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Sequential bioleaching of copper from brake pads residues using encapsulated bacteria

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ABSTRACT

Bioleaching of copper from pure granular shots and from a "pre-consumer" secondary resource from automotive industry (Brake Pads Powder, "BPP") was carried out in a comparative study with conventional planktonic and PVA-encapsulated micro-organisms using a mixed culture of iron oxidizing bacteria: *A. ferrooxidans*, *L. ferrooxidans* and *L. ferriphilum*. The global process is characterized as "sequential" since a preliminary acid leaching is performed for ferrous iron extraction from the BPP material before the copper bioleaching itself.

Prior to bioleaching experiments, resistance to dissolved copper has been quantified at various concentrations to assess a potential protective effect of the biomass by the PVA-matrix. PVA-encapsulated bacteria showed a better resistance to dissolved copper with a linear progression in the ferrous iron oxidation kinetic up to 40 gCu^{2+}/L concentrations while planktonic cells presented a drastic decrease in this kinetic between 10 and 40 gCu^{2+}/l, resulting in a 2 to 3-fold kinetic increase for the encapsulated bacteria depending on the strain. Bioleaching of elemental granular copper in 9 K medium (Fe^{2+}: 10 g/L) showed significant enhancement of copper leaching kinetic with encapsulated biomass ($1.9 \cdot 10^{-1}$ gCu^{2+}/L h) in comparison with the planktonic mode ($8.2 \cdot 10^{-2}$ gCu^{2+}/L h). These tests demonstrated thereby the role of ferrous iron bio-oxidation and acid consumption values close to the stoichiometric ratio. Finally, the bioleaching with encapsulated bacteria was applied to copper extraction from BPP and showed a 150% enhanced kinetic in comparison with equivalent planktonic system and a 300% increase vs abiotic mode with respective leaching rates of $4.43 \cdot 10^{-2}$; $2.9 \cdot 10^{-2}$ and $1.48 \cdot 10^{-2}$ gCu^{2+}/L h.

Cell immobilization offers complementary benefits as continuous process and permanent bacteria acclimation. This technology presents an interesting alternative to conventional planktonic and "two-step" processes.

1. Introduction

Bio-hydrometallurgy is a cost effective and eco-friendly technology complementary to hydro- and pyro-metallurgy for the circular economy of metals since it provides 20–25% of the total world copper production from low grade ores or primary resources (Brierley, 2008). It offers opportunities in recycling metals from metallurgy solid wastes and by-products (Solisio et al., 2002; Massinaie et al., 2006; Bakhtiari et al., 2008a,b; Vestola et al., 2010; Kaksonen et al., 2011) and from "post-consumer" secondary resources like WEEE (Brandl et al., 2001; Ilyas et al., 2007; Bi et al., 2011), printed circuit boards (Wang et al., 2009; Yang et al., 2009; Liang et al., 2013; Rodrigues et al., 2015) and end-of-life vehicles (Lewis et al., 2011; Lambert et al., 2015). Combined to engineering improvements for the design and control of bioreactors (Vakylabad et al., 2012; Mandl et al., 2014), biohydrometallurgy studies systematically aim to reach higher pulp densities (solids loadings) and to reduce energy consumption in order to bring the process to technical and economic feasibility.

Considering secondary resources, the basic principle of polymetallic wastes bioleaching consists in a combination of oxidation and acid leaching. Acid leaching is supported by a production of sulfuric acid by acidophilic autotrophs, e.g. *Acidithiobacillus thiooxidans*, in presence of thiosulfate/sulfur/sulfide; while oxidation is performed by ferric iron generated by acidophilic iron-oxidizing autotrophs, e.g. *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*.

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Amongst parameters limiting the process development and industrial scaling-up for secondary resources bioleaching, alkalinity of the wastes, toxicity of heavy metals or associated organic compounds (fire retardants, waxes, plastics, ...) and shear stress generated by fine solid particles were identified as major inhibitory effects limiting the maximal pulp density to 1–10% w/w depending on the residues nature (Rossi, 2001; Massinaie et al., 2006; Ilyas et al., 2010). In order to reach higher pulp densities, alternative operating modes have been introduced (Brandl et al., 2001; Tipre and Dave, 2004; Rodrigues et al., 2015).

With the same attempt, the concept of encapsulated microorganisms can be introduced since it showed advantages in various field: high microbial density, increased metabolic activity, protection against toxic compounds, (semi-)continuous process development (Cassidy et al., 1996; Martins et al., 2013). In the specific frame of bioleaching, this concept has shown long term performance and high ferrous iron oxidation activity in batch and continuous modes with reported oxidation rates ranging from 1.89 to 4.6 gFe^{2+}/L h (Long et al., 2004a,b; Yujian et al., 2006, 2007). In this framework, cell encapsulation could limit the diffusion of inhibiting organic compounds dissolved from the secondary resources from the medium into the near environment of the autotrophic bacteria. In the field of biohydrometallurgy, cell encapsulation appears as a potential method to build microbial communities leading to targeted and efficient population dynamics as reported in biofilm studies (Nemati et al., 1998; d'Hugues et al., 2008). Indeed, bacterial encapsulation could offer the opportunity to qualitatively and quantitatively control the development of a consortium through selected initial inoculation ratios and their preservation to a certain extent by a steric hindrance effect during cell growth in the matrix. This technique could allow, for example, the development of synergistic or mutualistic consortia as described in Johnson (2008).

This work reports an innovative approach combining bacterial cell encapsulation and bioleaching. It aims to validate the advantages and the protective effect of cell encapsulation in the bioleaching of secondary resources. In a first step, the protective effect is evaluated through dissolved copper resistance tests. Bioleaching efficiencies are then compared for planktonic and encapsulated bacteria in an intermediary experiment on granular copper shots and finally on a "model secondary resource": Brake Pads Powders, BPP (dust generated by the manufacturing of brake pads).

2. Materials and methods

2.1. Microorganisms and culture conditions

Mesophilic bacteria used in this study were obtained from the German National Culture Collection (DSMZ, Braunschweig, Germany) and include *Acidithiobacillus ferrooxidans* (DSM 11477), *Leptospirillum ferrooxidans* (DSM 2391) and *Leptospirillum ferriphilum* (DSM 14647). These strains were used as pure cultures in the copper resistance tests and as mixed culture combining the three strains in elemental copper and BPP bioleaching experiments. The nutrient medium used for cell growth and bioleaching experiments was the Silverman and Lundgren 9K liquor (1959). It contains (g/L): $(NH_4)_2SO_4$, 3.0; K_2HPO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.5; KCl, 0.1; $Ca(NO_3)_2$, 0.01. The basal salts solution was sterilized at 121 °C for 20 min. Ferrous iron concentration was adjusted at desired concentration by adding adapted volumes of a 25% $FeSO_4{\cdot}7H_2O$ solution at pH 1.70 ± 0.1 and filter-sterilized (0.22 µm, Chromfil Xtra; Macherey-Nagel).

Prior to bioleaching experiments, encapsulation and planktonic microorganisms were acclimated to dissolved copper or to the BPP. Before elemental copper bioleaching, microorganisms were subcultured at least 5 times in 9 K medium (10 g/L Fe^{2+}) supplemented with 5 g/l Cu^{2+}. The same method was applied prior to the BPP bioleaching with a pulp density of 0.5% of BPP.

2.2. Microorganisms encapsulation

Encapsulation mix: Polyvinyl alcohol or PVA (Kuraray POVAL 15–99, hydrolysis degree 99+ %mol), 9%; Na-Alginate (Manucol DM, ISP), 1%; distilled water 85% and concentrated bacterial cell suspension 5%. PVA and alginate were heated (85–100 °C) in water until complete dissolution; bacterial suspension was added after cooling down the solution to room temperature. Concentrated bacterial cell suspension was collected by ultrafiltration of late exponential phase cultures and were washed by two successive centrifugation (at 20,000*g*, Sorvall RC6+) and re-suspension (in 9 K medium) in order to discard ferric iron interfering with the hydrogel formation. The encapsulation cell suspension was extruded dropwise by gravity through 60 mL syringe opening (Omnifix, B-Braun) in the crosslinking solution ($CaCl_2{\cdot}2H_2O$, 2% (Merck); boric acid (>99.8%, Roth), 5% and gently stirred at room temperature during 1 h. The material was then stabilized during 1 h at room temperature by Walden inversion reaction (adapted from Zain et al., 2011) in a solution containing Na_2SO_4, 4.5% (Merck); $FeSO_4{\cdot}7H_2O$, 5.5% (Roth); pH adjusted at 2.5 with concentrated sulfuric acid. A volume of 2500 mL of microorganisms culture is used to produce 100 mL of PVA-Alginate beads, these beads were always cultured or used in bioreactors in 9 K medium (10 g/L Fe^{2+}) at a volumic ratio of 20% (v/v). This method implies a 5-fold microbial concentration in comparison with a planktonic late exponential culture. This "concentration factor can be considered as minimal since, after the encapsulation process, the inoculated PVA-beads are cultured in 9 K medium in order to promote internal cell growth. The maximal internal cell concentration in the encapsulation matrix is reached when time for 100% oxidation of ferrous iron in the medium was minimal and reproducible from one cultivation batch to the next one (typically 5–6 batches, minimal iron oxidation time: 24 h). This propagation step is achieved on orbital shaker (150 rpm, 30 °C) in 6 L spherical glass flasks.

Encapsulated biomass used in this study had performed 15 prior batches of ferrous iron oxidation/BPP bioleaching at several pulp densities over a 6 months period.

2.3. Copper resistance tests

Copper resistance test were performed with $CuSO_4$ (anhydrous, Merck). These tests were carried out in 125 mL cylindro-conical flasks containing 45 mL 9 K medium with 50 g/L $FeSO_4{\cdot}7H_2O$ Copper sulfate was added to reach selected Cu^{2+} concentrations ranging from 0 to 40 g/L. Inoculation of planktonic experiments was achieved by addition of 5 mL of late exponential phase pure culture (strain specific study). In encapsulated mode, with the objective of equivalent bacterial cell load as in planktonic experiments, the final volumic biomass load in PVA-beads after propagation steps was evaluated by specific oxygen consumption measurements. Indeed, due to irreversibility of PVA matrix, it is impossible to achieve conventional counting of cells after hydrogel depolymerization. Hence, the microbial load in a given mass of PVA matrix was evaluated by its specific volumic oxygen uptake rate (SOUR). The mass of beads involved in copper resistance tests (45 mL medium, 10 g/L Fe^{2+}) is then adapted to reach the same SOUR as the one measured for a 10% inoculated planktonic culture. From this method, it can be considered that the planktonic and encapsulated experiments start with the same inoculated cell concentration. This was achieved with the purpose of rational comparison of both systems: since ferrous iron oxidation kinetic was used