

A RAPID AND SIMPLE METHOD FOR DETECTION OF ACTIVE ACIDOPHILIC MICROORGANISMS IN COPPER BIOLEACHING PROCESSES

Davor Cotoras and Pabla Viedma
BIOHIDRICA
Biotecnologías del Agua Ltda.
Santiago, Chile

Introduction

- The whole bioleaching process depends principally on the presence and viability of microorganisms.
- Therefore, it is imperative to have methodologies that allow rapid, reliable and effective monitoring of the biological activity in commercial-scale biohydro-metallurgical processes.
- The objective of this work was to develop a rapid method for detection of the presence of acidophilic microorganisms in aqueous samples, such as bioleaching solutions or acid mine drainages.

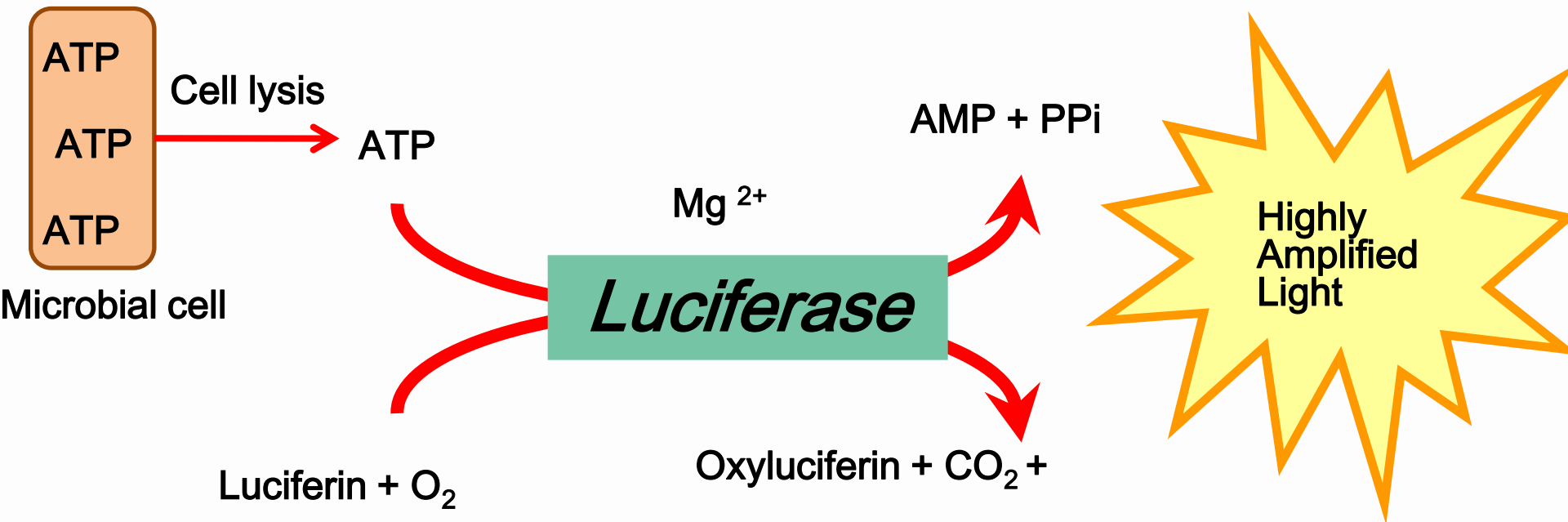
Current Methods

Method	Objective	Analysis Time (approx.)
Total bacterial count	Determination of viable + death microbial cells	24 hours
Culture-dependent viable count	Determination of viable bacteria	15 days
Fluorescent in situ hybridization (FISH) and (CARD-FISH)	Identification of bacteria and archaea directly from other cells present in a mixture (viable + death bacteria)	3 days
Real-time quantitative PCR	Quantification of microbial populations in community (viable + death bacteria)	20 days

Problem

- 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.

Principle of the test



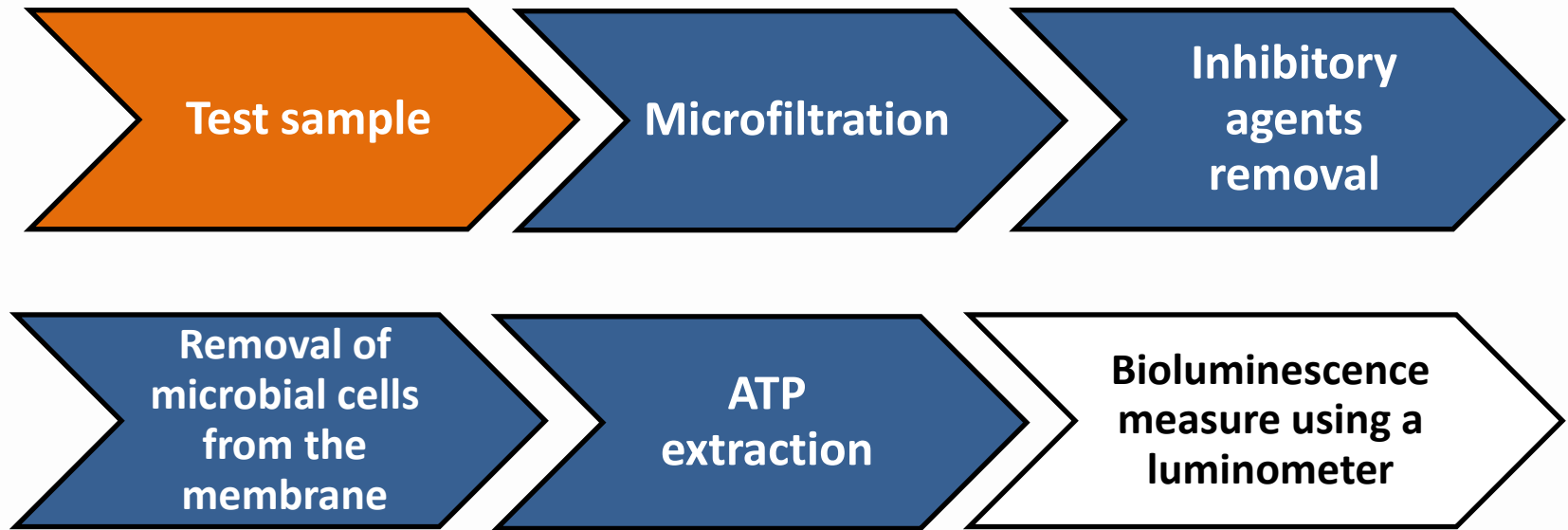


Figure 1. Method overview of the quantification of active bioleaching microorganisms using the LixKit[®].

Bioluminescence Method - LixKit[®]

The method comprises the following steps:

- a) concentrating acidophilic microorganisms from a given volume of aqueous sample
- b) removing the agents that are inhibitory for the bioluminescence reaction by washing the previously concentrated acidophilic microorganisms by means of two treatments with aqueous washing agents; and
- c) extracting adenosine-triphosphate (ATP) from the acidophilic microorganisms and measuring the light generated by such ATP by means of a bioluminescence detection system.

Table 1. Comparison of method of microscopic total bacterial counting in a Petroff-Hausser chamber and the bioluminescence method for the detection of *Acidithiobacillus ferrooxidans* at different culture times.

Culture time (days)	Total count method (bacteria/cm ³)	Bioluminescence method (RLU) ¹
3	3,4 x 10 ⁷	21.817
5	1,8 x 10 ⁸	21.000
12	1,2 x 10 ⁸	26.517
22	3,2 x 10 ⁸	2.703
48	2,9 x 10 ⁸	963
70	6,7 x 10 ⁷	849

¹ Triplicate average measurement

Table 2. Comparison of method of microscopic total bacteria counting in a Petroff-Hausser chamber and the bioluminescence method for the detection of *Acidithiobacillus thiooxidans* at different culture times.

Culture time (days)	Total count method (bacteria/cm ³)	Bioluminescence method (RLU) ¹
5	3,33 x 10 ⁸	114.833
12	1,85 x 10 ⁸	53.377
20	2,73 x 10 ⁸	36.637
27	2,00 x 10 ⁸	470

¹ Triplicate average measurement

Table 3. Comparison of method of microscopic total bacterial counting in a Petroff-Hausser chamber and the bioluminescence method for the detection of *Leptospirillum ferrooxidans* at different culture times.

Culture time (days)	Total count method (bacteria/cm ³)	Bioluminescence method (RLU) ¹
7	5,85 x 10 ⁸	17.324
13	1,93 x 10 ⁸	1.163

¹ Triplicate average measurement

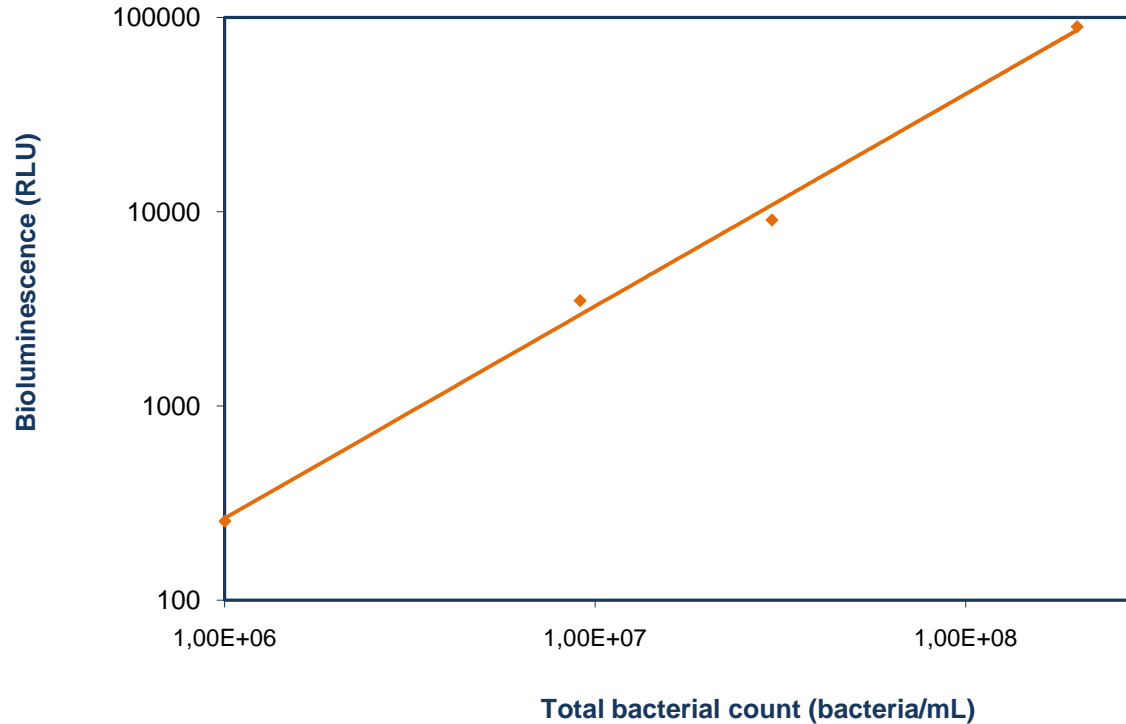


Figure 2. Plot of bioluminescence results (RLU) determined by means of the method for detection of the presence of *Acidithiobacillus ferrooxidans* by bioluminescence, compared to total bacterial count determined in a Petroff-Hausser counting chamber ($R^2 = 0.9980$)

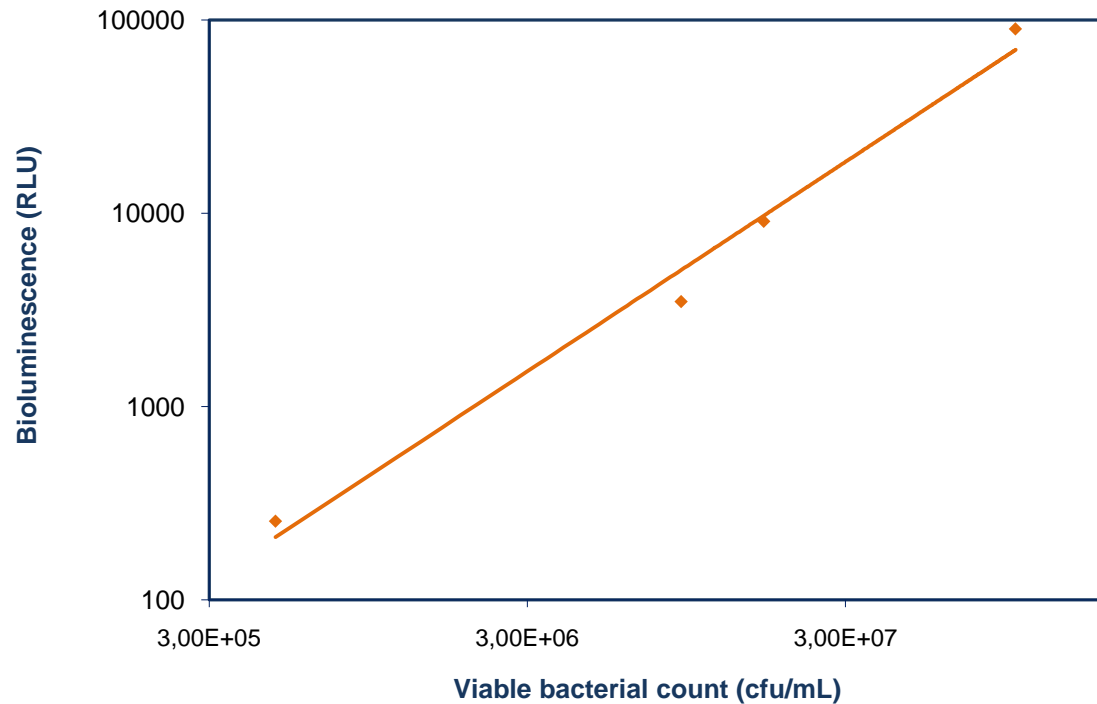


Figure 3. Plot of bioluminescence results (RLU) determined by means of the method for detection of the presence of *Acidithiobacillus ferrooxidans* by bioluminescence, compared to viable bacterial count (cfu/cm³) determined by the floating filter method (R2 = 0.9862)

Patents

Granted Patents

- USA: US Patent 7,851,177 (2010)
- Sudáfrica: N° 2007/06363 (2008)
- Germany: N° 102007035588.4-09 (2008)

Applied Patents

- Australia: AU2007203563
- Chile: N° 2002 -2006
- Canada: CA20072595392 20070731
- Peru: PE993-2007

Practical Application of the Bioluminescence Method - LixKit[®]



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

CONCLUSIONS



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- The LixKit[®] is an innovative technology and a powerful tool for monitoring the activity of microbial populations in a bioleaching heap at the mining plant.
- The bioluminescence method can measure the metabolically active microorganisms.
- This feature is very important because detection of metabolically active microorganisms is a relevant parameter for the operation and control of industrial scale bioleaching processes.