Influence of the nutrient medium composition during the bioleaching of polymetallic sulfidic mining residues

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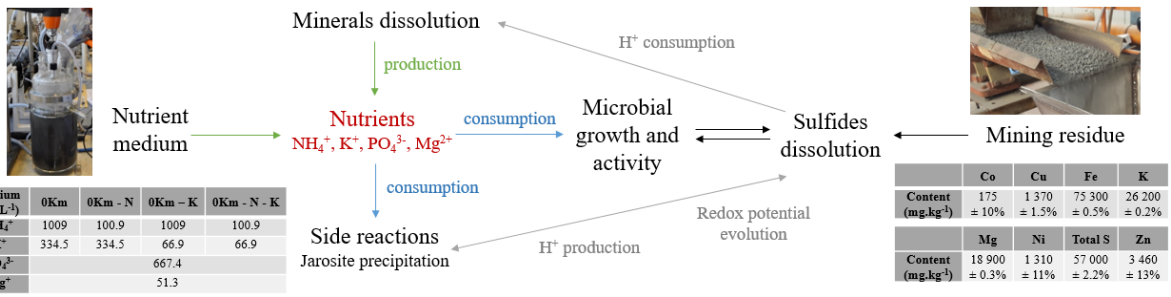
Abstract

Although bioleaching, i.e. the extraction of metals using microorganisms, remains a niche application, it has garnered growing interest as an ecologically acceptable and economic alternative to conventional processes such as pyrometallurgy or conventional hydrometallurgy. In particular, bioleaching is envisaged as a viable option to treat materials that in the past would have been considered as waste. The objectives of reprocessing mining wastes are both the recovery of remaining elements as well as preventing their potential environmental impacts. During such process, the requirements in carbon and nutrients for microbial growth and activity should be carefully determined, as they may be involved in side reactions or have non-negligible economic and environmental costs. This study aimed at determining the ammonium and potassium requirements to optimise the bioleaching performances of mining residues from Sotkamo (Terrafame, Finland). Co, Cu, Ni and Zn dissolution kinetics were evaluated in 2 L stirred tank reactors at 20% (w/w) solid with various nutrient media. By analysing the pregnant leach solutions and the residues, ammonium and potassium consumptions for microbial growth and activity were estimated. This study showed that using high concentration of potassium in the nutrient medium was not necessary, as there were some K-bearing minerals in the mining waste that could release potassium to enhance the microbial activity. On the contrary, amending the nutrient medium with high concentration of ammonium was required to ensure high bioleaching kinetics.

Graphical abstract



Medium (mg.L^{-1})	0Km	0Km - N	0Km – K	0Km - N - K
NH_4^+	1009	100.9	1009	100.9
K^+	334.5	334.5	66.9	66.9
PO_4^{3-}	667.4			
Mg^+	51.3			

	Co	Cu	Fe	K
Content (mg.kg^{-1})	175 ± 10%	1 370 ± 1.5%	75 300 ± 0.5%	26 200 ± 0.2%

	Mg	Ni	Total S	Zn
Content (mg.kg^{-1})	18 900 ± 0.3%	1 310 ± 11%	57 000 ± 2.2%	3 460 ± 13%

Statement of novelty

Bioleaching, as an alternative to conventional leaching processes, is based on the catalysis of the dissolution by microorganisms. They require carbon and nutrients to perform their activity. However, the presence of an excess of nutrients may affect the dissolution and the extraction yield due to side reactions (i.e. precipitation). The objective of this study was to determine the optimal nutrient concentrations to promote microbial activity, and thus the dissolution, while limiting the precipitation. In the past, bioleaching was mainly applied to ores and concentrates, and optimal nutrient concentrations were thus determined in this context. This study aims at determining such concentrations in a slightly different context: for the reprocessing of mining residues, to limit their environmental impact while valorizing the remaining metals they contain.

Keywords: bioleaching, mine residues, nutrients, nutrient medium, sulfides

1. Introduction

Biomining is the extraction of metals using microorganisms (bacteria, archaea or fungi). Although this technology remains a niche application, in the last decades it has garnered growing interest from both the academic community and the mining industry, who may consider biomining to be an ecologically acceptable and economic alternative to conventional processes such as pyrometallurgy or conventional hydrometallurgy. Thanks to its low capital costs and its flexibility, bioleaching is well suited to the treatment of metallic resources with complex composition and/or declining metal content (low-grade ores). In the current context, most primary resources of suitable grade that are reasonably accessible and are easy to process are quickly being depleted. Simultaneously, metal demand is growing exponentially. Bioleaching is thus envisaged as a viable option to treat materials that in the past would have been considered as waste.

Most familiar microorganisms involved in bioleaching processes grow autotrophically by fixing CO_2 from the atmosphere and do not require organic carbon. They have also very modest nutritional requirements. Usually, in industrial operations, nitrogen, phosphate, and potassium are provided to the microorganisms by adding small quantities of inorganic fertilizer. Even if microbial nutrient demand is very low, the nutrient speciation and concentration may highly influence the efficiency of bioleaching [1]. Nutrients are involved in biomass growth [2], but can also influence the solubility of oxygen [3] and take part in various side reactions that take place during bioleaching, such as the precipitation of jarosite [4]. Nutrients can also influence cell attachment onto the solid surface [5], which may enhance the dissolution kinetics [6]. Nutrients represent a cost for the process operation, in both heap and stirred tank reactors (STR) [7]. On the one hand, nutrients may limit the reactions kinetics if they are not added in sufficient quantity. On the other hand, they may require additional water treatment if they are added in large excess. Thus, optimizing the composition of the nutrient medium is primordial.

Among all nutrients, nitrogen requirement has been extensively studied, in particular for *Acidithiobacillus ferrooxidans* [8]. Nitrogen may be present in bioleaching environments in various forms. The commonly used nitrogen speciation for bacterial growth is ammonium. Usual nutrient media in bioleaching are the '0K' medium [9] and the '0Km' medium [10], but nutrients are reported to be often in large excess in these media, as described for the BIOX process [7]. In some studies dealing with the bioleaching of sulfidic minerals with acidophiles, a required ammonium concentration of 3.6 and 10-20 $mg.L^{-1}$ were reported [11-12] whereas ammonium concentration in 0K and 0Km media reaches 818 and 1009 $mg.L^{-1}$ respectively (Table 1). Apart from its consumption by the microbial activity and growth, ammonium may be removed from the liquid phase by precipitation into jarosite ($KFe_3(OH)_6(SO_4)_2$) or into ammoniojarosite ($NH_4Fe_3(OH)_6(SO_4)_2$) [13]. The use of atmospheric nitrogen has also been investigated [14]. Some bacterial strains (i.e. *Acidithiobacillus ferrooxidans*) have been reported to be able to fix atmospheric nitrogen and reduce it to ammonia in a process called 'diazotrophy' [14]. However, this ability may require specific operating conditions (such as reduced oxygen concentration) which are not met in highly aerated STR [7] and which differ from one bacterial strain to another [15]. Nitrogen may also be supplemented in the form of urea in the growth medium [16]. Urea is probably assimilated by the microorganisms as ammonium after hydrolysis or enzymatic activity [5]. However, the use of urea enables to reduce considerably the stability and the quantity of precipitates ([16]; no indication on the precipitates composition in this study). Finally, nitrate cannot be considered for bacterial growth as it has an inhibitory effect in low pH environments [11].

Table 1: Nutrient concentrations reported in the literature.

Nutrients	0K medium	0Km medium	BIOX process
NH_4^+	818 mg.L^{-1} *45.5 mmol.L^{-1}*	1009 mg.L^{-1} *56 mmol.L^{-1}*	273 mg.L^{-1} *15 mmol.L^{-1}*
PO_4^{3-}	349 mg.L^{-1} *3.7 mmol.L^{-1}*	667 mg.L^{-1} *7.0 mmol.L^{-1}*	279 mg.L^{-1} *2.9 mmol.L^{-1}*
K^+	144 mg.L^{-1} *3.7 mmol.L^{-1}*	335 mg.L^{-1} *8.6 mmol.L^{-1}*	115 mg.L^{-1} *2.9 mmol.L^{-1}*
Mg^{2+}	49 mg.L^{-1} *2.0 mmol.L^{-1}*	51 mg.L^{-1} *2.1 mmol.L^{-1}*	10 mg.L^{-1} *0.4 mmol.L^{-1}*
Reference	[9]	[10]	[7]

Phosphate, magnesium and potassium requirements have been discussed less than nitrogen in the literature, although their concentrations are highly variable in different nutrient media (Table 1). It has been reported that phosphate and magnesium concentrations from ores or concentrates are most of the time sufficient for bacterial requirements [17]. In the literature, phosphate requirements have been evaluated at 30-40 mg.L^{-1} and at 2 mg.L^{-1} for potassium [11-12]. Phosphate precipitation with Fe(III) was reported [18-19], as well as their sorption onto goethite if this mineral is present in the solid phase [20]. However, phosphate anion is less likely to precipitate than cationic nutrients which can be taken up in minerals such as jarosite [19,21]. Potassium is the cation that is preferentially incorporated in jarosite, compared to sodium, hydronium and ammonium [22]. Consequently, potassium availability is highly dependent on temperature and pH [4].

As a complement to these 'macronutrients', other trace elements (as Cl^-, Mn^{2+}, BO_3^{3-}, SeO_4^{2-}, Zn^{2+}, Na^+, MoO_4^{2-}, Co^{2+}, Cu^{2+}, …) can also be added to improve microbial growth. The quantities of nutrients to amend in the nutrient medium for optimal growth and activity is highly dependent on the composition of the materials and on the operating conditions.

In Sotkamo (Terrafame, Finland), the ore is bioleached in a primary heap and the residue, called 'secondary ore' is currently treated by secondary bio-heap leaching on site to recover remaining metals [23-24]. The objective of this secondary leaching is to improve the economic benefits of the mine while reducing the metal content of the residues. In the framework of European project NEMO ('Near-zero-waste recycling of low-grade sulfidic mining waste for critical-metal, mineral and construction raw-material production in a circular economy' [25]), experiments are being conducted with Sotkamo mine residues, known in our previous work [26] and herein as 'secondary ore', for potential reuse as building and construction material. The aim of the project is to reduce Europe's dependence on the imports of raw materials such as critical and valuable metals (Co, REEs, Cu, Ni, Zn…) and industrial minerals by reducing our waste volumes and thus by valorizing mine tailings. However, for reuse in building and construction, as proposed in the project, the residual content of metals in the tailings should be low to limit the risk of their release into the environment [27]. One objective of the NEMO project is thus to increase the bioleaching performances of the Sotkamo secondary ore, by studying different parameters such as the nutrients addition, that may limit the metal dissolution kinetics and yields of the process.

To date, some work has been carried out on the nutrient requirement of the Sotkamo primary heap [28]. The nutrient concentrations of the pregnant leach solutions from this process were also evaluated to determine if they were in sufficient quantities to support microbial Fe(II) bio-oxidation [1]. However, the amounts of

nutrients remaining in the secondary ore and the nutrient demand in the secondary heap have not yet been evaluated.

This study, performed in the framework of NEMO project, aimed to assess the influence of the nutrient medium composition on the bioleaching of Sotkamo secondary ore. Bioleaching experiments were first performed in 2-L STR operated in batch mode with 20% (w/w) solids. At this high solid concentration, the nutrient requirement for microbial growth and activity, as well as their potential precipitation, are more perceptible. Four nutrient media containing different concentrations of ammonium and potassium were compared. These elements were selected as variables since potassium is known to favour the precipitation of jarosite and ammonium is one of the predominant required elements for cell growth. The metal dissolution kinetics and physicochemical parameter evolution were evaluated over time. To better understand the precipitation, the mineralogy of solids was also investigated. Nutrient concentrations should be carefully defined as a compromise between a high microbial activity and a reduced precipitation. The determination of the optimal nutrient concentrations is helpful for the scale-up of the process, and particularly for its environmental and economic evaluation.

2. Materials and methods

2.1. Sulfidic material from Sotkamo mine

Experiments were performed using primary leaching residue from the Sotkamo mine (Terrafame, Finland). These residues are used as secondary ore in secondary heap leaching and, as outlined above, are named 'secondary ore' in our study. 30 kg were provided, from which 15 kg were ground to 500 µm with successive crushing and milling (Pinette and Henry jaw crusher, roller mill and rod mill). After milling, 80% of the particles were smaller than 235 µm in diameter. The chemical composition of the material has been fully described and is summarised in Table 2 [26]. Major elements (Al_2O_3, CaO, Fe_2O_3, K_2O, MgO, MnO, Na_2O and P_2O_5) were determined by X-Ray Fluorescence (XRF; Zétium from Panalytical), NH_4^+ by potentiometry, elemental S and SO_4^{2-} by gravimetry and total S by combustion. Co, Ni and Zn were determined by triacid leaching (HNO_3 – $HClO_4$ – HF) followed by inductive coupled plasma-optical emission spectrometry (ICP-OES, Horiba Jobin Yvon Ultima 2) and inductive coupled plasma mass spectrometry analysis (ICP-MS, Thermo Scientific X Series). Cu concentrations were determined with a 4-acid digestion followed by ICP-OES analysis (method ME-4ACD81 from ALS, Ireland). Sulfide concentrations were calculated by subtracting S° and SO_4 concentrations from total sulfur.

Table 2: Total elemental concentrations in the secondary ore from Sotkamo mine.

Element	Concentration (g.kg^{-1})	Element	Concentration (g.kg^{-1})
Al	49.9 ± 0.3%	Na	2.94 ± 4.8%
Org C	67.8 ± 0.2%	NH_4^+	1.51×10^{-3} ± 2.3%
Ca	21.1 ± 0.5%	Ni	1.31 ± 11%
Co	0.175 ± 10%	P	1.28 ± 0.7%
Cu	1.37 ± 1.5%	S as SO_4	21.5 ± 13%
Fe	75.3 ± 0.5%	S as S^0	8.33 ± 13%
K	26.2 ± 0.2%	S as $S^=$	26.1 ± 14%
Mg	18.9 ± 0.3%	Total S	57.0 ± 2.2%
Mn	3.18 ± 0.0%	Zn	3.46 ± 13%

The mineralogy of the solids was determined using a QEMSCAN® 4300 at Camborne School of Mines (University of Exeter, UK) following the methodology outlined in a previous study [30]. The QEMSCAN® is equipped with a Zeiss Eco SEM and energy-dispersive X-ray (EDX) spectrometer. X-ray data were collected every 10 µm across the sample surface. X-ray diffractometry (XRD; Siemens D5000 diffractometer) was performed [30]. The XRD profiles were interpreted using the JCPDS PDF-2 2004 database and the method developed to differentiate the altered Fe minerals from other sulfides [31]. The altered Fe minerals designates a mixture of elemental sulfur and sulfide and sulfate minerals (such as melanterite, coquimbite, schwertmannite and copiapite) resulting from the partial alteration of the material sulfides during bioleaching. The main S-minerals in the secondary ore were altered Fe minerals (12.0%), jarosite (11.2%) and gypsum (2.7%), as well as pyrite (2.5%), pyrrhotite (0.30%), sphalerite (0.20%), chalcopyrite (0.17%) and violarite (0.06%). Metal distribution in the sulfides showed that copper occurred in discrete particles of chalcopyrite (33.8 wt%) and in pyrrhotite (0.04 wt%) [26]. Violarite contained the highest concentration of Co (3.6 wt%) but only one grain of this mineral was found. Pyrite and pyrrhotite also contained Co (0.09–1.27 wt% and 0.07–0.13 wt%, respectively). Violarite and pentlandite were the main Ni-bearing minerals. The major Zn-bearing mineral was sphalerite, which also contained high concentrations of Mn (4.3–6.8 wt%) and Fe (7.1–9.8 wt%). All these metals were also detected in the altered Fe minerals and in the grains of Fe oxides, hydroxides or oxyhydroxides (all these minerals being inseparable during the analysis).

2.2. Consortia and nutrient medium

The cultures used for this study was the moderate thermophilic N55 consortium [26]. The predominant microorganisms of this consortium were affiliated to the genera *Acidithiobacillus* (or *Fervidacidithiobacillus* in new classification), '*Acidithiomicrobium'* and *Sulfobacillus*. N55 cultures were grown in a 0Km optimised for bacterial growth on sulfidic materials [10]. Its standard composition is the following: $(NH_4)_2SO_4$, 3.70 g L^{-1}; H_3PO_4 85%w, 0.81 g L^{-1}; $MgSO_4 \cdot 7H_2O$, 0.52 g L^{-1}; KOH, 0.48 g L^{-1}. To determine the influence of ammonium and potassium concentrations on bioleaching kinetics, the nutrient medium was modified as follows: either ten times less ammonium ('0Km – N'), five times less potassium ('0Km – K') or both ('0Km – N – K') (Table 3). Concentrated sulfuric acid (96%w/v) was used to adjust the pH of these media to 2.0.

Table 3: Nutrient concentrations used for bioleaching experiments.

	0Km	**0Km - N**	**0Km - K**	**0Km - N - K**
NH_4^+	1009.1 mg.L^{-1} *56 mmol.L^{-1}*	100.9 mg.L^{-1} *5.6 mmol.L^{-1}*	1009.1 mg.L^{-1} *56 mmol.L^{-1}*	100.9 mg.L^{-1} *5.6 mmol.L^{-1}*
K^+	334.5 mg.L^{-1} *8.6 mmol.L^{-1}*	334.5 mg.L^{-1} *8.6 mmol.L^{-1}*	66.9 mg.L^{-1} *0.9 mmol.L^{-1}*	66.9 mg.L^{-1} *0.9 mmol.L^{-1}*
PO_4^{3-}	667.4 mg.L^{-1} *7.0 mmol.L^{-1}*			
Mg^+	51.3 mg.L^{-1} *2.1 mmol.L^{-1}*			

2.3. Two L STR experiments in batch mode

2.3.1. Experimental setup

The experimental setup is fully described in a previously published article [29]. Bioleaching experiments were performed in batch mode in laboratory-scale glass bioreactors with a working capacity of 2 L and jacketed for

water circulation to maintain a constant operating temperature (55 °C). The temperature of the circulating water was regulated by a cryothermostat and thermocouples placed in the bioreactors. The bioreactors were equipped with four baffles mounted 90° apart and extended down to the base of the vessel to optimize the mixing of pulp. The agitation was performed using a dual impeller system (axial/radial) consisting of a standard 6-blade Rushton turbine combined with a 3-blade 45° axial flow impeller. The gas supply system was designed to accommodate air enriched with 1% CO_2 that was injected beneath the turbine at the bottom of the bioreactors via a stainless-steel pipe. The impellers and the gas injection pipe were positioned in order to respect the standard dimensions and thus, to optimize gas mass transfer and mixing in the bioreactors. The top of the reactors was connected to a gas cooling system to prevent excessive evaporation.

2.3.2. Bioreactor monitoring and operating conditions

Bioleaching experiments were first carried out with different solid concentrations ranging from 5% (w/w) up to 20% (w/w) in 0Km medium. For each test, the inoculum used was a sample of 200 mL of bioleached pulp taken at the end of the previous batch test. At least two successive subcultures were performed for each solid concentration before increasing the solid concentration. Experiments with four nutrient media described in section 2.2 were performed using the adapted microbial culture with a solid concentration of 20% (w/w). These tests were performed simultaneously and 200 mL of inocula were taken from the same previous batch, to ensure complete reproducibility between the four conditions.

For all tests, the gas flow rate was maintained at 120 $L.h^{-1}$, with 1% CO_2, to avoid any oxygen or carbon limitations in the system. At the beginning of each test, the secondary ore was added to the nutrient medium and pH was adjusted to 2.0 with sulfuric acid. The inoculum was then added to the pulp. The pH was adjusted to 1.7. The stirring speed was maintained at 700 rpm. Daily monitoring was performed, including pH, redox potential, biomass concentration and metal concentrations measurements. Metal concentrations were measured with a 4210 MP-AES (microwave plasma atomic emission spectroscopy) from Agilent Technologies. The pH was manually adjusted to 1.7 with concentrated sulfuric acid when needed. Water was also added to counterbalance water losses due to evaporation. All the tests lasted two weeks, unless specified. At the end of each experiment, the solid and the liquid phases of the bioleached pulp were separated by filtration on glass microfiber filters (from Ahlstrom, with pore size from 0.7 to 2.7 µm); the bioleached residues were rinsed with acidified water (pH 1.7, sulfuric acid). A solution containing 1 $g.L^{-1}$ of flocculant (FA920) was added to the pulp to improve the filtration. The quantity was adjusted to reach a ratio of 400 mg of flocculant per kg of materials.

2.3.3. Leachates and residues characterization

Bioleaching residues and filtrates were analysed to perform mass balances. At the end of the experiments, the filtrate compositions were determined using inductive coupled plasma-optical emission spectrometry (ICP-OES, Horiba Jobin Yvon Ultima 2) for Mg, Ca, Fe, Na, K, S, P, Al, Co, Cu, Mn, Ni and Zn, by ion chromatography (Metrohm 940 professionnal IC Vario) for SO_4^{2-} and by UV-visible spectrophotometry (Thermo Scientific Gallery) for NH_4^+.

The chemical composition of the residues and their mineralogy were determined with the same methodology than the secondary ore (detailed in section 2.1).

Ammonium and potassium mass balances were calculated with the following equation (by taking into account

the inoculum, the nutrient medium, the initial solid, the leachate and the residue) :

$$m_{solid,f} - m_{solid,i} = m_{liq,i} - m_{liq,f} \qquad \text{[Eq. 1]}$$

With $m_{solid,f}$ and $m_{solid,i}$ the mass of the nutrient in the solid at the end and at the beginning of the experiment, respectively, and $m_{liq,i}$ and $m_{liq,f}$ the mass of the nutrient in the liquid phase at the beginning and at the end of the experiment, respectively.

For both ammonium and potassium, $m_{solid,f}$ was calculated from the analysis of the residues. However, it should be noticed that potassium concentration used in this calculation of the solid phase takes into account all the potassium in the solid, while ammonium concentration only reports exchangeable ammonium due to the method used for analysis (potentiometry). This means that the ammonium from the jarosite is not detected with this method.

2.4. Bacterial community monitoring

The number of microorganisms in the liquid phase was monitored by counting using a Thoma cell. For DNA-based gene quantification and fingerprinting methods, 2 mL of pulp were taken from the 2 L-reactors and were centrifuged for 10 minutes at 14,000 g. Pellets were washed by re-suspension in 1 mL Tris buffer (100 mM, pH 8) until pH reached around 7 and stored at -20 °C prior to DNA extraction. Microbial DNA was extracted from the frozen pellets with the FastDNA Spin Kit for Soil and using the manufacturer's protocol (MP Biomedicals) with a FastPrep®-24 instrument at a speed of $5 ms^{-1}$ for 30 s. To complement microscopic cell counts, quantitative *real-time* PCR (qPCR) assays targeting the 16S rRNA gene were used to estimate bacterial biomass, using primers 341F and 534R [32]. Real-time data and gene copy numbers were obtained with the CFX Manager software (BioRad), and the results were expressed as gene copies per mL of pulp. The CE-SSCP fingerprinting technique was applied on the V3 region of 16S rRNA genes, amplified by PCR with w49 forward primer and 5′-end FAM-labeled w34 primer, to obtain a diversity profile and to determine relative abundances of the detected bacterial strains [32]. The software Bionumerics (Applied Maths) was used to realign the peak profiles and assign peak position based on internal standard migration, calculate relative abundances on the basis of peak heights, and compare with peak profile of reference bioleaching strains.

3. Results

The influence of the nutrient medium composition on metal dissolution kinetics and physicochemical parameters was evaluated in 2-L STR operated in batch mode on four nutrient media differing in potassium and/or ammonium concentrations: (i) the so-called ‘0Km’ medium as the reference, as this medium contains high concentrations of ammonium and potassium compared to other media reported in the literature (Table 1); (ii) the 0Km medium with the ammonium concentration divided by 10 (‘0Km – N’); (iii) the 0Km medium with the potassium concentration divided by 5 (‘0Km – K’) and (iv) the 0Km medium with ammonium and potassium concentrations divided by 10 and 5, respectively (‘0Km – N – K’).

3.1. Proton consumption and sulfides oxidation

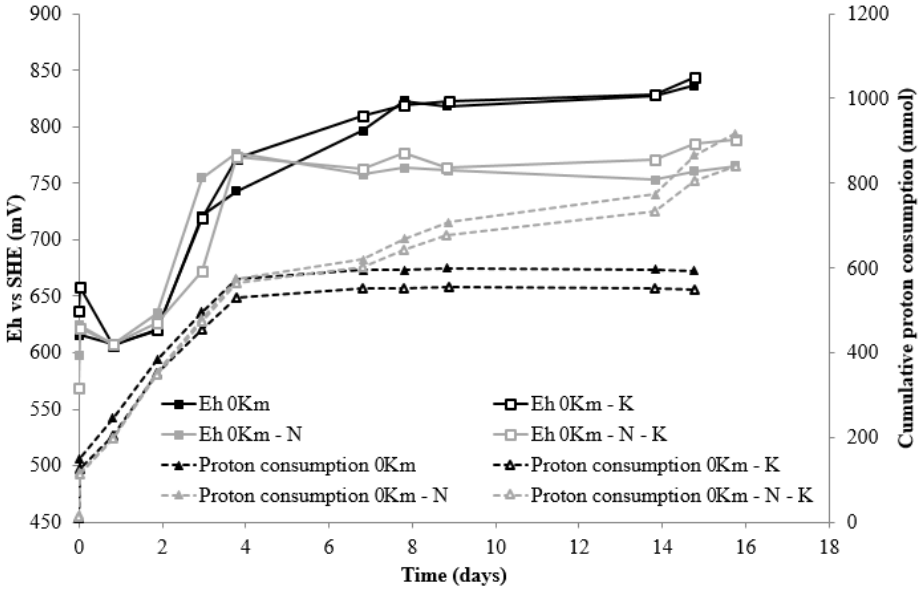


Fig. 1 Redox potential and cumulative proton consumption versus time with a solid content of 20% (w/w) and different nutrient media

In the reference medium (0Km), the first 3 days were characterized by low Eh conditions (around 620 mV vs SHE, Fe mainly as Fe(II), see supplementary material) and high acid consumption was observed (Fig. 1). During this period, the pH increased between daily acid additions (see supplementary material). After this first period, Eh increased sharply whereas pH decreased and acid addition was no longer necessary. The Eh and cumulative proton consumption then stabilized around 850 mV (Fe mainly as Fe(III)) and 600 mmol, respectively. Redox potential and proton consumption evolution over time obtained with the 0Km - K medium were similar to the results achieved with the 0Km medium.

By contrast, a decrease of the ammonium concentration appeared to have a substantial impact on redox potential and proton consumption. The redox potential was similar for all nutrient media until day 4, and then increased up to 830 mV vs SHE for experiments with 3.7 $g.L^{-1}$ $(NH_4)_2SO_4$ (0Km and 0Km - K), whereas it remained steady (around 770 mV vs SHE) when the ammonium concentration was divided by ten (0Km - N and 0Km – N - K). The same trend was observed for the cumulative proton consumption until day 4. This trend is similar to the one observed in abiotic experiments (experiments without bacteria; Hubau et al., 2020). As a result, it was not necessary to add any sulfuric acid for the tests performed with the 0Km and 0Km - K media, whereas the acid consumption continued to increase in the tests carried out with the media with lower ammonium concentration (0Km - N and 0Km – N - K).

3.2. Metal and gangue material dissolution kinetics

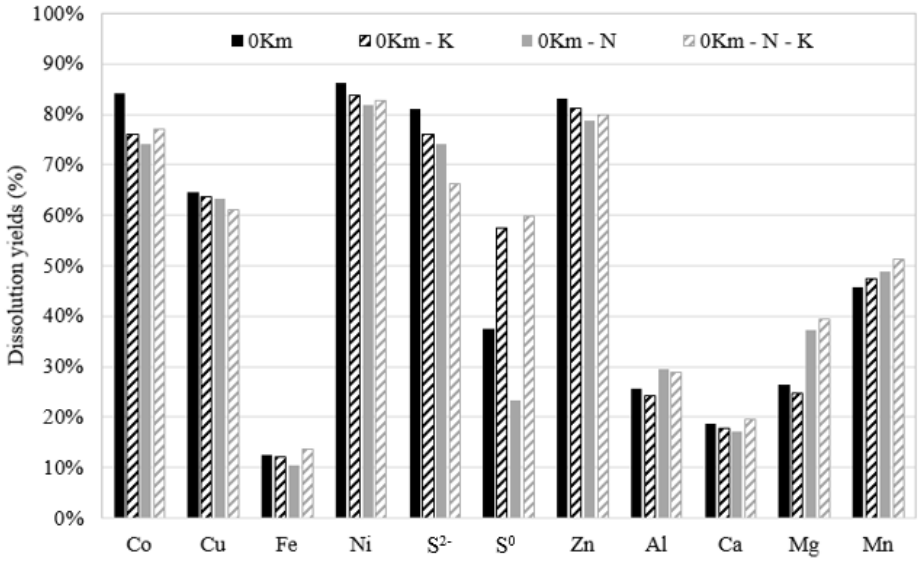


Fig. 2 Dissolution yields at the end of the experiments with 4 nutrient media with a solid concentration of 20% (w/w)

For most metals (Cu, Fe, Ni and Zn), the same dissolution yields were obtained at the end of the experiments whatever the nutrient medium (Fig. 2). At the beginning of the bioleaching experiments, substantial and rapid release of metals (55% of Ni, 25% of Co, 20% of Zn and 10% of Cu) occurred (see supplementary material). This initial and fast dissolution was similar for all the nutrient media, and was then followed by a slower dissolution phase in which Ni, Zn and Cu were released into solution. Cobalt dissolution ceased until a sharp increase of the redox potential up to 800 mV was reached and then its dissolution yield increased again. Similar dissolution kinetics were obtained for all the nutrient media. They are highly comparable to the kinetics that were obtained with the same solid at lower solid concentration (10%w/w) in the previous study done by the authors [26]. For Co, the dissolution yield was slightly higher in the presence of 0Km medium (84%) than when the media contained less ammonium (75% Co extraction yield; Fig. 2).

For sulfur species, large differences in their leaching amenability were noticed in the different nutrient media. Sulfides were oxidized to a greater extent in the 0Km medium (81%) than in the other media (around 75%), with a lower oxidation yield in the case of the 0Km-N-K medium (66%). Elemental sulfur dissolution reached 38% in 0Km medium. When the ammonium concentration was low, the elemental sulfur dissolution decreased down to 23%. The reduction of potassium concentration coincided with the dissolution of elemental sulfur up to 60%, whatever the ammonium concentration.

The dissolution yield of Al and Mg from the gangue material was higher when the NH_4^+ concentration was lower. For Mn and Ca, similar dissolution yields were observed whatever the nutrient medium.

3.3. Fe and nutrient concentrations evolution

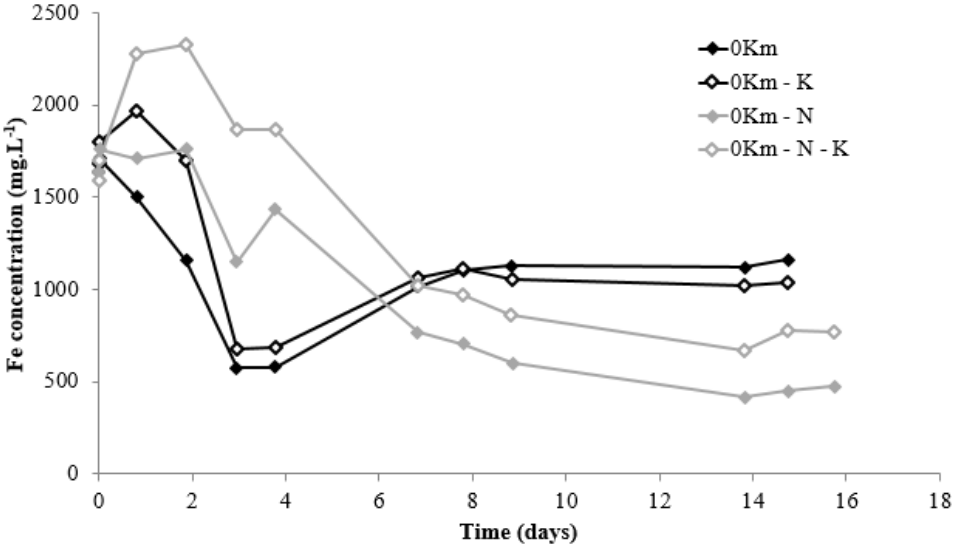


Fig. 3 Fe concentration versus time at 20% (w/v) solid with different nutrient media

In the reference medium (0Km), Fe concentrations decreased sharply at the beginning of the experiment and until day 3 and then started to increase slowly (Fig. 3). Concentrations reached around 1.1 $g.L^{-1}$ at the end of the experiment. Iron concentration decrease was much slower when the concentration of ammonium was ten times lower (0Km – N). The decrease was even slower when ammonium and potassium concentrations were low (0Km – N – K). It is worth mentioning that the filtration of the pulp was much more difficult in the two experiments with lower ammonium concentrations (0Km – N and 0Km – N – K).

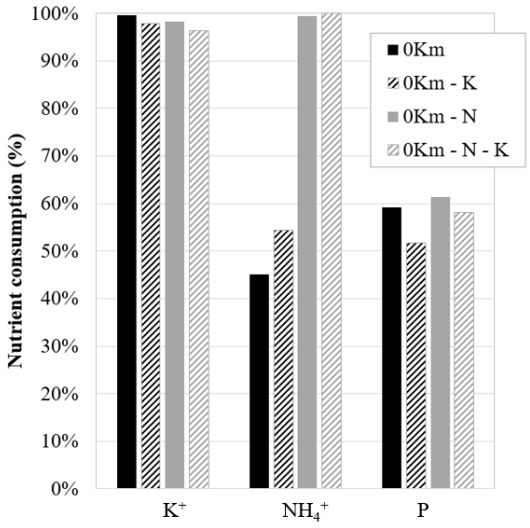


Fig. 4 Nutrient consumption (calculated from the liquid phase) at the end of the experiments performed with the 4 different nutrient media

Fig. 4 presents the portion of nutrients consumed from the liquid phase at the end of the different experiments. This was calculated as the difference of nutrient quantities in the liquid phase between the beginning and the end of experiments, reported to the initial quantity. Potassium was almost entirely removed from the liquid

phase, whatever its initial concentration in the nutrient medium was. This may come from the microbial consumption and/or precipitation. Around 60% of PO_4^{3-} quantity was also removed from the liquid phase in all experiments.

Contrary to phosphate and potassium, ammonium behaviour was strongly linked to its initial concentration in the nutrient medium. When ammonium was highly concentrated in the medium (0Km and 0Km - K), around 50% of the initial ammonium quantity was removed from the liquid phase. When ammonium concentration was low (0Km – N and 0Km – N – K), all the ammonium was removed from the liquid phase.

3.4. Mass balances on K^+ and NH_4^+

In the 0Km medium, the quantity of potassium removed from the liquid phase, $m_{liq,i} - m_{liq,f}$, was 15.4 mmol. Simultaneously, the quantity of potassium removed from the solid phase, $m_{solid,f} - m_{solid,i}$, was 12.3 mmol, meaning that a loss of 27.7 mmol of potassium occurred during bioleaching (Table 4). As potassium analysis in the solid phase takes into account the potassium that is incorporated as jarosite and other phases such as K-bearing clay minerals such as illite (see section 3.5, below), it seems that this loss is only related to its incorporation for microbial growth and activity. In the literature on bioleaching with acidophiles, the quantity of potassium that can be incorporated in cell synthesis is, to our knowledge, not detailed.

Table 4: Mass balances (in mmol) on K^+ and NH_4^+ nutrients.

	0Km	**0Km - N**	**0Km - K**	**0Km – N - K**
K^+ removed from liquid phase	15.4	14.6	3.2	3.0
K^+ removed from solid phase	12.3	15.8	22.6	31.3
K^+ total loss	27.7	30.4	25.8	34.3
NH_4^+ removed from liquid phase	47.4	14.3	57.3	14.4
NH_4^+ accumulated in the solid	4.8	0.3	4.9	0.3
NH_4^+ total loss	42.6	14.6	52.4	14.7

Slightly higher losses of potassium were obtained for 0Km - N medium. In the case of the low potassium concentration media (0Km – K and 0Km – N – K), the losses from the liquid phase were lower, while the ones from the solid phase were higher. Thus, the total loss between the beginning and the end of the experiment was of the same order of magnitude whatever the nutrient medium composition (around 30 mmol of K^+). In the low potassium concentration media experiments (0Km – K and 0Km – N – K), the potassium may have come from the dissolution of K-rich phases (such as biotite, K-feldspar or muscovite) that were initially present in the secondary ore.

The same calculations were performed for ammonium. This nutrient was removed from the liquid phase and was slightly accumulated on the solid phase. By comparing the quantity of ammonium removed from the liquid phase, $m_{liq,i} - m_{liq,f}$, (47.4 mmol and 57.3 mmol in 0Km and 0Km – K, respectively) to the quantity of ammonium accumulated in the solid phase, $m_{solid,f} - m_{solid,i}$, (4.8 mmol and 4.9 mmol, respectively), a loss of 42.6 and 52.4 mmol of ammonium, respectively, was denoted (corresponding to 21.3 and 26.2 $mmol.L^{-1}$, respectively). This ammonium loss may be linked to its precipitation as ammoniojarosite or ammonium-jarosite (which is not detected in the final residue due to the analysis methodology used) or its consumption for microbial growth and activity, thus transforming it in another nitrogen species. The speciation of ammonium was not monitored during the experiments to discriminate between both hypotheses.

In the literature, to our knowledge, no data are available on the quantification of ammonium consumption for biomass growth in sulfide bioleaching reactors. However, some authors propose the following reaction for cell synthesis with ammonium as nitrogen source [33]:

$$\frac{1}{5}CO_{2(g)} + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^- \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O \quad \text{[Eq. 2]}$$

Carbon dioxide uptake rate has been estimated to be close to 40 $mg.L^{-1}.h^{-1}$ by several authors in the case of the bioleaching of sulfidic minerals with acidophilic microorganisms [5,29]. Using this value and the stoichiometric ratio between C and N (5:1) from Eq. 2, the ammonium consumption linked to biomass growth and activity was estimated to be close to 20 $mmol.L^{-1}$ (cumulative value between the beginning and the end of the bioleaching experiment). This estimation, which is consistent with the loss of ammonium reported above, suggests that there was no ammonium incorporated in the jarosite and that the loss of ammonium was only due to the microbial consumption.

When low ammonium concentration media were used (0Km - N and 0Km – N - K media), no residual ammonium was detected in the liquid phase at the end of the experiments, and almost no ammonium was detected in the solid phase at the end of the experiments, so the initial amount of ammonium was entirely lost (7.3 $mmol.L^{-1}$). Once again, this may result from the transformation of ammonium into another N-species or its precipitation as ammoniojarosite or ammonium-jarosite. Another hypothesis could explain the loss of ammonium: in the 0Km – N and 0Km – N – K, the filtration of the pulp was disturbed, probably due to colloids and/or small precipitates. To remove these very fine particles, a centrifugation was performed after the filtration. The centrifugation pellet was not analysed but may contain ammonium precipitates and thus explain part of the undetected ammonium.

3.5. Solid residue characterization

The analysis of the secondary ore and of the residues was performed with the QEMSCAN. No new phases were detected, while some phases completely disappeared. There were some changes in the distribution of these phases. Phase dissolution yields were thus calculated (Fig. 5) and compared to those calculated from the elemental analysis (see Section 3.2).

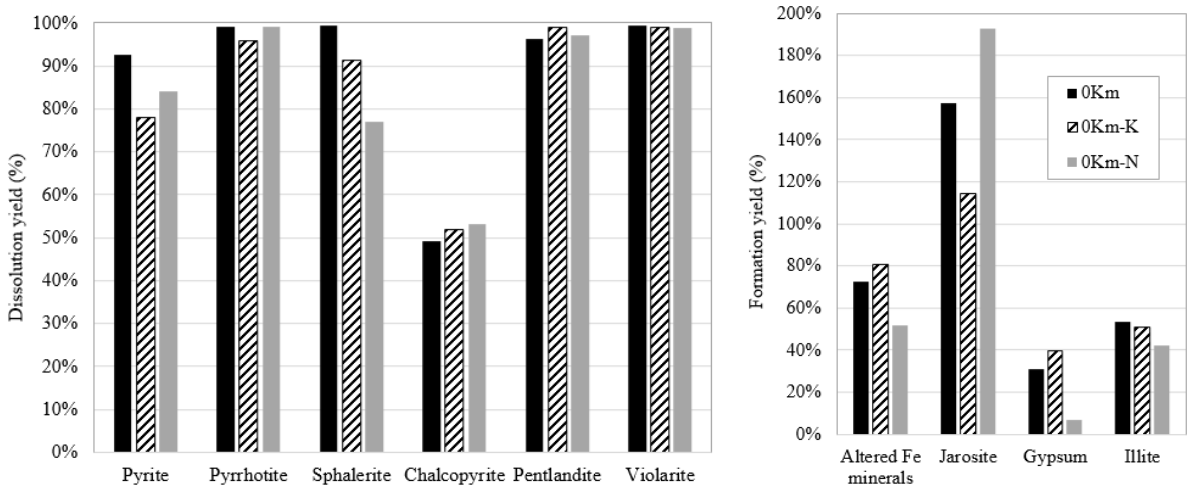


Fig. 5 Mineralogical phase dissolution (left) and formation (right) yields calculated from QEMSCAN analysis of the solid residues

The bioleaching efficiency that was inferred from the QEMSCAN mineralogical analysis was consistent with the metal dissolution yields obtained from the metal analysis performed (section 3.2): the highest dissolution yield of sulfides was reached with the 0Km medium. The Co final dissolution yield (85% in 0Km medium) was similar with pyrite dissolution yield (90% in 0Km medium), which is consistent with the fact that Co is mainly hosted by the pyrite (see Section 2.1). In a similar manner, the chalcopyrite dissolution yield (around 50%) was consistent with Cu (around 60%) and the pentlandite (around 96%) and violarite (around 98%) dissolution yields were consistent with Ni high dissolution yield (more than 80%). The calculation of dissolution yields from the mineral analysis (QEMSCAN) gave generally higher dissolution yields than the calculation from the metal analysis (ICP-OES and XRF). This may arise from normalisation of the QEMSCAN data, from a bias in the count of the minerals during the QEMSCAN analysis and the uptake of some of the dissolved metals in secondary phases. With partial transformation of easily-characterized 'pure' phases (as pentlandite or violarite) in the secondary ore into altered phases in the residues, the 'pure' phases may not have been detected, thus leading to a calculated 100% dissolution yield. However, this does not mean that total dissolution of the initial 'pure' phases occurred, as the oxidation may only be partial. This partial oxidation is highlighted by the altered Fe minerals contents (Table 5) that were higher in the residues than in the initial solid.

Table 5: Altered Fe minerals and jarosite content (in %w) in the solid.

	Initial	**0Km**	**0Km - N**	**0Km - K**
Altered Fe minerals	11.97	20.93	18.61	21.70
Jarosite	11.21	29.24	33.64	24.12

The QEMSCAN results suggested that sphalerite dissolution varied from 100 to 75% from 0Km to 0Km - N, while it was unchanged whatever the nutrient medium when calculated from Zn elemental analysis (around 80%). This difference may arise from the accuracy of QEMSCAN analysis, as the sphalerite content reached the limit of quantification, with around 0.00%, 0.02% or 0.05% of sphalerite in the residues, compared to 0.20% in the secondary ore, or from the possible uptake of released Zn in secondary jarosite or other secondary phases identified in the QEMSCAN analysis as 'altered Fe minerals'.

Fe dissolution yields calculated from elemental analysis are low (reaching around 12% in all the nutrient media), which may be consistent with the jarosite formation and altered Fe minerals production (Table 5). It appeared that Fe was not dissolved but was retained in the solid phases as they transformed from sulfides to altered minerals.

The QEMSCAN analysis suggested that the mineral biotite was not dissolved, but instead formed, in the experiments. This is unlikely since biotite is a metamorphic phyllosilicate mineral that cannot be formed at the temperatures and pressures in these experiments. It has a very similar formula $[K(Mg,Fe)_3(Si_3AlO_{10}(OH)_2]$ as the mineral illite $[(K,H_3O)(Al,Mg,Fe)_3(Si_3AlO_{10}(OH)_2]$, which is also a phyllosilicate mineral, but is formed in lower-temperature environments from the weathering of K-feldspar and muscovite. Both of the latter minerals occur in the secondary ore, and their QEMSCAN mass percentages decreased during the experiments, suggesting they were weathered. We therefore propose that the biotite identified by the QEMSCAN was not biotite, but instead was illite (Fig. 5). Therefore, both illite and K-jarosite could have taken up dissolved potassium during the bioleaching. The lowest amounts of jarosite formed in the 0Km - K experiment (Fig. 5). This is consistent with the lower supply of K in the media and the moderate uptake of potassium in illite in

these experiments. The highest amounts of altered Fe minerals also occurred in this experiment; these may have been non-stoichiometric jarosite that were not identified as such in the QEMSCAN analysis. By contrast, the highest amounts of jarosite, and lowest amounts of illite and altered Fe minerals, formed in the 0Km - N experiment. This suggests that there was excess potassium available to make these secondary phases. This may have been accommodated by a higher degree of leaching of the K-bearing minerals in the secondary ore (K-feldspar, muscovite, illite) in this experiment compared to the other two experiments for which QEMSCAN data were available. Alternatively, the potassium may have been provided by the 0Km - N medium that was not used by the bacteria. This is possible, given that the microbial activity and metal dissolution was lower in this experiment, which we assumed was due to the lower amount of ammonium available.

3.6. Microbial growth and bacterial community structure in different nutrient media

When considering cell counting, it appeared that microbial growth in the liquid phase during the beginning of the experiments was not influenced by the composition of the nutrient medium (data not shown). However, part of the cells may have been attached on the solid particles and the composition of the nutrient medium may have affected the bacterial attachment [11]. The use of nitrogen in the form of ammonium improves bacterial attachment to the solid substrate, compared to the use of urea [5]. In our study, the ammonium concentration may also have influenced the quantity of attached biomass. Consequently, the microbial biomass was not only evaluated in the liquid phase but also in the pulp, comprising planktonic cells (cells suspended in the liquid phase) and bacteria attached to the solids.

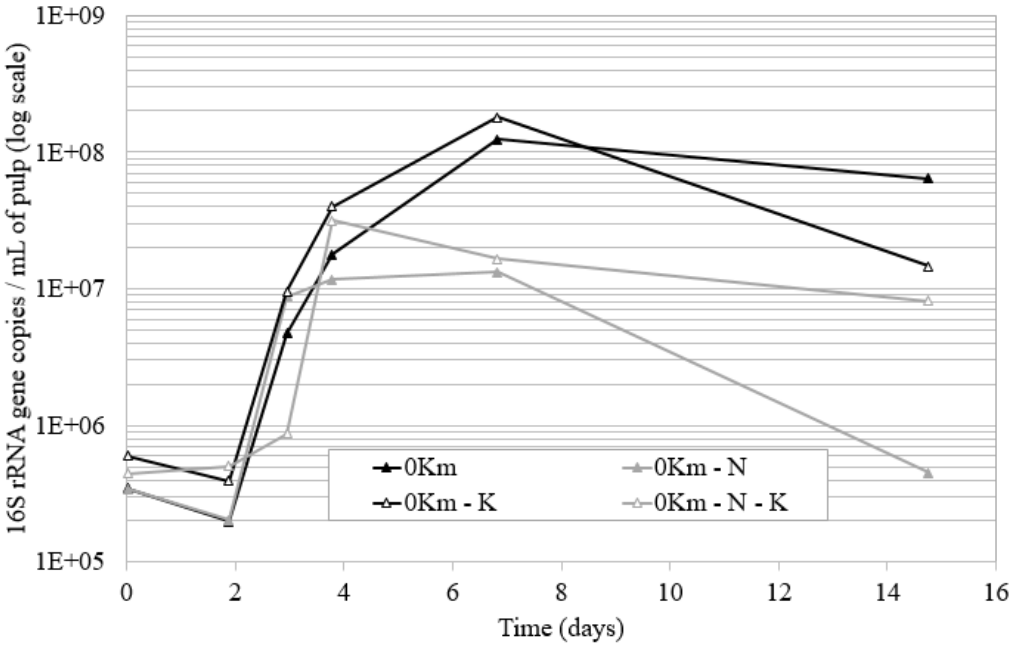


Fig. 6 Concentration of 16S rRNA gene copies in the pulp (in log scale) versus time at 20% (w/w) solid and different nutrient media

The results of the gene abundance over time were comparable in 0Km medium and in the medium with less potassium (0Km - K) during the first 8 days of the experiment (Fig. 6) although the amount of biomass tended to decrease more rapidly in the media with less potassium. The amount of biomass was clearly lower in the

pulp when the media contained less ammonium (0Km - N and 0Km – N - K). In all the media, an exponential phase was detected, during which the concentration of 16S rRNA gene copies was multiplied by more than 10. This exponential phase was not so marked in the liquid phase, confirming that a substantial part of the biomass is attached onto the solid particles.

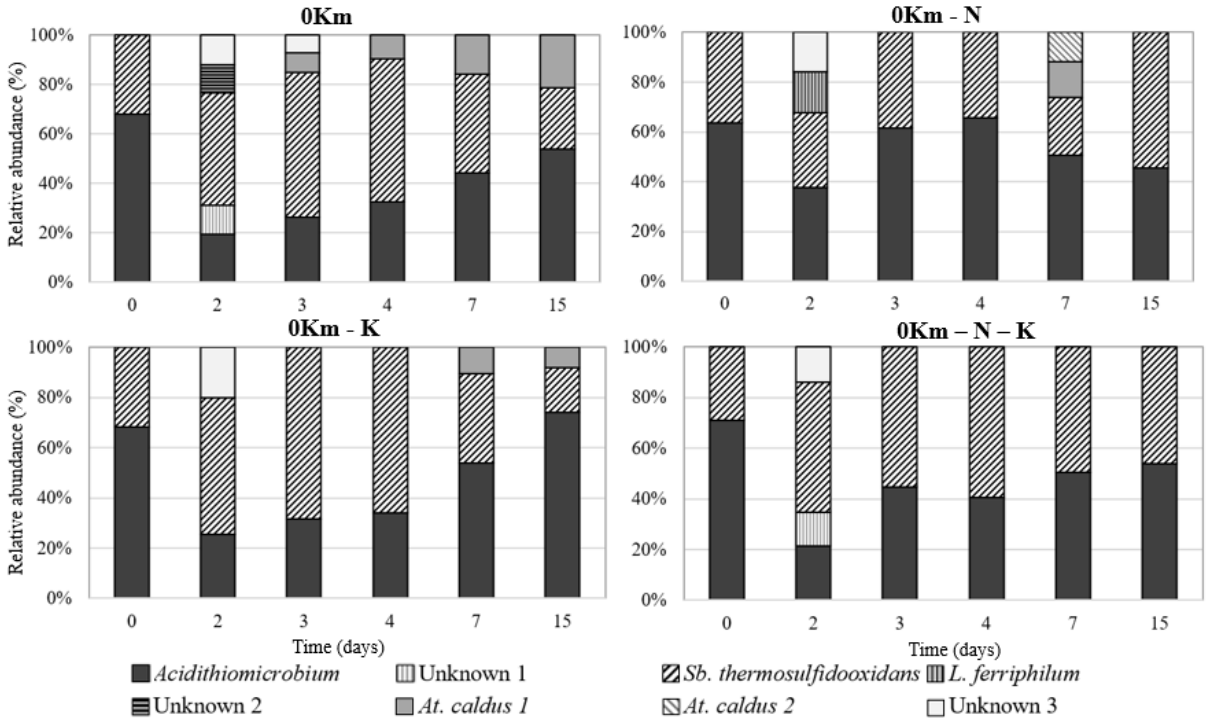


Fig. 7 Structure of the bacterial communities obtained in bioleaching reactors with different nutrient media

The diversity fingerprints obtained with the different nutrient medium compositions are presented in Fig. 7. Two signals corresponding to *At. Caldus* (recently reclassified as *Fervidacidithiobacillus caldus*) were found, suggesting the presence of two strains of the species. Some species were not identified, thus described as 'unknown' in the figure. The structures of the communities followed the same trend whatever the nutrient concentrations: '*Acidithiomicrobium*' P2 and *Sb. thermosulfidooxidans* were the main bacteria found. However, the 0Km medium sustained a larger diversity along the experiment compared to media containing lower concentration of nutrients. Differences in the nutrient medium composition also resulted in variations of the proportions of the two dominant strains between the experiments. In the experiments using 0Km and 0Km - K, *Sb. thermosulfidooxidans* became dominant over '*Acidithiomicrobium*' P2, especially during the Eh increase phase (days 3 and 4, see Fig. 1). At the end of the run, both bacteria were well represented and largely dominant. However, the opposite was true in the 0Km with low ammonium concentration (0Km - N) experiments, in which *'Acidithiomicrobium'* P2 was favoured during the period of Eh increase. Low ammonium and potassium concentration medium (0Km – N - K) resulted in equal proportions of these two species at the end of the experiment.

4. Discussion

Regarding the metal dissolution kinetics, it appeared that the increase of the solid concentration from 10% to 20% of solids did not change the order of the successive mineral dissolutions that were described in a previous

study [26]. At the beginning, substantial metal dissolution suggested the presence of soluble salts in the secondary ore. The remaining pyrrhotite and acid soluble minerals were dissolved at low redox, releasing Ni, Zn and Cu in solution. When most of the acid soluble minerals were leached, the Eh increased due to microbial activity, leading to the dissolution of acid insoluble minerals such as Co-bearing pyrite, thus causing Co release. As metals were mostly chemically leached, except for Co [26], the use of different nutrient media did not affect the Ni, Zn and Cu dissolution kinetics and yields. This was consistent with the observed pyrrhotite, pentlandite, violarite and chalcopyrite dissolution yields, which were similar whatever the nutrient medium composition used during the experiments. The amounts of Co, sulfide and pyrite dissolution were high in the 0Km medium but were lower in the low ammonium and potassium concentration media (0Km – N, 0Km – K and 0Km – N – K). This means that the microbial growth and activity was reduced in the case of the nutrient-poor media, as the Co release was directly linked to the pyrite oxidation, which in turn was linked to the microbial activity. This is consistent with a previous study that demonstrated that an excess of nutrients led to better biomass development, and thus higher dissolution of sulfide minerals [19].

It however appeared that the reduction of potassium and ammonium concentrations did not have the same influence on the bioleaching performances. In the low potassium concentration medium (0Km – K), the redox potential evolution, proton consumption, Fe dissolution yield and nutrient removal from the liquid phase were similar to the ones observed in the 0Km medium (Fig. 1, 3 and 4). The microbial activity was thus slightly affected by the decrease of potassium in the initial medium, as the final Co dissolution yield was lower, but only slightly, as the production of protons from the sulfur oxidation happened, as well as iron oxidation. In the low potassium concentration medium, jarosite production was noticed to be lower than in the 0Km medium: less jarosite was produced when potassium was missing in the liquid phase. Mass balances highlighted that the same quantity of potassium was removed in the 0Km and 0Km – K media between the beginning and the end of the experiments. These similar losses in both media, attributed to microbial consumption, revealed that potassium dissolved from the solid phase may be sufficient for microbial development and activity and it may counterbalance the initial low potassium concentration in the 0Km - K medium. This implies that the potassium microbial assimilation may have shifted the potassium solid / liquid concentration equilibrium toward a release of potassium from the solid phase. From this, the consumption of potassium for microbial growth and activity could be estimated to around 15 $mmol.L^{-1}$. It could be hypothesized that the slight difference in the final 16S rRNA gene copies concentration could be associated to a difficulty for the cells to maintain when potassium was almost absent in the liquid phase. Lowering the potassium concentration may not limit the growth but rather the maintenance of the cells during the stationary phase.

In the low ammonium concentration medium (0Km – N), the low redox potential and high acid consumption (similar to the one observed in abiotic tests [26]) showed that the microbial activity was much more affected than in the medium with low potassium concentration. Moreover, the 16S rRNA gene copies concentration was lower in the low ammonium concentration medium, which is probably associated to a lower biomass concentration. This was consistent with the lower ammonium consumption noticed (around 7 $mmol.L^{-1}$ vs 21 $mmol.L^{-1}$ in 0Km - N and 0Km, respectively), probably mainly related to the microbial growth and activity. In the low ammonium concentration medium, ammonium was completely removed from the solid and liquid phases (no ammonium detected at the end of the experiment in the 0Km – N medium) due to the microbial consumption. As ammonium was not available in the solid, its initial low concentration in the nutrient medium

could not be counterbalanced, opposite to potassium that was released from the K-containing minerals in the secondary ore. A previous study demonstrated the effect of ammonium limitation on bioleaching efficiency in continuous reactors using 0Km medium as reference [34]. In their study, although the concentration of ammonium sulfate was only reduced from 3.7 $g.L^{-1}$ to 1 $g.L^{-1}$, they measured a detrimental effect on the uptake of oxygen and carbon and, assuming that the latter contributes mainly to bacterial growth, they hypothesised a decrease in the number of bacteria attached to the solid particles since no change in bacteria on the liquid phase occurred. These results highlight the importance of nitrogen availability for bacterial growth during bioleaching. The need for ammonium amendment demonstrated on Sotkamo primary ore [28] is confirmed on the secondary ore.

To maintain the electrical neutrality in the absence of ammonium and potassium in the liquid phase, the gangue mineral dissolution may be favoured, explaining the higher dissolution of Al and Mg in the presence of low amounts of NH_4^+. Precipitation was also affected by the lack of ammonium (Table 5 and Fig. 5). At the end of the experiment, larger quantity of jarosite was produced in 0Km – N medium compared to 0Km medium. It may be hypothesized that, as biomass was less active and its growth was reduced, two effects were produced: (i) potassium was less consumed that in the 0Km medium and potassium was thus more available to precipitate; (ii) sulfide oxidation occurred at a lesser extent, which increased the external acid addition for longer time to maintain a similar pH. These differences may have also caused a slower precipitation rate than in the 0Km medium (Fe concentration decrease was slower).

Moreover, in the poorest nutrient medium (0Km – N – K), the lack of ammonium affected bioleaching performances as in the 0Km – N medium, with an additional difficulty for the biomass to maintain in the pulp in the stationary phase (as seen in the 0Km – K medium).

To conclude, it seemed that the decrease of ammonium concentration was more detrimental to the bioleaching performances than the decrease of potassium concentration (Fig. 6). This may be attributed to the fact that potassium concentration was only reduced five times (from 334.5 $mg.L^{-1}$ to 66.9 $mg.L^{-1}$), compared to a ten-fold reduction of ammonium (from 1009.1 $mg.L^{-1}$ to 100.9 $mg.L^{-1}$). It could be also attributed to the potassium content in the secondary ore (2.62%w), that can counterbalance the lack of potassium with an equilibrium between solid and liquid phase (see section 3.5), whereas ammonium content in the solid is very low. In a previous study, similar conclusions were drawn on phosphate [28]: in the Sotkamo primary ore, the P_2O_5 content reach 0.11%w and ammonium is almost absent from the solid. Phosphate concentration variation in the nutrient medium did not influence the bioleaching performances in their study, whereas ammonium amendment resulted in higher dissolution kinetics of the black-schist ore.

5. Conclusion

Thanks to its low capital costs and its flexibility, bioleaching can be seen as an opportunity for the reprocessing of mining residues to limit their environmental impacts while recovering the remaining elements. One of the operating parameters that should be determined is the composition of the nutrient medium as it may have a high influence on the bioleaching kinetics. Bioleaching of Sotkamo secondary residues in 2 L-stirred tank reactors with different nutrient media was evaluated and Co, Cu, Ni and Zn dissolutions were followed. Among all nutrients, ammonium and potassium were selected as variables since potassium is known to favour the precipitation of jarosite and ammonium is one of the predominant required elements for cell growth.

The use of different nutrient media did not affect the Ni, Zn and Cu dissolution kinetics and yields, as their host minerals were mostly chemically leached. On the contrary, Co and pyrite dissolutions were high in the medium with the highest concentration of nutrients (0Km) but were lower in media with low concentration of nutrients, this is thought to be due to a reduced microbial growth and activity. It appeared that the reduction of potassium and ammonium concentrations did not have the same effect on the bioleaching performances. In low potassium concentration media, the microbial activity was only slightly affected by the decrease of potassium. Mass balances highlighted that the same quantity of potassium was consumed for microbial growth and activity in all the different media, around 15 $mmol.L^{-1}$. The potassium microbial assimilation may have shifted the equilibrium toward a release of potassium from the solid phase. Moreover, lowering the potassium concentration did not limit the growth but rather the maintenance of the cells during the stationary phase.

The microbial activity was much more affected by limited ammonium concentration. A lower biomass concentration was detected as well as a lower activity. In the presence of an excess of ammonium, an ammonium consumption of 21 $mmol.L^{-1}$ was noticed, probably mainly due to the microbial growth and activity. The absence of available ammonium in the mining residue cannot counterbalance the lack of ammonium in the nutrient medium, thus leading to limited bioleaching performances. Consequently, it seemed that in this study, the decrease of ammonium concentration was more detrimental to the bioleaching performances than the decrease of potassium concentration.

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Declarations

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Data availability: The datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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