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Anne-Gwénaëlle Guezennec[1]*, C. Joulian[1], J. Jacob[1], A. Archane[2], D. Ibarra[3], R. Gregory[3], F. Bodénan[1], P. d'Hugues[1]

[1] BRGM -Water, Environment & Ecotechnologies Division, 3, av. Claude Guillemin, BP 36009, 45060 Orléans Cedex 2, France - +33 (0)2 38 64 31 36, a.guezennec@brgm.fr

[2]Milton Roy Mixing, 10 rue du Bois Gasseau, 77210 Samoreau, France

[3]Air Liquide, CRCD, 1 chemin de la porte des Loges, BP 126, 78354 Jouy en Josas, France

ABSTRACT

The use of oxygen enriched air is a common practice in high-temperature bioleaching tests (> 70°C) to overcome oxygen solubility limitation and reduced the energy costs of the process. Air is usually preferred in medium and low-temperature operations mainly for technical and economic constraints. Nevertheless, under high-sulfide loading conditions - high-grade metal sulfide concentrates and high solids concentration - the microbial and chemical demand for oxygen is significantly increased during the bioleaching process. If not satisfied, this high oxygen demand might limit the oxidation efficiency. Therefore it requires the injection of large amounts of air. Sparging with oxygen enriched gas instead of air may offer an interesting alternative process option to improve gas transfer in the bioleaching reactor and to provide an adequate oxygen supply in order to satisfy the oxygen demand. It might be useful to develop innovative alternative to the classical stirred tank reactor (STR) technology. However, the use of such conditions can lead to much higher dissolved oxygen (DO) concentrations than those encountered with air. Very few papers have been devoted to the study of the optimal range of DO concentrations for bioleaching processes. Most of them reported an inhibitory effect of DO concentrations above 5 ppm. The purpose of this study was to investigate the influence of DO on the bioleaching efficiency under oxygen-enriched atmosphere in 21 L stirred tank reactor at 42°C. Bioleaching experiments were performed in continuous mode with sulfide-rich tailings wastes composed mainly of pyrite (51%) and quartz using the "BRGM-KCC" bacterial consortia. The solid load was close to 20% (w/w). Using various oxygen supply conditions (partial pressure, gas rate), the DO concentration in the reactor varied between 4 and 17 ppm. For a DO ranging from 4 to 13 ppm, a good bacterial oxidizing activity was observed and the sulfide dissolution efficiency increased with the DO concentration. It is assumed that this improvement of the bioleaching efficiency was linked to an increase of the oxygen transfer rate from the gas phase to the liquid phase rather than a direct effect of the DO level. When the DO concentration reached 17 ppm a significant decrease of the microbial activity and consequently of the oxygen consumption was noticed. These results show that there is a critical value above which the DO concentration is detrimental to the activity of the bioleach microorganisms present in the "BRGM-KCC" consortia but this value is much higher than the one usually mentioned in the literature.

Key-words: bioleaching, oxygen, dissolved oxygen concentration, sulfide, bacteria monitoring, stirred tank reactor

1- INTRODUCTION

Biohydrometallurgy is well established for the treatment of certain sulfide minerals, where iron and sulfur-oxidising bacteria are used for the leaching of low grade copper ores and the pretreatment of pyritic gold ores and concentrates. However part of the mining industry remains skeptical and reluctant to adopt biohydrometallurgical techniques as a reliable alternative option. Heap leaching is sometimes considered unsuitable due to space constraints, slow leaching kinetics and low recovery rate. The interest of using stirred tank reactor (STR) for the treatment of other minerals than refractory gold ores, such as the base metal sulfides, has already been demonstrated but some improvements are still needed to meet economic viability (d'Hugues *et al.*, 2008; Spolaore *et al.*, 2009 ; Kutschke *et al.* 2015). The main costs of bioleaching operations in STR are the costs associated with the leaching tanks and the gas mass transfer to the pulp. The main capital costs are the ones for the agitators and for the gas injection devices. The main operating costs are associated with the energy consumption required for slurry agitation and air compression, since air is usually used to provide oxygen in bioleaching operations (Rossi, 2001; Morin et d'Hugues, 2007; van Aswegen *et al.*, 2007). The microorganisms involved in bioleaching processes get their energy through the oxidation of reduced sulfur compounds and iron (II). In these reactions oxygen is used as electron acceptor. In the case of pyrite bioleaching mechanisms involve the following oxidation reactions:

$$FeS_2 + 2\ Fe^{3+} \rightarrow 3\ Fe^{2+} + 2\ S^0 \qquad \textit{Eq. 1}$$

$$2\ Fe^{2+} + 2\ H^+ + 1/2\ O_2 \rightarrow 2\ Fe^{3+} + H_2O \qquad \textit{Eq. 2}$$

$$S^0 + 3/2\ O_2 + H_2O \rightarrow H_2SO_4 \qquad \textit{Eq. 3}$$

The reactions (2) and (3) are biologically catalyzed by acidophilic Fe- and S-oxidizing bacteria, whereas the reaction (1) occurs through chemical oxidation. The combination of the three reactions leads to the overall bioleaching reaction (Eq. 4) which shows that 3.5 mols of oxygen is required to oxidize 1 mol of pyrite.

$$2\ FeS_2 + 4\ H^+ + 7\ O_2 + 2\ H_2O \rightarrow 2\ Fe^{2+} + 4\ H_2SO_4 \qquad \textit{Eq. 4}$$

Oxygen supply is thus a key issue, particularly in bioleaching processes with high-sulfide loading conditions where the microbial and chemical demand for oxygen is significantly increased. As a consequence, there is a need to inject large amounts of air which can be technically difficult and increases the costs of the process. Sparging with oxygen enriched gas instead of air may offer an interesting alternative process option to improve gas transfer and bioleaching efficiency by providing an adequate oxygen supply in order to satisfy high oxygen demand.

Gas mass transfer in bacterial leaching systems has been widely studied and is well documented (Bailey and Hansford, 1993a; Bailey and Hansford, 1994; Boon and Heijnen, 1998; Savic *et al.*, 1998; Veglio *et al.*, 1998). Transfer to liquid theory (illustrated schematically in Fig. 4) indicates that the O_2 mass transfer rate, called R_{O2} (quantity of oxygen transferred in liquid phase per unit of time) is given by:

$$R_{O2} = K_La \times (C^* - C_L) \qquad \textit{Eq. 5}$$

where: R_{O2} is O_2 transfer rate (mol m^{-3} s^{-1})
K_La is the volumetric oxygen mass transfer coefficient (s^{-1})
C^* is the oxygen solubility in reactor conditions (mol m^{-3})
C_L is the oxygen concentration in the liquid phase (mol m^{-3})

Several authors (Lui *et al.*, 1987; Bailey and Hansford 1994; Jordan *et al.*, 1995; Myerson, 1981; Bailey and Hansford, 1993b) have pointed out that the lack of adequate gas mass transfer is a rate limiting step in many bacterial leaching processes. The gas-liquid mass transfer rate depends on a number factors (Van Weert *et al.*, 1995, d'Hugues *et al.*, 1997, Boon and Heijnen, 1998) such as the reactor type, geometry and size, the gas-flow rate, sparger design and depth, the stirring speed, particle shape and size, pulp density and viscosity. However oxygen can become a limiting factor in bacterial leaching

because of its low solubility: only 0.26 mM O_2 (8.32 ppm) can dissolve per liter of water at 25°C in an air/water mixture. As mentioned by Witne and Phillips (2001), one way of increasing the solubility of oxygen in water or media solution is by increasing the driving force, i.e. raising the oxygen partial pressure in the gas stream supplied to the leach pulp. This mechanism is described by Henry's gas law which gives the solubility of oxygen in solution in relation to the oxygen partial pressure in the gas phase:

$$C^* = (P^\circ/H) \qquad \textit{Eq. 6}$$

where: C^* is the oxygen concentration of the nutrient solution;
P° is the partial pressure of the gas in the gas phase;
H is Henry's constant, which is specific for the gas and the liquid phase.

Henry's gas law shows that the solubility of O_2 in the leach pulp increases with increasing oxygen partial pressure in the gas stream. Higher O_2 partial pressures could be attained when the bioreactor air or gas stream is enriched with added pure oxygen. This approach was used for the development of processes using high thermophiles culture by teams working on the development of STR processes for the bioleaching of chalcopyrite concentrates (Dew *et al.*, 1999; d'Hugues *et al*, 2002). In these studies, oxygen was used instead of air because of the low solubility of O_2 at high temperature. When working with mesophilic or moderately thermophilic microorganisms, they might be various interests of using O_2 enriched air such as (i) to decrease the flow of gas stream injected in the pulp, reducing energy consumption linked to agitation and compression of air (ii) to improve gas transfer to satisfy higher oxygen demand associated with higher sulfide concentration. Using O_2 enriched air is a promising alternative that might be useful to develop innovation to improve the classical STR technology. Air Liquide, Milton Roy Mixing and BRGM are currently testing an innovative bioleaching process using floating agitators to mix and to suspend solids in the solution as well as to inject gases in the pulp. This new concept enables to decrease the costs of bioleaching processes by operating in lagoons or ponds instead of using costly tanks, and at higher solid loading (>20%) than in conventional stirred tank bioreactors. In these conditions of high solid load, the microbial and chemical demand for oxygen is significantly increased and air could be replaced by oxygen enriched gas to provide an adequate oxygen supply in order to satisfy the oxygen demand.

However the use of such conditions can lead to much higher dissolved oxygen (DO) concentrations than those encountered with air sparging. Very few papers have been devoted to the study of the optimal range of DO concentrations for bioleaching processes. However most of them reported an inhibitory effect of DO concentrations above 5 ppm (de Kock *et al.*, 2004; Wang *et al.*, 2015).

The purpose of this study was to investigate the influence of DO on the bioleaching efficiency of a mesophile to moderate thermophile consortium. The bioleaching tests were carried out under oxygen-enriched atmosphere in a 21L stirred tank reactor at 42°C in continuous mode with sulfide-rich tailings wastes composed mainly of pyrite (51%) and quartz. The solid load was closed to 20% (w/w). The DO concentration in the reactor was varied between 4 and 18 ppm by increasing the gas flow rate. The influence of the DO level on the bioleaching efficiency was investigated through the monitoring of the sulfide leaching kinetics, the sulfide dissolution yield and the structure and the abundance of the microbial community.

2- MATERIALS AND METHODS

Characterization of the sulfidic materials

The experiments were performed using flotation tailings coming from a European copper mine. The mineral of economic interest in the ore body is chalcopyrite ($CuFeS_2$). At site, the ore is ground and valuable chalcopyrite is then separated from pyrite by flotation. Copper contained in the chalcopyrite is recovered by smelting whereas pyrite is discharged in tailings, from which the material used in this

study was sampled. The tailings are mainly composed of pyrite (51%) containing cobalt (0.06%), copper (0.19%) and gold (1 g/t). This waste has been chosen as test materials for its high content of pyrite, which makes it particularly suitable for bioleaching.

Bacterial culture and nutrients

The experiments were carried out using the BRGM-KCC microbial consortium, which has already been fully described (d'Hugues *et al.*, 2003). The predominant organisms in the culture are affiliated to *Leptospirillum (L.) ferriphilum, Acidithiobacillus (At.) caldus* and *Sulfobacillus (S.) benefaciens*. The culture used as an inoculum originated from BRGM stock culture, stored at −80 °C. The culture was subcultured several times in batch mode from 2 mL up to 21 L prior to the beginning of the test. The culture was grown in a nutrient medium called "0Km". This is a modified "9K" medium (9K without iron, "m" indicating modification of the basal salts) and it was optimised for bacterial growth on cobaltiferous pyrites. Its standard composition is the following: $(NH_4)_2SO_4$, 3.70 g L^{-1}; H_3PO_4, 0.80 g L^{-1}; $MgSO_4 \bullet 7H_2O$, 0.52 g L^{-1}; KOH, 0.48 g L^{-1}.

Laboratory apparatus and experimental conditions

Bioleaching tests were carried out in continuous mode using a 21 L (working capacity) laboratory-scale stirred tank. This tank is made from 316L stainless-steel and has a height/diameter ratio equal to 1. The top of the reactors was connected to a gas cooling system to prevent excessive evaporation. The bioleach slurry was mechanically stirred by a mixed (axial/radial) system (so-called BROGIM® — BRGM/MRM) mounted on a rotating shaft. The gas stream made of N_2, CO_2 and O_2 supplied by Air Liquide gas cylinder was injected beneath the turbine at the bottom of the reactor via a stainless steel pipe. Gas mixture composition was controlled by valves and mass flow regulators for each gas. Gases went through a gas mixer before injection in the reactor. The feed was made up of a high density pulp flow (40% wt/wt of sulfidic materials in water) and a concentrated nutrient medium flow. The two feed flows were pumped separately and injected into the tank in a ratio corresponding to the solids ratio (20% w/w) required for the feeding pulp. The combination of the two flows resulted in nutrient concentrations corresponding to 0Km medium. The residence time of the pulp in the tank was closed to 2.2 days. The pH was regulated between 1.2 and 1.5 by adding limestone slurry of 500 g L^{-1} $CaCO_3$. The standard temperature was 42°C and was maintained constant by circulating cold water through an internal stainless-steel coil.

Five conditions of gas flow rates and compositions were tested (Table 1). CO_2 was maintained close to 1% during the whole assay. O_2 partial pressure was first set to 56% and the gas flow rate was progressively increased from 50 to 500 NL h^{-1} (conditions 1 to 4, see Table 1) in order to increase the concentration of dissolved oxygen in the liquid phase. In the last condition the gas flow rate was decreased to 250 NL h^{-1} and the oxygen partial pressure was set to 70%. For each condition, the experiment was carried out for an average time of 20 days, which enabled to monitor the bioleaching test during several residence times and reach a steady state.

Table 1: Main operating conditions for the gas injection (the partial pressures are measured in the gas inlet after mixing of the N_2, O_2 and CO_2 streams)

	Gas flow rate	**O_2 partial pressure**	**CO_2 partial pressure**
	NL h^{-1}	%	%
Condition 1	50	57.4	1.10
Condition 2	100	56.9	1.10
Condition 3	250	55.4	0.99
Condition 4	500	57.1	1.12

Condition 5	250	70	1.0

Analytical techniques

Daily monitoring

Electrochemical potential, pH and dissolved oxygen (DO) were measured directly in the pulp. Samples of pulp were collected daily in the bioreactor and filtered (syringe filters in cellulose acetate, diameter 30 mm, porosity 0.45 µm). Cobalt and total iron concentrations were measured by atomic absorption spectroscopy (Varian SpectrAA-300) in the filtered fraction. The O_2 and CO_2 concentrations in the inlet and outlet gas of the reactor were measured using a paramagnetic analyser and an infrared analyser (ADC 7000 — Analytical Development Company Ltd.) respectively. The gas balancing method was used to calculate the oxygen uptake rate (OUR). The oxygen transfer rate (OTR) and the OUR are linked by the following equation:

$$\boldsymbol{OTR = OUR + \frac{dC_{O2}}{dt} = \frac{Q_{O2}^{in} - Q_{O2}^{out}}{V}} \qquad \textit{Eq. 7}$$

where: dC_{O2}/dt is the accumulation oxygen rate in the liquid phase,

Q_{O2}^{in} and Q_{O2}^{out} are the oxygen gas flow at the bioreactor inlet and outlet,

V the volume of the liquid phase in the bioreactor.

At steady state, when the DO concentration is constant, there is no accumulation of oxygen in the reactor and the OUR is equal to the OTR. Assuming that the nitrogen gas flow is the same at the inlet and outlet of the reactor (i.e. is not consumed neither transferred to the liquid phase), Eq. 7 becomes:

$$\boldsymbol{OTR = OUR = \frac{Q^{in} * \left(P_{O2}^{in} - {P_{N2}^{in}}/{P_{N2}^{out} \cdot P_{O2}^{out}} \right)}{V}} \qquad \textit{Eq. 8}$$

where: Q^{in} is the gas flow rate at the inlet of the bioreactor,

P_{N2}^{in} and P_{N2}^{out} are the partial pressure of nitrogen in the gas at the inlet and outlet of the bioreactor.

Analysis of the solid fractions and mass balances

When the bioleaching reactor was operating at steady state, samples were collected from the overflowing pulp at the exit of the reactor and from the initial pulp feed. The samples of pulp were first filtered with a Büchner funnel to separate liquid and solid phases. The filtered solid material was then rinsed with a sulfuric acid solution at pH 1.8 and dried. Representative aliquots of the dried materials (<80 µm) were analysed for metal contents (namely Fe, Co, Cu), total carbon and sulfur speciation. Major and trace metal contents were determined by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometry) after oxidizing digestion according to protocols dedicated to ore analysis (degradation of all sulfides). Total carbon and total sulfur were determined using a Leco analyzer: i.e. non-dispersive infrared analysis of CO_2 and SO_2 gas respectively, after heating and passing of an oxygen stream. The same technique was used for sulfide (S^{2-}) determination, once the sample has been leached in warm sodium carbonate solution to convert the metal sulfate into insoluble carbonates and soluble sulfate. Sulfate was determined by gravimetry after the sample being leached at boiling temperature with a sodium carbonate solution, ferric ion reduced to ferrous iron by the addition of hydroxylamine hydrochloride, and the filtrate being precipitated with barium chloride. The analytical data were then used to calculate the mass balance of the operation and to assess the bioleaching performances (sulfide oxidation rates and yields).

Bacterial community monitoring

Homogeneous 2 mL pulp samples were taken at the end of the steady state of each of the five operating conditions. After centrifugation, the pellet was washed in Tris buffer (100 mM, pH 8) until acidity was neutralized, and genomic DNAs were extracted with the FastDNA Spin Kit for Soil and manufacturer's protocol (MP Biomedicals), except for a speed of 5 for 30 s for mechanical lyses. DNA

was also extracted with the same procedure from filtrate (2 mL) and solids (about 0.7 g) obtained after filtration of homogeneous pulp, in order to characterize bacteria located in the liquid phase or associated with the solids. The abundance of each inoculated strains, *i.e. L. ferriphilum*, *At. caldus*, and *S. benefaciens*, was determined by measuring abundance of their 16S rRNA gene using species-specific real-time quantitative PCR (qPCR) performed with the newly developed assays described by Hedrich *et al.* (2016). Results were expressed as numbers of gene copies per mL of bioleaching pulp.

3- RESULTS

3.1- Influence of the dissolved oxygen on the dissolution rate and the OUR

The oxygen partial pressures analyzed in the gas inlet and outlet for the five tested conditions and the corresponding oxygen solubility calculated using Henry's law (Eq. 6) are gathered in Table 2. This table presents also the DO concentration measured in the reactor. The gas injection conditions selected enabled to test a wide DO concentration range from 4.7 ppm to 17.7 ppm.

Table 2: Oxygen concentration in the gas phase and in the liquid phase in continuous pyrite bioleaching experiments (O_2 partial pressure measured in the gas inlet (P_{O2} in) and outlet (P_{O2} out) and corresponding O_2 solubility calculated using Henry's law; DO concentration measured in the reactor)

	P_{O2} in	O_2 solubility* corresponding to P_{O2} in	P_{O2} out	O_2 solubility* corresponding to P_{O2} out	measured DO concentration
	%	*ppm*	%	*ppm*	*ppm*
Condition 1	57.4	17.6	41.1	12.6	4.7
Condition 2	56.9	17.5	48.3	14.8	5.0
Condition 3	55.4	17.0	51.7	15.9	9.5
Condition 4	57.1	17.5	55.2	16.9	12.9
Condition 5	70	21.8	69	21.3	17.7

The variation in OUR and pyrite dissolution rate for the five tested conditions is shown in Fig. 1. Both increased when the DO concentration is increased from 4.7 ppm to 12.9 ppm, which corresponds to the conditions 1 to 4. OUR values are close to the values of pyrite dissolution rate as expected from Eq. 4 which shows that the bioleaching of 1 kg of pyrite consumes 1 kg of oxygen.

For the condition 5 where the DO concentration reached 17.7 ppm a sharp decrease of the pyrite dissolution rate was observed. In that case it was not possible to calculate the corresponding OUR because of the lack of precision in the analysis of the gas composition.

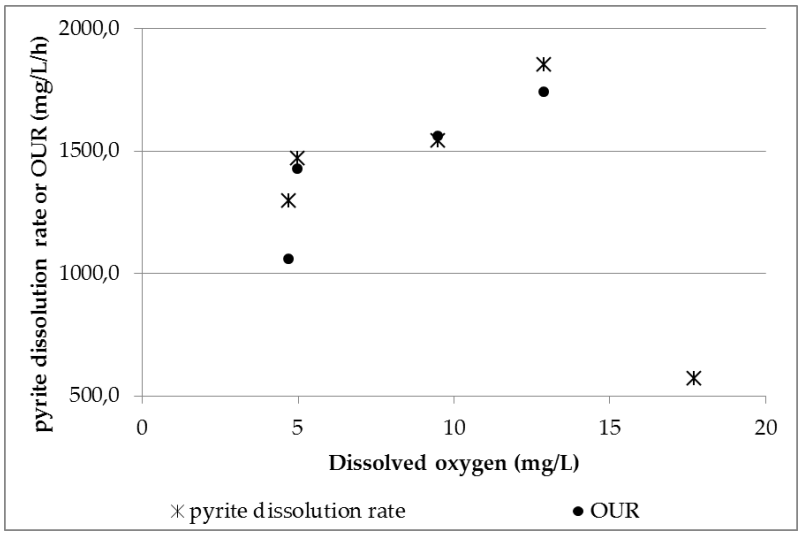

Figure1: Pyrite dissolution rate and OUR vs. DO concentration in continuous pyrite bioleaching experiments

3.2- Influence of the dissolved oxygen on the dissolution yields

In table 3 are reported the sulfide dissolution yields obtained for each tested conditions and the corresponding residence time. The slight variation in residence time can be explained by variation in the pumps flows for the calcite as it varies with the leaching efficiency and the corresponding production of sulfuric acid (see Eq. 4). It explains why the residence time is not constant especially for the conditions 1 and 5 for which the oxidizing activity was lower (as shown by the lower values of OUR). In both cases the amount of calcite introduced in the reactor was much lower and the residence time increased.

Table 3: Residence time and sulfide dissolution yield

	measured DO concentration	**Residence time**	**Sulfide dissolution yield**
	ppm	*d*	%
Condition 1	4.7	2.5	63%
Condition 2	5.0	2.2	56%
Condition 3	9.5	2.2	61%
Condition 4	12.9	2.2	77%
Condition 5	17.7	2.4	29%

When looking at the conditions 2, 3 and 4 for which the residence time is the same, the sulfide dissolution yield increased with the DO concentration. For the condition 4 the sulfide dissolution yield reached 77% for a DO concentration of 12.9 ppm. These results as well as the corresponding values of OUR and pyrite dissolution rates show (i) that the oxidizing activity in the bioleaching reactor is high from 5 to 12.9 ppm DO concentration and (ii) this activity is enhanced when the DO concentration increases.

For the condition 1 the sulfide dissolution yield is higher than for the conditions 2 and 3. But it is likely that this result is linked to the higher residence time in the reactor rather than to an effect of the DO considering the OUR and the pyrite dissolution rate which are both lower for the condition 1 than for the conditions 2 and 3. For the condition 5 the sulfide dissolution yield drops to 29% despite the

higher residence time. This result is in accordance with the decrease of pyrite dissolution rate observed for this condition and seems to confirm the appearance of an inhibitory effect of the DO concentration on the biological activity when the DO becomes too high.

3.3- Influence of the dissolved oxygen on the microbial community (abundance and structure)

The bacterial community composition shows that the strains present in the bioleach pulp were the same as the three main strains which make up the BRGM-KCC consortium used to inoculate the bioreactors. They are all found in the five tested condition, *L. ferriphilum* being dominant and accounting for up to 80% of the total community (Fig 2).

The influence of DO on the biomass was studied, using 16S rRNA gene abundances as a measure of total biomass as well as strain specific abundances. The total biomass was negatively affected by an increase of DO, as shown by the decrease especially marked at a DO of 17.7 ppm where about one log of gene copy numbers was lost (Fig. 2). The strains composing the community followed the same trend. Especially, *At. caldus* abundance, although high at low DO (4.7 and 5 ppm), showed a regular diminution all along the experiment as well as the sharper decrease (1 log) as soon as DO increased from 5 to 9.5 ppm. Interestingly, *At. caldus* represented the large majority of planktonic bacteria recovered from the liquid compartment (see Fig. 3) and accounted for more than 96% of the total planktonic community at DO of 4.7 and 5 ppm, respectively. At 17.7 ppm however, the biomass of all strains had decreased, and *L. ferriphilum* and *S. benefaciens* appeared to be only slightly less affected (1.9 log between conditions 1 and 5) than *At. caldus* (2.3 log).

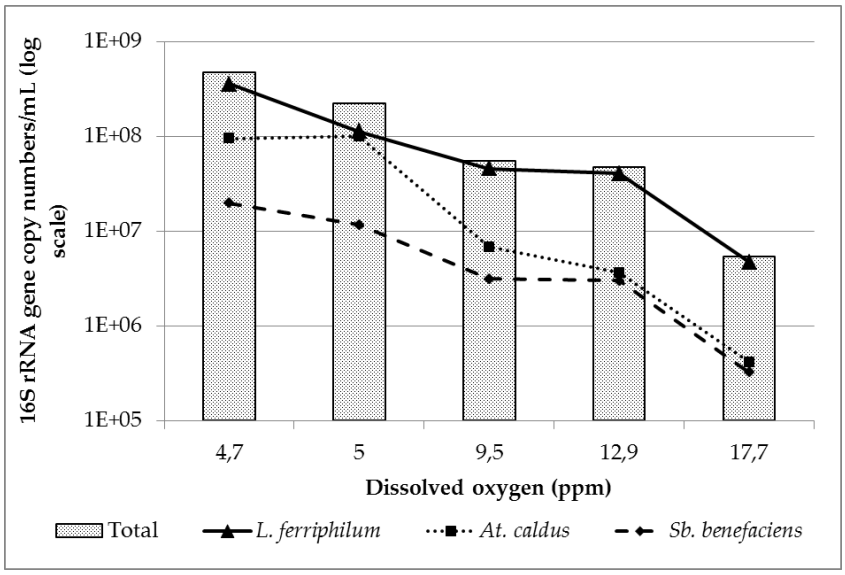

Figure 2: Influence of DO concentration on the microbial community structure and the quantity of biomass present in the reactor (assessed using the number of 16S rRNA gene copy)

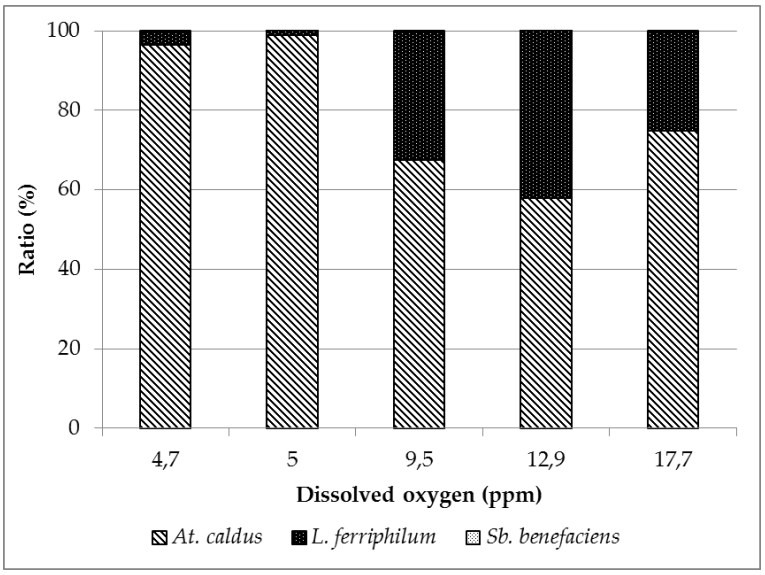

Figure 3: Structure of the microbial community in the liquid compartment

DISCUSSION

It is likely that a dissolved oxygen concentration between 13 and 17 ppm is critical for the bioleaching efficiency. Below this critical value bioleaching efficiency increases with the DO concentration. It is assumed that this improvement of the bioleaching efficiency is linked to an increase of the oxygen transfer rate from the gas phase to the liquid phase rather than a direct effect of the DO level. This hypothesis is partially confirmed by the increase of the oxygen uptake rate which is equal to the oxygen transfer rate in steady state mode. Above this critical value the DO concentration starts to be detrimental to the microbial activity and a sharp decrease of bioleaching efficiency (associated with a significant decrease of the bacterial population) is observed. This critical value is difficult to determine since the DO concentration depends on the rate of oxygen transferred from the gas phase to the liquid phase and from the oxygen consumed by the bio-oxidation. As soon as the DO concentration is above this critical value, the microbial activity decreases as well as the oxygen consumption, which entails an increase of the DO concentration.

The DO level which affects negatively the efficiency of bioleaching with the KCC consortium is quite different from those previously mentioned in the literature which reports an inhibitory effect for much lower DO levels. Especially this study can be compared to the results published recently by Wang *et al.* (2015) which studied the effect of dissolved oxygen on bioleaching using a sulfidic material and a microbial community similar to the ones used in this study. The optimum activity of the microbial population was obtained at 3.75 ppm concentration of DO. Above and below this value the OUR, the oxidation rate and the oxidation ratio decreased. It must be noted that Wang *et al*. carried out their experiments at 10% solids and in batch mode. The maximum OUR measured was closed to 160 mg L^{-1} h^{-1}, which is more than ten times lower than the maximum OUR measured in this study. The microbial oxidizing activity was thus much lower. In the absence of data regarding the gas flow rate and its composition (especially the partial pressure of oxygen) it is difficult to explain the discrepancies observed between both studies, but it can be assumed that the influence of DO concentration on the bacterial activity is very dependent on the other operating conditions such as the solid concentration and the culture mode. It might also be bacterial strain dependent.

The increase of DO concentration has also a noticeable influence on the biomass which progressively decreases when the DO concentration increases. Below 13 ppm *At. caldus* seems to be the most affected by the increase of DO concentration. This might suggest that *At. caldus* is less tolerant to high DO concentrations than the other strains of the KCC consortium. Nevertheless other factors must also be

considered and especially the fact that *At. caldus* is the most abundant strain in the liquid phase compared to *L. ferriphilum* and *S. benefaciens* which are mainly located on the particles. Because of the presence of these bacteria on the solid phase it can be assumed that the oxidation of FeII and sulfur occur also at the surface of the pyrite particles. Oxygen is thus consumed at the liquid/solid interface and its transfer in the liquid/solid boundary layer is governed by the same theory as the one presented in the introduction:

$$R_{O2} = K_s \cdot a_s \cdot (C_L - C_s) \qquad \textit{Eq. 7}$$

where: R_{O2} is O_2 mass transfer rate (mol m^{-3} s^{-1})
K_s is the mass transfer coefficient (m s^{-1})
a_s is the solid specific area (m^{-1})
C_L is the oxygen concentration in the liquid phase (mol m^{-3})
C_s is the oxygen concentration at the solid surface (mol m^{-3})

This phenomenon is illustrated in Fig. 4 and shows that the oxygen concentration in the liquid phase is higher than the one at the surface of the particles. The transfer rate being closely linked to the oxygen consumption in the system the difference (also called "driving force") is higher when the oxygen consumption rate increases. Planktonic cells are thus submitted to higher DO concentrations than the cells attached to the particles, which might explain that *At. caldus* abundance decreases more quickly than *L. ferriphilum* and *S. benefaciens.*

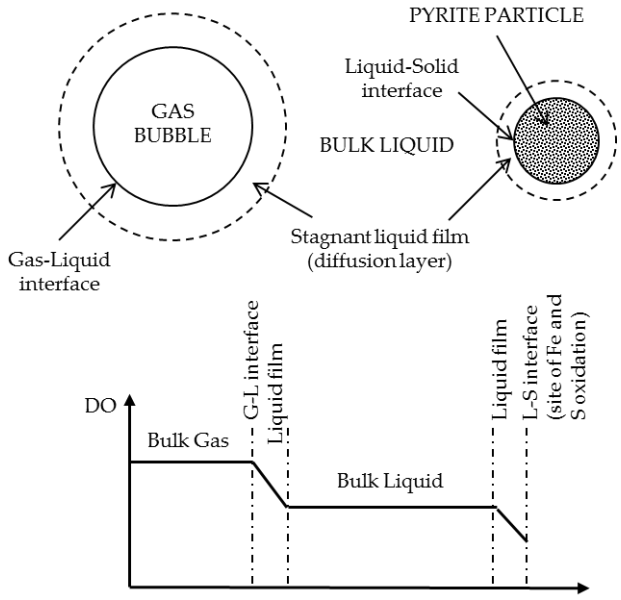

Figure 4: Schematic description of oxygen transfer and oxygen concentration profile in a triphasic media with reactions consuming oxygen at the solid surface

The level of detrimental effect may also be linked to the capacity of a cell to handle high DO concentration. The toxicity of the oxygen towards the bacteria involved in bioleaching might be linked to the formation of reactive oxygen species (ROS) by a mechanism involving Fenton and Haber-Weiss reactions. By switching oxidation states metal ions further activate species like hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot}$) to the highly reactive hydroxyl radical ($^{\cdot}OH$) (Imlay, 2008). ROS are known to have deleterious effects on cells through a mechanism called oxidative stress (Halliwell 2007). Several authors (Borda *et al.*, 2003; Cohn *et al.*, 2006; Schoonen *et al.*, 2006; Schoonen *et al.*, 2010) have shown that the oxidation of pyrite in solutions containing dissolved oxygen leads to the formation of ROS through several mechanisms that are synthetized in Fig. 5. As can be seen the ROS

concentration in the leaching solution would increase with the DO concentration. Recently, evaluations in tank reactors have also shown the negative influence of ROS generation (due to fine grinding of pyrite) on bioleaching (Jones *et al.*, 2011), suggesting the direct effect of external ROS generation on bioleaching organisms. Bioleaching bacteria harbor genetic systems for the defense against oxidative stress by the production of various proteins involved in anti-oxidative protection and biomolecules repair mechanisms (Cardenas *et al.*, 2012). If there is evidence that *Leptospirillum*, *Sulfobacillus* and *Acidithiobacillus* strains possess such systems, the mechanisms are not fully understood yet and their efficiencies depending on genus or strain during bioleaching are not known.

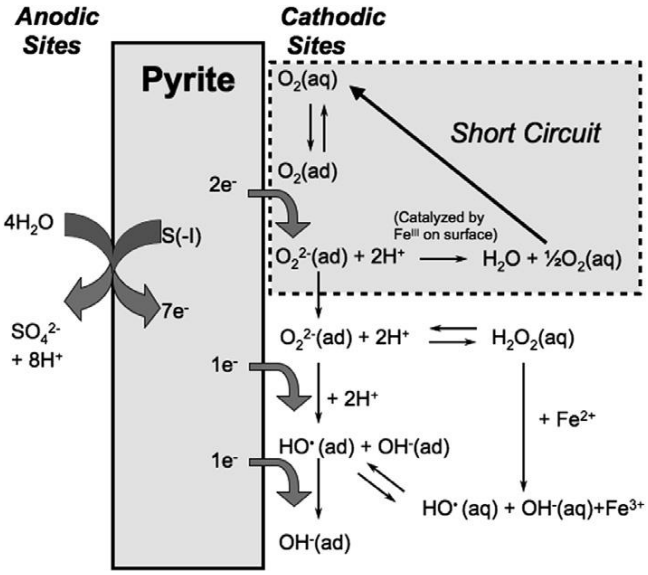

Figure 5: ROS formation mechanisms at pyrite surface (Schoonen *et al.* 2010)

CONCLUSION

The BRGM-KCC microbial consortium can tolerate DO levels up to 13 ppm, and probably higher level with a critical limit between 13 and 17.7 ppm, during continuous bioleaching and still maintain a high bioleaching activity. This value is much higher than the maximal DO levels reported in previous papers and is also higher than the solubility of oxygen in an air/water mixture (8.3 ppm at 25°C, 6 ppm at 40°C). The bioleaching efficiency (oxidation rate and dissolution yield) increases with the increase of the oxygen supply (and oxygen transfer) up to a DO concentration of 13 ppm. However the microbial activity and consequently the oxygen consumption significantly decreased at DO concentration of 17 ppm. These results confirm the applicability of using oxygen enriched gas instead of air to efficiently supply oxygen in bioleaching reactors without inhibitory effect on the microbial oxidizing activity. However the oxygen partial pressure and the gas flow must be carefully adapted to the oxygen requirements of the system to avoid too high DO concentrations in the bioreactors.

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