MICROBIOLOGICAL LEACHING; AN ENVIRONMENTALLY FRIENDLY AND COST EFFECTIVE METHOD FOR EXTRACTION OF METALS

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ABSTRACT

Finding a cleaner, environmentally friendly and cost-effective way of metal and mineral extraction has a great importance in today's world. Using microorganisms in bio-leaching and bio-oxidation process is of great value. From Archaea to bacteria and fungi, microorganisms can play an important role in extraction of metals from mine drainage and un-accessible sources, both in aquatic and terrestrial environments. Optimization of environmental factors such as the temperature, pH and substrate concentration is crucially important to access the optimum extraction of selected metals from an ore or mine drainage. The present paper will review the bio-leaching and bio-oxidation process of minerals with emphasis on the most well-known species of bacterial communities of such ability, through the literature.

RESUMEN: La lixiviation microbiologique; une méthode écologique et rentable pour l'extraction des métaux.

Trouver une façon plus propre, respectueuse de l'environnement et économique de l'extraction des métaux et des minéraux est d'une grande importance dans le monde d'aujourd'hui. L'utilisation de microorganismes dans le processus de bio-lixiviation et de bio-oxydation est d'une grande valeur. De l'Archaea aux bactéries et aux champignons, les microorganismes peuvent jouer un rôle important dans l'extraction des métaux du drainage des mines et des sources non accessibles, tant dans les milieux aquatiques que terrestres. L'optimisation des facteurs environnementaux tels que la température, le pH et la concentration du substrat est cruciale pour accéder à l'extraction optimale des métaux sélectionnés à partir d'un minerai ou d'un drainage minier. Le présent document examinera le processus de bio-lixiviation et de bio-oxydation des minéraux, en mettant l'accent sur les espèces les plus connues de communautés bactériennes de cette capacité, à travers la littérature.

REZUMAT: Lixivierea microbiologică; o metodă ieftină și ecologică de extragere a metalelor.

În contextul internațional actual, găsirea unei metode mai ecologice, mai puțin poluante și ieftine de extragere a metalelor și minereurilor este de mare importanță. O deosebită valoare este reprezentată de utilizarea microorganismelor în procese bio-oxidative și de bio-lixiviere. De la Archaea la bacterii și fungi, microorganismele pot juca un rol important în extragerea metalelor din apele de mină și din surse inaccesibile atât în mediu terestru cât și acvatic. Optimizarea factorilor ambientali precum temperatura, pH-ul și concentrația substratului sunt cruciale pentru o extragere optimă a metalelor selecționate din zăcământ sau din apele de mină. Articolul de față trece în revistă procesele de extragere a mineralelor prin bio-oxidare și bio-lixiviere, evidențiind cele mai bine cunoscute specii din comunitățile bacteriene cu astfel de abilități, așa cum sunt ele prezentate în literatura de specialitate.

INTRODUCTION

Excavation of mines for different minerals extraction is one of the most destructive anthropogenic activities for terrestrial and aquatic ecosystems. Such activities affect the topography, turbidity, concentration of dissolved particles and minerals, pH, etc. and could affect the life of inhabited organisms directly or indirectly (Ashton et al., 2001).

Microorganisms are one of the most abundant living organisms of all ecosystems from terrestrial to aquatic habitats. Different classes of such organisms have biological, chemical, and physical effects on their surrounding environments. Using microorganisms for extraction of wide range of minerals and biological products is an effective and environmentally friendly way to use the new sources of minerals and metals which cannot be extracted using common and ordinary methods (Edwards et al., 2013).

Using the microorganisms, especially the bacteria and fungi, is a clean way of extracting minerals and metals from the wastewater of industries as well. Thereby the process called bio-leaching, which means the dissolution of metals from their mineral source using microorganisms, or using microorganisms to transform elements of certain ore to a kind of soluble form which could easily accessible by washing the ore by aqueous solutions. These processes also could be named as bio-oxidation (Rajkumar et al., 2010).

Many sulphide ores known as refractory ores e.g. pyrite (FeS_2), arsenopyrite (FeAsS) and pyrrhotite (FeS) include gold as fine dispersed particles and encapsulated in the sulphide matrix (Fig. 1), so that the gold cannot be recovered by conventional leaching. Roasting as a traditional method for oxidizing sulphides is not acceptable due to economic (high consumption of heat energy and need expensive equipment) and environmental (release of SO_2 gas) considerations. In recent years, the development of microbiological leaching has been led to the effective recovery of precious metals from sulphide ores. Using iron and ulphursulphur oxidizing bacteria can accelerate the oxidation of sulphides and the liberation of gold. The bacteria gain energy by oxidizing Fe^{2+} and S^0 that produce Fe^{+3} and H_2SO_4. However, this process needs a long time (often one week) and precise control to provide the bacterial survival (Bierlein and Wilde, 2010; Liu et al., 2015; Zhang et al., 2016).

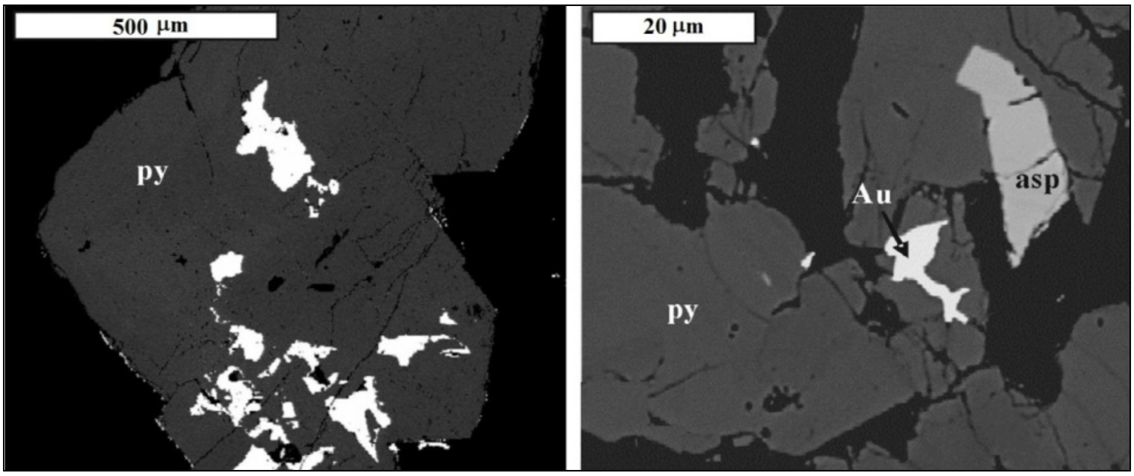

Figure 1: Gold particles encapsulated in the sulphide minerals (Bierleine and Wilde, 2010).

Bio-oxidation reactions of ulphursulphur ores

In bio-oxidation of pyrrhotite ore, the process begins by a chemical step:

$$FeS + 2H^{+} \rightarrow Fe^{2+} + H_2S$$

Then, bacteria oxidise pyrrhotite according to the following reactions:

$$2FeS + 4.5O_2 + 3H^{+} \rightarrow 2Fe^{3+} + SO_4^{2-} + HSO_4^{-} + H_2O$$

$$2FeS + 1.5O_2 + 6H^{+} \rightarrow 2Fe^{3+} + 2S^{0} + 3H_2O$$

Sulphur is also produced by H_2S decomposition:

$$H_2S + 2Fe^{3+} \rightarrow 2Fe^{2+} + 2H^{+} + S^{0}$$

Then a chemical reaction between FeS and Fe^{3+} takes place:

$$FeS + 2F^{3+} \rightarrow 3Fe^{2+} + S^{0}$$

Finally, bacteria oxidise ulphursulphur:

$$2S^{0} + 3O_2 + 2H_2O \rightarrow 2SO_4^{-} + 4H^{+}$$

Therefore, overall bio-oxidation reaction of pyyrotite is:

$$FeS + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + Fe^{2+} + 2H^{+}$$ (Ubaldini et al., 2000)

Overall bio-oxidation reaction of pyrite is similar to above reaction:

$$FeS_2 + 3.5O_2 + H_2O \rightarrow 2SO_4^{2-} + Fe^{2+} + 2H^{+}$$ (Ciftci and Akcil, 2010)

In bio-oxidation of arsenopyrite, following reactions take place:

$$4FeAsS + 11O_2 + 2H_2O \rightarrow 4HAsO_2 + 4FeSO_4$$

$$4FeAsS + 13O_2 + 6H_2O \rightarrow 4H_3AsO_4 + 4FeSO_4$$

Then Fe^{2+} is oxidized to F^{3+}:

$$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2 (SO_4)_3 + 2H_2O$$

Chemical oxidation of arsenopyrite by ferric sulphate is another reaction along with bio-oxidation process:

$$4FeAsS + Fe_2(SO_4)_3 + 10.5O_2 + 3H_2O \rightarrow 6FeSO_4 + 4HAsO_2 + H_2SO_4$$

$$2FeAsS + Fe_2(SO_4)_3 + 6O_2 + 4H_2O \rightarrow 4FeSO_4 + 2H_3AsO_4 + H_2SO_4$$

As^{3+} is also oxidized to As^{5+} as follows:

$$2HAsO_2 + O_2 + 2H_2O \rightarrow 2H_3AsO_4$$

$$HAsO_2 + Fe_2(SO_4)_3 + 2H_2O \rightarrow H_3AsO_4 + 2FeSO_4 + H_2SO_4$$

Changing As^{3+} to As^{5+} is necessary for decreasing the toxicity of media, improving bacterial activity and thus increasing leaching efficiency (Langhans et al., 1995; Cheng et al., 2010).

Bio-oxidation reaction parameters

The effective parameters of a bioleaching (or bio-oxidation) process have been described as follows: temperature, pH, culture media, bacterial species and concentration of the ore pulp (Elrich and Brierly, 1990; Elrich, 2001). The main pollutant product of chemical leaching is SO_2 gas which is consumed by bacteria in bioleaching process and this latter processes is one of the environmental friendly characteristics of bioleaching (Elrich, 2001).

On the other hand, in chemical leaching, using high concentrations of strong acids such as Nitric Acid, Hypochlorus Acid and Sulphuric Acid and also providing a tank made up of stainless steel and in some cases using a high temperature autoclave for running the chemical reaction were the expensive and complicated parameters of such leaching process. Compared to that, without any needs to chemical hazards or special tank or extreme temperatures, bioleaching could only be conducted by optimizing the culture media for selected organisms, which makes it the most cost-effective and environmentally friendly way for extraction of metals from sulphide ores (Elrich, 2001).

Also, bio-extraction of metals from waste water of industries, using mesofilic and thermofilic ulphursulphur bacteria for extraction of ulphursulphur from waste water has been previously studied (Elrich and Brierly, 1990). In such experiments, ulphursulphur bacteria such as *Acidiothiobacillus ferroxidans* and *Acidiothiobacillus thiooxidans* were used for bioextraction of sulphide from a waste water to clean up the sewage (Fuseler et al., 1996).

Microbial diversity for bioleaching habitats

A large variety of microorganisms has been segregated from the mining and environmental bio-leaching situations, from bacteria, to fungi and algae. Diverse species have been segregated from a copper mine from different classes of microorganisms such as bacteria (*Acidiothiobacillus* sp.), yeasts (*Rhodotorula* sp., *Trichosporon* sp.), flagellates (*Eutrepia* sp.), amoebas and protozoa. One of the most important species in bioleaching habitats is *Acidiothiobacillus ferrooxidans* which is occasionally exchange with *Leptospirillum* sp. based on environmental elements (Acosta et al., 2014).

Also, a variety of thermophilic microorganisms (especially *Sulfolobus* sp.) have been enriched and isolated from bio-leaching environments. Table 1 shows a selection of abundant microorganisms of bio-leaching habitats.

Table 1: Some microorganisms of bio-leaching habitats.

Domain	Organism	Main leaching agent
Archea	*Acidianus* ssp.	ulphursulphuric acid
	Ferroplasma acidiphilum	ferric iron
	Metallosphaera ssp.	ferric iron, ulphursulphuric acid
	Sulfolobus ssp.	ferric iron, sulphuric acid
	Sulfurococcus ssp.	ferric iron, sulphuric acid
Bacteria	*Acetobacter methanolicus*	gluconate
	Acidiphilium ssp.	organic acids
	Bacillus megaterium	citrate
	Chromobacterium violaceum	cyanide
	Crenothrix ssp.	ferric iron
	Gallionella ssp.	ferric iron
	Leptospirillum ferrooxidans	ferric iron
	Leptothrix discophora	ferric iron, sulphuric acid

Table 1 (continued): Some microorganisms of bio-leaching habitats.

Domain	Organism	Main leaching agent
	Pseudomonas putida	Citrate, gluconate
	Siderocapsa ssp.	ferric iron
	Sulfobacillus thermosulfidooxidans	ferric iron, sulphuric acid
	Thermothrix thiopara	sulphuric acid
	Thiobacillus ssp.	sulphuric acid
	Thiomonas cuprinus	sulphuric acid
Eukarya	*Actinomucor* sp.	succinate
Fungi	*Alternaria* sp.	citrate, oxalate
	Aspergillus ssp.	oxalate, citrate, gluconate, malate, tartrate, succinate
	Fusarium sp.	oxalate, malate, pyruvate, oxaloacetate

Bacteria

Among the all classes of microorganisms, bacteria are the most observed group of bio-leaching communities. The most well-known and first identified bio-leaching bacteria are *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Acidithiobacillus thiooxidans* and *Thiobacillus thiooxidans* (Harneit et al., 2006; Lei et al., 2007).

– *Acidithiobacillus ferrooxidans*

Acidithiobacillus ferrooxidans belongs to the group of chemolithotrophic organisms. The organism is rod-shaped (usually single or in pairs), non-spore forming, gram-negative, motile, and single-pole flagellated (Jerez, 2009).

Acidithiobacillus ferrooxidans is an acidophilic bacterium. It has obligate autotrophic way of gaining energy, using elementary sulphur, ferrous iron and tetrathionate as electron donors during ATP production. *Acidithiobacillus ferrooxidans* also is a rod-shaped motile bacterium living in acidic or neutral environments (Fig. 2). Due to its unique characteristics, it is well-known as an economically significant species for bio-leaching process of low- grade sulphide ores. The species is naturally inhabitant of mine drainage and tailings. Also due to high ability of this species in bio-oxidation process, it has been described as a significant species for bioremediation process in contaminated aquatic and terrestrial environments (Sun et al., 2012; Fomchenko et al., 2016).

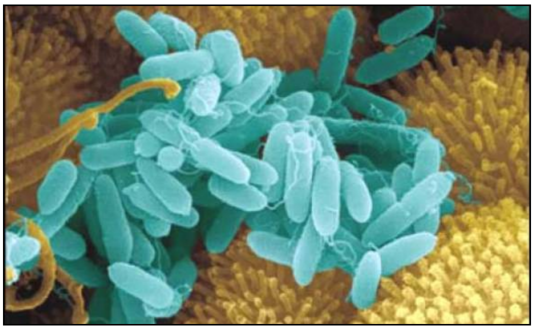

Figure 2: SEM image of *Acidithiobacillus ferrooxidans* bacteria (Ribeiro et al., 2011).

– Leptospirillum ferrooxidans

Leptospirillum ferrooxidans is an obligate aerobic bacterium with high ability of iron-oxidization and has an important role in bio-leaching and bio-oxidation of industrial activities (Fig. 3). The species is acidophilic and has been known as main component of mine drainage (Corkhill et al., 2008; Liu et al., 2017).

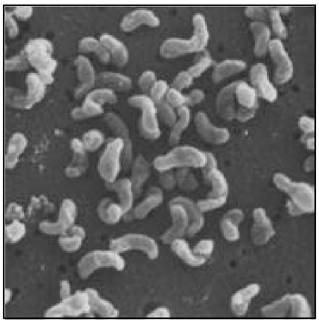

Figure 3: SEM image of *Leptospirillum ferrooxidans* (right) and drainage containing *L. ferrooxidans* (left) (Chapana and Tributsch, 2004).

– Acidithiobacillus thiooxidans

Acidithiobacillus thiooxidans is a sulphur bacteria belongs to gram negative group and has rod-shaped cells which uses sulphur compounds as energy source (Fig. 5). Due to mesophilic properties of this spices and being a natural inhabitant of diverse ecosystems, *A. thiooxidans* has important activities (*A. thiooxidans* (mesophilic) and *A. caldus* (thermophilic) oxidize elemental sulphur and sulphur reduced compounds) in bio-oxidation and bio-leaching process (Liu et al., 2003; Harneit et al., 2006; Leng et al., 2009; Khan et al., 2012).

Figure 5: SEM image of *Acidithiobacillus thiooxidans* bacteria.

CONCLUSIONS

Due to increasing risk of anthropogenic activities on biological characteristics of aquatic and terrestrial ecosystems, finding novel methods that are cost-effective and environmentally friendly ways of metal extraction has a great importance in today's world.

Natural microorganism communities of mines and drainage systems are the first object of application as a new method of metal extraction through bio-leaching and bio-oxidation pathways.

Diverse variety of such microorganism from Archaea to bacteria and fungi are among the observed species. The most important factors of application of the microorganism in bio-leaching process are the pH, temperature and the concentration of metals and leaching agents in the ecosystem.

Setting up the optimum environmental factors for selected species could lead to a successful extraction of valuable amounts of metals and minerals from mine drainage or inaccessible sources.

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