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Sequential bioreduction–bioleaching and bioreduction–chemical leaching hybrid tests for enhanced copper recovery from a concentrator ball mill reject sample

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ABSTRACT

Dumping of poor but metal containing industrial waste is associated with several environmental issues. Exposure of these wastes to the natural environment offers serious concerns for the mineral processing industries to utilize them for metal recovery and check environmental pollution. In the present study, a novel sequential bioreduction–bioleaching and bioreduction–chemical leaching route as a hybrid process is compared and discussed for the enhanced recovery of copper from an industrial concentrator plant ball milling unit rejected sample. A mixed consortium of metal reducing bacteria (DMRB) initially adapted to high Fe(III) concentrations was found to cause mineralogical/matrix alteration (possibly silicate weathering) including Fe(III) bioreduction in the sample and dissolute 29.73% copper during the first 35 days under facultative anaerobic conditions. Sequential leaching of the bioreduced waste sample (generated from the first step) using a mixed meso-acidophilic bacterial consortium predominantly *Acidithiobacillus ferrooxidans* showed additional 28.72% copper dissolution within 2 days using 1 gL^{-1} Fe(II). On the other hand, a comparative chemical leaching of the same bioreduced sample using 0.5 M H_2SO_4 yielded additional 32.17% copper within 4 days of leaching and indicated better performance than the bioleaching tests.
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1. Introduction

Several management strategies as means of metal recoveries have been adopted to utilize various industrial and mining wastes generated globally with poor but valuable metal content (Baba et al., 2011; Panda et al., 2012a,b,c). Interestingly, the mineralogical complexities within the low grade ores offer strong challenges to economically reuse them through the conventional and feasible methods available for metal extraction. It is very interesting to note that the demand for microbial processing of metals from industrial wastes is rapidly increasing. Over the past few decades, application of specific microbial agents have not only been successful in finding application for recovering metal values from low grade ores, secondary metal sources but also removal of sulphur from coals in an economic and eco-friendly way (Ahmadi et al., 2015; Akcil and Deveci, 2010; Esther et al., 2015; Liu et al., 2015; Mishra et al., 2014; Panda et al., 2014). Amongst all the reported microorganisms used for metal recovery, the meso-acidophilic iron- and sulfur-oxidizing γ-proteobacterium *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*) is the most widely studied and industrially accepted microorganism (Rawlings, 2005; Sand et al., 2001).

Of all the copper minerals, chalcopyrite ($CuFeS_2$) is the primary and accounts to about 70% of the world's total copper reserves. Owing to its recalcitrant behavior towards the conventional methods of metal recovery, the use of microbial applications have been very successful for treating such low grade wastes bearing chalcopyrite and several other associated minerals (Panda et al., 2015b and references therein). Owing to the rapid demand of copper on one hand and faster depletion of high grade ore deposits on the other, it has become very essential to process the low grade or run-of-mine ores for metal recovery. One such waste material from the copper industry that needs proper attention is the concentrator plant ball milling unit reject which is generated during grinding of high copper grade ores. The high grade ores obtained directly from the mines appears in sizes of huge boulders which require size reduction through 1°, 2° and 3° level crushers followed by fine grinding steps (to reduce the ore size to some microns suitable for flotation process) in industrial ball mill units. Most interestingly, some of the material subjected to ball milling is unable to break down resulting in the generation of ball mill reject. The material is thus dumped for its lower metal content and unsuitability in further subjection to the

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conventional method. Simultaneously, dumping of such waste rejects of industry cause several environmental issues. Most importantly, extensive exposure leads to changes in the atmospheric weather conditions over longer periods of time enabling chemical or bio-chemical weathering of these dumped samples (Nugraha et al., 2009; Uzarowicz, 2013) which in turn causes release of toxic metals to the environment. Apart from metal contamination of soil and water bodies, occupation of extra land is one of the major concerns due to such higher dumping activities. The above issues have been drawing considerable attention of the industries for an appropriate recycling method that can reuse the discarded material for recovery of copper. This in turn can contribute a significant portion to the overall copper production on one hand and simultaneously utilize and check environmental pollution on the other.

Bio-weathering is a natural degradation phenomenon wherein biogeochemical transformation of rock and mineral occurs by the bio-mechanical and bio-chemical activity of living organisms. Bio-chemical mode of weathering by microorganisms involves the micro topographical changes of the minerals through secretion of EPS (exopolysaccharides) and non-EPS metabolites that cause pitting and etching, mineral displacement reactions and even complete dissolution (Barker et al., 1998; Adeyemi and Gadd, 2005). Excretion of mucopolysaccharides, inorganic and organic acids, ligands and enzymes by microorganisms accelerate mineral weathering (Luttge, 2005). The low pH in the micro-environment of the biofilm (EPS) on the mineral surface also mediates mineral dissolution (Liermann et al., 2000). The predominance of silicate minerals in the earth's crust are the main focus of the weathering studies (Barker et al., 1998; Schippers et al., 2013).

It is interesting to note that very few studies are available in the literature for processing of ball mill rejects. In our previous fundamental study (Panda et al., 2013b), thermal activation of a representative ball mill sample of M/s Malanjkhand Copper Project (MCP) was seen to enhance the bio-recovery rate of copper. However, without thermal activation the recovery from the sample was seen to be comparatively lower. It therefore becomes very essential to work on a suitable method that can naturally treat the sample and enhance the copper recovery rates. As discussed in our previous study, the ball mill reject contained higher percentage of silicate minerals i.e. around 78%, which makes it an attractive sample for microbial degradation/weathering studies (Barker et al., 1998). In addition, iron was seen to be present in higher quantity as indicated by previous XRD and XRF analysis/studies in form of goethite (FeOOH) with relatively few hematite (Fe_2O_3) phases in the sample. Furthermore, mineralogical identification through microscopic studies have also indicated other Fe(III) rich silicate minerals such as biotite, epidotes, hornblende or the iron phases associated with other accessory or clay minerals. Hence, it was decided to attempt bioreduction experiments for a possible reduction in the iron (III) states in view of any possible mineralogical alternation that can open up doors to facilitate enhanced recovery rates of copper naturally thereby providing an alternative to thermal activation.

In view of the above, a fundamental two step sequential leaching method involving both bioreduction and biooxidation principles have been proposed in the present study for enhanced copper recovery. In addition to the above, a direct comparison has also been made for the recovery of copper using a hybrid bioreduction–chemical leaching method. Furthermore, to put better insights into the mineralogical changes occurred in the sample as a result of the application of such hybrid methods, the industrial waste samples were analyzed and characterized through the sensitive Fourier transform infrared spectroscopy (FTIR).

2. Materials and methods

2.1. Industrial concentrator ball milling unit waste sample

In the present study, a representative ball mill waste reject previously generated during an industrial ball milling operation at MCP, India was obtained for experimental purposes. The waste that was dumped over a period of time was subjected to sampling through the conventional coning and quartering method. The chemical analysis of this waste is presented in Table 1. Mineralogical analysis of the ball mill reject has been discussed in our earlier study (Panda et al., 2013b).

2.2. Metal reducing microorganisms

A laboratory stock culture of a dissimilatory metal reducing microbial consortium (DMRB) initially adapted to high Fe(III) concentration was used for bioreduction experiments. For the adaptation and growth of this consortium, a 2% (w/v) pulp density of the grounded (<750 μm) ball mill waste (serving as an electron acceptor) was suspended and autoclaved in the mineral salt media containing (gL^{-1}) KH_2PO_4 — 0.8; K_2HPO_4 — 3.0; KCl — 0.2; NH_4Cl — 1.0; $MgCl_2$ — 0.2; $CaCl_2$ — 0.1 and yeast extract — 0.05 along with 10 mM glucose as carbon source (electron donor). Adaptation is quite an essential feature that improves heavy metal tolerance and improves metal dissolution by metal reducers under facultative anaerobic conditions (Esther et al., 2013). To the sterile media, 10% (v/v) of the adapted DMRB consortium was added to each of the experimental flasks as inoculum which was further layered with paraffin oil to maintain facultative anaerobic conditions. The flasks were incubated in dark at ambient temperature. Every 7 days, the reduction in Fe(III) concentration as a result of microbial activity was monitored by titration method.

Following adaptation to the low copper containing waste sample, the cultivable DMRB from the seed culture was streaked onto nutrient agar (NA) plates and incubated for 24–48 h to obtain isolates. Five colonies of different size and pigmentation were re-streaked onto fresh NA plates to obtain pure culture isolates. For identification of the strains we followed the conventional molecular approach of PCR amplification of *16S rRNA* gene using universal primers. The PCR product was analyzed by gel electrophoresis using 0.8% agarose gel followed by sequencing using ABI Genetic Analyzer 3500. Based on *16S rRNA* sequence, homology search was performed using the NCBI BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). The isolates were identified based on the neighbor-joining method (Saitou and Nei, 1987) using bootstrap values based on 1000 replications (Felsenstein, 1985).

2.3. Meso-acidophilic iron-and-sulfur oxidisers

A laboratory stock of mixed meso-acidophillic chemolithotrophic microbial consortium was used for bioleaching studies (discussed in Section 2.4.2). Since, mixed consortium is considered to be more effective than a pure culture (Ciftci and Akcil, 2010; Akcil et al., 2007), a consortium predominantly comprising *At. ferrooxidans*, *Leptospirillum ferrooxidans* and *Acidithiobacillus thiooxidans* strains were used in the present study. The standard 9 K^+ media containing $(NH_4)_2SO_4$ — 3 gL^{-1}, KH_2PO_4 — 0.5 gL^{-1}, $MgSO_4{\cdot}7H_2O$ — 0.5 gL^{-1}, KCl — 0.1 gL^{-1} and $FeSO_4$ — 44.2 gL^{-1} was used for microbial growth (Silverman and Lundgren, 1959). The pH of the medium was maintained at 1.8 ± 2 using dilute sulfuric acid. Repeated sub-culturing for activation of the microbial strains was performed at ambient temperatures in sterilized 9 K^+ media. Following activation, the meso-acidophiles were adapted to Cu (1 gL^{-1}) with an incremental increase to improve their metal tolerance capacity. The Cu adapted strains with iron oxidation rate (IOR) of >600 kg m^{-3} h^{-1} was used during the entire leaching period.

Table 1
Chemical composition of the ball mill reject sample.

Composition	Cu	Fe	S	Mo	Acid insoluble
Percentage	0.24	1.8	0.6	Traces	97

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