**Novel biosupport material for *A. ferrooxidans* immobilization**

Arrate Santaolalla1*\**, Naiara Rojo1, Juncal Gutierrez1, Gorka Gallastegui1, Astrid Barona2

Department of Chemical and Environmental Engineering; University of the Basque Country UPV/EHU

1 Faculty of Engineering Vitoria-Gasteiz. Nieves Cano, 18. 01006 VITORIA-GASTEIZ (SPAIN)

 2 Faculty of Engineering of Bilbao. Torres Quevedo, 1. 48013 BILBAO (SPAIN)

*\*Corresponding author:* *arrate.santaolalla@ehu.eus*

Highlights

* Bacterial cellulose (BC) is an innovative support for biomass immobilization.
* The active material *A. ferrooxidans*+BC can be used in continuous bioleaching processes.
* The immobilized biomass remains active even at 15 g Cu2+/L.

**1. Introduction**

Bioleaching is a microorganism-assisted process used to dissolve metals through the action of oxidizing agents. It has been proposed as a sustainable alternative to conventional micromachining and extracting processes for the recovery of valuable metals [1].

Several authors have investigated the immobilization of the bacterium *Acidithiobacillus ferrooxidans* (the most widely studied microorganism for metal bioleaching) on different support materials with a view to increasing microbial density inside the bioreactor and facilitating biomass replacement when required [2,3]. Bacterial cellulose (BC) is a promising candidate to be used as a support material for biomass immobilization due to its peculiar 3D network structure of ribbon-shaped cellulose nanofibrils (2-4 nm in diameter) and its high specific surface [4].

This study’s main objective was to assess the response of an innovative active material when used to immobilize *A. ferrooxidans* bacteria. An immobilization methodology was proposed, and the study focused on the influence that the number of attached bacteria and the concentration of dissolved copper had on the time required to recover the oxidant.

**2. Methods**

Bacterial cellulose (BC) was synthesized following the procedure described by Retegi et al. [5]. Bacteria were immobilized on BC by immersing the biopolymer into an Erlenmeyer flask with a nutrient medium containing 6 g Fe2+ L-1. A 5% inoculum of *A. ferrooxidans* in the exponential growth phase was added, and cells were cultivated until the complete oxidation of the Fe2+ to Fe3+ (Figure 1a). Thus, the active BC was obtained (*A. ferrooxidans*+BC).

The relationship between the number of immobilized bacteria and Fe2+ oxidation time was studied by culturing 4, 6 and 8 pieces of the active BC (20x20x7mm) in a fresh medium. In addition, a study was conducted on the effect of copper concentration on the activity of the immobilized bacteria by cultivating active BC pieces in nutrient media containing 0, 5, 10 and 15 g Cu2+ L-1.

1.  b- 

**Figure 1.** a- Preparation of the active BC; b- SEM analysis of A. *ferrooxidans* attached to the BC.

**3. Results and discussion**

*A. ferrooxidans* cells successfully colonized the support material, and the time required for the microorganisms to oxidize the Fe2+ was not affected by the presence of the BC. It is noteworthy that only one growth cycle was required to obtain the active material (Figure 1b).

The average Fe2+ oxidation rate with eight BC pieces was about 25% higher than with four pieces. Regarding the inhibitory effect of dissolved copper on the biological activity of A. *ferrooxidans*, the oxidation time increased linearly with copper content (up to threefold for the highest metal concentration). Nevertheless, when the immobilized bacteria previously exposed to the three concentrations of copper were cultivated in a second cycle, the time needed for the complete conversion of Fe2+ was significantly reduced, being similar in all the bioreactors. The most significant difference was recorded in the flask with 15 g Cu2+ L-1, where the oxidation time was reduced by 68% compared to that in the first oxidation cycle. These results suggest that the attached bacteria gradually adapted to the presence of Cu2+. Moreover, active BC pieces adapted to 5 and 10 g Cu2+ L-1 were not inhibited when cultivated in media containing 15 g Cu2+ L-1 (in a third cycle). These results confirm the successful adaptation of the immobilized bacteria regardless of the initial copper concentration.

**4. Conclusions**

Bacterial cellulose is an outstanding innovative support material for the immobilization of *A. ferrooxidans,* and therefore a promising carrier for continuous bioleaching processes. Oxidation time was reduced according to the number of active BC pieces, and the immobilizedbacteria gradually adapted to the toxicity of high concentrations of dissolved copper, even at 15 g Cu2+/L.

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