Global J. Environ. Sci. Manage.,4(3): 373-386, Summer 2018
DOI: 10.22034/gjesm.2018.03.010

REVIEW PAPER

A delve into the exploration of potential bacterial extremophiles used for metal recovery

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Received 5 February 2018; revised 28 March 2018; accepted 29 May 2018; available online 1 July 2018

ABSTRACT: A multitude of microbes are involved in the solubilisation of minerals and metals as this approach offers numerous advantages over traditional methods. This strategy is preferred as it is eco-friendly and economical, thus overcoming the drawbacks of the traditional approach of pyrometallurgy. Many different types of bacteria are employed in the process of Bioleaching, which are collectively grouped under chemolithotrophs, as they derive their energy from inorganic compounds. Bioleaching is the mobilization of metal cations from insoluble ores by microorganisms. All chemolithotropic bacteria are extremophiles since they have the ability to survive in extreme conditions. They carry out the process of Bioleaching through three mechanisms: Indirect, contact/ direct and cooperative bioleaching. This review gives a sneak peek into the different strains of chemolithotrophs which are used in bioleaching, and some recent work in the field. It also gives an insight into the general process and mechanism of Bioleaching, the study of which will pave way for developing better and efficient industrial bioleaching operations.

KEYWORDS: *Acidithiobacillus; Bioleaching; Chemolithotrophs; Leptospirillum; Metal recovery mechanism; Minerals.*

INTRODUCTION

The most dominant and diverse group of organisms on this planet are microbial communities (Dinsdale, *et al.,* 2008). Bacteria can be found in any part of the world and in any climate, including the highest peaks, deepest oceans, at extreme temperatures and even in polluted soils. Soil hosts phylogenetic groups of bacteria which are abundant and distributed globally. All ecologically relevant biogeochemical processes occurring in soils are mediated by microorganisms (Watt, *et al.,* 2006). Chemolithotrophic bacteria are a group of important microbes which obtain energy by oxidation of some reduced forms of inorganic compounds like sulphide, ammonia, ferrous and hydrogen ions. They are the primary microbes used in the process of bioleaching. Since they obtain cellular carbon through carbon dioxide, these bacteria can thrive in the absence of light and organic compounds (Oren and Aharon, 2009). They are split into two groups on the basis of their electron donors- Obligate and Facultative lithotrophs. Ammonium, nitrite, sulphur and sulphide oxidising bacteria come under the category of obligate lithotrophs while aerobic hydrogen and carbon monoxide oxidisers are included in the list of facultative lithotrophs (Tolli and King,

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Note: Discussion period for this manuscript open until October 1, 2018 on GJESM website at the "Show Article".

2005). Most of the chemolithotrophic bacteria use Calvin-Benson-Bassham pathway for incorporation of carbon dioxide (Fig. 1), even though chemolithotrophs are known to exhibit a broad range of ecological and physiological traits (Alfreider, *et al*., 2003; Dunfield and King, 2004; Ellis, 1979; Raven, 1996). The Calvin-Benson-Bassham pathway in chemolithotrophs can be divided into three phases. Carboxylation of ribulose 1,5-bisphosphate is the first phase which is catalysed by ribulose-1,5-bisphosphate carboxylase. The primary fixation product is 3-phosphhoglyceric acid. Reduction is the second phase, which is catalysed by 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase. The two carboxyl groups are reduced to the aldehyde level. Regeneration is the third phase in which fructose 6-phosphate is formed. Pentose 5-phosphate is formed following that by unidirectional rearrangement reactions. Finally, phosphorylation occurs, to regenerate ribulose 1,5-bisphosphate, which is the original carbon dioxide acceptor. The total cost of this cycle in chemolithotrophs is high. The cycle requires 3 molecules of ATP and 2 of NADH to produce organic compounds for each CO_2 that is fixed (Lengeler *et al*., 1999). Biological metal recovery is the oxidative and selective solubilisation of minerals facilitated by the activity of chemolithotrophic microbes. It is also known as microbial leaching, bacterial leaching or biooxidation and is a part of the broad field biohydrometallurgy or biomining. In this process, insoluble sulphides and oxides of metals are treated by hydrometallurgy, instead of using the traditional method pyrometallurgy. Some significant advantages of bioleaching include low power requirement, eco-friendly, suitable for treatment of low grade or waste ores and simple and affordable equipment. Some common drawbacks of this technique are that it is inadequate for treatment of primary sulphides, requires vast grounds and has low reaction rates and productivities (Gentina and Acevedo, 2013). Microbes are also actively used in the rehabilitation of mining sites. In any abandoned mine site, the microbial activity could be found in tailings, sediments, drainage water, top soil environment, rhizosphere environment found in association with roots and plants and phyllosphere environment of plants in the form of epiphytes or endophytes. The use of microorganisms in rehabilitation of mining site is an eco-friendly, economical and a sustainable approach (Nirola, *et al*., 2016; Sheoran, and Sheoran, 2006; Venkateswarlu, *et al*., 2016). This review study has been carried out at Vellore Institute of Technology, Tamil Nadu in 2017-18.

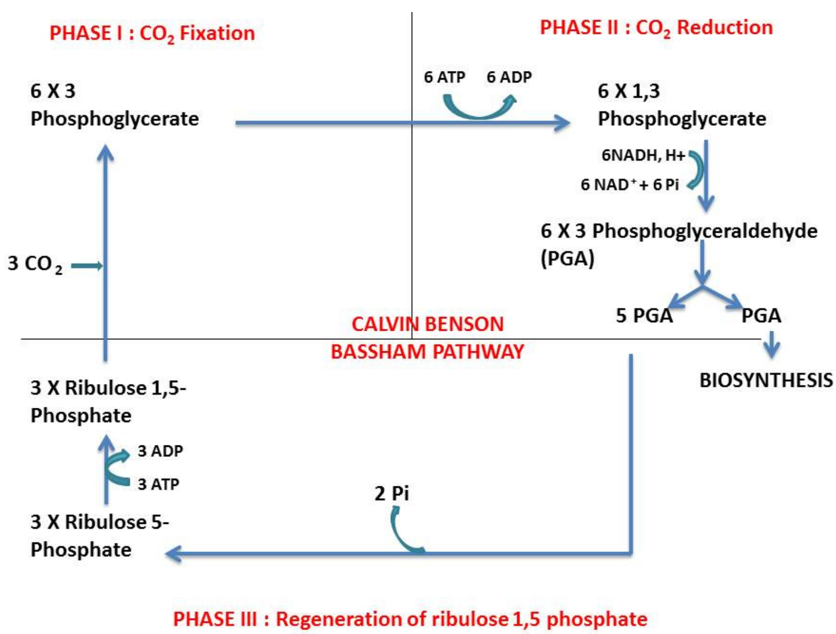

Fig. 1: The Calvin-Benson-Bassham cycle used by Chemolithotrophs (Bertrand, 2015)

Overview of metal recovery and bioleaching process

Three types of metal recovery methods exist-Pyrometallurigical methods, Hydrometallurgical methods and Bioleaching. Pyrometallurgical methods include smelting, melting, sintering, dossing etc [Shamsuddin, 1986]. On the other hand, Hydrometallurgy involves the use of leaching agents such as halide, thiosulfate, thiourea and cyanide for metal recovery (Veglio, *et al*., 2003). Since high grade ores are exhausted, the traditional approaches for metal recovery such as pyrometallurgy seem to have become less economically feasible, and newer processes are being researched for the lower grade deposits. Microbe-based metal extraction processes, that is, bioleaching and biosorption offer several benefits in the extraction from lower-grade ores since they are comparatively more environmentally friendly than other physico-chemical processes, consume less energy than is required for smelting or roasting, avoid the emission of harmful gases such as sulphur dioxide and produce chemically inactive tailings (Rawlings, *et al*., 2003). The process of bioleaching involves the acidification by products of bacterial metabolism or the oxidation by leaching bacteria to remove heavy metals. Thus, the acid production and oxidation activity of the bacteria act as mechanisms for the process of bioleaching (Chen and Lin, 2004). The metals which are commonly bioleached include cobalt, copper, zinc, nickel and uranium (Table 1). The extraction of these metals is done from insoluble sulphides or from oxides (in case of uranium). In case of gold and silver recovery, leaching bacteria are employed only before cyanidation treatment to eliminate interfering metal sulphides from the ores. In this case, it is preferred to use the term bio-oxidation since there is no recovery of the metals which are bioleached such as iron and arsenic. Biomining is a broad term which covers both bio-oxidation and bioleaching (Rawlings, 2002; Rohwerder, *et al*., 2003; Bosecker, 1997; Ehrlich, *et al*., 2015; Olson, *et al*., 2003). Chemolithotrophs play a vital role in metal bioleaching from rocks, ores or metal bearing wastes. The first process described here, occurs by ferric ion and proton reactions. The cells growing in the biofilms in the extracellular polysaccharide perform these reactions. Microbes are involved in the production of sulphuric acid used for proton attack as well as to contain iron in ferric form for oxidative attack on metal (Rawlings, 2005; Suzuki, 2001). Tributsch in 1999 described three different strategies for bioleaching. In Contact/ Direct Bioleaching (Eqs. 1 and 2), microorganisms attach to the surface of minerals and are involved in mineral preparation to facilitate mineral attack by electrochemical dissolution which involves Fe^{3+} present in the extracellular polymeric substances (EPS) of microbes (Tributsch, 1999).

Direct mechanism of bioleaching (Sand, et al., 2001)

$$2FeS_2 + 3.5O_2 + H_2O \xrightarrow{\text{Bacteria}} Fe^{2+} + 2H^+ + 2SO_4^{2-} \quad (1)$$

$$2Fe^{2+} + 0.5\,O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (2)$$

The second strategy is indirect Bioleaching in which microbes are involved in renewing the bioleaching reagent Fe^{3+}. There are two mechanisms of indirect oxidation for metal sulphides. In the first mechanism (Eqs. 3 and 4), acid insoluble metal sulphides such as FeS_2, MoS_2 and WS_2 undergo oxidative attack by Fe^{3+} ions. Thiosulfate is formed as sulphur intermediate. In the second mechanism (Eqs. 5, 6 and 7), there is an attack of Fe^{3+} ions or protons which allows dissolution. Here, Polysulfide and finally elemental sulphur is formed as an intermediate. The two mechanisms are illustrated below: (Sand, *et al*., 2001)

Bioleaching mechanism (Thiosulfate mechanism) for Pyrite (FeS_2), Molybdenite (MoS_2), Tungstenite (WS_2):

$$FeS_2 + 6Fe^{3+} + 3\,H_2O \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+ \quad (3)$$

$$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \rightarrow 2\,SO_4^{2-} + 8Fe^{2+} + 10H^+ \quad (4)$$

Bioleaching mechanism (Polysulfide mechanism) for chalcopyrite ($CuFeS_2$), sphalerite (ZnS), galena (PbS), orpiment (As_2S_3), hauerite (MnS_2), realgar (AsS):

$$MS + Fe^{3+} + H^+ \rightarrow M^{2+} + 0.5H_2S_n + Fe^{2+}\ (n\geq 2) \quad (5)$$

$$0.5\,H_2S_n + Fe^{3+} \rightarrow 0.125\,S_8 + Fe^{2+} + H^+ \quad (6)$$

$$0.125\,S_8 + 1.5\,O_2 + H_2O \xrightarrow[\text{Bacteria}]{} SO_4^{2-} + 2H^+ \quad (7)$$

The last strategy is Cooperative bioleaching, whereby there is cooperation between free bacteria present in solution and microbes found attached to mineral surface. Through contact bioleaching, the attached microorganisms are involved in the release of chemicals in the solution which serve as the energy source for free microbes in solution. Apart from these, there are two other mechanisms which contribute to bioleaching: galvanic leaching and acid leaching (Tributsch, 1999).

Overview of chemolithotrophs found in mineral mines

All primary biomining microbes are chemolithotrophs which can use reduced inorganic sulphur or ferrous as donors of electrons. They thrive in a pH range of 1.5-2.0. This acidophilic nature is attributed to the fact that sulphuric acid is formed as the by-product of oxidation of sulphur. Biomining bacteria grow well in aerated conditions and fix carbon dioxide, though the efficiency of fixation may vary (Rawlings, 2002). Some important chemolithotrophic bacteria important in mineral bio-oxidation include *Acidithiobacillus thiooxidans, Acidithiobacillus ferrooxidans, Acidithiobacillus caldus, Leptospirillum ferrooxidans, Leptospirillum ferriphilum and Acidiphilium* (Coram and Rawlings, 2002; Hallberg and Johnson, 2001; Kelly and Wood, 2000; Rawlings, 2002). At temperatures higher than 70 degrees, biomining is carried out mainly by archaea such as *Sulfolobus sp*. and *Metallosphaera sp*. (Norris, *et al*., 2000). Some additional microorganisms associated with different minerals are listed in Table 1.

Acidithiobacillus thiooxidans

Acidithiobacillus thiooxidans (A. thiooxidans) can be described as an aerobic and mesophilic bacterium which belongs to the genus *Acidithiobacillus* and plays a vital role in industrial bioleaching (Yin, *et al*., 2014). Three genomes of the *Acidithiobacillus thiooxidans* strains have been sequenced and deposited in National Center for Biotechnology Information (NCBI) (Travisany, *et al*., 2014; Valdes, *et al*., 2011; Yin, *et al*., 2014). In 2016b, Zhang *et al* extracted and sequenced six new genomic DNA of the strains which were isolated from various acidic places in China, with the aim to understand the niche adaptation and genetic diversity within different strains of the bacterium. The genetic and functional characteristics were studied by comparative genomics. Phylogeny of core genome was studied and it was identified that genetic diversity among the strains was dependent on geochemical conditions of habitat as well as geographic distribution. The genes involved in metabolism were uncovered by functional assignment of common genes. Lastly, comprehensive analysis was used to evaluate the intraspecific diversification in relation to the predicted metabolic profiles (Zhang, *et al*., 2016b). Jang and Valix, 2017 performed the adaptation of this bacterial strain to heavy metals by gradual acclimatisation to overcome the heavy metals' bacteriostatic effects on the strain. Cultivation of the bacterium was done in heavy metals such as cobalt, nickel, iron, chromium, manganese and magnesium in the concentration range of 2400- 24000 ppm. Acid generation, adaptation period and concentration of metal were three factors on which adaptation evolution was found to be dependent. It was found that heavy metal stress promoted the production of acid and biostimulation of growth of cell. When metal tolerance was pre-established in the bacterial strain, it was found that the leaching rate increased in early phase: around 20% increase in Ni and 7% increase in Co recovery by using adapted bacteria. It was concluded that *Acidithiobacillus thiooxidans* is suitable for direct bioleaching of nickel ores (Jang and Valix, 2017). Nguyen *et al.,* 2016 described the biosorption property of *Acidithiobacillus thiooxidans* by testing the capability of the strain to remove sulphur blue 15 dye from water. The process of biosorption was shown to follow pseudo second order rate kinetics. In the study of response surface methodology, they used a central composite design in order to analyse independent variables. They concluded that the strain also oxidises sulphur compounds to sulphuric acid in addition its biosorption property (Nguyen *et al*., 2016). *Acidithiobacillus thiooxidans* has been used for the bioleaching of various metals in the past, including Arsenic, Magnesium, Zinc, Copper, Chromium (Hocheng, *et al*., 2014; Lee, *et al*., 2015, Wang, *et al*., 2007). *Acidithiobacillus thiooxidans* along with *Acidithiobacillus ferroorxidans,* finds applications in the solubilisation of metals from printed wire boards. The mixed culture can also effectively bioleach lead and zinc along with the copper (Wang, *et al*., 2009).

Acidithiobacillus ferrooxidans

Acidithiobacillus ferrooxidans is a γ-proteobacterium which is gram negative and has optimum growth temperature of 30°C, at pH 2, though it could also thrive at lower values of pH (Rohwerder, *et al*., 2003). It is found in association with pyrite ores and coal deposits in addition to their acidic drainages (Davis, *et al*., 2000; Gonzalez-Toril, *et al*., 2003) and is chiefly used in the recovery of copper through the process of biomining or bioleaching (Rawlings, 2002). Carbon dioxide fixation is carried out via Calvin-Benson-Bassham cycle (Fig. 1) by utilising the energy which has been obtained by iron and sulphur oxidation (Friedrich, *et al*, 2001). *Acidithiobacillus ferrooxidans*, in addition to cooper recovery, has been known to be actively involved in the bioleaching of Arsenic (Park, *et al*., 2014), Ferrous (Zhu, *et al*., 2017), Nickel, Cadmium, Cobalt (Bajestani, *et al*.,

Table 1: Different minerals found in nature and bacteria associated with bioleaching (Ardnt, *et al.,* 2015)

Class	Element	Mineral	Composition	Bioleaching microbes associated with the mineral	References
Ferrous metals	Iron (Fe)	Hematite	Fe_2O_3	*A.thiooxidans, A.ferrooxidans*	Huang, *et al*., 2013
	Manganese (Mn)	Pyrolusite	MnO_2	*A.ferrooxidans ,Penicillum citrinum*	Acharya, *et al.,* 2003; Feng, *et al*., 2017
	Chromium (Cr)	Chromite	$FeCr_2O_4$	*Lactobacillus acidophilus, Staphylococcus lactis, Lactobacillus sp., Propionibacterium acnes*	Acharya, *et al*., 1998
	Nickel (Ni)	Pentlandite	$(Fe,Ni)_9S_8$	*A. ferrooxidans, A. thiooxidans, L. ferrooxidans*	Brierley and Brierley, 2001
		Garnierite	$(Ni, Mg)_3Si_2O_5(OH)_4$	*A.niger*	Castro, *et al*., 2000
	Molybdenum (Mo)	Molybdenite	MoS_2	*Acidianus brierleyi, Acidithiobacillus ferrooxidans , Acidithiobacillus thiooxidans, Leptosporillum ferrooxidans, Acidianus ambivalens and Sulfolobus solfataricus*	Rastegar, *et al*., 2014; Pistaccio, *et al*., 1994; Roshani, *et al*., 2017; Romano, *et al*., 2001
	Vanadium (V)	Magnetite	$(Fe, V)_3O_4$	*Acidithiobacillus ferrooxidans*	Liu, *et al*., 2013
Aluminium	Aluminium (Al)	Gibbsite	$Al(OH)_3$	*Paenibacillus polymyxa*	Natarajan, 2016
Base metals	Copper (Cu)	Chalcopyrite	$CuFeS_2$	*Acidithiobacillus ferrooxidans, Leptospirrilum ferriphilum, Ferroplasma acidiphilum, Acidithiobacillus thioxidans, Acidithiobacillus caldus, Metallosphaera sedula, Sufobacillus sp., Acidianus brierleyi, Acidianus infernus, Sulfolobus shibatae , Sulfolobus acidocaldarius, and sulfolobus metallicus*	Panda, *et al*., 2015; Stott, *et al*., 2003
		Chalcocite	Cu_2S	*Leptospirillum ferriphilum, Acidithiobacillus caldus, Acidithiobacillus thiooxidans, Acidithiobacillus albertensis, Sulfobacillus thermotolerans, Sulfobacillus thermosulfidooxidans*	Xingyu, *et al*., 2010
		Cuprite	Cu_2O	*Alicyclobacillus sp.*	Chaerun, *et al*., 2017
		Azurite	$Cu_3(CO_3)_2(OH)_2$	*Sulfobacillus thermosulfidooxidans strain-RDB, Thermoplasma acidophilum*	Ilyas, *et al*., 2012
	Zinc (Zn)	Sphalerite	(Zn, Fe)S	*A.thiooxidans, A.ferrooxidans, Leptospirrilum sp.*	Rodríguez, *et al*., 2003b; Xia, *et al*., 2008
	Lead (Pb)	Galena	PbS	*A.ferrooxidans*	Adekola, *et al*., 2011
Metalloid	Arsenic (As)	Realgar	AsS	*A.ferrooxidans, A.thiooxidans*	Zhang, *et al*., 2007
		Orpiment	As_2S3	*A.ferrooxidans, Sulfobacillus sibiricus*	Zhang, *et al*., 2015
Energy sources	Uranium (U)	Pitchblende	UO_2	*A.ferrooxidans, A. acidophilus, A. thiooxidans, Leptospirillum ferrooxidans , Leptospirillum ferriphilum*	Chen, *et al*., 2016
High-technology metals	Titanium (Ti)	Ilmenite	$FeTiO_3$	*Aspergillus niger, Penicillium citrinum, Bacillus megaterium*	Jonglertjunya and Rubcumintara, 2013

2014), Vanadium, Nickel and Copper (Rastegar, *et al*., 2015). Recent research on this bacterium has been focused on increasing the efficiency of bioleaching by using catalysts. Zhang *et al.,* 2016a used polyethylene glycol (PEG) as a catalyst to increase the output of chalcopyrite bioleaching with *Acidithiobacillus ferrooxidans.* They concluded that when PEG was added, the bioleaching of chalcopyrite was enhanced as PEG increased the oxidation of sulphur by the bacterium as a result of enhancement in attachment of bacteria. Also, PEG could remove elemental sulphur from the surface of chalcopyrite, leading to increase in the efficiency of bioleaching (Zhang, *et al*, 2016a). Gu *et al.,* in 2017 explored the effect of Graphene as a catalyst in copper bioleaching by *Acidithiobacillus ferrooxidans* from waste printed circuit board (WPCB). They performed various tests such as graphene optimal dosage test, compatibility test of graphene and *Acidithiobacillus ferrooxidans*, graphene mechanism analysis, orthogonal experiment design and recycling test for used graphene to conclude that the catalysis was promising and could enhance the bioleaching efficiency (Gu, *et al*., 2017). Research on the bacterium has also been revolving around the possession of metal active properties. Arshadi and Mousavi, 2015 confirmed that *Acidithiobacillus ferrooxidans* is resistant to heavy metal toxicity by obtaining a simultaneous recovery of up to 99% copper and nickel from mobile phone printed circuit board (MPPCB) through optimization procedures. The bioleaching from printed circuit board was carried out using adapted *Acidithiobacillus ferrooxidans.* It was shown that four factors influenced that rate of bioleaching- initial iron concentration, initial pH, particle size and density of pulp (Arshadi and Mousavi, 2015). *Acidithiobacillus ferrooxidans*, being the most common bioleaching organism, has wide commercial applications. It has been used extensively in commercial bioleaching and biooxidation of pyrite and other related ores [Rawlings, *et al*., 1999b]. It is also the predominant organism in the consortia of microbes used in industrial copper recovery by bioleaching or biomining [Valdés, *et al*., 2008]. Finally, it plays a major role in E-waste management since it is the most important organism used in bioleaching of metals from printed circuit boards (Wang, *et al*., 2009).

Acidithiobacillus caldus

This bacterium thrives in acidic environments of pH 1-3 and is active in bioleaching of copper and natural acid drainage systems (Gonzalez-Toril, *et al*., 2003; Rawlings, 2002; Rohwerder, *et al*., 2003). It is acidophilic, moderately thermophilic, gram negative and a sulphur oxidiser (Hallberg and Lindstrom, 1994). It is unable to perform nitrogen fixation and iron oxidation (Norris *et al*., 1995) and can grow at temperatures of up to 45 to 50°C, as opposed to the other two species of *Acidiothiobacillus* genus. Valdes *et al* in 2009 carried out the generation and annotation draft genome sequence of this strain and metabolic reconstruction to identify the strategies that this strain uses for the assimilation of nutrients and energy (Valdes, *et al*., 2009). *Acidithiobacillus* has the potential to be used in marketable arsenopyrite bio-oxidation tanks along with the iron oxidiser *Leptospirillum ferriphilum* (Rawlings, *et al*., 1999b). These tanks are aerated and used in decomposition and opening of the structure of gold containing arsenopyrite ores for cyanide mediated gold extraction (Rawlings, *et al*., 2003). Recent research has explored the bioleaching potential of mixed culture which includes *Acidithiobacillus caldus*. Ngoma *et al.,* 2017 investigated the arsenic leaching kinetics of arsenopyrite containing South Korean mine tailings using a mesophilic culture of *Acidithiobacillus Caldus* (56%), *Leptospirillum ferriphilum* (29%) and other Archaea (15%). The evaluation of the efficiency of leaching was done using iron oxidation rates, arsenic release in solution, redox potential and pH. They obtained Arsenic solubilisation between 94%-97% around 12.5 weeks after inoculation (Ngoma, *et al*., 2017).

Leptospirillum ferrooxidans

Leptospirillum ferrooxidans is an aerobic, gram negative, non-spore forming microorganism, which grows in a pH range of 1.5-4 on a mineral medium rich in iron. Use of sulphur as energy source is not reported (Donati and Sand, 2007; Harrison, 1984; Harrison and Norris, 1985; Harneit, *et al*., 2006). Rawlings *et al.,* 1999b reported that *Leptospirillum ferrooxidans* and *Thiobacillus thiooxidans* are used very commonly in biooxidation processes for copper treatment by heap leaching and zinc-lead or arsenopyrite ores through continuous flow tank leaching (Rawlings, *et al*., 1999b). Giaveno *et al.,* 2007 carried out bioleaching of complex low-grade sulphide ores using a native strain of *Leptospirillum ferrooxidans* and found that the zinc recovery from the ores was higher than that obtained with bioleaching by *Acidithiobacillus*

Table 2: Summary of results of recent research work carried out by various researchers on different bioleaching bacteria

Sr. no.	Organism	Researcher	Year	Result
1	*Acidithiobacillus thiooxidans*	Zhang, *et al.*	2016b	Six new genomic DNA of strains of *Acidithiobacillus thiooxidans* were sequenced Phylogeny of core genome revealed that genetic diversity among the strains was dependent on geochemical conditions of habitat as well as geographic distribution
		Jang and Valix	2016	When metal tolerance was pre-established in *Acidithiobacillus thiooxidans*, the leaching rate increased in early phase: around 20% increase in Ni and 7% increase in Co recovery by using adapted bacteria.
		Nguyen, *et al.*,	2016	*Acidithiobacillus thiooxidans* oxidises sulphur compounds to sulphuric acid in addition its biosorption property
2	*Acidithiobacillus ferrooxidans*	Zhang, *et al.*	2016a	When PEG was added, the bioleaching of chalcopyrite by *Acidithiobacillus ferrooxidans* was enhanced as PEG increased the oxidation of sulphur by the bacterium as a result of enhancement in attachment of bacteria.
		Gu, *et al.*	2017	Using grapheme as catalyst in copper bioleaching by *Acidithiobacillus ferrooxidans* was promising and could enhance the bioleaching efficiency
		Arshadi and Mousavi,	2015	Recovery of 99% copper and nickel from mobile phone printed circuit board (MPPCB) using *Acidithiobacillus ferrooxidans* through optimization procedures were done
3	*Acidithiobacillus caldus*	Ngoma, *et al.*	2017	Obtained Arsenic solubilisation between 94%-97% around 12.5 weeks after inoculation using a mesophilic culture of *Acidithiobacillus Caldus* (56%), *Leptospirillum ferriphilum* (29%) and other Archaea (15%)
4	*Leptospirillum ferrooxidans*	Giaveno, *et al.*	2007	The strain of *Leptospirillum ferrooxidans* was very efficient in complex sulphides processing even in the absence of sulphur oxidisers
		Corkhill, *et al.*	2008	*Leptospirillum ferrooxidans* can withstand over hundred ppm of As(III) and As(V)
5	*Leptospirillum ferriphilum*	Smith and Johnson	2017	*Leptospirillum ferriphilum,* when cultivated in conjunction with *Acidithiobacillus caldus,* could survive for long time on the sulphur medium containing sparse soluble iron concentration, suggesting the occurrence of dynamic cycling in the co-culture of these bacteria.
		Wang, *et al.*	2017	In the bioleaching of bornite by *Leptospirillum ferriphilum*, efficiency of copper extraction was increased to 95.9% when pyrite was added as compared to a mere 19% without the addition of pyrite
		Wang, *et al.*	2018	In the analysis of the diversity and dynamics of microorganisms in leachates and ore surfaces in copper sulphide bioleaching, *Leptospirillum ferriphilum* was present in maximum proportion almost all the times when pH was operated during the bioleaching
6	*Acidiphilum*	Merino, *et al.*	2016	A synergistic interaction existed between *Ferroplasma acidiphilum* and *Leptospirillum ferriphilum*
7	*Sulfolobus sp.*	Roshani, *et al.*	2017	Recoveries of molybdenum and uranium in bioleaching experiments resulted in 43.2% and 79.1% respectively using *Acidianus ambivalens* and *Sulfolobus solfataricus*
8	*Metallosphaera sp.*	Ai, *et al.*	2017	Increase in the bioleaching capacity of a consortium which consisted of genetically engineered copper sensitive strain and naturally occurring strain of *Metallosphaera sedula*

ferrooxidans. They concluded that the strain of *Leptospirillum ferrooxidans* was very efficient in complex sulphides processing even in the absence of sulphur oxidisers (Giaveno, *et al*., 2007). Corkhill *et al., 2008* characterised Arsenopyrite (FeAsS) and enargite (Cu_3AsS_4) after oxidative dissolution both in the absence and presence of *Leptospirillum ferrooxidans* and showed that the bacteria can withstand over hundred ppm of As(III) and As(V). Coexisting aqueous solution was analysed to monitor the dissolution, and it was found that arsenopyrite and enargite dissolution reactions released 917 and 180 ppm arsenic in solution respectively. The researchers also identified that arsenopyrite bioleaching proceeds via direct oxidation mechanism or combination of direct and indirect mechanisms while enargite bioleaching follows indirect mechanism since cells did not have any contact with the surface (Corkhill, *et al*., 2008). *Leptpspirillum ferrooxidans* finds application in commercial bioleaching and biooxidation of pyrite and other related ores (Rawlings, *et al*., 1999a).

Leptospirillum ferriphilum

A gram negative, iron oxidising, spore forming, thermotolerant, motile bacteria (Rawlings, *et al*., 1999b; Liu, *et al*., 2007) and can grow at an optimum pH and temperature of 1.68 and 40°C respectively (Liu, *et al*., 2007). According to previous studies, *Leptospirillum ferriphilum* is unable to fix atmospheric nitrogen (d'Hugues, *et al*., 2008; García-Moyano, *et al*., 2008; Tyson, *et al*., 2004), but latter studies revealed that certain strains can switch to nitrogen fixation when NH^{4+} becomes scarce (Galleguillos, *et al*., 2013; Issotta, *et al*., 2016). Research on this bacterium has been proceeding in different directions, from using mixed culture containing this microbe to individual leaching by the organism. Smith and Johnson, 2018 cultivated *Leptospirillum ferriphilum* in conjunction with *Acidithiobacillus caldus* and demonstrated that *Leptospirillum ferriphilum* could could survive for a long period of time on the sulphur medium containing sparse soluble iron concentration, thus suggesting the occurrence of dynamic cycling in the co-culture of these bacteria. When the mixed culture did not contain sulphur, the population of *Leptospirillum ferriphilum* was reduced at a rapid rate. Moreover, the presence of *Leptospirillum ferriphilum* partially inhibited the growth of *Acidithiobacillus caldus* as the sulphur oxidising bacteria was more sensitive to ferric ion rather than ferrous ion, and also to positive redox potentials (Smith and Johnson, 2018). Wang *et al.,* 2017 explored the bioleaching of bornite by *Leptospirillum ferriphilum* and pyrite effect on bioleaching. Through the bioleaching experiments, they reported that efficiency of copper extraction was increased to 95.9% when pyrite was added as compared to a mere 19% without the addition of pyrite. Another effect of pyrite addition was decrease in acid consumption during bioleaching. Apart of that, electrochemical experiments carried out suggested the existence of a galvanic effect between pyrite and bornite. To show that pyrite addition enhanced bornite dissolution, Tafel plot (a graph of overpotential against log current (Zhou *et al*., 2013) as well as galvanic corrosion test was carried out. A model mechanism of how bornite dissolution was enhanced by pyrite was proposed (Wang, *et al*., 2017). Wang *et al.,* 2018 analysed the diversity and dynamics of microorganisms in leachates and ore surfaces in copper sulphide bioleaching at variable pH values using mesophiles and moderate thermophiles. They found that *Leptospirillum ferriphilum* was present in maximum proportion in the community almost all the times when pH was operated during the bioleaching (Wang, *et al*., 2018). Leptospirillum ferriphilum is a part of the mixed culture of bacterial consortium in industrial pyrite biooxidation plant which enhances dissolution of pyrite (Okibe and Johnson, 2004).

Acidiphilum

The bacteria belonging to this genus are rod shaped, gram negative, obligate acidophilic and grow in aerobic environment. It is believed that they increase the growth of lithotrophs by eliminating excreted inhibitory organic compounds (Johnson and Hallberg, 2009). Merino *et al.,* 2016 posed a hypothesis that *Ferroplasma acidiphilum* takes up the organic matter which *Leptospirillum ferriphilum* secretes and that maintains a low level of the compounds in the mixed culture, thus saving *Leptospirillum ferriphilum* from their toxic effects. They showed that the strain of *Ferroplasma acidiphilum* they were working on was chemomixotrophic and that yeast extract at 0.04% weight/volume concentration maximised its growth. Their results from the experiments of one organism growing in the supernatant of another proved a synergistic interaction between the two species (Merino, *et al*., 2016).

Sulfolobus sp.

Sulfolobus metallicus is a thermophillic Archaea used in agitated tank bioleaching reactors. They act as catalysts in iron and sulphur oxidation, thus resulting in beneficiation or the dissolution of the metals like cobalt, copper, gold or nickel (Rawlings, *et al*., 2003; Norris, *et al*., 2000; Batty and Rorke, 2006). They can grow autotrophically in a temperature range of 65- 80 °C and are mostly used for the bioleaching of chalcopyrite (Vilcáez *et al*., 2008; Blázquez *et al*, 1999; Muñoz *et al*, 1998; Rodríguez *et al*., 2003a, Stott *et al*., 2003). When *Sulfolobus metallicus* is used for chalcopyrite bioleaching, it oxidises residual sulphur compounds (Gautier, *et al*., 2008). The rate of copper dissolution increases dramatically when compared to dissolution of chalcopyrite during simple indirect bacterial action due to this oxidative process (Jordan *et al*., 2006). Roshani *et al.,* 2017 isolated and characterized the strains *Acidianus ambivalens* and *Sulfolobus solfataricus* which were able to bioleach molybdenum ore. They carried out an experimental design to optimize bioleaching process by these strains. The selected responses were recovery of uranium and molybdenum and important factors such as initial pH, pulp density and ferric ion concentration were variables. After the bioleaching experiments, they obtained recoveries of molybdenum and uranium as 43.2% and 79.1% respectively (Roshani, *et al*., 2017). The first industrial use of the strain was detected by Demergasso et al in 2005 when they found hyperthermophilic archaeal organisms which were related to *Sulfolobus* to be present in the process of industrial heap bioleaching of low grade copper ore which operated at a temperature lesser than 60 °C (Demergasso, *et al*., 2005).

Metallosphaera sp.

It is a thermoacidophilic crenarchaeote and lithoautotroph that can grow best at 75°C and pH 2.0 (Huber, *et al*., 1989; Auernik, *et al*., 2008). It can oxidise sulphur and iron and is used in copper extraction from low-grade copper sulphide ores and tailings (Brierley and Brierley, 2013). Ai *et al.,* 2017 isolated Cu^{2+} and As^{5+} cross resistant strains of *Metallosphaera sedula* using Adaptive laboratory evolution (ALE). They observed an increase in the bioleaching capacity of a consortium which consisted of genetically engineered copper sensitive strain and naturally occurring strain. The predominant species was *Metallosphaera sedula* ARS50-2 and after its introduction, there was modulation in the proportion of other strains (Ai, *et al*., 2017).

CONCLUSION

Among extremophiles, Chemolithotrophic bacteria play a very important role in nature. Their metal solubilising ability is mainly used in bioleaching process and holds applications in electronic waste management, metal pollution reduction, bioremediation etc. Vast amount of research has been conducted in this field with respect to diversity of chemolithotrophs and their application in bioleaching. From the review, it can be seen that in the past few years, many different chemolithotrophs have been identified and characterized. These chemolithotrophs are involved in the bioleaching of various metals. However, there is still ample scope for exploring the vast diversity of bacteria found in mining sites. Research till date has been mainly focused on common chemolithotrophs belonging to *Acidithiobacillus* and *Leptospirillum* genus. Isolation and characterization of new bacteria and understanding the mechanism of bioleaching could aid in developing efficient and more productive procedures for commercial scale bioleaching process.

ACKNOWLEDGEMENTS

This work was supported by Science and Engineering Research Board by funding the project “Differential membrane lipid profile and fluidity of *Acidithiobacillus ferrooxidans* during the process of adhesion to minerals” Department of Sciences and Technology, India [grant number DO No. SR/S3/ME/0025/2010], which helped Dr. Anand Prem Rajan to establish Geo-microbiology laboratory at VIT, laid foundation to explore industrially important elite chemolithotrophs. All authors would also like to thank management of VIT for all the necessary facilities provided. Dr. Anand Prem Rajan thank Prof K. A Natarajan, IISc Bangalore and Dr. Preston Devasia, Singapore for their immense inputs for successful completion of this project.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

ABBREVIATIONS

%	Percentage
$(Fe,V)_3O_4$	Magnetite
$(Fe_2Ni)_9S_8$	Pentlandite
$(Ni,Mg)_3Si_2O_5(OH)_4$	Garnierite
(Zn,Fe)S	Sphalerite
°C	degree Celcius
$Al(OH)_3$	Aluminium hydroxide (Gibbsite)
ALE	*Adaptive Laboratory Evolution*
As(III)	*Trivalent arsenic*
As(V)	*Pentavalent arsenic*
As_2S_3	Arsenic trisulfide (Orpiment)
As^{5+}	*Arsenic*
AsS	Arsenic monosulfide (Realgar)
ATP	Adenosine triphosphate
Co	Cobalt
CO_2	Carbon dioxide
Cu^{2+}	*Copper ion*
Cu_2O	Cuprous oxide (Cuprite)
Cu_2S	Copper(I) sulphide (chalcocite)
$Cu_3(CO_3)_2(OH)_2$	Azurite
Cu_3AsS_4	Copper Arsenic Sulfide (enargite)
$CuFeS_2$	Copper iron sulphide (Chalcopyrite)
DNA	Deoxyribo Nucleic Acid
DO	Dispatch Order
EPS	Extracellular polymeric substances
Fe^{2+}	Ferrous ion
Fe_2O_3	ferric oxide
Fe^{3+}	Ferric ion
FeAsS	iron arsenic sulphide (Arsenopyrite)
$FeCr_2O_4$	iron chromium oxide (Chromite)
FeS_2	Iron sulphide
$FeTiO_3$	titanium-iron oxide (Ilmenite)
H^+	Proton
H_2O	Water
H_2S_n	Hydrogen polysulphide
M^{2+}	Metal ion
MnO_2	Manganese dioxide
MnS_2	Manganese(II) sulphide
MoS_2	molybdenum disulphide (Molybdenite)
MPPCB	Mobile phone printed circuit board
MS	Metal sulphide
NADH	Nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information
NH^{4+}	Ammonium cation
Ni	Nickel
O_2	Oxygen
PbS	Lead sulphide (Galena)
PEG	Polyethylene glycol
pH	Potential of hydrogen
Ppm	parts per million
S_8	Octasulfur
SO_4^{2+}	Sulfate
Sp.	Species
UO_2	Uranium dioxide (Pitchblende)
VIT	Vellore Institute of Technology
WPCB	Waste printed circuit board
WS_2	Tungsten disulphide (Tungstenite)
ZnS	Zinc sulphide
γ	Gamma

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HOW TO CITE THIS ARTICLE

Deshpande, A.S., Kumari, R., Prem Rajan, A., (2018). A delve into the exploration of potential bacterial extremophiles used for metal recovery. Global. J. Environ. Sci. Manage., 4(3): 373-386.

DOI: 10.22034/gjesm.2018.03.010

url: http://www.gjesm.net/article_31211.html