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Comparison of Cu, Zn and Fe bioleaching from Cu-metallurgical slags in the presence of *Pseudomonas fluorescens* and *Acidithiobacillus thiooxidans*

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A B S T R A C T

Metal leaching from metallurgical wastes (slags) by means of environmentally friendly approaches is promising for practical applications. The goal of this study was to compare the feasibility of metal bioleaching from Cu slags by means of *Pseudomonas fluorescens* and *Acidithiobacillus thiooxidans*. Two size particles (<0.3 mm and 1–2 mm) of two types of Cu slags (massive crystalline slag and granulated amorphous slag) were used to study metal (Cu, Zn and Fe) bioleaching. The 40-days bioleaching experiments with *P. fluorescens* began at circumneutral pH (7.0), whereas the experiments with *A. thiooxidans* were started under acidic (pH 2.5) conditions. The results demonstrated that *A. thiooxidans* catalyzes metal leaching from both slag types investigated. After 21 days of incubation, optimal leaching was achieved and up to 79% Cu, 76% Zn and 45% Fe could be extracted from crystalline slag under conditions of 1 wt.% pulp density and particle size <0.3 mm. The optimal efficiency achieved with amorphous slag was 81% Cu, 79% Zn and 22% Fe when 1% pulp density and 1–2 mm particle size were used. The use of *P. fluorescens* resulted in poor leaching efficiencies as compared to the performance of *A. thiooxidans*, presumably due to the higher pH conditions maintained during the *P. fluorescens* incubations. The maximum metal leaching efficiencies with *P. fluorescens* were achieved at 1% pulp density and particle size <0.3 mm and did not exceed 10% Cu, 4% Zn, 0.3% Fe for crystalline slag and 4% Cu, 3% Zn, 0.7% Fe for amorphous slag. Both slags exhibited a good potential for bioleaching with *A. thiooxidans*, however; further optimization of the process parameters (*e.g.* pulp density, particle size and pH) is needed to improve the efficiency.

1. Introduction

Industrial smelting activities have produced huge quantities of by-product slags that still contain significant amounts of residual metals (*e.g.* Cu > 1 wt.%) of environmental concern. Currently, these wastes are used as supplementary materials for civil engineering applications such as cement and concrete additives, road bed filling materials or as hydraulic construction materials (Ettler et al., 2001; Shi et al., 2008; Ettler et al., 2009; Piatak and Seal, 2010; Harish et al., 2011; Chen et al., 2012; Schmukat et al., 2012). In contrast, the use of former pyrometallurgical techniques has led to the disposal of metal-laden slags nearby smelter complexes. Any management practice carries the risk of the release of metallic elements to the environment (Gee et al., 1997; Manz and Castro, 1997; Sobanska et al., 2000; Parsons et al., 2001; Lottermoser, 2002; Ettler et al., 2003; Piatak et al., 2004; Kierczak et al., 2013; Ettler and Johan, 2014). The presence of metal at excessively high concentration levels in the environment is undesirable, because this may lead to deleterious effects on living organisms and bioaccumulation of metals at higher levels of the food chain. Therefore, slags must be managed in a mode that ensures maximum

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environmental protection (Schmukat et al., 2012, 2013).

The quantities of metals present in Cu slags are usually too low and are considered to be economically non-viable for recycling by any traditional recovery process. On the other hand, the slags would be more appropriate for building and construction materials or for any other reuse strategies after their metal content is reduced. Thus, strong emphasis has recently been placed on novel leaching and recovery technologies.

Leaching is a promising treatment and metal recovery route for Cu slags. An important advantage of this approach is the potential yield of Cu and other metals whose natural reserves are progressively depleting (Prior et al., 2012). Several approaches for metal leaching from Cu slags have been reported in the literature (Potysz et al., 2015: references therein). Chemical extraction with various leaching agents such as sulfuric acid, ferric chloride, ammonium chloride, ferric sulfate, ammonium sulfate as well as hydrochloric and nitric acids were found to rapidly and efficiently solubilize metals (up to 99% of bulk content) from slags. An important disadvantage of chemical processes is the costs involved, mainly associated with chemical reagents and energy demands (Yang et al., 2010). For this reason, the use of microorganisms (bioleaching) for metal extraction has been investigated as an environmentally friendly and economically viable alternative (Banza et al., 2002; Vestola et al., 2010; Erüst et al., 2013; Vainshtein, 2014; Sukla et al., 2015).

The bioleaching process is based on the ability of microbial mediated biochemical reactions to dissolve phases present in slags and solubilize valuable metals (Ehrlich, 1998; Suzuki, 2001; Watling, 2006; Gadd, 2010). The processes involved in bioleaching include acidolysis, complexation and redox transformations. Autotrophic bacteria such as *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferroxidans* have been used commonly for bioleaching various materials such as slags, fly ashes and electronic wastes (Krebs et al., 2001; Brandl et al., 2001; Ishigaki et al., 2005; Vestola et al., 2010). The effectiveness of those bacteria was mainly attributed to their ability to lower the pH through sulfuric acid production. Acidic conditions are favorable for metal extraction (Suzuki, 2001; Lee and Pandey, 2012) due to proton replacement of metals entrapped in phases. *A. thiooxidans* and *A. ferroxidans* utilize elemental sulfur as an energy source and the bioprocess corresponds to the following acid producing reaction:

$$S + 1.5O_2 + H_2O \rightarrow H_2SO_4 \rightarrow SO_4^{2-} + 2H^+ \quad (1)$$

Another important factor that renders acidophilic bacteria efficient is their tolerance to harsh environmental conditions including low pH (*e.g.* pH < 1) and high metal concentrations (Bosecker, 1997; Leduc et al., 1997; Watling, 2006; Erüst et al., 2013).

The ability of heterotrophic bacteria such as *Pseudomonas* sp. to leach the metals has also been reported (Brandl and Faramarzi, 2006; Cheng et al., 2009; Aouad et al., 2006; Aouad et al., 2008; Chen et al., 2014; Yin et al., 2014; van Hullebusch et al., 2015; Potysz et al., 2016a). Circumneutral pH conditions required for the growth of *Pseudomonas* sp. could imply a lower efficiency in terms of metal leaching because of the lower solubility of metals under these conditions. For example, 112-days exposure of Cu slags to *Pseudomonas aeruginosa* (pH 7.8) led to the extraction of Cu at levels not exceeding 8.9% (503 mg/kg) (Potysz et al., 2016a). However, excretion of extracellular organic metabolic compounds, for example siderophores, may be a factor playing a role in the extraction (Bosecker, 1997; Gadd, 2004; Sand and Gehrke, 2006). Microbially-derived metabolites assist the extraction process through proton detachment from the functional groups of the organic compounds creating binding sites with high complexation affinity towards metals. Proton attack of the mineral phase may lead to metal release and its subsequent complexation by the organic molecules.

Although the efficiency of bioleaching is known to be restricted by the tolerance of bacteria towards metals and longer treatment time as compared to chemical leaching, high metal extraction efficiencies can be achieved when process conditions are maintained at optimal levels for high microbial activity (Sukla et al., 1995; Brandl et al., 2001; Vestola et al., 2010). Optimal conditions for bioleaching require a number of parameters to be evaluated because the best leaching approach is specific to the chemical and mineralogical composition of the material subjected to the process. Therefore, the performance of different bacterial strains as leaching agents as well as operating parameters such as pulp density and particle size are important to ascertain the bioleaching performance. In this regard, an exploration of effective bioleaching methods and estimation of underlying benefits should be prioritized.

The objective of this study was to compare the ability of two different bacterial strains to be used as metal leaching agent for Cu metallurgical slags. *Pseudomonas fluorescens* (DSM 50091) was employed as the representative of heterotrophic bacteria, known to produce siderophores, whereas *A. thiooxidans* (DSM 9463) was selected as lithoautotroph known for its ability to produce sulfuric acid from reduced sulfur compounds. Two experimental designs were used for each microorganism. The experimental approaches considered the effect of the particle size (PS) (<0.3 mm and 1–2 mm) and pulp density (PD) (amount of slag [kg] per volume of solution [L]) (1 and 3%) on metal (Cu, Zn and Fe) bioleaching efficiencies. The following questions were addressed in this study: i) what is the efficiency of *P. fluorescens* and *A. thiooxidans* in terms of metal leaching from Cu slags?, ii) what conditions are appropriate for further process development?, and iii) which slag type exhibits greater potential for bioleaching?

2. Materials and methods

2.1. Cu slags

Two representative samples of Cu pyrometallurgical slags were chosen to test the feasibility of a bioleaching process. Granulated amorphous (modern: XXI century) slag (AS) represented glassy material, whereas massive crystalline (historical Medieval) slag (CS) was mainly composed of fayalite. Bulk concentrations of metals of interest (Cu, Zn and Fe) and overview of phase compositions are presented in Table 1. A detailed characterization of the slags is given elsewhere (Potysz et al., 2016b).

2.2. Bacterial strains

2.2.1. Acidithiobacillus thiooxidans

The gram-negative *A. thiooxidans* (DSM 9463) bacterial strain was grown in salt medium composed of 2 g ammonium sulfate ($(NH_4)_2SO_4$), 0.25 g of magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 0.1 g dipotassium hydrogen phosphate (K_2HPO_4), 0.1 g potassium chloride (KCl) and 1% (*wt./v*) of elemental sulfur (S) per 1 L of ultrapure water. The pH of the growth medium was adjusted to 2.5 and bacteria were grown two times over one week in order to adjust to pH conditions lower than its optimal (pH = 3.5). 2% (*v/v*) of pregrown inoculum was introduced to each biotic batch flask.

2.2.2. Pseudomonas fluorescens

The gram-negative *P. fluorescens* (DSM 50091) bacterial strain was purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen). This bacterium was initially grown in