Effects of elevated pressures on the activity of acidophilic bioleaching microorganisms

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Abstract

This study reports effects of elevated pressures on the oxidation of a soluble ferrous iron and low-grade sulphidic ore as little is known about biological iron and sulphur oxidation under these conditions. Pressure effects were studied in a pressurised batch-operated stirred tank reactor using acidophilic enrichment cultures. The oxidation of soluble Fe^{2+} by enrichment culture dominated by *Leptospirillum ferriphilum*, *Sulfobacillus* sp. and *Ferrimicrobium acidiphilum* increased with increasing pressure induced by technical air to up to +3 bar (0.63 bar P_{O2}) and was inhibited at +7 bar (1.47 bar P_{O2}). Elevated pressures induced by nitrogen (low oxygen partial pressure) were tolerated up to +40 bar. Another enrichment culture dominated by *Acidithiobacillus ferrivorans*, *Sulfobacillus* sp. and *F. acidiphilum* partially oxidised the ore at pressures up to +20 bar induced with air (4.2 bar P_{O2}). This is the first study reporting activity of acidophiles under pressurised conditions in a stirred tank reactor.

Keywords: Acidophiles; Biooxidation; Iron oxidation; Pressure tolerance; Pressurised stirred tank reactor

1. Introduction

There is a growing need to develop environmentally sustainable mining operations and decrease the overall footprint of metal recovery and processing (for reviews, see [1–6]). The importance of biomining is continuously increasing because many of the available resources are not amenable to metal recovery by any other means [3]. Biological approaches such as heap and tank bioleaching are efficient for metal recovery from many low-grade ores and concentrates, respectively (for a review, see [6]).

It is now generally accepted that bioleaching of sulphide minerals occurs through indirect mechanisms and that bioleaching microorganisms are not able to enzymatically oxidise the sulphur moiety of metal sulphides. The “indirect mechanism” can be divided into three submechanisms, which are contact, non-contact or cooperative leaching. All of these mechanisms involve biological ferrous iron oxidation to ferric iron which is followed by ferric iron initiated oxidation of metals sulphides. Sulphur oxidising microorganisms are involved in subsequent oxidation of the solubilised sulphur compounds to sulphuric acid. Majority of the cells typically attach to the surface of the mineral, in which case the bioleaching occurs via the contact mechanism, while in the non-contact process bioleaching occurs via the metabolic activity of planktonic cells. The third process, cooperative bioleaching, refers to the dissolution of mineral fragments, sulphur intermediates and sulphur colloids by protons and ferric iron released by planktonic cells. (for reviews, see [6-10])

Bioleaching has considerably high ecological footprint because of generation of large quantities of acidic concentrated metal solutions during the processing (for a review, see [6]) as well as the reliance on the crushing of the metal containing ores [11] (for reviews, see [12,13]). As the ore grades become lower, the energy input and quantity of waste rock associated with generation of a ton of usable metal increase even further [14]. Therefore, process developments both in heap and tank leaching are in demand.

The recovery of metals from deep subsurface deposits is not possible by mining operations, which require drilling, blasting, excavation and hauling the metal-containing rock above ground (for review, see [3]). *In situ* recovery (ISR) has been used since 1960s and enables metal recovery from deep deposits without conventional underground mining by circulating the (bio)leach solutions through the deep deposit materials [3,15,16]. Bioleaching has the potential to enable recovery of metals from deep subsurface deposits with or without fracturing the ore [17] (for reviews, see [3,15]). Biooxidation of gold-based concentrates is the most common application of tank bioleaching [18]. Another option for gold recovery is autoclave leaching, which relies on pressurised steam to initiate the oxidation and oxygen as the principal oxidant of sulphidic minerals [16,19]. Tank bioleaching works at lower temperatures than autoclave leaching and does not require elevated pressure (Table 1). However, the retention times of bioleaching processes are much longer than those of autoclave leaching [7,21–24].

[Table 1 here]

One way of further developing gold recovery in tanks could be combining the benefits of biooxidation with those of physico-chemical treatment. However, combining the benefits of pressure oxidation and biooxidation has not been considered in experimental or commercial gold pretreatment processes. For both deep subsurface and tank leaching, a better understanding of microbial tolerance to harsh environmental conditions such as elevated temperature and pressure are needed. The response of mineral-oxidising microorganisms towards elevated temperatures has been widely studied [6,25,26]. However, very little is known about the effect of elevated pressure on bioleaching microorganisms as it has not been an issue in existing biomining processes. Only a few studies have addressed the pressure dependence of bioleaching. Davidson et al. [27] studied the carbon dioxide fixation by *Acidithiobacillus ferrooxidans* (formerly known as *Thiobacillus ferrooxidans)* in pressure cylinders after decompression and showed that it was retarded at 1-689 bar (0.1- 68.9 MPa) hydrostatic pressures in 48 h. They also reported that hyperbaric oxygen tension at 1-69 bar (0.1-6.9 MPa) absolute pressures had only minor effect on the Fe^{2+} oxidation of washed suspension of *Acidithiobacillus ferrooxidans* whereas the growth, sulphur oxidation and carbon dioxide fixation were strongly inhibited. Another study showed that the Fe^{3+} reduction and oxidation of reduced sulphur compound by acidophiles (*Acidithiobacillus ferrooxidans, Sulfobacillus thermosulfidooxidans* and the archaeon *Ferroplasma acidiphilum*) were not inhibited at 360 bar (36 MPa) hydrostatic pressure [28]. The growth and Fe^{3+} reduction of the mixed acidophilic iron oxidising culture (*Acidianus brierleyi*, *Thermoplasma acidophilum*) was only partially hindered at 100 bar hydrostatic pressure [29].

The aim of this work was to delineate the effects of pressure / oxygen partial pressure on the activity of bioleaching microorganisms. The effects of elevated pressures on the iron oxidation and sulphidic ore oxidation by two acidophilic enrichment cultures were studied in a stirred tank pressure reactor. The activity of the microorganisms after incubation under pressurised conditions was determined in shake flask batch assays. However, it should be noted that the stirred tank pressure reactor was not designed to simulate ISR or tank bioleaching.

2. Materials and methods

2.1. Growth medium

The growth medium used in the experiments included 10% v/v (10 mL and 100 mL with shake flask and reactor incubations, respectively) mineral salts medium (MSM), 1% v/v (1 mL and 10 mL with shake flask and reactor incubations, respectively) trace element solution (TES) and Milli-Q water [30]. In shake flask incubations, the nutrient solutions, Milli-Q water and the flasks were sterilised by autoclaving at 121°C for 20 min. In reactor experiments, only the MSM and TES were autoclaved before adding them to the reactor. Either ferrous iron (Fe^{2+}) or sulphidic ore (see Section 2.2) was used as electron donor in the experiments. In case of Fe^{2+}, ferrous sulphate stock solution containing 22.5 g/L of Fe^{2+} (pH 1.7) dissolved in Milli-Q water was sterile-filtrated (0.2 µm polyethersulfone membrane syringe filter, VWR International, North America) and supplemented to the media to reach an initial concentration of 5.6 g/L Fe^{2+}. The ore was added to the medium crushed and ground to 70 µm. The pH of the medium was adjusted to 1.3 (experiments with Fe^{2+}) or 1.8 (experiments with sulphidic ore) with H_2SO_4.

2.2. Ore composition

The ore used in the experiments originated from a gold ore deposit located in Eastern Finland. The deposit forms the North-East part of the 140 km long late Archaean Suomussalmi greenstone belt. The main phases of the ore were silicates and sulphides (Table 2). Iron and sulphur contents in the ore sample were 6.5 and 3.0 wt-%,

138 respectively. The ore contained only few gold grains with a fine particle size (ranging
139 from a few µm up to 15 µm) that existed as inclusions in pyrite and the silicates. No
140 native gold was observed in the ore sample. Most of the gold-bearing grains contained
141 above 80% of gold and less than 20% of silver. The average gold content of the ore was
142 3.5 g/t. The ore also contained some petzite (Ag_3AuTe_2) and hessite (Ag_2Te) grains, the
143 latter of which is commonly associated with gold-bearing minerals.
144
145 [Table 2 here]
146
147 **2.3. Acidophilic enrichment cultures**
148
149 Two different microbial enrichment cultures originating from samples from mining
150 environments were used. The cultures had been enriched and maintained using different
151 growth conditions as shown in Table 3. The enrichment culture 2 was enriched for
152 sulphidic ore bioleaching, which requires the activity of both iron and sulphur oxidising
153 microorganisms, while the enrichment culture 1 was enriched solely for Fe^{2+} oxidation.
154 The enrichment culture 1 grown on soluble Fe^{2+} originated from a tailings pond at a
155 Finnish talc mine site and had been used in iron-oxidising bioreactors [31,32] whereas
156 the enrichment culture 2 used for oxidation of the sulphidic ore originated from an
157 open-pit mine situated at the Karelian Gold Line in Eastern Finland. The enrichment
158 cultures were characterised by polymerase chain reaction – denaturing gradient gel
159 electrophoresis (PCR-DGGE) followed by sequencing (Fig. S1). The enrichment
160 cultures were maintained in duplicate shake-flasks (150 rpm) at a constant temperature
161 by subculturing (10% v/v inoculum) into fresh medium at regular intervals. When

subculturing, the initial pH of the medium was adjusted to 1.3 (in experiments with Fe^{2+}) or 2.0 (in experiments with sulphidic ore) with H_2SO_4.

[Table 3 here]

2.4. Stirred tank pressure reactor experiments

All pressure experiments were carried out in a 2 L (total working volume 1 L) stirred tank pressure reactor (4524 bench top reactor, Parr Instrument Company, USA) operated in batch mode (Fig. 1). The reactor and its ports were made of titanium and Teflon, respectively, to prevent the material from dissolving even at a highly acidic pH and oxidative conditions. The pressure reactor allowed studies on the effects of pressure / oxygen partial pressure on bioleaching microorganisms but was not designed for simulating or optimising tank bioleaching under pressurised conditions.

[Fig. 1 here]

Two sets of pressure experiments were performed: one with soluble Fe^{2+} as the electron donor and the other with the sulphidic ore (Table 4). The respective enrichment culture 1 and 2 (see Table 3) were used to inoculate the reactors (10% v/v). To ensure that the activity of the inoculum was similar for all the replicates, the age of the inoculum cultivated in batch in shake flasks (days of incubation after subculturing) was always maintained the same. The effect of different pressures on the oxidation of Fe^{2+} and sulphidic ore was investigated by inducing the gas phase of the closed reactor with

either compressed technical air (AGA, Finland) or a mixture of nitrogen gas, oxygen and carbon dioxide as presented in Table 4. The pressure was increased and decreased manually using 10 bar/min for the Fe^{2+} oxidation experiments and of 1.3 bar/h for the sulphidic ore experiments. During operation, 10 mL liquid sample was removed from the reactor daily for analysis. No additional gas was added to the reactor during the experiments except when needed to readjust the pressure after sampling. The reactor had to be pressurised to obtain samples. Therefore, +1 bar above atmospheric pressure was the lowest pressure studied. Uninoculated control experiments were performed at each pressure to determine possible abiotic oxidation of the soluble Fe^{2+} and the ore.

[Table 4 here]

The ferrous iron oxidation rate (g/L/d) for the experiments with Fe^{2+} was determined from the slope of the linear regression line of the Fe^{2+} oxidation curves. The exponential part of the Fe^{2+} oxidation curves were used for the linear trend lines and the correlations R^2 were > 0.93.

The calculated initial dissolved oxygen (DO) concentration inside the reactor at different pressures was estimated using a thermodynamic equation by Tromans [33]:

$$c_{aq} = P_{O_2} exp\left\{\frac{0.046T^2+203.357Tln\left(\frac{T}{298}\right)-(299.378+0.092T)(T-298)-20.591x10^3}{8.3144T}\right\} \quad (1)$$

where c_{aq} is DO concentration [mol/L], P_{O2} is oxygen (O_2) partial pressure [atm] and T is temperature [K].

2.5. Microbial Fe^{2+} oxidation after pressure exposure

The activity of enrichment culture 1 in the pressure experiments with soluble Fe^{2+} was determined after releasing the pressure from the stirred tank pressure reactor. The activity tests were conducted in 250 mL (100 mL working volume) shake flasks with 10% v/v culture suspension samples from the pressure reactor. As a positive control, 10% v/v of the original enrichment culture 1 (see Section 2.3) was used to compare the Fe^{2+} oxidation rate and efficiency of the culture before and after being exposed to elevated pressures. The incubations were performed in triplicate. An uninoculated control using Milli-Q water instead of inoculum was also performed to distinguish between abiotic and biotic iron oxidation. All the shake flasks were incubated for 7-8 days at 35 ± 1°C and 150 rpm on an orbital shaker.

2.6. Analyses

In the shake flask and stirred tank pressure reactor experiments, pH was measured with a pH 3210 meter (WTW, Germany) equipped with a SenTix 81 pH-electrode (WTW, Germany). Redox potential was determined with a pH 315i meter (WTW, Germany) and a Blue Line 31 Rx redox-electrode (SI Analytics, Germany) with Silamid® reference system (Ag/AgCl, 3 mol/L KCl). DO concentration was measured using a HQ 40d multi meter (Hach, Germany) equipped with an LDO 101 probe (Hach, Germany). With the stirred tank pressure reactor, DO was measured immediately after sampling, while pH and redox potential were measured within 10 minutes after the sampling. Fe^{2+}

concentration was determined with a Shimadzu UV-1601 spectrophotometer using the modified 3500-Fe *ortho*-phenantroline method [34]. Total Fe concentration was determined with atomic adsorption spectroscopy (AAS) as reported by [35]. Sulphate (SO_4^{2-}) was analysed by ion chromatography (IC) as described by [36]. Before Fe^{2+}, total Fe and SO_4^{2-} analysis, the samples were filtered through 0.45 µm polyethersulfone membrane filters (Pall Corporation, USA) and diluted with 0.07 M HNO_3 (Fe^{2+} and total Fe analysis) or deionised Milli-Q water (SO_4^{2-} analysis) when necessary. Chemical composition of the ore was analysed by X-ray fluorescence (XRF).

3. Results and discussion

3.1. Oxidation of Fe^{2+} in stirred tank pressure reactor

The effect of pressure from +1 to +30 bar on Fe^{2+} oxidation was studied in the stirred tank pressure reactor. The results for +1 to +3 bar and for +7 to +30 bar experiments were as shown in Fig. 2 and 3, respectively.

At +1, +2 and +3 bar pressures induced with technical air (corresponding P_{O2} of 0.21, 0.41 and 0.63 bar, respectively), oxidation of ferrous iron was faster with enrichment culture 1 than in the uninoculated controls (Fig. 2a). At +1, +2 and +3 bar, the percent Fe^{2+} oxidations with enrichment culture 1 were 73, 97 and 99% in 7 days, respectively, while at the same pressures with uninoculated controls the percent Fe^{2+} oxidations were 23, 32 and 25%, respectively (Fig. 2a). Iron oxidation rates with the enrichment culture were 0.6, 0.98 and 1.28 g/L/d at +1, +2 and +3 bar, respectively, while the corresponding oxidation rates with uninoculated controls were 0.35, 0.26 and 0.28 g/L/d, respectively. The microbial activity at these three pressure levels was also demonstrated by the increase of both redox potential and pH (Fig. 2b and 2c) in all three experiments with the enrichment culture compared to the uninoculated controls. Fig. 2c shows that at +1, +2 and +3 bar in uninoculated controls the pH remained stable (~1.3) while it increased to 1.6, 1.7 and 1.6, respectively, in the incubations with the enrichment culture. The pH increase was associated with the proton consumption of the iron oxidation reactions ($2Fe^{2+} + 1/2O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$). The remaining DO in all pressures as measured after releasing the samples (Fig. 2d) from the

pressurised system, were approximately 1-9 mg/L. These results indicate that oxygen was not limiting the iron oxidation. The calculated initial DO values (based on the P_{O2} using Eq. 1) were 17, 35 and 52 mg/L at +1, +2 and +3 bar, respectively.

[Fig. 2 here]

At +7, +15 and +30 bar pressures (corresponding P_{O2} of 1.47, 3.15 and 6.3 bar, respectively) the Fe^{2+} oxidation was similar with enrichment culture 1 and uninoculated controls (Fig. 3a). This indicates that iron oxidation was mostly chemical not biological. At +7, +15 and +30 bar, the Fe^{2+} oxidation rates with the enrichment culture were 0.42, 0.38 and 0.32 g/L/d, while with the uninoculated controls they were 0.28, 0.33 and 0.32 g/L/d, respectively. High Fe^{3+} to Fe^{2+} ratios were also indicated by the increased redox potentials (Fig. 3b). The DO concentration at +7, +15 and +30 bar pressures (Fig. 3c) varied between ~9-12 mg/L as measured immediately after sampling. The calculated initial DO concentration (based on Eq. 1) of the +7, +15 and +30 bar experiments were approximately 121, 260 and 521 mg/L, respectively. The pH (Fig. 3d) at +15 and +30 bar remained similar (pH 1.3-1.6) in all pressurised experiments demonstrating chemical iron oxidation. The total Fe concentrations remained stable in all experiments (results not shown).

[Fig. 3 here]

Chemical Fe^{2+} oxidation rate in uninoculated controls was not affected by the increasing pressure induced with technical air in the stirred tank pressure reactor (Fig. 4). With the

enrichment culture 1 (Fig. 4), Fe^{2+} oxidation rate increased with pressure increase up to +3 bar. At the higher pressures, the oxidation rates with the enrichment culture were similar to the ones obtained with uninoculated controls. Thus, the results show that microbial iron oxidation dominated up to +3 bar, whereas at +7 bar and above chemical iron oxidation dominated.

[Fig. 4 here]

3.2 Activity of iron oxidisers after pressurisation

After the pressure experiments, the effect of pressurisation on Fe^{2+} oxidation activity was tested in shake flasks at atmospheric pressure. The activity tests after +2 and +3 bar experiments showed biological Fe^{2+} oxidation (Fig. 5a). The Fe^{2+} oxidation was completed in two days with enrichment culture 1 maintained constantly under atmospheric pressure, while it took four days with enrichment cultures from the +2 and +3 bar stirred tank pressure reactor. No Fe^{2+} was oxidised by the enrichment culture 1 in shake flasks with inoculum from +7, +15 and +30 bar stirred tank pressure reactor (Fig. 5b). This confirms the inhibition of the organisms at +7 bar and above. Although biological Fe^{2+} oxidation was not inhibited by +40 bar pressure with very low initially added O_2 and CO_2 condition (Fig. 5b), it took around 4 days longer to reach >90% oxidation than with the original enrichment culture. Furthermore, the results show that the 10 bar/min rate of pressure increase and decrease was tolerated by the enrichment culture 1. The Fe^{2+} oxidation in the shake flasks was considerably slower by the

enrichment cultures exposed to elevated pressures than by the original enrichment culture (Fig. 5a).

[Fig. 5 here]

3.3. Oxidation of sulphidic ore at elevated pressures

Biological oxidation of the sulphidic ore in the stirred tank pressure reactor was monitored by analysis of soluble constituents including Fe^{2+} and SO_4 (Fig. 6) and the oxidised ore residue (Table 5). The studied pressures were +1, +10 and +20 bar induced with technical air, corresponding to P_{O2} pressures of +0.2, +2.1 and +4.2 bar. Pressure experiments were continued until no further Fe^{2+} oxidation was detected. Fig. 6a shows that initial dissolution of Fe from the ore was similar with the enrichment culture 2 and uninoculated controls regardless of the pressure used. From day 3 onwards, Fe was oxidised followed by partial precipitation in the incubations with the enrichment culture, while Fe^{2+} concentrations continued to increase in the uninoculated controls (Figs. 6a and 6b). Iron oxidation and precipitation was fastest at +1 bar and decreased with increasing pressure. Redox potential (vs. Ag/AgCl) at +1, +10, and +20 bar pressure with the enrichment culture increased from the initial 430-460 mV to 560, 520, and 480 mV, respectively, by the end of the experiments (Fig. 6c). In the uninoculated controls, redox potential remained at 430 mV and below. The pH increased from the initial 1.8 to approx. 2.5 - 2.7 (Fig. 6d). SO_4^{2-} was formed at +1 and +10 bar but not at +20 bar (Fig. 6e).

As an *Acidithiobacillus* strain has been shown to commence sulphur oxidation only after all available Fe^{2+} had been oxidised [37], the slow SO_4^{2-} generation at +20 bar may have been due to the high Fe^{2+} concentration. At +10 and +20 bar, DO concentrations as measured after releasing the samples from pressurised environment were ~ 9-10 mg/L (Fig. 6f), indicating oxygen saturation. The calculated initial DO concentration at +1, +10, and +20 bar (using Eq. 1) was approximately 16, 90, and 170 mg/L, respectively.

[Fig. 6 here]

Modal mineralogies of the biooxidised ore residues show that pyrrhotite was the only mineral, the share of which decreased notably during biooxidation under pressurised conditions in the stirred tank pressure reactor (Table 5). The ore residue analyses also demonstrate biooxidation at up to +20 bar air pressures. To our knowledge, pressure biooxidation of sulphidic ore has not been previously reported.

[Table 5 here]

3.4 Effect of pressure and P_{O2} on bioleaching microorganisms

Pressurisation with technical air increases the P_{O2} and thus also the dissolved oxygen concentration. Guezennec et al. [38] showed that the oxidation rate and dissolution yield of sulphide increased with increasing DO up to ~ 13 mg/L, by a mixed culture containing *Leptospirillum ferriphilum*, *Acidithiobacillus caldus* and *Sulfobacillus benefaciens*. At ~ 18 mg-DO/L, however, the microbial activity decreased. They also

reported that *L. ferriphilum* and *S. benefaciens* in biofilm were less inhibited by high DO concentrations than the suspended cultures. Our experiments also demonstrated that biological oxidation of Fe^{2+} is more inhibited by high DO than that of sulphidic ore. In our experiments, biological Fe^{2+} oxidation was inhibited at +7 bar air pressure (P_{O2} +1.5 bar) based on both pressure experiments (Fig. 4) and activity tests (Fig. 5). However, with the sulphidic ore, biooxidation was demonstrated at up to +20 bar (P_{O2} +4.2 bar) pressure (Fig. 6). This corresponds to a calculated DO concentration of 170 mg/L. High O_2 solubility at elevated pressures likely decreased the biological oxidation rates in our study. However, our experimental system did not allow disclosure of the actual DO's inside the stirred tank pressure reactor and their effects on the kinetics of the studied reactions.

Davidson et al. [27] used pressure cylinders (bombs) to expose bioleaching organisms in cell suspensions for 4 to 6 hours to elevated pressures induced by compressed air. Their experimental design did not allow sampling during the experiments, so the determination of iron and sulphur oxidation was done by analysing soluble constituents following decompression of the systems. With uncharacterised facultatively thermophilic microbe TH3 the Fe^{2+} oxidation was reduced over 17 bar (1.7 MPa) and completely inhibited at 69 bar (6.9 MPa) air pressure. They also showed that sulphur oxidation by *A. ferrooxidans* decreased when air pressure increased from 1 to ~ 14 bar (0.1-1.4 MPa), whereas sulphur oxidation by *A. thiooxidans* was inhibited at 17 bar (1.7 MPa) air pressure. The results by Davidson et al. [27] are in accordance with the present study in that SO_4^{2-} production in the incubations with the sulphidic ore did not proceed at +20 bar (Fig. 6). We further demonstrated that the Fe^{2+} oxidation by our enrichment

385 culture 1 was not inhibited at +40 bar pressure, when the P_{O2} was very low. As
386 compared to the findings of Davidson et al. [27] the novelties of this study were that we
387 used acidophilic enrichment cultures instead of pure cultures, actual sulphidic ore in
388 addition to model compounds and stirred tank pressure reactor allowing monitoring the
389 oxidation kinetics. This study indicated improved P_{O2} tolerance of sulphidic ore
390 attached microorganisms compared to cell suspensions.
391
392 The pressure tolerance of acidophilic bioleaching microorganisms may have practical
393 implications both in tank biooxidation and ISR. In this study, the highest air pressure
394 where biooxidation of sulphidic ore occurred was +20 bar (4.2 bar P_{O2}) in the stirred
395 tank pressure reactor at 27±2°C. Although the retention time of commercial autoclave
396 leaching is 0.66-3.33 hours, it requires temperatures as high as 135-240°C (Table 1). For
397 example, Parga et al. [21] reported effective pressure oxidation of refractory gold
398 concentrates at 6 bar P_{O2} and 80 °C. Our results indicate that biooxidation under
399 pressurised conditions would be possible at milder temperature and pressure conditions
400 than used in traditional autoclave leaching. However, the retention times would likely
401 be prolonged. One suggested approach for ISR applications is to utilize the activity of
402 acidophilic bioleaching microorganisms under pressurised conditions in the deep ore
403 deposits. The results of this study demonstrated bioleaching activity at elevated
404 pressures and highlighted the importance to maintain the P_{O2} at tolerable level for the
405 acidophiles.
406

4. Conclusions

In stirred tank pressure reactor, biological soluble Fe^{2+} oxidation rate with acidophilic enrichment culture increases with pressure increase from +1 bar to +3 bar and is inhibited at higher pressures. Partial biooxidation of sulphidic ore is possible at up to +20 bar whilst the oxidation rates decrease with increasing pressure. At very low P_{O2}, elevated pressures are tolerated at up to +40 bar. Thus, the decreased bioactivity at elevated pressures induced by technical air is due to increased dissolved oxygen concentration rather than pressure or the rate of pressure change. Ore surface attached microorganisms tolerate higher P_{O2} than suspended ones.

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Appendix A. Supplementary data

E-supplementary material (PCR-DGGE profiling of the microbial community) of this work can be found in the online version of this paper.

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Figure captions

Fig. 1. 4524 bench top reactor (Parr Instrument Company, USA) used during all stirred tank pressure reactor experiments. The cylinder with the culture and an internal stirrer is inside the heating blanket. The agitation and temperature are controlled and controlling unit that is connected to power supply (on left). The gas supply on the top right is used to increase the pressure and the gas outlet to decrease the pressure inside the closed reactor. On the top is the sampling port that is used to withdraw sample during operation. The heating and cooling of the reactor is done by injection of water to the heating blanket.

Fig. 2. Fe^{2+} oxidation in the stirred tank pressure reactor at +1 (black sphere), +2 (green rhombus) and +3 bar pressure (orange square) showing **a)** Fe^{2+} concentration, **b)** changes of DO concentration, **c)** pH and **d)** redox potential. Continuous lines show iron oxidation by enrichment culture 1 and dashed lines uninoculated controls. The pressures were induced with technical air and the experiments conducted at 35±2ºC and 150 rpm.

Fig. 3. Fe^{2+} oxidation in the stirred tank pressure reactor at +7 (blue sphere), +15 (red rhombus) and +30 bar pressure (black x) showing **a)** Fe^{2+} concentration, **b)** redox potential, **c)** DO concentration and **d)** pH. Continuous lines indicate enrichment culture 1 and dashed line uninoculated controls. The pressures were induced with technical air and the experiments conducted at 35±2ºC and 150 rpm.

Fig. 4. Fe^{2+} oxidation rates with enrichment culture 1 dominated by *Leptospirillum ferriphilum*, *Sulfobacillus* sp. and *Ferrimicrobium acidiphilum* (red rhombus) and uninoculated controls (green square) as a function of pressure and oxygen partial pressure (P_{O2}). The pressures were induced with technical air and the conditions were 35±2ºC, 150 rpm and initial pH 1.3 during the pressure experiments in the stirred tank pressure reactor.

Fig. 5. Fe^{2+} oxidation in shake flasks with enrichment culture 1 dominated by *Leptospirillum ferriphilum*, *Sulfobacillus* sp. and *Ferrimicrobium acidiphilum* inoculated from **a)** atmospheric pressure (black triangle), +2 bar (green rhombus) and +3 bar (orange square), and in **b)** +7 bar (black x), +15 bar (red rhombus), +30 bar (blue sphere) induced by air, and +40 bar induced by nitrogen and very low p_{O2} (purple +). Continued lines indicate experiments with enrichment culture 1 performed in triplicate and dashed lines with uninoculated controls. The activity tests were conducted at 35±2ºC, 150 rpm and initial pH 1.3.

Fig. 6. Biological oxidation of the sulphidic ore in the stirred tank pressure reactor at +1 (in blue), +10 (in red), and +20 bar pressure (in green) showing **a)** Fe dissolution, **b)** Fe^{2+} concentration, **c)** redox potential, **d)** pH, **e)** SO_4^{2-} production, and **f)** DO concentration. Continued lines indicate experiments with enrichment culture 2 dominated by *Acidithiobacillus ferrivorans*, *Sulfobacillus* sp. and *F. acidiphilum* performed in duplicates and dashed lines with uninoculated controls. Error bars show the standard error (n = 2). The pressures were induced with technical air and the experiments conducted at 27±2ºC and 100 rpm.

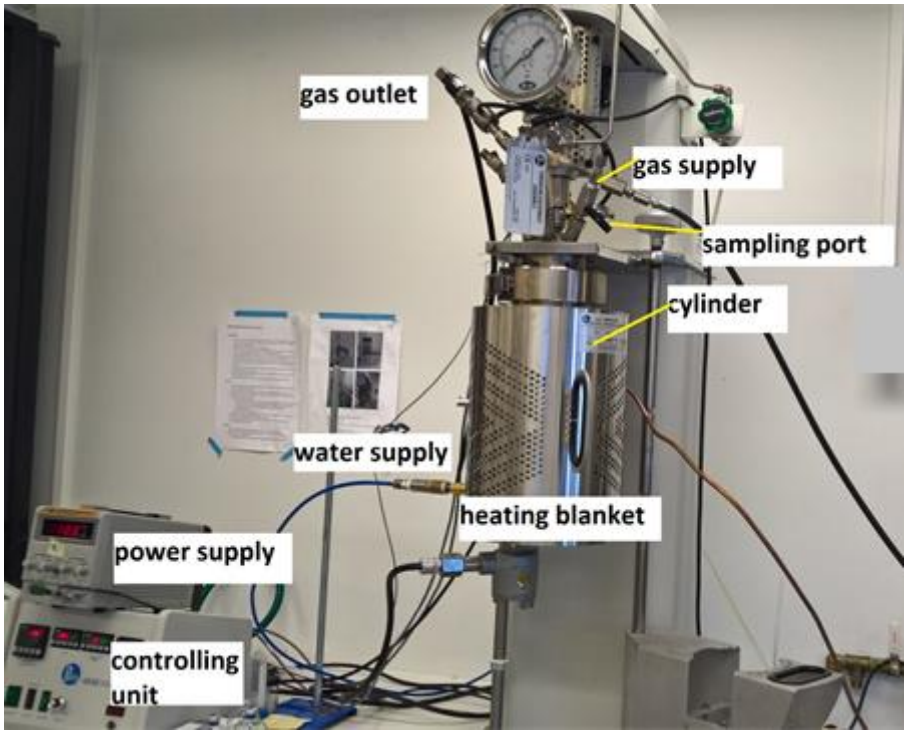
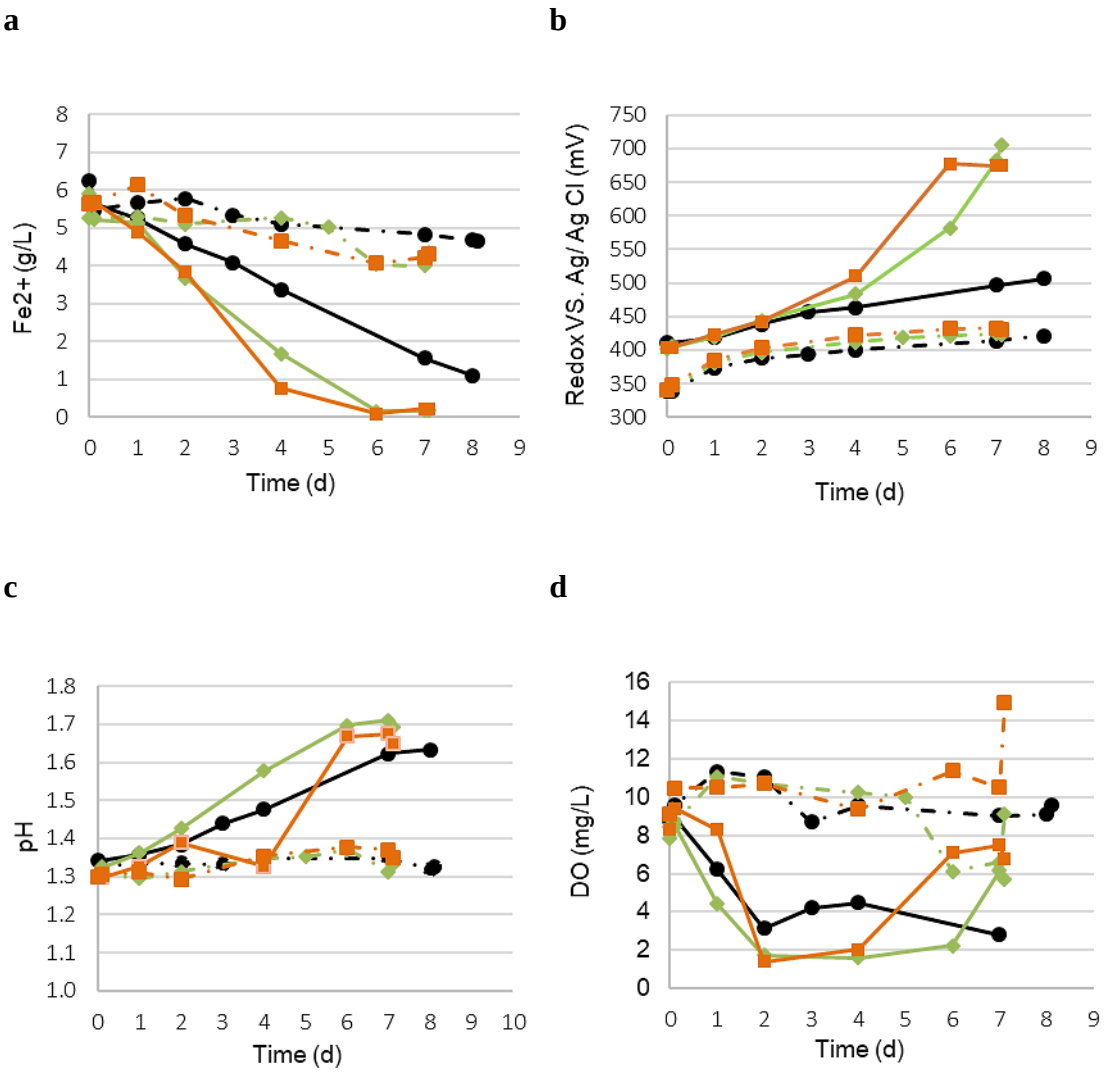

gas outlet
gas supply
sampling port
cylinder
water supply
heating blanket
power supply
controlling unit

Fig. 1

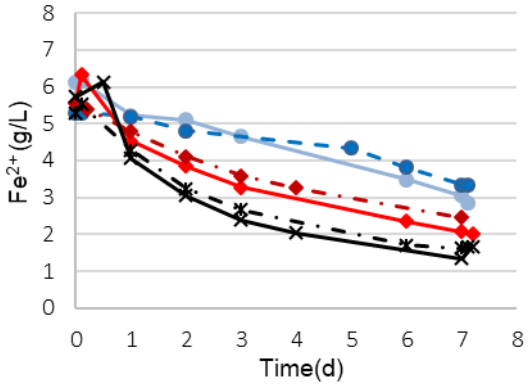
616



617 **Fig. 2**

618

a



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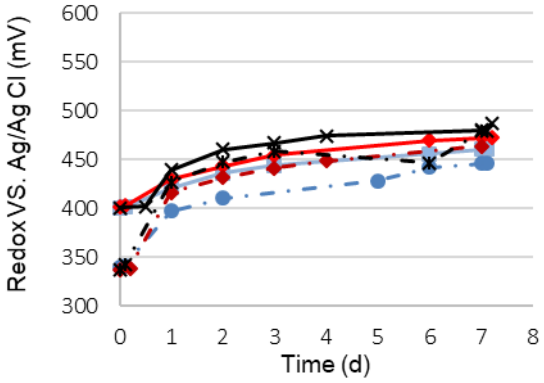

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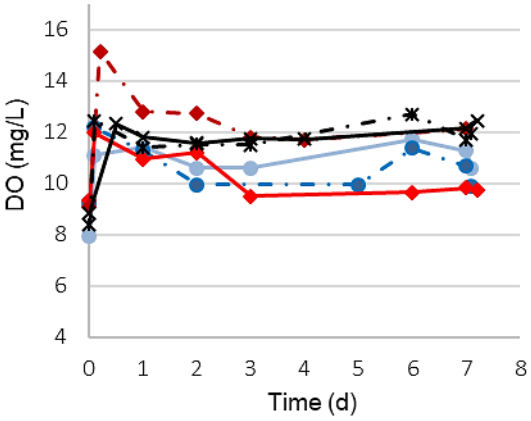

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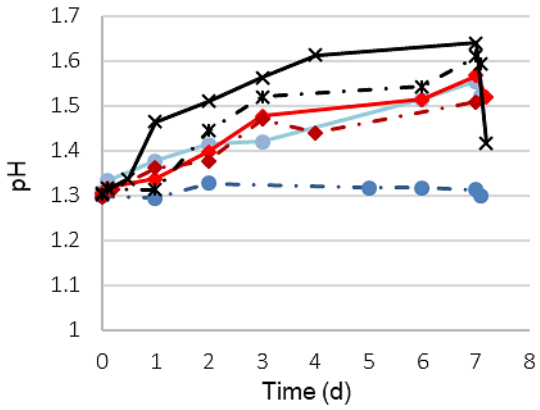
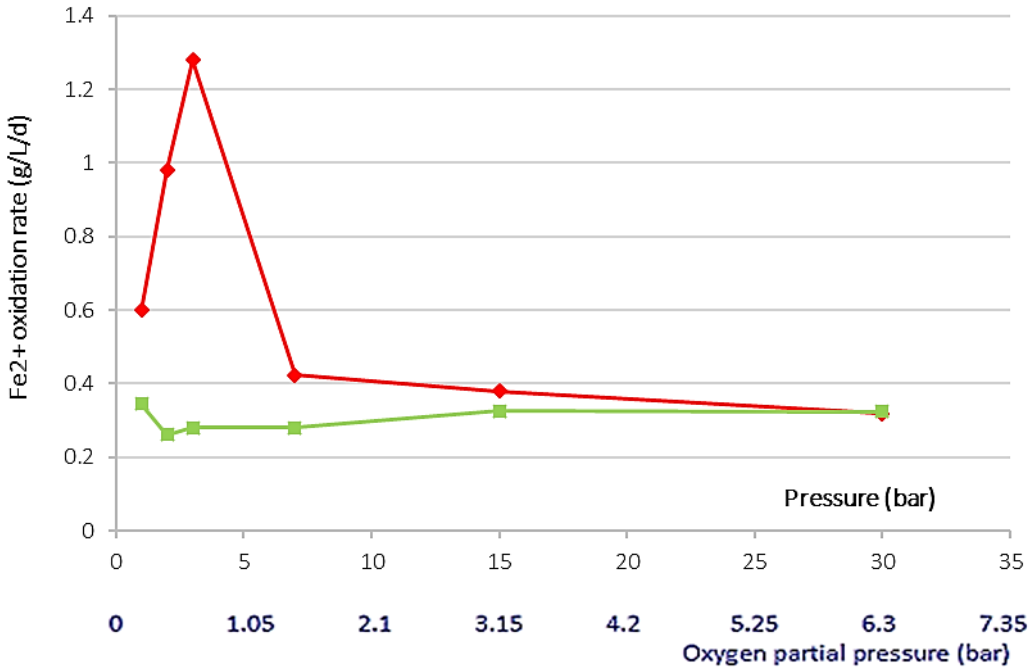


619 **Fig. 3**

620

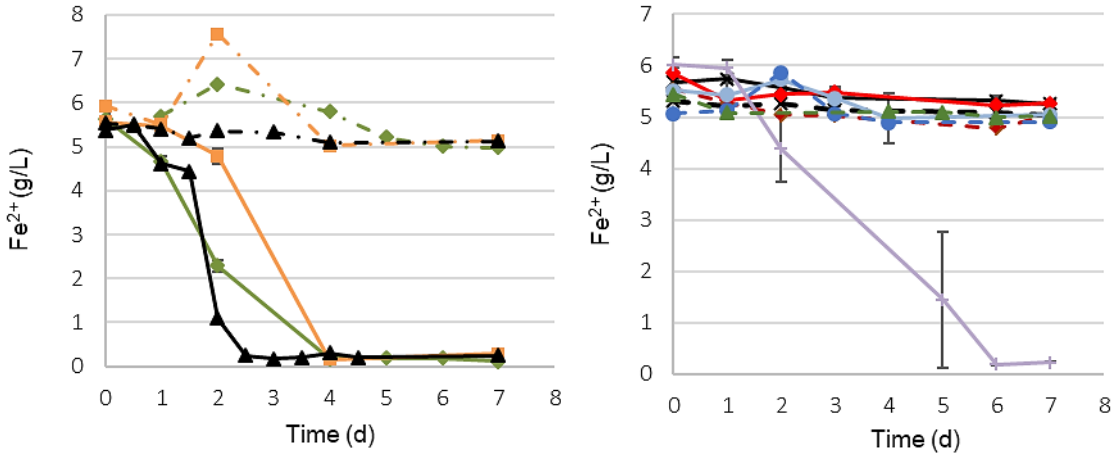


621

622 **Fig. 4**

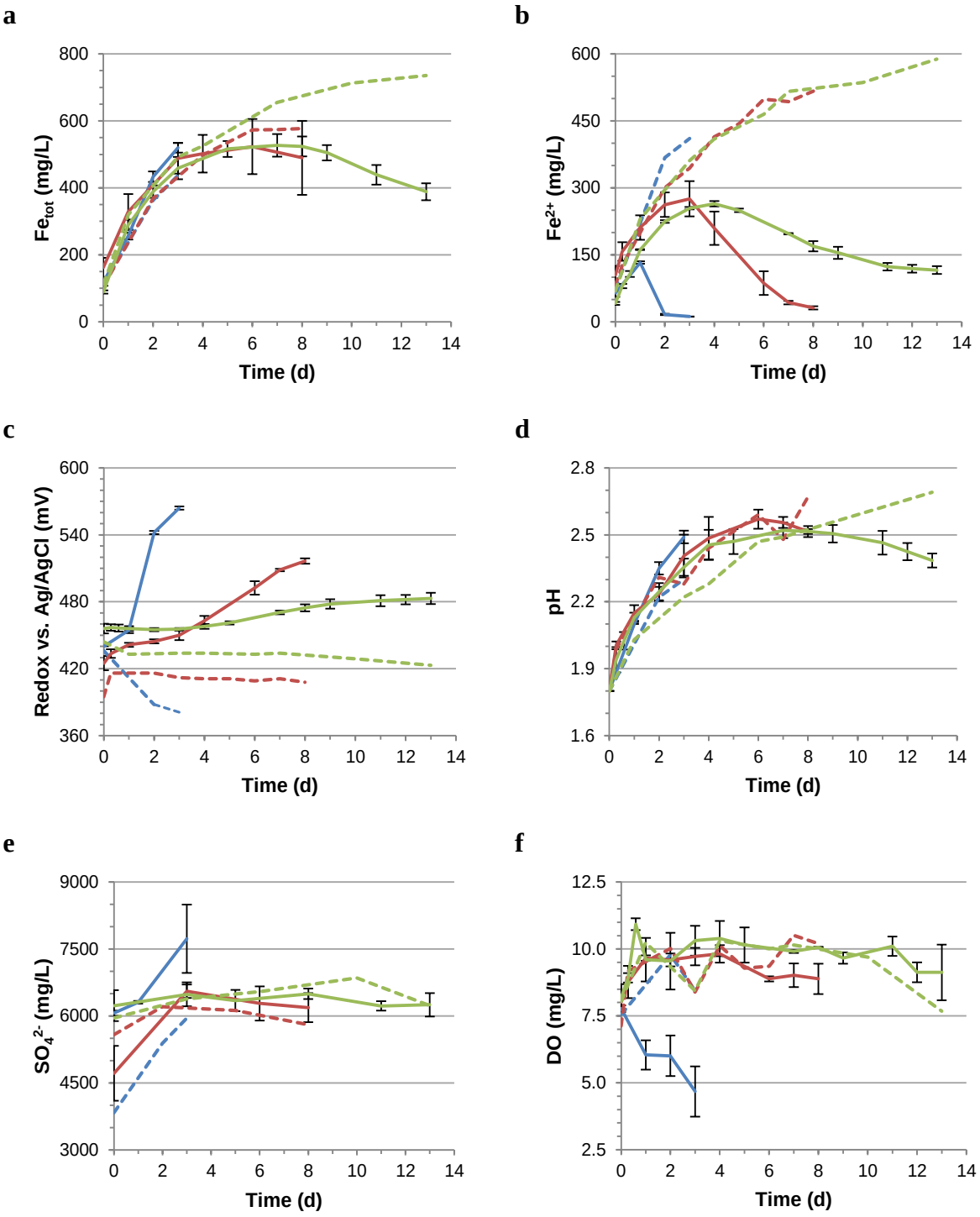
623

a **b**



624 **Fig. 5**

625


a
b
c
d
e
f
Fe$_{tot}$ (mg/L)
Fe^{2+} (mg/L)
Redox vs. Ag/AgCl (mV)
pH
SO_4^{2-} (mg/L)
DO (mg/L)
Time (d)

626 **Fig. 6**

627

Table 1

Typical conditions applied during tank bioleaching and autoclave leaching

Conditions	Tank bioleaching	Autoclave leaching	Reference
Pressure (bar)	atmospheric	7-29	[7]; [22]
P_{O2} (bar)	0.213	6-20	[22]
Temperature (°C)	30-45	135-240	[7]; [22]
Retention time (hours)	96-360	0.66-3.33	[7]; [24]

* This was calculated based on that 1 atm is approximately 1.013 bar and the air contains 21% oxygen.

Table 2

Modal mineralogy of the sulphidic ore used in the experiments.

Mineral	**Wt-%**
Silicates	92.6
Pyrite	4.7
Pyrrhotite	1.6
Carbonates	0.3
Others	0.7
Total	100.0

637 **Table 3**

638 Dominant microorganisms in the enrichment culture 1 and 2 as characterised by polymerase
639 chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) followed by sequencing
640 (Fig. S1.) and culturing conditions for the enrichment culture 1 and 2

Acidophilic enrichment cultures (similarity to culture collection type strains)	**Electron donor (g/L)**	**Initial pH**	**Temperature (˚C)**
Enrichment culture 1 dominated by *Leptospirillum ferriphilum* (100%) *Sulfobacillus* sp. (99.8%) *Ferrimicrobium acidiphilum*. (99.2%)	Fe^{2+} (5.6)	1.3	35 ± 1
Enrichment culture 2 dominated by *Acidithiobacillus ferrivorans* (99.8%) *Sulfobacillus* sp. (98.7%) *Ferrimicrobium acidiphilum* (99.2%)	Sulphidic ore (10)	2.0	27 ± 1

641

642

643 **Table 4**

644 Conditions applied during stirred tank pressure reactor experiments. Uninoculated control

645 experiments were also done at each pressure.

Experiment	Electron donor (g/L)	Temperature (°C)	Initial pH	Mixing rate (rpm)	Pressure (bar) above atm	Oxygen partial pressure (bar)	Rate of pressure change	Gas used for pressure increase
Fe^{2+} oxidation	Fe^{2+} (5.6)	35 ± 2	1.3	150	+1	+0.2	10 bar/min	Technical air[a]
					+2	+0.4		Technical air
					+3	+0.6		Technical air
					+7	+1.5		Technical air
					+15	+3.2		Technical air
					+30	+6.3		Technical air
					+40[b]	-		Nitrogen
Sulphide oxidation	Sulphidic ore (100)	27 ± 2	1.8	100	+1	+0.2	1.3 bar/h	Technical air
					+10	+2.1		
					+20	+4.2		

646 [a] 79% nitrogen and 21% oxygen

647 [b] Before pressure increase, 1% v/v oxygen and 0.01% v/v carbon dioxide were injected to the closed reactor system to
648 enable growth of the microorganisms.
649

650

Table 5

Modal mineralogy of the ore compared to biooxidised ore residues at different pressures.

NOTE: Fe-sulphates are oxidation products and are, therefore, not present in the non-pretreated ore.

Mineral	**Wt-%**			
	Ore	**+1 bar**	**+10 bar**	**+20 bar**
Fe-sulphates	-	0.3	0.3	0.4
Pyrite	4.7	4.5	4.3	5.1
Pyrrhotite	1.6	0.8	0.9	0.6