IJEAST

INTERNATIONAL JOURNAL OF ENGINEERING APPLIED SCIENCE AND TECHNOLOGY

VOLUME : 6 ISSUE : 3 Print / Issue Publication Date: 16-Sep-2021

ISSN : 2455-2143

DOI : 10.33564/IJEAST.2021.v06i03.037

Indexed In

Google SCHOLAR OPEN ACCESS doi Crossref

WWW.IJEAST.COM

editor@ijeast.com

International Journal of Engineering Applied Sciences and Technology, 2021
Vol. 6, Issue 3, ISSN No. 2455-2143, Pages 231-237

A COMPREHENSIVE REVIEW ON THE USE OF MICROBES IN MINERAL RECOVERY AND ACID MINES

Rakshita Sawani
Amity Institute of Biotechnology
Amity University, Noida, Uttar Pradesh, India

Abstract- **Biomining is successful on the commercial scale for the recovery of various metals such as copper and ores from their ores. The methodology involved in this is primarily chemistry-driven and is a combination of ferric and hydrogen ions which varies on the type involved. Hydrogen ions present here are produced by the activity of chemolithotrophic bacteria growing in a highly acidic environment. Bioleaching reactions, on the other hand, the role of microorganisms involved, and whether the reaction carried out are direct or indirect are discussed below. In places where the availability of oxidants to sulfide mineral surfaces is exposed due to mining, the acid mine drainage tends to contaminate the surroundings as well. Microorganisms that mainly consist of autotrophic and heterotrophic archaea and bacteria, take part in catalyzing iron and sulfur oxidation which determines the release of metals and sulfur into the surrounding. Indication towards the physiological synergy in sulfur, iron, and carbon flow in the microbial community is assessed. With this, the development, and future aspects of this with the challenges in mind are described as well.**

Keywords- **Biomining, Acid mine drainage, Bioleaching, Iron oxidation, Chemolithotrophic bacteria**

~~I.~~ INTRODUCTION

Biomining is a substantially used term to depict the usage of microbes in order to expedite the descent of metals from sulfide or iron-containing ores or its distills [1]. As the metal is extracted by the conversion of solid metal values into their water-soluble forms by the action of these microorganisms, this procedure is referred to in the direction of bioleaching or bio-oxidation (in the case of gold recovery as the metal remains in the mineral form) [2].At present, in mineral extraction, in particular, microorganism usage plays an escalated role [3]. If the given mineral is responsive towards bioleaching, the metal is extracted economically even if the metal grade of the ore is comparatively low, for example the leaching procedure of copper from waste copper dumps [4]. The higher-grade minerals get cracked due to which there is an elevated need to undergo metal recovery from lower-grade minerals. Additionally, the process of bioleaching is more conservational when compared to other physiochemical metal extraction processes. As it's a naturally occurring phenomenon, whenever a sulfide- or iron-containing mine dump is unveiled to precipitation, metal-laden, acid solutions tend to leach out of the dump yard and cause pollution in the surrounding habitat [5]. When spraying of the dump yard is done as an intended extraction procedure, the metal source is recovered and the acid is counterbalanced, along with proper discarding of the solutions which results in a protected environment. As bioleaching is considered more of an economic process than alternative methods, it's used in metal recovery from higher-grade ores or their concentrates.

When chemical weathering of metal sulfide-rich rocks takes place, acidic, metal-rich fluids are formed that further result in hot acid rock drainage solutions. This is since metal sulfide oxidation reactions are greatly exothermic in nature. Predominantly, pyrite-rich deposits are mined to extract metals namely, Au, Cu, Pb, Zn, which as available as impurities in pyrite or concerning sulfide minerals for instance galena (PbS), chalcopyrite ($CuFeS_2$). Mining ultimately results in exposure of greater surface area of sulfide ores to air that causes an increase in acid generation. The zone where the rocks have low buffering ability tend to produce highly acidic toxic solutions that are known as acid mine drainage or AMD. This AMD environment is densely populated with a variety of microorganisms despite the acute heat, acidity, and high concentration of toxic metals and sulfate. The organisms tend to form a chemo autotrophically - based biosphere in their subsurface that is sustained by the help of electron donors. These electron donors have been derived from sulfide minerals, CO_2, O_2, and N_2 that are obtained from the atmosphere, and phosphate liberated by the water-rock interaction. The production of AMD at large is due to the increase in AMD formation because of the microbial activity [6].

II. MICROORGANISMS INVOLVED IN MINERAL DECOMPOSITION

2.1 General Characteristics:

To define a generalized microbial population, an analysis of the ecological system is important. Basically, iron- and sulfur-oxidizing chemolithotrophic bacteria and archaea have been involved in producing ferric iron and sulfuric acid which is utilized in bioleaching reactions and bio-oxidation of minerals [2]. These microorganisms are ideal for mineral solubilization and share a few common features between them. They can grow autotrophically while fixing atmospheric CO_2, hence it's not predetermined to sustain them on any carbon-based source. It's known that the vast majority of autotrophic systems use luminescent energy generated through sunlight, chemolithotroph, on the other hand, derive their energy by utilizing either ferrous iron or reduced inorganic sulfur compounds as electron donors and oxygen as electron acceptor. Usually, the mineral bio-oxidation process takes place at low pH between 1.4 and 1.6 where the sulfuric acid is obtained during the oxidation of inorganic sulfur. This acidic, low pH allows the microorganisms to utilize the iron cycle where the ferrous and ferric iron produced are soluble. The ferrous iron produced acts as an electron donor for iron oxidizers while the ferric iron is operated by sulfur-oxidizing organisms in lieu of oxygen as electron acceptor [7]. The nutritional requirements of the microbes are met by the aeration process done to iron- and sulfur-containing mineral suspension in a water source. As a result, the microbes that grow in a mineral-rich ecosystem are relatively tolerant to a wide array of metal ions [8], with a dissimilarity occurring within and between the species.

2.2 Types of Microorganisms:

As might be gathered from above, the intensity of iron- and sulfur-oxidizing microorganisms vary depending upon the temperature scale and the bioleaching procedures that could be carried out on this temperature scale. These microorganisms that take part in the bioleaching processes are analogous and intervene from ambient to 40°C within the temperature scale 45-55°C and 65-80°C.

When mineral bio-oxidation practices that specifically intervene at 40°C or less than that are deemed to be, an association of gram-negative bacteria is found to be the principal microorganisms taking part in it. These microorganisms comprise the iron- and sulfur-oxidizing *Acidithiobacillus ferrooxidans* (formerly, *Thiobacillus ferrooxidans*), the sulfur-oxidizing *Acidithiobacillus thiooxidans* (formerly, *Thiobacillus thiooxidans*), and *Acidithiobacillus caldus* (formerly, *Thiobacillus caldus*), and the iron-oxidizing *Leptospirillum ferrooxidans and Leptospirillum ferriphilum* [9-13].

Microbes working around 50°C in bioleaching methods are much lesser-known. Though, *At. caldus*, some *Leptospirillum* spp, bacteria that are part of the gram-positive genera *Sulfobacillus* and *Acidimicrobium* [14], and members of the archaeal genus, *Ferroplasma* [15] are considered to be ideal in this operating temperature. Archaea tends to become more prominent than bacteria where the temperature is greater than 65°C. Here, the species of *Sulfolobus* and *Metallosphaera* are more notable [16]. The archaea belong to the genus *Acidianus* such as *Ad. ambivalensi* and/or *Ad. Infernus* tends to grow on reduced sulfur and at low pH as well as high heat. But, are not considered ideal to participate in bioleaching procedures as these microbes grow at 50°C or less.

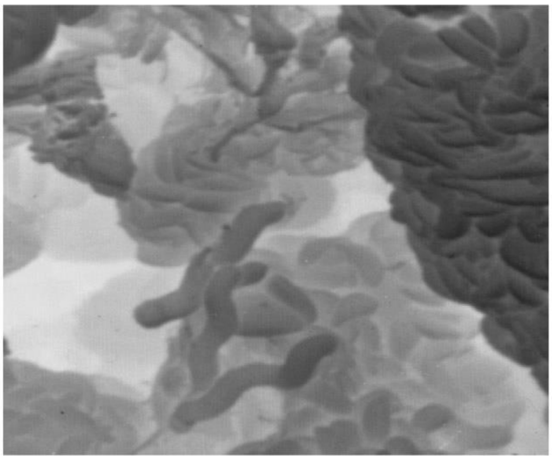

Fig 1. A scanning electron microscope photograph illustrating the typical spiral shape of a strain of Leptospirillum. Rod-shaped bacteria in different stages of entrapment in a biofilm on ore particles are also visible.

2.3 Are bioleaching reactions direct or indirect:

It's a persistent quarrel revolving around whether the microbial-assisted bio-oxidation of minerals taking place follows a "direct" or "indirect" mechanism [17]. To be absolute, a direct mechanism occurs in such a manner where the constituent within the bacterial membrane interacts directly with the sulfide or metal portion of the mineral by utilizing the enzymatic form of the mechanism [2]. Contrary to this, the indirect mechanism involves a chemical attack by protons or ferrous iron on leads to acid mines' production mineral sulfide whose consequence is the dissolution of the mineral and forming ferrous iron and several types of sulfur.

This ferrous iron is utilized by iron-oxidizing microorganisms as electron donors, which re-oxidizes it to ferric iron and thus regenerating the reactant.

The question here arises is whether the microorganisms play any role in the solubilization of the metals from minerals that is more than their capability to generate ferric iron and acid? This is part of the confusion in the direct vs. indirect dispute was caused when strong evidence was witnessed that involved attachment of microorganisms did, in fact, enhance the rate of leaching. Bacteria such as *At. ferrooxidans* [18-20] or *L. ferrooxidans* [21,22] show a strong affinity towards mineral surfaces like pyrite on which it attaches itself. When seen at face value, it appears that the microorganisms react on the mineral directly. Though, when studied through reaction kinetics, reaction stoichiometry [23], it suggested that the procedure involving mineral solubilization is physiochemical and indirect. In terms of efficiency, leaching reaction occurs inside the exopolysaccharide or EPS layer that envelops the microbial cells, is mainly by the indirect procedure. This EPS layer is generated by the microbial cells when they are multiplying on a mineral surface and not in the solution. This EPS is iron-impregnated and acts as the "reaction space" where the reactants are closely concentrated towards the surface of the mineral, resulting in a highly efficient chemical attack on the valence bonds of the mineral [24].

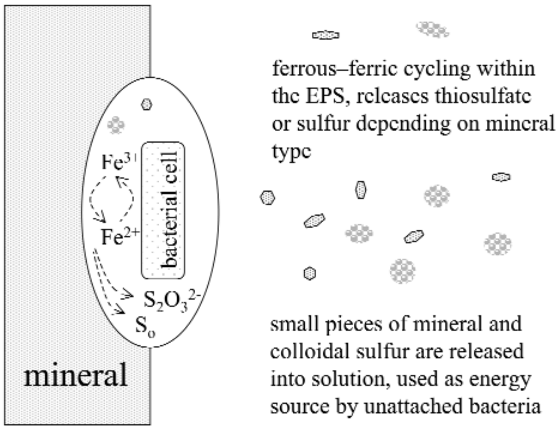

Fig 2. Schematic diagram illustrating the indirect leaching reactions believed to take place in the reaction space provided by the exopolysaccharde (EPS) layer that surrounds mineral attached microbial cells.

Keeping this in mind, there does exist potential indirect enzymatic attack. In a study carried out by Tributsch and co-workers [22.25], cysteine, an amino acid, can rapidly oxidize pyrite in the absence of bacteria or oxygen [26]. The pyrite has free SH groups that react with the sulfhydryl group present on the cysteine. This entire thiol-disulfide reaction results in the cysteine getting utilize by the pyrite with iron-sulfur species getting released. This direct attack on minerals by enzymes is evident as cysteine is present in most of them.

In short, when microorganisms get attached to a mineral surface ends up in the production of EPS and gives a reactive space to expedite an increase in mineral solubilization rate. This showcases a false appearance of the bioleaching process is direct. However, the essential reactions are chemically indirect where the chemical turnover occurs over short distances between the surface of the mineral and the surface of the bacterial cell.

2.4 Abundance, community structure, and physical/chemical regimes:

Predominately, the microbial community in an acidic environment mainly comprises *Thiobacilli*. Schrenk et al. [27] and Edwards et al. [28] reported that *A. ferrooxidans* were close to undetectable within the Richmond Mine at Iron Mountain (pH being 0.3-0.7 at 30-50°C) and in pyrite-dominated bioreactor systems [29]. This failure of *A. ferrooxidans* not being able to thrive in extreme AMD environments is characterized by their mesophilic optimal growth (26°C) and moderate acidophilic nature (pH 1.3-4.5). However, Druschel et al. [30] demonstrated that an *A. ferrooxidans* strain was present at large in an oxidized pool with pH 1.4.

There are *Leptospirillum* strains also present in these AMD environments that were portrayed by a wide range of temperatures and pHs. They are present in abundance as compared to *A. ferrooxidans* over its growth range. Sand et al. [31] noted that in the bioreactor systems, a high amount of Fe^{3+} to Fe^{2+} appeared to be less inhibitory to *Leptospirillum* than to *A. ferrooxidans*. Other than this, several other groups of bacteria are present in abundance in AMD environments. *F. acidophilus* has a pH scale ranging from 1.3 to 4.8 with temperatures varying from <20 to 40°C [32]. With the help of probe-based studies, *Ferromicrobium* spp. was found to be in minority [33]. *Acidimicrobium ferrooxidans* have been reported from a diverse range of environments [34] and are easily cultivated between 34 and 57°C [35].

Sulfobacillus spp. has a broad array of physical growth regimes with a few isolates capable of growing at 65°C [36]. The strains isolated from geothermal sites and studied by Yahya et al [37] were found to be effective pyrite oxidizers at pH<1. This concluded that thermophilic species can be found in AMD systems. *Sulfobacillus* clones were also recovered from pH 0.7-0.9 and 35-43°C [38] with around 6.8% microbial communities present [30].

Six members of the genus *Acidiphilum* are adapted to temperatures ranging from 17 to approximately 45°C and pH values from 1.5 to 60 [32]. Peccia et al. [39] found that genus *Acidiphilum* outnumbered *Acidithiobacillus ferrooxidans* in their mixed culture bioreactors with their population equal in the sediment samples. In broad, *Acidiphilum* sp. occupies at lower temperature and higher pH in microenvironments. Under anaerobic conditions, it contributes towards iron cycling by redissolving ferric iron-based minerals precipitated when the pH is increased with mixed with groundwater and in streams. The environmental distribution of *Thermoplasmatales* indicates adaptations towards high biomass, metal-enriched, pH 0.5-1.4, 30-50°C habitats close to air-biofilm interface [30]. *Metallosphaera* spp. (*Sulfolobales* order) tend to impact pyrite dissolution via catalysis of ferrous iron oxidation [40] and is isolated from an acidic uranium mine [41] and a bioleaching reactor [44].

III. ENVIRONMENTAL IMPACT OF BIOMINING ACTIVITIES

Acidophilic microorganisms tend to mobilize the metals and acid mines that can leave a negative impact on the environment. This acid mine drainage needs to be remediated. The contaminated locations containing acidic fluid barriers are sealed off and approaches are adopted to avoid spillage of the acidic effluents. These acidic effluents are monitored with the help of chemical treatments such as neutralizing the acidic pH with the help of calcium oxide [42]. These acidophilic microorganisms can be inhibited with the help of organic acid, sodium benzoate, sodium lauryl sulfate.

The process of bioremediation or removal of the toxic metals from the contaminated soil can be done by utilizing two opposite biological activities: sulfur-oxidizing bacteria with sulfur-reducing microbes. The sulfur-oxidizing bacteria produce sulfuric acid that bioleaches or solubilizes the metals in the solid phase. The leached metals are precipitated in a bioreactor where the hydrogen sulfide is eliminated. The hydrogen sulfide was produced in the presence of sulfate-reducing bacteria under anaerobic and neutral circumstances from insoluble metal sulfides. Metal contaminants such as Cu, Cd, Ni are leached from the contaminated environment and the effluent thus obtained is clean enough to be reused [42].

IV. SYNERGISTIC INTERACTION INVOLVING IRON, SULFUR, AND CARBON OXIDIZERS

The microbial community fluctuates with pH and temperature, and the concentration of the metal. Though, in the majority of the subsurface environments, within this microbial community individual species or their combination tend to perform iron oxidation, carbon fixation, sulfur oxidation, nitrogen fixation, in some cases extracellular polymeric slime production, along with iron and sulfur reduction.

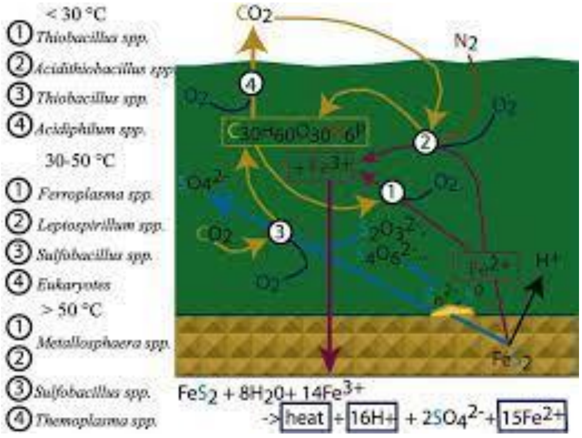

Fig 3. Potential iron, sulfur, and carbon cycling based on known metabolic capabilities (1, 2, 3, and 4) associated with AMD members. Crystalline pyrite (Fe_2S) is in yellow at the bottom and green is representing AMD solution. Elemental sulfur is shown at the pyrite-water interface as a possible inhibitor of surface dissolution. The overall oxidation of pyrite is shown at the bottom, with Fe^{3+} indicated as the primary oxidant. Intermediate sulfur compounds are indicated as follows: $S_2O_3^{2-}$ being thiosulfate and $S_4O_6^{2-}$ is tetrathionate. $C_{30}H_{60}O_{30}N_6P$ indicates organic carbon compounds.

Any interaction taking place between elements of microbial associations plays a key role in the optimization of the activity showcased by the AMD microbial community. For instance, a symbiotic relationship exists between heterotrophic and some autotrophic types: autotrophs happen to co-exist with heterotrophs to dispose of toxic organic compounds. In return, the heterotrophic acidophiles utilize the organic materials that are produced by the autotrophic acidophiles [43,44]. Autotroph *Acidithiobacillus* ferrooxidans subdued enough organic matter in its culture filtrate to support the growth of heterotrophic *S. thermosulfidooxidans* [45]. An experiment carried out by Clark and Norris [35] involved the mixing of *Acidimicrobium ferrooxidans. S. acidophilus* and *S. thermosulfidooxidans.* This concluded the substantial iron oxidation taking place in the mixed culture of the *Sulfobacillus* spp. and *Acidithiobacillus ferrooxidans* as compared to those of the pure culture accommodating the isolate.

V. ACID MINES TAILING WASTE AND ITS EFFECTS ON MICROORGANISMS

Acid mines tailings wastes or AMTW are the different minerals present in the mined rocks. Once the excavation is done and the sulfide minerals are exposed to atmospheric oxygen and water, a succession of bio-geochemical processes takes place that leads to the production of acid mines. The naturally occurring bacteria play a major role in this process and helps in the acceleration of acid mins generation by breaking down sulfide minerals. Factors that influence the rate of acid generation are the degree of saturation with water; oxygen, pH, temperature, chemical activity of Fe^{3+}, iron-oxidizing bacteria, the total surface area of the exposed metal sulfide [46].

Metal sulfides are reduced to their ferrous form in a reaction carried out with pyrite, a sulfide mineral, by ferric iron. This particular reaction occurs in the absence of oxygen and hence is a crucial step. The oxidation of ferrous iron is mediated either biologically with the help of iron-oxidizing bacteria such as *Gallionella ferruginea* or chemically by molecular oxygen at pH above 4 [47].

Acid mines tailing waste's chemical composition is usually ascertained by the minerals, microbiological, chemical as well as physical properties of the particular mining site. Here, the physical property consists of density, size, and the supply of the waste material along with the hydrological properties of the mining site. The chemical composition comprises the elevated concentration of the various metals and in some cases the dissolved salts other than sulfates [48].

When exposed to heavy metals extracted from acid mine wastes, several leads to acid mines' production further to disorders and diseases follow [49]. This is due to the synergistic effect between acidic pH and heavy metals that increases the bioavailability, thus increasing biotoxicity. The assembly of reactive oxygen species, due to this, damages the cellular plasma membrane. The damage includes alteration of biophysical parameters, the sudden increase in membrane permeability, enhanced build-up of the extracellular ions [50]. This AMTW mainly aids the heterotrophic microbial communities that undergo severe stress. As a result, the diversity of carbon utilization and the species richness is comparatively low among the microbial communities while iron- and sulfur-oxidizing autotrophs become prominent [51]. Other effects on microorganisms consist of apoptosis, ATP synthesis inhibition, damages nucleic acid, denaturation of proteins, inhibition of cellular division and transcription, impairment of DNA repair [52,53].

VI. FUTURE OUTLOOK

In today's time, it is a well-established methodology that involves the use of microbes to extract metal from ores and concentrates. The organisms required should be capable of producing ferric iron and act as acid leaching reagents at higher workable temperatures. The majority of the microorganisms growing at such temperatures are bacteria in appearance, but instead, are single-celled microorganisms called archaea. At higher temperatures, the solubility of air reduces, and oxygen gas is used as an oxidant.

There's an increased concern with regards to the effect of mining on the environment which is likely to improve the advantage of microbially based metal recovery processes. The enforcement of legislation to limit environmental pollution would make the bioleaching processes more attractive. Acid mine drainage communities tend to provide a deeper understanding of self-contained biomes that are independent of sunlight and other life forms. Various culture-independent methodologies involved giving an in-depth study of the organisms' diversity and phylogeny that populate the AMD systems. The low species richness of the AMD communities helps in reconstructing the genomes of the population at large. By integrating the geochemical and biological knowledge, a comprehensive model for AMD production may be possible. The simplicity showcased by these acidophilic habitats provides a fundamental understanding of how microbial communities work which is possible through knowledge of more complex ecosystems.

VII. REFERENCES

1. D. E. Rawlings (Ed). Biomining: Theory, Microbes and Industrial Processes, Springer-Verlag, Berlin (1997)
2. D. E. Rawlings. Annu. Rev. Microbial. 56, 65–91 (2002)
3. D. E. Rawlings and S. Silver. Bio/Technology 13, 773–778 (1995).
4. C. L. Brierley. Sci. Am. 247 (2), 42–51 (1982).
5. P. R. Norris. In Microbial Mineral Recovery, H. L Erlich and C. L. Brierley (Eds.), pp. 3–27, McGraw-Hill, New York (1990).

6. Silverman M.P. Ehrlich H.L. (1964) Microbial formation and degradation of minerals. In: Advances in Applied Microbiology (Umbreit W.W. Ed.), Vol. 6, pp. 153–206. Academic Press, New York.

7. T. Sugio, C. Domatsu, O. Munakata, T. Tano, K. Imai. Appl. Environ. Microbial. 49, 1401–1406 (1985).

8. L. G. Leduc and G. D. Ferroni. FEMS Microbial. Rev. 14, 103–120 (1994).

9. B. M. Goebel and E. Stackebrandt. Appl. Environ. Microbial. 60, 1614–1621 (1994).

10. D. P. Kelly and A. P. Wood. Int. J. Syst. Evol. Microbiol. 50, 511–516 (2000).

11. K. B. Hallberg and E. B. Lindström. Microbiology 140, 3451–3456 (1994).

12. N. J. Coram and D. E. Rawlings. Appl. Environ. Microbiol. 68, 838–845 (2002).

13. K. B. Hallberg and D. B. Johnson. Adv. Appl. Microbiol. 49, 37–84 (2001).

14. D. A. Clark and P. R. Norris. Microbiology 142, 785–790 (1996).

15. O. V. Golyshina, T. A. Pivovarova, G. Karavaiko, T. F. Kondrat’eva, E. R. B. Moore, W.-R. Abraham, H. Lünsdorf, K. N. Timmis, M. M. Yakimov, P. N. Golyshin. Int. J. Sys. Evol. Microbiol. 50, 997–1006 (2000).

16. P. R. Norris, N. P. Burton, N. A. M. Foulis. Extremophiles 4, 71–76 (2000).

17. D. G. Lundgren and M. Silver. Ann. Rev. Microbiol. 34, 263–283 (1980).

18. R. C. Blake, K. Sasaki, N. Ohmura. Hydrometallurgy 59, 357–372 (2001).

19. J. Rojas, M. Giersig, H. Tributsch. Arch. Microbiol. 163, 352–356 (1995).

20. T. Gehrke, J. Telegdi, D. Thierry, W. Sand. Appl. Environ. Microbiol. 64, 2743–2747 (1998).

21. R. Hallmann, A. Friedrich, H.-P. Koops, A. Pommerening-Röser, K. Rohde, C. Zenneck, W. Sand. Geomicrobiology. J. 10, 193–206 (1992).

22. H. Tributsch. Hydrometallurgy 59, 177–185 (2001).

23. F. K. Crundwell. In Biohydrometallurgy: Fundamentals, Technology and Sustainable Development, Part A, V. S. T. Ciminelli and O. Garcia Jr. (Eds.), pp. 149–158, Elsevier, Amsterdam (2001).

24. T. Gehrke, R. Hallmann, K. Kinzler, W. Sand. Water Sci. Technol. 43, 159–167 (2001).

25. J. Rojas, M. Giersig, H. Tributsch. Arch. Microbiol. 163, 352–356 (1995).

26. J. A. Rojas-Chapana and H. Tributsch. Hydrometallurgy 59, 291–300 (2001).

27. Schrenk, M.O., Edwards, K.J., Goodman, R.M., Hamers, R.J. and Banfield, J.F. (1998) Distribution of Thiobacillus ferrooxidans and Leptospirillum ferrooxidans: implications for generation of acid mine drainage. Science 279, 1519-1522.

28. Edwards, K.J., Gihring, T.M. and Banfield, J.F. (1999) Seasonal variations in microbial populations and environmental conditions in an extreme acid mine environment. Appl. Environ. Microbiol. 65, 3627-3632.

29. Rawlings, D.E., Tributsch, H. and Hansford, G.S. (1999) Reasons why ‘Leptospirillum’-like species rather than Thiobacillus ferrooxidans are the dominant iron-oxidizing bacteria in many commercial processes for the bio-oxidation of pyrite and related ores. Microbiology 145, 5-13.

30. Druschel, G.K., Baker, B.J., Gihring, T. and Banfield, J.F. Acid mine drainage biogeochemistry at Iron Mountain, California, in review.

31. Sand, W., Rohde, K., Sobotke, B. and Zenneck, C. (1992) Evaluation of Leptospirillum ferrooxidans for leaching. Appl. Environ. Microbiol. 58, 85-92.

32. Johnson, D.B. and Roberto, F.F. (1997) Heterotrophic acidophiles and their roles in the bioleaching of sulfide minerals. In: Biomining: Theory, Microbes, and Industrial Processes (Rawlings, D.E., Ed.), pp. 259-279. Springer, Berlin.

33. Bond, P.L., Druschel, G.K. and Banfield, J.F. (2000) Comparison of acid mine drainage microbial communities in physically and geochemically distinct ecosystems. Appl. Environ. Microbiol. 66, 4962-4971.

34. Rheims, H., Rainey, F.A. and Stackebrandt, E. (1996) A molecular approach to search for diversity among

bacteria in the environment. J. Ind. Microbiol. 17, 159-169.

35. Clark, D.A. and Norris, P.R. (1996) Acidimicrobium ferrooxidans gen. nov., sp. nov. mixed-culture ferrous iron oxidation with Sulfobacillus species. Microbiology 142, 785-790.

36. Atkinson, T., Gairns, S., Cowan, D.A., Danson, M.J., Hough, D.W., Johnson, D.B., Norris, P.R., Raven, N., Robinson, C., Robson, R. and Sharp, R.J. (2000) A microbiological survey of Montserrat Island hydrothermal biotopes. Extremophiles 4, 305-313.

37. Yahya, A., Roberto, F.F. and Johnson, D.B. (1999) Novel mineral oxidizing bacteria from Montsterrat (W.I.): physiological and phylogenetic characteristics. In: Biohydrometallurgy and the Environment Toward the Mining of the 21st Century Process Microbiology 9A (Amils, R. and Ballester, A., Eds.), pp. 729-740. Elsevier, Amsterdam

38. Bond, P.L., Smriga, S.P. and Banfield, J.F. (2000) Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site. Appl. Environ. Microbiol. 66, 3842-3849

39. Peccia, J., Marchand, E.A., Silverstein, J. and Hernandez, M. (2000) Development and application of small subunit rRNA probes for assessment of selected Thiobacillus species and members of the genus Acidiphilum. Appl. Environ. Microbiol. 66, 3065-3072.

40. Boogerd, F.C., Vandenbeemd, C., Stoelwinder, T., Bos, P. and Kuenen, J.G. (1991) Relative contributions of biological and chemical reactions to the overall rate of pyrite oxidation at temperatures between 30‡C and 70‡C. Biotechnology. Bioeng. 38, 109-115

41. Fuchs, T., Huber, H., Teiner, K., Burggraf, S. and Stetter, K.O. (1995) Metallosphaera prunae, sp. nov., a novel metal-mobilizing, thermoacidophilic archaeon, isolated from a uranium mine in Germany. Syst. Appl. Microbiol. 18, 560-566.

42. Jerez, C.A., 2009. Biomining microorganisms: Molecular aspects and applications in biotechnology and bioremediation. In: Singh, A., Kuhad, R.C., Ward, O.P. (Eds.),Advances in Applied Bioremediation. Berlin: Springer, pp. 239–256.
43. Das, A. and Mishra, A.K. (1996) Role of Thiobacillus ferrooxidans and sulphur (sulphide)-dependent ferric-ion-reducing activity in the oxidation of sulphide minerals. Appl. Microbiol. Biotechnology. 45, 377- 382.
44. Huber, G., Spinnler, C., Gambacorta, A. and Stetter, K.O. (1989) Metallosphaera sedula gen. and sp. nov. represents a new genus of aerobic, metal-mobilizing, thermophilic archaebacteria. Syst. Appl. Microbiol. 12, 38-47.
45. Norris, P.R. and Kelly, D.P. (1980) Dissolution of pyrite (FeS2) by pure and mixed cultures of some acidophilic bacteria. FEMS Microbiol. Lett. 4, 143-146.
46. Akcil, A., and Koldas, S. (2006). Acid mine drainage (AMD): causes, treatment, and case studies. *J. Clean. Prod.* 14, 1139–1145. doi: 10.1016/j.jclepro.2004.09.006
47. Hallberg, K. (2010). New perspectives in acid mine drainage microbiology. *Hydrometallurgy* 104, 448–453. doi: 10.1016/j.hydromet.2009.12.013
48. Kuyucak, N. (2002). Role of microorganisms in mining: generation of acid rock drainage and its mitigation and treatment. *Eur. J. Mineral Proc. Environ. Prot.* 2, 179–196.
49. Ayangbenro, A. S., and Babalola, O. O. (2017). A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *Int. J. Environ. Res. Public Health* 14:94. doi: 10.3390/ijerph14010094
50. Poljsak, B., Pócsi, I., and Pesti, M. (2011). “Interference of chromium with cellular functions,” in *Cellular Effects of Heavy Metals*, ed. G. Bánfalvi (Berlin: Springer Science Business Media B.V), 59–86. doi: 10.1007/978-94-007-0428-2_3
51. Mendez, M. O., and Maier, R. M. (2008). Phytoremediation of mine tailings in temperate and arid environments. *Rev. Environ. Sci. Biotechnology*. 7, 47–59. doi: 10.1007/s11157-007-9125-4
52. Wysocki, R., and Tamás, M. J. (2011). “*Saccharomyces cerevisiae* as a model organism for elucidating arsenic tolerance mechanisms,” in *Cellular Effects of Heavy Metals*, ed. G. Bánfalvi (Berlin: Springer Science Business Media B.V), 87–112. doi: 10.1007/978-94-007-0428-2_4
53. Fashola, M., Ngole-Jeme, V., and Babalola, O. (2016). Heavy metal pollution from gold mines: environmental effects and bacterial strategies for resistance. *Int. J. Environ. Res. Public Health* 13:1047. doi: 10.3390/ijerph13111047

IJEAST

INTERNATIONAL JOURNAL
OF ENGINEERING APPLIED SCIENCE
AND TECHNOLOGY

ABOUT IJEAST

International Journal of Engineering Applied Science and Technology (IJEAST) is a peer-reviewed, open access journal that publishes high-quality research papers in the field of Engineering, Applied Science and Technology.

IJEAST aims to provide a platform for researchers, academicians, and professionals to share their innovative ideas, research findings, and practical experiences with the global scientific community.

FOCUS AREAS

- Engineering
- Applied Science
- Technology
- Innovation & Development
- Interdisciplinary Studies

PEER REVIEWED
All submissions are rigorously peer reviewed to ensure quality.

OPEN ACCESS
Free and unrestricted access to research for all.

GLOBAL REACH
Connecting researchers and professionals worldwide.

TIMELY PUBLICATION
We ensure a swift and efficient publication process.

For more information, visit our website
www.ijeast.com

INTERNATIONAL JOURNAL
OF ENGINEERING APPLIED SCIENCE
AND TECHNOLOGY

editor@ijeast.com
www.ijeast.com
India

2455-2143