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Review

Advances in bioleaching for recovery of metals and bioremediation of fuel ash and sewage sludge

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ABSTRACT

Bioleaching has been successfully used in commercial metal mining for decades. It uses microbes to biosolubilize metal-containing inorganic compounds such as metal oxides and sulfides. There is a growing interest in using bioleaching for bioremediation of solid wastes by removing heavy metals from ash and sewage sludge. This review presents the state of the art in bioleaching research for recovery of metals and bioremediation of solid wastes. Various process parameters such as reaction time, pH, temperature, mass transfer rate, nutrient requirement, pulp density and particle size are discussed. Selections of more effective microbes are assessed. Pretreatment methods that enhance bioleaching are also discussed. Critical issues in bioreactor scale-up are analyzed. The potential impact of advances in biofilm and microbiome is explained.

1. Introduction

Many industrial activities generate solid wastes that are toxic to the environment. They pollute air, water and soil. Removing organic matters from solid wastes is relatively easy using biological, chemical and physical means. However, the degradation of inorganic matters, especially those compounds containing heavy metals is usually difficult (Kulshreshtha et al., 2014; Lee and Pandey, 2012). There are various methods used to extract metals from metal oxides and sulfides such as hydrometallurgy and pyrometallurgy. These classical methods for metal extraction face problems such as environmental pollution, low recovery yields and high operating costs (Pathak et al., 2009). Bioleaching uses microorganisms to solubilize metal oxides and sulfides. This process is sometimes called biosolubilization, which is simple and low cost (as low as 1/3–1/2 of the costs in conventional processes) (Bosecker, 2001; Watling, 2006, Motaghed et al., 2014). It is also environmentally friendly as long as the leachate is contained and acidic wastewater is neutralized before discharging (Kulshreshtha et al., 2014; Lee and Pandey, 2012).

Bioleaching has been used to recover various metals from low-grade mineral ores and tailings for many years at industrial scales using processes such as dump bioleaching, heap bioleaching and *in situ* bioleaching (Watling, 2006; Cox and Bryan, 2017). Researchers have investigated bioleaching for bioremediation of solid wastes, but this application is not yet ready for deployment for practical bioremediation. The slow reaction kinetics is a major bottleneck (Pathak et al, 2017). Optimizations of operating parameters such as reaction time, pH, temperature, mass transfer rate, nutrient requirement, pulp density and particle size are needed to improve bioleaching process efficiency (Rastegar et al., 2014b; Arshadi and Mousavi, 2014; Bosecker, 1997; Ilyas et al., 2014). A proper pretreatment method is also an effective way to improve bioleaching. This review discusses various issues in bioleaching for the bioremediation of solid wastes with recovery of useful metals as value-added products.

2. History of bioleaching

Since the 1940s many researchers have contributed to the clarification of the mechanisms of specific microorganisms used in the biosolubilization of metal oxides and sulfides (Mishra et al., 2005). Most notably, sulfide ores have been bioleached in copper mining commercially in several countries (Ehrlich, 2001). The term biohydrometallurgy is used to describe a subfield in hydrometallurgy that involves biotechnology. It uses microbes to interact with insoluble metal oxides/sulfides to convert them into soluble metal ions for further recovery. It was first practiced in Rio Tinto mines in southwestern Spain

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in the beginning of the 1890s (Mishra et al., 2005). Bioleaching has been used for decades at industrial scales without operators knowing the exact roles of microorganisms in metal solubilization until 1961 when *Acidithiobacillus ferrooxidans* was discovered in the leachates (Brandl, 2008). This bacterium oxidizes Fe^{2+} to Fe^{3+} and then using Fe^{3+} as an electron acceptor (i.e., oxidant) to solubilize metal sulfides. In 1965, *Acidianus brierleyi* as the first iron and sulfur-oxidizing archaeon was discovered (Brierley, 2007). Since 1980, bioleaching has been effectively practiced at industrial scales for mining in various locations owing to the better understanding of the microbes involved (Mishra et al., 2005). Bioleaching of numerous copper ores such as chalcopyrite concentrates has been carried out since 1997 in Chili, Mexico, USA, Australia and South Africa. Dump bioleaching at a high altitude was found to be a very low cost process for extracting copper from ores (Watling, 2015). Other successful commercial operations including bioheap leaching and *in situ* bioleaching have been reported as well (Watling, 2006, 2015).

To increase bioleaching efficiency, some pretreatment methods were used. For example, the Fairview Mine in South Africa has the longest history of using the BIOX process for biooxidation pretreatment of sulfidic gold ores (Kaksonen et al., 2014). The Youanmi project in Australia utilized moderately thermophilic bacteria including *Sulfobacillus thermosulfidooxidans* and biooxidation with operating temperatures between 45 and 55 °C . Another notable process was developed by GeoBiotics. It is called GEOCOAT for the biooxidation of refractory gold ores by coating the concentrate slurry onto a support rock or a substrate material, and stacking this coated material in a biooxidation heap (Mishra et al., 2005; Rawlings, 2013).

3. Microbes used in bioleaching

3.1. Mesophilic bacteria

Most industrial microorganisms used in the bioleaching are mesophilic bacteria. Microbes in the *Acidithiobacillus* and *Leptospirillum* genera are mesophilic that prefer temperatures between 25 and 35 °C for growth.

3.1.1. Acidithiobacillus

Bacteria in the *Acidithiobacillus* genus are rod-shaped, Gram-negative and non-spore forming. They can grow under aerobic condition. They derive energy by oxidizing sulfur compounds such as elemental sulfur, sulfides and thiosulfate that are either reduced or partially reduced electron donors. Sulfate is usually the final oxidation product. Most *Acidithiobacillus* species are chemolithotrophic species that use CO_2 in the air as the carbon source. This means that they do not need an organic carbon supplement in bioleaching operations, which is very advantageous. *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans, Acidithiobacillus caldus*, *Acidithiobacillus albertis*, *Acidithiobacillus acidophilus, Acidithiobacillus concretivorus*, *Acidithiobacillus prosperus* are the most important *Acidithiobacillus* species in bioleaching. All of them are rod shaped. These bacteria can oxidize elemental sulfur and generate sulfuric acid that can reduce the culture medium pH to as low as 1, making them appropriate in the bioleaching of metal oxides and sulfides to recover metals (Hoque and Philip, 2011).

A. ferrooxidans, *A. thiooxidans* bacteria can grow well at an acidic pH between 1 and 3, while other *Acidithiobacillus* genus require a higher pH that is not sufficiently acidic for bioleaching (Hoque and Philip, 2011). Thus, these two microbes are the most popular *Acidithiobacillus* species in bioleaching. *A. ferrooxidans* is a more important species in bioleaching because in addition to harvesting energy by oxidizing reduced sulfur compounds, Fe^{2+} is used as an energy source. In the absence of oxygen, *A. ferrooxidans* is able to grow on reduced inorganic sulfur compounds using ferric ion as an alternative electron acceptor with CO_2 as the carbon source. Like *A. thiooxidans, A. caldus* does not use Fe^{2+} as an electron donor in its metabolism.

3.1.2. Leptospirillum

Leptospirillum ferrooxidans is an acidophilic obligatory chemolithotroph that uses Fe^{2+} as an energy source. This bacterium can tolerate a lower pH (about 1.2), and higher concentrations of uranium, molybdenum and silver than what *A. ferrooxidans* can, but it is more sensitive to copper and is also unable to oxidize sulfur compounds. *L. ferrooxidans* has a lower growth rate than *A. ferrooxidans*, but it can be accelerated by the addition of Zn^{2+}. Furthermore, *L. ferrooxidans* is less widespread than *A. ferrooxidans*, and this may reflect an inability to compete in the natural environment (Ewart and Martin, 1991). Because of this, a co-culture of *L. ferrooxidans* with *A. ferrooxidans* or *A. thiooxidans* is sometimes used to solubilize metals in a sulfidized solid matrix (Bosecker, 1997; Hoque and Philip, 2011). With regard to growth temperature, *Leptospirillum* sp. have an upper limit of around 45 °C and a lower limit of around 20 °C (Rawlings, 2013).

3.2. Thermophilic bacteria and archaea

Due to high microbial activities with heat generation in exothermic biooxidation in stirred tank reactors and in heaps, thermophiles are desired in bioleaching to tolerate the high temperatures. Increased operating temperatures and the use of thermophilic bacteria improve not only reaction rates but also yields of extracted metals from some minerals. Moderately thermophilic bacteria grow at a temperature around 50 °C. Extreme thermophiles which are usually archaea (Kaksonen et al., 2017) can grow at a temperature above 60 °C. *A. brierleyi* is an archaeon formerly classified under genus *Sulfolobus*. *Sulfolobus* species are capable of utilizing Fe^{2+}, S^0 and sulfides as energy sources that are also used by *Sulfobacillus thermosulfidooxidans*, which is a spore-forming facultative autotrophic bacterium. However, in lab tests, its growth will only occur in a culture medium enriched with yeast extract (Bosecker, 1997; Rastegar et al., 2014a). This hampers its use in the field unless it is grown in a mixed culture with other microbes that supply its nutritional needs.

3.3. Heterotrophic bacteria and fungi

Heterotrophic bacteria and fungi rely on organic compounds as energy sources in their metabolism. In the growth phase, they secrete different organic acids such as lactic, citric, oxalic and gluconic acids as well as enzymes. These compounds can solubilize metals from a solid matrix by forming soluble metal complexes and chelates. *Bacillus* species are found to be the most effective bacteria, while some fungal species in the *Aspergillus* and *Penicillium* genera are often used in bioleaching (Rastegar et al., 2014a, Rasoulnia et al., 2016; Vakilchap et al., 2016).

3.4. Mixed culture consortia

It is simple to operate a pure-culture bioreactor in a lab because there is no shift in the microbial population structure over time. It is also less complex to study the intrinsic bioleaching mechanisms in such a bioreactor. Due to the strong acidity produced by bioleaching microbes, often one microbe continuously thrive in the bioreactor even without sterilization of the culture medium and solids, unless another organism can tolerate the low pH. In recent years, some researchers used mixed culture communities for bioleaching (Feng et al., 2013, Latorre et al., 2016). In one study, the use of a co-culture of *A. thiooxidans* and *A. ferrooxidans* considerably increased the recovery yields of Cu, Ni, Pb and Zn from waste printed circuit boards compared with results using the single cultures of these bacteria. Much more research will be done using mixed cultures in the future due to its potential to make bioleaching more practical in the field that often harbors mixed culture communities (Li et al., 2014, Ma et al., 2017).