAS. Chloride concentrations were determined using a turbidometric technique (Freier, 1974).

2.7. Microbiological and biomolecular analyses

Metabolically-active microorganisms were isolated on selective solid overlay media (Johnson and Hallberg, 2007). Plates were incubated at both 30° (for mesophiles) and 45 °C (for thermo-tolerant species) for 10 days, and isolates identified based on their colony and cellular morphologies. Terminal restriction enzyme fragment length polymorphism (T-RFLP) analysis of their 16S rRNA genes was used to confirm their identities (Kay et al., 2013). The compositions of planktonic prokaryotic populations were also analyzed using semi-quantitative T-RFLP, using two restriction enzymes for both bacterial (*HaeIII* and *CfoI*), and archaeal (*CfoI* and *AluI*) populations (Kay et al., 2013).

3. Results

3.1. Performance of the FIGB using saline and non-saline influent liquors

Biomass accumulated on the porous glass beads within the FIGB, as evidenced by increasing rates of ferrous iron oxidation at constant flow rates and hydraulic retention times (HRTs). With HRTs of 10 h or more, > 99% of the 50 mM ferrous iron present in salt-free influent

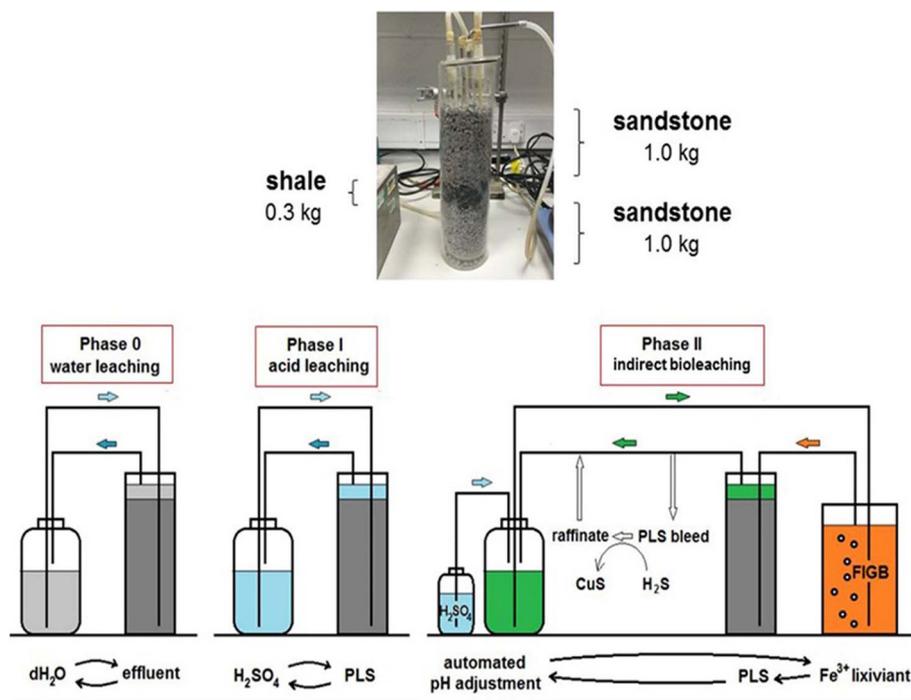


Fig. 1. Image of the column used to leach composite *kupferschiefer* ore (top) and a schematic representation of the set ups used for water leaching (Phase 0), acid leaching (Phase I) and indirect bioleaching (Phase II).

liquors was oxidized to ferric, but this declined to ~55% when the HRT was reduced to 5 h. Larger HRTs were required to fully oxidize solutions when the ferrous iron concentration was increased to 180 mM (data not shown). Soluble iron (~180 mM) in the lixivants used to indirectly bioleach ores in the first column experiments was always > 99% ferric, and had E_H values of ~ +880 mV and pH values of ~1.7. Relatively few of the bacteria that were used to inoculate the FIGB were detected in T-RFLP profiles of lixiviant liquors, which were dominated by *L. ferriphilum*, *Sb. thermosulfidooxidans* and *At. ferridurans/ferrooxidans* (which shared the same T-RF sizes in digested genes). Archaeal 16S rRNA genes were also amplified from DNA extracted from the acidic ferric lixivants, and these corresponded to the iron-oxidizer *F. acidophilum*.

Chloride concentrations of up to 3 g/L (84.5 mM) in the influent liquor had no detectable effects on rates of ferrous iron oxidation by the FIGB, but 10 g/L (282 mM Cl⁻) and 20 g/L (563 mM Cl⁻) lowered them to 70% and 60%, respectively, of the rate obtained with no salt addition (Fig. 2a). There were also corresponding decreases in redox potentials and increased pH values of the lixivants generated when salt was added (Fig. 2). All measured parameters (Fe²⁺ oxidation rates, pH values and redox potentials) recovered to their initial values after using a salt-free influent medium at the end of this experiment.

Only two bacterial species (*L. ferriphilum* and *Sb. thermosulfidooxidans*) were identified in lixiviant solutions generated by the FIGB by T-RFLP analysis during the course of this experiment. These changed in relative proportion as the feed liquor became increasingly saline, *L. ferriphilum* falling from 65% to 35% relative abundance and *Sb. thermosulfidooxidans* increasing from 35% to 65% relative abundance between the start of the experiment (with salt-free influent liquor) to when 20 g/L of Cl⁻ was added. Interestingly, reverting to a salt-free influent liquor resulted in *L. ferriphilum* being detected at 95% relative abundance. Amplified 16S rRNA genes corresponding to the archaeon *F. acidophilum* were detected in all lixiviant solutions generated, irrespective of salt content.

3.2. Sequential leaching of sandstone and black shale *kupferschiefer* fractions

Acid leaching of *kupferschiefer* sandstone and black shale fractions carried out in separate columns showed different and distinct trends. With the former, consumption of sulfuric acid stopped after 6 days whereas acid continued to be consumed in the black shale column up until (and beyond) the point that the next phase of indirect bioleaching was initiated (Fig. 3). The acid leachates from the sandstone column were bright blue in colour, reflecting the fact that ~13% of the copper (from solution chemistry data) had been extracted by sulfuric acid. In contrast, negligible amounts of copper were acid-leached from the black shale sample. Changing from acid leaching to indirect bioleaching resulted in large increases in copper solubilized from both the sandstone and black shale columns (Fig. 4). While copper was successfully acid- and bio-leached from both fractions of *kupferschiefer* ore, this was more rapid and extensive with sandstone (~87% copper extracted within 32 days) than black shale (~50% copper extracted within 49 days).

Redox potentials of the lixivants decreased markedly (to < +600 mV) during indirect bioleaching, as a consequence of ferric iron being consumed by oxidizing sulfide minerals. This occurred more rapidly in the earlier sub-phases of indirect bioleaching. Analysis of PLS generated by acid leaching and indirect bioleaching showed that they contained significant concentrations of chloride, especially those from the sandstone fraction (Fig. 5). The summated concentrations were equivalent to total leachable NaCl of 3.8% (by wt.) in the sandstone fraction, and 0.82% in the black shale.

3.3. Sequential leaching of composite *kupferschiefer* ore

Column leaching of the three-layered ore (a single layer of black shale sandwiched between two layers of sandstone gravel) reflected the layered nature of *in situ kupferschiefer*. This experiment also differed from the previous column leaching experiment by incorporating a preliminary water washing stage (Phase 0) ahead of acid leaching. As before, acid was consumed and soluble copper was produced during

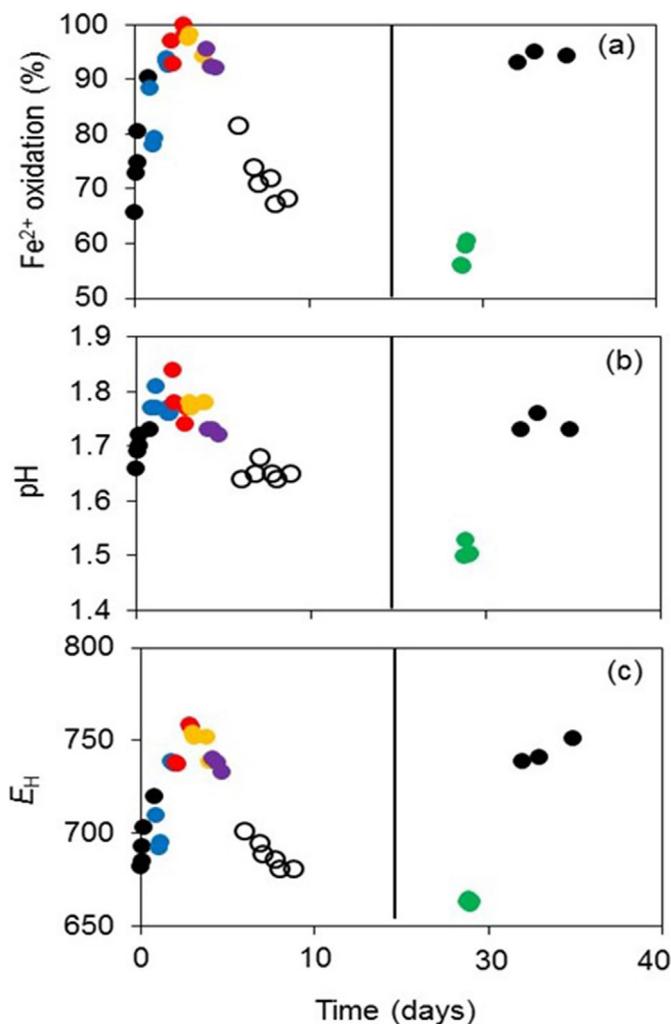


Fig. 2. Changes in (a) percentage of ferrous iron oxidized, (b) pH, and (c) redox potentials in lixiviant solutions generated by the FIGB, using influent liquors containing different concentrations of chloride. Key: (●) 0 g/L Cl⁻, (●) 0.5 g/L Cl⁻, (●) 1 g/L Cl⁻, (●) 2 g/L Cl⁻, (●) 3 g/L Cl⁻, (○) 10 g/L Cl⁻, (●) 20 g/L Cl⁻.

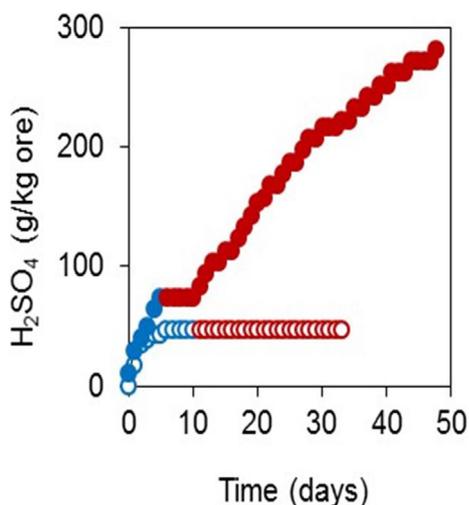


Fig. 3. Consumption of sulfuric acid during sequential leaching of *kupferschiefer* sandstone (○, ○) and black shale (●, ●). Blue symbols show data from the acid leaching phase, and red symbols from the indirect bioleaching phase.

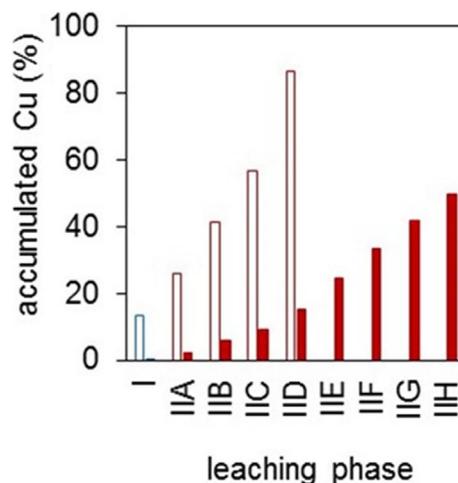


Fig. 4. Accumulated percentages of copper extracted during acid leaching (phase I; blue bars) and indirect bioleaching (phase II; red bars) of separate *kupferschiefer* strata, sandstone (hollow bars) and black shale (filled bars). Each sub-phase of indirect bioleaching (IIA to IIIH) involved using a fresh, fully oxidized lixiviant solution.

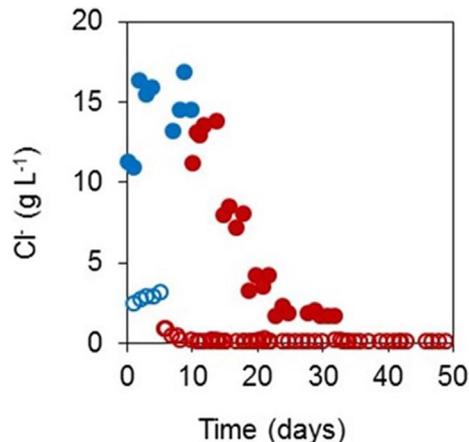


Fig. 5. Changes in concentrations of chloride during sequential leaching of separate *kupferschiefer* sandstone (○, ○) and black shale (●, ●). Blue symbols show data from the acid leaching phase, and red symbols from the indirect bioleaching phase.

both the acid-leaching and indirect bioleaching phases. Ferric iron consumption (by the ore) slowed down rapidly with time, while ferric iron regeneration by the FIGB was highly efficient throughout, as in previous experiments (data not shown). The total amount of copper extracted (59%; calculated from that in all of the leachates obtained), even taking into account that removed in the bleed stream, was less than that from the sandstone (87%) but slightly more than that from the black shale (50%) in the first column leaching experiment. Most (~63%) of the chloride present in the composite ore was extracted during the water washing phase (Fig. 6). Initially this was rapid, but slowed markedly after day 12.

However, about an additional 20% of the chloride present in the ore was solubilized during acid leaching. Very little additional chloride was removed from the composite ore during the bioleaching phase (Fig. 6).

Other transition metals were also leached from the composite *kupferschiefer* ore (Table 3), though this varied between metals, and concentrations of nickel in leachates were always below detection limits. The data for cobalt were rather anomalous, with detectable amounts being extracted from the sandstone fraction but not from the composite ore.

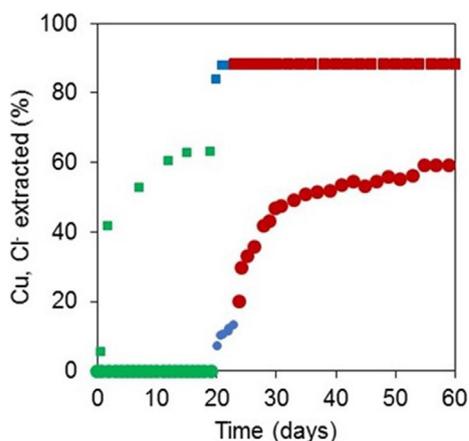


Fig. 6. Cumulated percentages of copper (●, ○, ●) and chloride (■, ■, ■) leached from the composite *kufverschiefer* ore during Phase 0 (water washing; green symbols), Phase I (acid leaching, blue symbols) and Phase II (indirect bioleaching; red symbols).

Table 3

Total amounts of transition metals (other than copper) extracted from separate fractions (sandstone and black shale) and composite *kufverschiefer* ore by sequential acid leaching and indirect bioleaching.

Metal extracted (%)	Ag	Co	Cr	Zn
Sandstone	0.3	9.4	<	<
Black shale	0.2	<	6.9	> 99
Composite ore	0.2	<	0.4	> 99

< , below levels of detection.

3.4. Effect of water washing *kufverschiefer* sandstone on the integrity of the ore

Chloride was rapidly leached from the sandstone ore by immersion in water, particularly when the gravel-water mixes were agitated by aeration (Fig. 7a). Analysis of soluble cations in water leachates showed that the ratio of sodium to chloride was about 1:1.2, and that negligible amounts of magnesium and calcium were present, implying that only halite was solubilized in significant amounts during water washing. After 14 days of being immersed in standing water, 99.2% of the sandstone recovered was still present as gravel-size particles, and 0.8% as fine-coarse grain sand particles. Agitating the water with air greatly increased the amount of sand particles released from the gravel (5.3%). Crush tests showed that sandstone gravel which had previously been

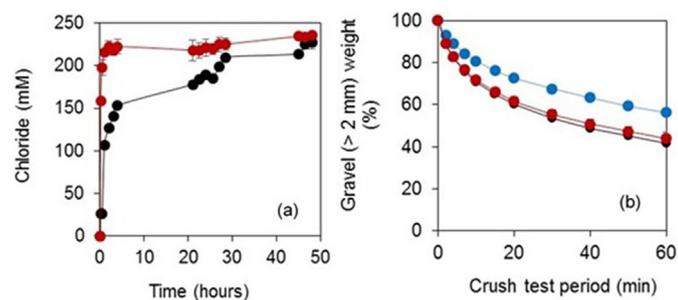


Fig. 7. (a) Changes in chloride concentrations in solutions of *kufverschiefer* sandstone immersed in water with (●) and without (●) agitation; (b) disintegration of *kufverschiefer* sandstone gravel (> 2 mm diameter) in a rock-crusher, following active (●) and passive (●) immersion in water, and compared with that of untreated sandstone (●). The error bars represent data ranges (n = 2).

soaked in water had less physical strength than untreated sandstone, but that agitating the water-gravel mix had no effect on this (Fig. 7b).

4. Discussion and conclusions

In situ bioleaching of deep buried ore bodies has the potential to have a major impact on metal mining in the 21st century, making accessible reserves that are currently not economically viable because of their physical remoteness and offering possible environmental benefits over conventional mining (Johnson, 2015). However, there are many obstacles to overcome before DISB would be considered to be a feasible technology by mining companies and one that would be acceptable to society in general. A great deal of fundamental research is needed to allow sufficient understanding of the complexities of extracting base metals from an in-place, fractured sulfidic ore body.

BIOMORE is a multidisciplinary research project addressing some of these fundamental issues (Matthies et al., 2017; Filippov et al., 2017). The ore body selected for the project, *kufverschiefer*, is not particularly suited to low pH biohydrometallurgical processing as it is both calcareous and saline. It is, however, a well characterized and widely distributed ore, and is exploited by the largest primary producer of copper (KGHM); *kufverschiefer* has also been the focus of previous bioleaching projects (e.g. Kutschke et al., 2015). It occurs as a relatively thin ore layer at ~1 km depth in deep mines in Poland, and its accessibility at one of these (the Rudna mine) has facilitated a pilot-scale trial to be set up without the large costs involved in drilling from the land surface.

The calcareous nature of *kufverschiefer* is well documented (e.g. Vaughan et al., 1989) and this prompted the two-stage leaching protocol used in the first column experiment. Using acidic ferric iron-rich lixivants directly would have led to extensive formation of ferric carbonate precipitates, which would have blocked fissures and formed passivation layers on mineral surfaces, impeding their dissolution. Experiments carried out separately with sandstone and black shale showed that these behaved very differently. Sulfuric acid solubilized about 13% of copper present in the sandstone but liberated very little from the black shale. This was not due to different copper mineralogies, as chalcocite (Cu_2S)-like minerals are the most abundant in both fractions. The difference was probably due more to the fact that the sandstone fraction was much more porous than the black shale. Chalcocite is an “acid soluble” mineral (Vera et al., 2013), though it is more readily solubilized by acidic ferric iron solutions than by acid alone, and the likely source of most of the copper that was leached from the sandstone by sulfuric acid. Atacamite (present in relatively small amounts) would also probably have contributed to that leached by sulfuric acid. It was interesting to note, however, that oxidative leaching with an acidic ferric iron solution was superior to acid leaching alone, more notably in the case of the black shale fraction.

The fact that large concentrations of sodium chloride were also readily leached from *kufverschiefer* (both the sandstone and black shale fractions) was not appreciated until the PLS from the first column experiment were more extensively analyzed. Acidic, saline ferric iron-rich liquors are highly corrosive and would need to be avoided in a pilot- or full-scale operation in order not to damage metal components of pumps and other equipment. Moreover, the concentrations of salt in many of the PLS were well above those reported to partially or completely inhibit the growth and activities of most iron-oxidizing acidophilic prokaryotes (Zammit and Watkin, 2016). These have central roles in DISB as they are required to continuously regenerate oxidized lixivants. Anionic chloride, rather than cationic sodium, is known to be the problematic ion in this context (Shiers et al., 2005). In the first column experiment, this potential impasse did not arise, as the lixivants generated by the FIGB recirculated through the ore columns until they were fully reduced and were not biologically regenerated. However, in an actual DISB operation this would not be the case, and the second column experiment (shown schematically in Fig. 1) involved a flow

circuit that incorporated both the FIGB and the ore column. Leaching *kupferschiefer* with water removed most of the halite from the ore, and incorporating this stage (Phase 0) ahead of an acid-leaching stage (Phase I, which removed most of the residual chloride) was effective in desalinating the ore sufficiently ahead of the critical indirect bioleaching Phase II. Indirect bioleaching of the ore would have essentially been an anoxic process (as evidenced that there was net reduction of iron in the columns), as the concentration of dissolved oxygen in the lixiviant (estimated to be between 1 and 5 mg/L) would only be enough to regenerate 125–625 μM ferric iron within the ore column, which is a tiny fraction of that present in the lixiviant (180 mM). The integrated set up used in the second column experiment mimicked an actual operation by incorporating a bleed stream where copper was effectively removed (by sulfide precipitation) and the raffinate returned to the flow circuit. This stage also helped decrease net acid consumption, as off-line precipitation of copper sulfide generated protons ($\text{Cu}^{2+} + \text{H}_2\text{S} \rightarrow \text{CuS} + 2\text{H}^+$), producing raffinates that were more acidic than the PLS.

The FIGB is an integral component of a DISB system (Johnson, 2015). A laboratory-scale FIGB was commissioned and used in the current study, in which iron-oxidizing acidophiles were immobilized in biofilms that colonized porous glass beads, facilitating their retention within the bioreactor. Only three of the acidophiles used to inoculate the FIGB were detected in later experiments: *L. ferriphilum* and *Sb. thermosulfidooxidans* (both bacteria) and the archaeon *F. acidophilum*. All of these catalyze the dissimilatory oxidation of ferrous iron, but only one (*L. ferriphilum*) is an obligate chemoautotroph and is likely to have provided the organic carbon required by *F. acidophilum* and preferred by *Sb. thermosulfidooxidans*. Community analysis was only carried out with planktonic cells (in order not to impair the FIGB) and it is possible that biofilm communities were more complex. It is interesting to note however that a similar consortium of iron-oxidizing acidophiles were found in a laboratory-scale FIGB set up elsewhere (Kinnunen and Puhakka, 2004). More germane to the present study was the fact that the FIGB continued to oxidize ferrous iron, albeit at slower rates, when the influent liquor contained > 500 mM sodium chloride, a concentration that is known to completely inhibit the growth of almost all known iron-oxidizing acidophiles, with the notable exception of *Acidihalobacter prosperus* (Dopson et al., 2016) which was not included in the inoculum. This unexpected result could be due to the greater resilience of bacteria within biofilm communities.

One of the more unexpected findings of this study was that the sandstone fraction underwent partial disintegration when immersed in water, and that gentle agitation accentuated this process. Water-soaked sandstone gravel also displayed lower resistance to crushing than unwashed ore. Calcite and dolomite (which may have acted as cements holding sand particles together) did not appear to be solubilized, though large amounts of halite were. This intriguing observation could be significant for any hydro-metallurgical processing of *kupferschiefer* ore.

Results from this study have greatly influenced the planning and operation of the pilot-scale tests carried out deep within the Rudna mine. This is now adopting the same three phase (water, acid, and indirect bioleaching) approach as that used in the second column test, which successfully leached most of the copper from the ore with no impairment to the performance of the FIGB. Given the results from the first column experiment, most of this copper was considered to have been leached from the sandstone rather than from the black shale fraction. The copper contents of the sandstone and black shale fractions used in the current experiments were similar, though in most *kupferschiefer* strata the copper content of the thinner black shale layer is generally much greater than that of the thicker sandstone layers. In addition, sandstone was more readily leached, both with acid and acidic ferric iron lixiviant, than the black shale component of the ore.

Although the results from the current work suggest that *kupferschiefer* could be mined using DISB, whether this would be economically viable is more questionable. Large amounts of water would be required in the initial stage, producing waste water that requiring desalination. Acid leaching produces copper-containing PLS, but the large amounts of sulfuric acid required to solubilize carbonate minerals would probably exceed the value of the copper extracted. More efficient copper extraction would be achieved by indirect bioleaching, though the relatively low iron content of the ore (~1–3%) would require additional iron (e.g. as ferric sulfate) to be brought on site to allow this phase to operate. In addition, the raffinates present at the end of the process would require treatment (e.g. oxidation and liming) to immobilize the iron present. Non-calcareous and non-saline metal ores, containing larger amounts of iron sulfide minerals such as pyrite and pyrrhotite, would therefore be more suitable candidates for DISB.

Acknowledgements

This work was supported by the European Union Horizon 2020 project “BIOMore” (Grant agreement # 642456). We are grateful to Axel Schippers (BGR) and colleagues at KGHM for providing samples of *kupferschiefer* ore, and to Sylvain Guignot (BGRM) for analytical data of the composition of the sandstone and black shale samples.

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