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A real-time method for detecting active microorganisms in commercial-scale biohydrometallurgical processes

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ABSTRACT

Due to the fact that the entire bioleaching process depends strictly on the presence and viability of microorganisms, it is capital to have methodologies that allow rapid, reliable and effective monitoring of the biological activity in commercial-scale biohydrometallurgical processes. The existing biological activity must be known at different sampling points in the leaching heap to ensure efficient metal recovery and to take corrective measures whenever necessary. However, there are, currently, no useful methodologies that can effectively replace classic techniques for determining total viable bacteria, which have a very long analysis time (between 7 and 14 days). In a former presentation, we described the development of a rapid and simple method for detecting the presence of acidophilic microorganisms in aqueous samples. This method is based on concentration and washing of the microbial cells, and detection of active microorganisms by means of its cellular ATP using a bioluminescent reaction. Unlike conventional methods, this technology enables the determination of active microorganisms in just 10 minutes. To facilitate implementation of this method a simple kit was designed. This kit, called LixKit®, contains all materials needed to make such determination. This work aimed at demonstrating the application of LixKit® as a real-time method for detecting the active microorganisms in commercial-scale biohydrometallurgical processes. LixKit® has been applied to 85 samples of 6 mining companies and 8 samples of acid mine drainage. The determination of microbial activity by LixKit® was performed at the mining site. In parallel, the same samples were transported to the laboratory for analysis with conventional methods of total count and viable count by the Most Probable Number technique. These results showed a significant correlation between this real time method and conventional methods. The simplicity of LixKit® allows it to be used without problem by an operator at the mining site.

Key words: bioleaching, bioluminescent, monitoring, ATP, bacteria, acidophiles

INTRODUCTION

Due to the fact that the entire bioleaching process depends strictly on the presence and viability of microorganisms, it is capital to have methodologies that allow rapid, reliable and effective monitoring of the biological activity in commercial-scale biohydrometallurgical processes. Therefore, during monitoring of sulphide leaching heaps, the existing biological activity must be known at different sampling points in the leaching heap to ensure efficient metal recovery and to take corrective measures whenever necessary. However, currently there are no modern methodologies that can effectively replace classic techniques for determining total viable bacteria, which have a very long analysis time (between 7 and 14 days) and therefore do not allow carrying out the required corrective actions in time.

Microorganism detection in bioleaching heaps are currently carried out by using different classic methods for determining total and viable leaching bacteria. The total microbial count can be determinate by direct count using a phase contrast microscope and a Petroff-Hausser chamber. The culture-dependent viable count of microorganisms is based on determining microbial growth in different adequate culture media (Harrison, 1984; Johnson, 1995; Lafleur *et al.*, 1993).

In the last years, the use of molecular biology techniques has become a very powerful tool to identify leaching microorganisms without microorganism culturing (Pizarro *et al.*, 1996; Vásquez, Moore and Espejo, 1999; Bond, Smriga and Banfield, 2000 Coram and Rawling, 2002; Demergasso *et al.*, 2005 Remonsellez *et al.*, 2009).

Despite these techniques give valuable qualitative and quantitative information about the diversity of microbial communities that take part in leaching processes or are responsible for acid mine drainage formation, such techniques are not able to give an account of the activity or viability of such microorganisms.

An interesting alternative is the detection of intracellular microbial ATP by means of an enzyme reaction (luciferin-luciferase) according to Reaction 1.

$$\text{D-Luciferin} + \text{ATP} + O_2 \xrightarrow{\text{Luciferase}} \text{Oxyluciferin} + \text{AMP} + \text{PPi} + CO_2 + \text{light} \quad (1)$$

The amount of light is proportional to the concentration of ATP in the original sample. Due to all metabolic active microorganisms show practically constant concentration of ATP, the reaction has been proven to be proportional to the amount of microorganisms present in solution (Hammes *et al.*, 2010; He et al., 2009).

Despite the relevant development of the bioluminescence-based methodology, none of these publications describe a technique that could be applied to detect the presence of active acidophilic microorganisms that are important in bioleaching processes or responsible for the production of acid mine drainage. Measurement of active acidophilic microorganisms present in these samples has always had significant difficulties. The main problems for the analysis using this technology are the presence of inorganic compounds (toxic metals, high ionic strength etc.), the extreme conditions in which these microorganisms proliferate (particularly dominated by very low pH values) and the low

cell density attained by these microorganisms in their growth. This has made their detection by means of regular bioluminescence techniques very difficult.

In a former presentation, we described the development of a rapid and simple method for detecting the presence of acidophilic microorganisms in aqueous samples (Cotoras and Viedma, 2011). This method is based on concentration and washing of the microbial cells, and detection of active microorganisms by means of its cellular ATP using a bioluminescent reaction. Unlike conventional methods, this technology enables the determination of active microorganisms in just 10 minutes. To facilitate implementation of this method a simple kit was designed. This kit, called LixKit®, contains all materials needed to make the determination. This work aimed at demonstrating the application of LixKit® as a real-time method for detecting the active microorganisms in commercial-scale biohydrometallurgical processes.

METHODOLOGY

Microorganisms and culture conditions

The microorganisms used in this study were *Acidithiobacillus ferrooxidans* strain ATCC 23270 and *Acidithiobacillus thiooxidans* strain DSMZ 14887. *Acidithiobacillus ferrooxidans* strain was grown in ferrous iron-containing modified 9K medium as described previously (Viedma, 2010), *Acidithiobacillus thiooxidans* strain was cultured in culture medium 71 in accordance with German Collection of Microorganisms and Cell Cultures (DSMZ) recommendations. 250 mL Erlenmeyer flasks containing 100 mL of the culture medium were used. Each flask was inoculated with 0.5 mL of a culture. Subsequently, the flasks were incubated in an orbital shaker at 30°C and 120 rpm.

Sampling of bioleaching solutions

Bioleaching solutions were obtained from the following mines: Minera Los Bronces, Minera Escondida, Minera Carmen de Andacollo, Minera Barrick Zaldivar, Minera Spence and Minera Quebrada Blanca. Each sample batch consisted of 4-8 samples each time. Samples were collected in 500 mL polypropylene clean jars. The luminescence assays were performed in the field site. Later on the samples were brought back to the laboratory for the following tests: total count of microorganisms, viable bacterial counting by the most probable number count (MPN) (Lafleur *et al.*, 1993).

Bioluminescence-based bacterial counting

The quantification of active bioleaching microorganisms was performed using the LixKit® (Biohidrica, Chile), according to the manufacturer's instructions. A volume of 10 mL of the microorganism culture to be assayed was passed through a filter holder with a 0.22 μm membrane using a syringe. The second stage of this assay consists in the removal of the agents that are inhibitory for the bioluminescence reaction. It was carried out by sequentially washing of the previously concentrated acidophilic microorganisms. In order to do that, 20 mL of solution 1 were passed through the filter and after that the filtrate was discarded. Then the membrane was rinsed with 20 mL of solution 2 and the filtrate was discarded again. Finally, using moderate pressure over a syringe plunger air is allowed to pass through the membrane to remove the remaining solution). A swab was

wetted by immersion in solution 2, and was rubbed against the surface of the membrane, keeping a constant pressure. The third stage of the assay corresponds to the extraction of intracellular adenosine-triphosphate (ATP) from the acidophilic microorganisms and the performance of the bioluminescence reaction. Finally, emitted light was immediately measured in a luminometer (Kikkoman Lumitester PD-20, Japan). The values obtained are expressed in relative light units (RLU). Figure 1 shows a schematic representation of the procedure applied for the quantification of active bioleaching microorganisms using the LixKit®

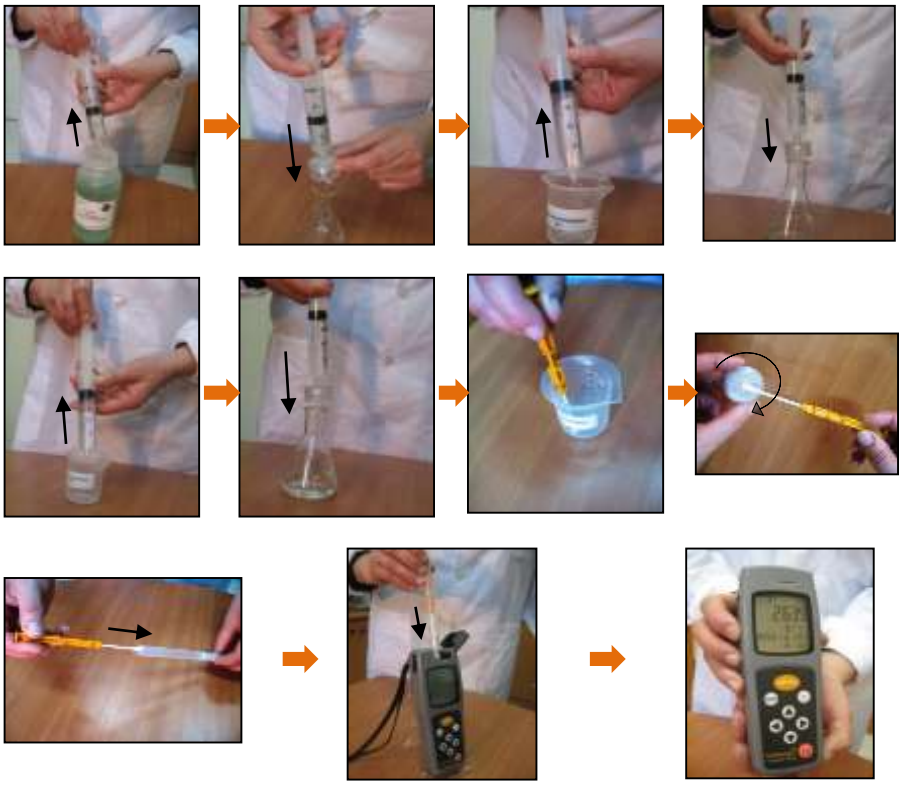

Figure 1 Steps of the quantification of active bioleaching microorganisms using the LixKit®

RESULTS AND DISCUSSION

Detection of active microorganisms in samples of commercial-scale heap and dump bioleaching processes using the LixKit® method

The goal of this work was to demonstrate the application of LixKit® as a real-time method for detecting the active microorganisms in heap or dump bioleaching samples. The main factors that could interfere with the analysis of these samples are low microbial concentration and presence of high concentrations of toxic metals and low pH. A total of 85 bioleaching solution samples were collected from different heap and dump bioleaching of six mining companies (Minera Los Bronces, Minera Escondida, Minera Carmen de Andacollo, Minera Barrick Zaldivar, Minera Spence and Minera Quebrada Blanca). These samples include 40 heap leaching solutions, 14 dump leaching

solutions, 10 raffinate solutions, 7 pregnant leaching solutions, 5 intermediate leaching solutions, 6 bioreactor solutions and 3 bioleaching column solutions. The purpose of this is to demonstrate the applicability of the method in different types of samples. The analysis by LixKit® was conducted immediately at the sampling place. This operation was done by a researcher. However, in some cases, the challenge for this new kit was even greater, since due to safety regulations, the operation had to be done only by professionals or technicians of the mining company. Subsequently, the same samples were analyzed in the laboratory by conventional methods of total count and viable count.

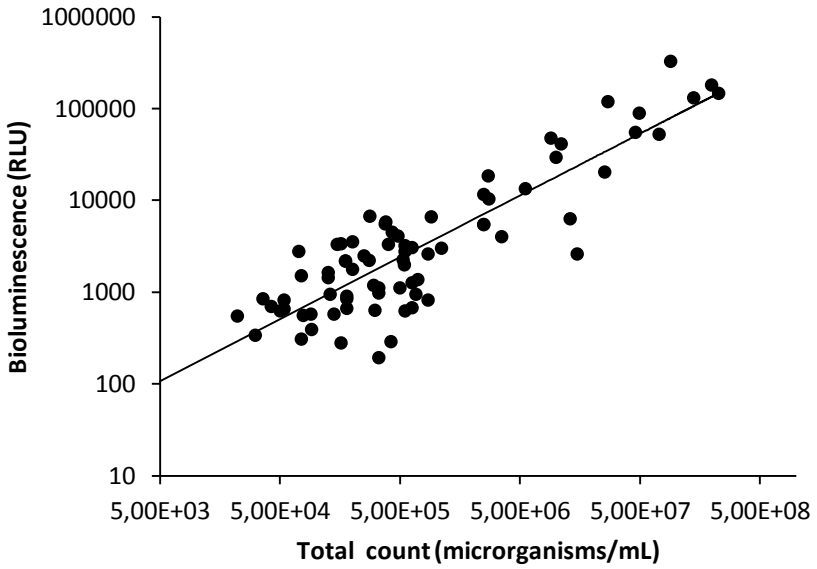

Figure 2- Correlation between the LixKit® bioluminescence method and total cell concentrations of heap and dump bioleaching solution samples and pure cultures of *A. ferrooxidans* and *A. thiooxidans* (R^2=0.774)

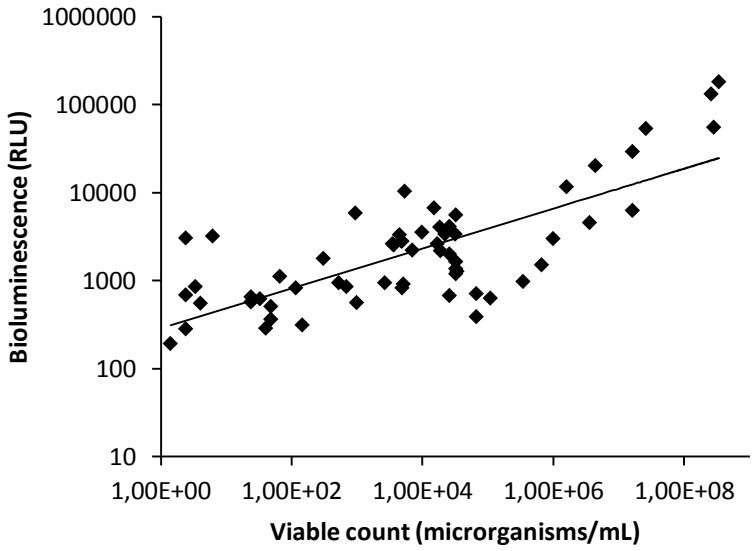

Figure 3- Correlation between the LixKit® bioluminescence method and viable cell concentrations (iron- and sulphur-oxidizing bacteria) of heap bioleaching solution samples and pure cultures of *A. ferrooxidans* and *A. thiooxidans* (R^2=0.565)

Figure 2 shows that although occurs dispersion in analyzing actual samples of different origin, there is a linear relationship between the LixKit® method based on bioluminescence and total count of microorganisms. The data show a correlation coefficient of 0.774, which is normally considered adequate when validating a new analysis technique. In this case, it is important to realize that they are being compared two methods of analysis based on completely different principles.

In a second comparison between the LixKit® method based on bioluminescence and the viable counting of microorganisms it is also possible to find a linear relationship (Figure 3). To obtain an estimate of total viable microorganisms, the viable counting iron- and sulphur-oxidizing bacteria microorganisms by MPN were added. The data show a correlation coefficient of 0.565. This correlation may be considered appropriate, since the comparison is between two methods, which are based on totally different principles of analysis. Furthermore, it should be noted that the conventional methodology for analysing viable microorganisms based on MPN has a high experimental error of around 30% (Lafleur *et al.*, 1993), significantly increases the deviation of the experimental data.

These results showed a significant correlation between this real time method and conventional methods even though the samples had very different origin, such as bioleaching solutions from heaps of different operating times, PLS or raffinate. The experience gained in the various mining companies also showed that LixKit® can be used without problem by an operator at the mining site.

Detection of the effect of environmental temperature on the microbial activity in samples of commercial-scale dump bioleaching processes and acid mine drainages using the LixKit® method

Detecting microbial activity in ROM dump leaching solutions operations represents a particular challenge because of the low concentration of microorganisms commonly found in these samples. A

similar situation occurs when trying to determine the microbial activity in acid mine drainage. It is important to note that in Chile dump bioleaching processes and generation of acid mine drainage occurs mainly in the high mountains. They are open systems that are directly affected by environmental temperature changes.

In this work, we sampled solutions that percolate from two ROM dump leaching operations and two solutions of the acid drainage in Minera Los Bronces. It was found possible to determine microbial activity of these samples with the LixKit®. Figure 4 shows the seasonal change of the monthly average temperature and microbial activity. All determinations of microbial activity followed the same trend than the average environmental temperatures. In July (monthly average temperature: -6 °C) it was found the lowest bioluminescence, which clearly shows an effect of environmental temperature on the microbial metabolic activity. This shows the good performance of the kit in extreme conditions where the microbial detection is difficult due to low population densities.

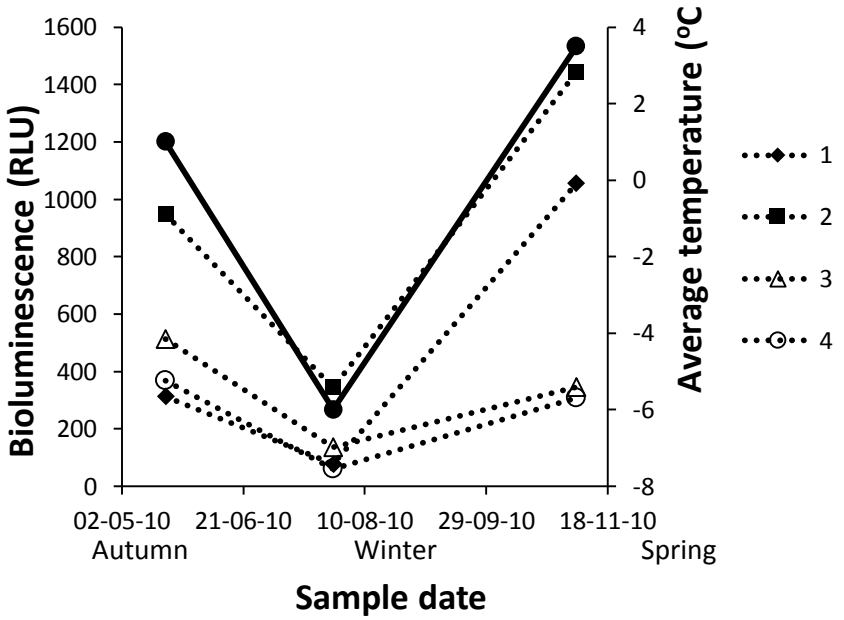

Figure 4 Seasonal change of the monthly average temperature and microbial activity, measured as bioluminescence (LixKit® method) in two samples of ROM dumps leaching solutions and two samples of acid mine drainage. (1) Acid mine drainage 1, (1) acid mine drainage 2, (3) dump leaching solution 1, (4) dump leaching solution 2

CONCLUSIONS

These results showed a significant correlation between this real time method and conventional methods. The simplicity of LixKit® allows it to be used without problem by an operator at the mining site.

In conclusion, the following are the advantages of LixKit® for monitoring the activity of microbial populations in a bioleaching plant:

- Rapid determination (10 minutes)
- Easy operation (kit ready for use)
- High reproducibility
- Allows measurements in the field
- Determines the metabolic activity of leaching microorganisms -"health" of the microflora
- Facilitates the metallurgical and operational decisions

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NOMENCLATURE

AMP	Adenosine monophosphate
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
MPN	Most Probable Number
PCR	Polymerase chain reaction
PPi	Inorganic pyrophosphate
RLU	Relative light units
ROM	Run of Mine

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