Minerals Engineering 75 (2015) 70–76

Contents lists available at ScienceDirect

Minerals Engineering

journal homepage: www.elsevier.com/locate/mineng

SEM and EDS observations of carrollite bioleaching with a mixed culture of acidophilic bacteria

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ARTICLE INFO

Article history:
Received 21 July 2014
Revised 23 November 2014
Accepted 5 December 2014
Available online 8 January 2015

Keywords:
Carrollite
Bioleaching
Mesophilic bacteria
SEM
Mineralised polymer substances

ABSTRACT

Bioleaching of high purity carrollite minerals with mesophilic bacteria was carried out and monitored by observations in scanning electron microscopy (SEM) and elemental X-ray microanalysis (EDS) to provide evidence of the interaction pattern between carrollite and microorganisms. A bacterial consortium involving three different acidophilic chemolithotrophs was adopted. The evolution of the surface topography, inside alteration effects and elemental composition of the mineral with leaching time was followed. It could be postulated that bacterial adhesion takes place on the mineral surface, resulting in the formation of dissolution pits of various shapes and continues by boring elongated channels deep inside the mineral grains. Enhanced concentration of ferric iron and sulphur could be assumed in vicinity of the zones where mineralized polymer substances are precipitated. It could be inferred that carrollite dissolution is governed by cooperative bioleaching involving oxidation induced by bacteria attached to the surface and ferric iron re-oxidized by planktonic bacteria in suspension.

1. Introduction

The ore deposits belonging to the Central African Copperbelt situated between the Democratic Republic of Congo (DRC) and Zambia are known to host vast amounts of cobalt. About half of the global production of cobalt originates from this region (Yager, 2010). These deposits are mainly of sulphide types with carrollite being the major cobalt bearing mineral. Hydrometallurgy is by far the most common way of treating cobaltiferous ores encompassing copper sulphides with cobalt and copper recovered from the pregnant leach solutions through solvent extraction. The metal bearing sulphate solutions are purified and high-purity copper and cobalt are electrowon (Habashi, 1997).

Beyond being a natural phenomenon, bioleaching has turned into a technique which is based on the ability of microorganisms (bacteria) to transform solid phases into soluble and extractable compounds which can be subsequently recovered (Akcil and Deveci, 2010; Ehrlich, 2004). Recent trends, like growing demand for metals coupled with the increasing complex mineralogy of the ore deposits leading to high exploitation costs with conventional methods are additional factors that promote bioleaching as an attractive hydrometallurgical process. Plenty of studies have proved that the oxidation rate of many sulphide minerals is markedly accelerated when mesophilic bacteria are present (Dziurla et al., 1997; McGuire et al., 2001; Rawlings, 2002). Recently published figures indicate that about 20–25% of the world copper production is derived from bioleaching (Brierley, 2008). Apart from copper, uranium and cobalt, other metals such as nickel and zinc are potential candidates for bioleaching (Brierley, 2010; Gericke et al., 2009). As of recently, biomining has been expanding into full-scale operation in Talvivaara, Finland for recovery of nickel and cobalt (Riekkola-Vanhanen, 2012).

Microorganisms related to the species *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* are characterized as Gram-negative, aerobic and chemoautotrophic, operating at ambient temperatures (mesophilic bacteria). They are known to play an important role in the leaching and recovery of valuable metals from sulphide ores due to their acidophilic character (Brierley, 1997; Krebs et al., 1997). To explain degradation of sulphides, two indirect mechanisms have been proposed (Schippers et al., 1996; Schippers and Sand, 1999). The first one is based upon the oxidative attack of ferric iron on acid-insoluble metal sulphides involving thiosulphate as the main intermediate compound. The second one supposes likewise proton and/or ferric iron attack on acid-soluble metal sulphides, but with polysulphides and sulphur as intermediates.

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http://dx.doi.org/10.1016/j.mineng.2014.12.005
0892-6875/

Deeper understanding of the bioleaching phenomena requires fundamental studies not only on physiology, biochemistry and genetics of the microorganisms, but on the nature of reactions occurring at bacteria–mineral interface as well. Therefore, bacterial activity on sulphide mineral surfaces has been investigated with the ultimate aim of overcoming some process-limiting factors for rendering bioleaching as attractive alternative to conventional roasting or pressure oxidation. For instance, the observations that bacteria attach on the pyrite surface by means of a secreted biofilm encapsulating the mineral, as shown in the studies by Mustin et al. (1993), Rojas-Chapana et al. (1995, 1996) and Tributsch et al. (1998), imply that bacterially assisted dissolution of sulphides does not involve "non-contact" mechanism only with leaching by bacterially regenerated iron (III), but a "contact" bioleaching as well involving electrochemically supported dissolution occurring at the interface bacterial cell/mineral. The latter assumes a physical attachment of bacterial cells to the surface of sulphide minerals under aerobic conditions (Silverman, 1967). Scanning electron microscopy (SEM) and energy dispersive spectroscopy of X-rays (EDS X-ray microanalysis) have been intensively used to investigate the attachment patterns of bacteria on mineral surfaces (Jordan, 1993; Gómez et al., 1996; Sampson et al., 2000; Liu et al., 2011; Jiang et al., 2009).

However, regardless the number of studies performed so far, research focusing on carrollite behaviour during leaching virtually does not exist. To fulfil this need, the objective of the present study is to contribute in advancing the knowledge on bacteria–substrate interaction through observations into mineral surfaces following their contact with bacteria and to interpret the results in view their relevance to Co and Cu bioleaching.

2. Materials and methods

2.1. Mineral samples

High purity carrollite samples accompanied by their dolomitic gangue were handpicked from rich mineralized zones of the Kamoya deposit located in Katanga province of DRC. Selected samples between 25–50 mm in size have been then gently sliced in order to separate intact carrollite crystals from their gangue. The thus obtained mono-crystals of carrollite have been dry-ground using disc mill to obtain material with particle size below 0.053 mm for the leaching.

The elemental composition of both solid and liquid samples was determined by atomic absorption spectroscopy (Analytic Jena CONTRAA 300). X-ray diffraction unit Bruker D8-Advance was employed to obtain the mineralogical characteristics of the pure carrollite.

The chemical analysis gave the following concentration for the main elements: Co 36%, Cu 19.8%, Ni 1.3%; Fe 2.18%, S 21.5%. The mineralogical composition given in Fig. 1 indicated carrollite as major mineral phase with a small amount of chalcopyrite, bornite and pyrite being detected.

2.2. Microorganisms and culturing medium

A bacterial consortium involving three different mesophilic chemolithotrophic bacterial strains belonging to the species *A. ferrooxidans*, *L. ferrooxidans* and *A. thiooxidans* was used. The native strains have been isolated from various acid mine drainage waters and dumps in Bulgaria and kindly supplied for this study by Prof. S. Groudev (UMG – Sofia). The isolation, identification and enumeration of the microorganisms were carried out by described methods (Karavaiko et al., 1988). Bacteria have been successively adapted through several stages on carrollite substrate in iron-free 9 K medium (Silverman and Lundgren, 1959), pH 1.8–2.0 and temperature of 33 °C. Once the inoculum has reached its log phase indicated by redox potential in the range 620–640 mV, the suspension was filtered and the resulting solution used as lixiviant during all the experiments.

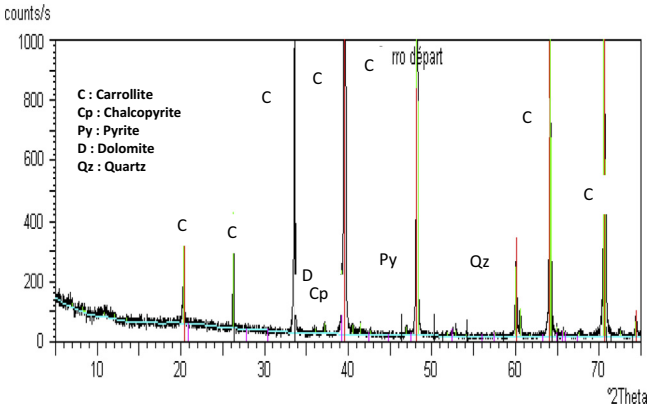

Fig. 1. XRD pattern of pure carrollite.

2.3. Bioleaching procedure

Bioleaching of carrollite samples was carried out in triplicate inside shake flasks of 250 mL (working volume) at pulp solids loading of 2% (w/v). The shaker has been placed in a thermostatized room at 33 °C and agitated at 120 rpm. At predetermined time intervals, 2 mL have been sampled from the bioleaching solutions, filtered and delivered for Co and Cu analysis. Eh and pH of the suspension have been monitored on a regular basis and losses due to evaporation compensated by addition of distilled water. Concentrated sulphuric acid has been used to keep pH value between 1.8 and 2.

For microscopical study of the evolution of carrollite surface during bioleaching, 10 mL of the pulp was collected at day 5, 10, 15, 20 and 30 and subsequently filtered through filter paper. The remaining solids on the filter surface have been gently washed with 9 K solution and transferred into glass tubings containing 10 mL of 9 K solution before being delivered to SEM-preparation.

2.4. Sample preparation for SEM and EDS analysis

The filtered-solids from the bioleached material have been recovered from the glass tubings by sedimentation and pipetting, then glutaraldehyde-fixed for 2 h at 20 °C in 1 mL of iron-free 9 K solution with addition 100 μL of 25% glutaraldehyde, and finally rinsed in iron-free 9 K medium then in distilled water before being separated into two sets. The samples from the first set have been freeze-dried, glued on conductive carbon tape (on aluminium stubs) and coated with 20 nm Pt in a sputtering unit (Balzers SCD-030) before SEM observation. Samples from the second set have been "en bloc" stained in aqueous 2% uranium acetate for 2 days, rinsed in distilled water, dehydrated through an ethanol series and embedded in Epofix resin (Struers, cat no. 40200029). Mirror-polished slices have been realized by hand polishing with SiC grit papers (up to P4000) followed by final polishing with a non-aqueous 1 μm-sized diamond suspension (ESCIL, 1PS-1MIC).

SEM observations were carried out by use of secondary electron (SE) and backscattered electron (BSE) detectors on bulk samples and polished slices respectively in a FEI ESEM-FEG XL-30 system working in high vacuum mode and at 15 kV of accelerating voltage. The ESEM was coupled with an EDAX energy dispersive X-ray spectrometer (EDS) for elemental microanalysis and with a sapphire