COMMENTARY

Insights into differential biomining traits of Indian copper sulphides

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Metal solubilization in microbiological leaching of copper sulphides is promoted by synergism of microbe consortium. Low-grade copper sulphides (chalcopyrite, pyrite) in India, when subjected to bacterial leaching/biomining in acidic medium show differential responses. Such reaction occurs due to geological/geochemical environments of ore formation. The co-existence/symbiotic interactions of bacterial species along with P–T conditions are equally responsible for their characteristic behaviour. Igneous activities associated with formation of Singhbhum (Jharkhand)–Malanjkhand (Madhya Pradesh) copper deposits influence the rate of bioleaching of refractory chalcopyrite. This rate is higher in sedimentary remobilized deposits of Khetri–Rajpura Dariba–(Rajasthan) Ambamata (Gujarat). It is found that microbes handle leaching problems within genetically defined abilities.

World over, 80% copper is produced through conventional processing (crushing, grinding, floatation, fusion-conversion of concentrates, electrolytic refining). However, this is limited to high-medium grade ores, specificities of ore deposits and processing plants. There are none-the-less valuable, relatively low-grade resources which are sub-economic and remain unexploited for lack of effective technology. However, these ores could be recovered through recent developments in microbial ecology and metabolic processes of biomining/bioleaching, using tools of comparative genomics/metagenomics/bioinformatics. Complete genomic sequences of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* have led to a better understanding of biochemical pathway predictions and physiology of iron–sulphur oxidation for higher copper solubilization.

Low-grade copper in chalcopyrite of Malanjkhand–Khetri–Amba–Mata–Singbhum may be subjected to bioleaching through direct/indirect microbial actions. Microbes essential for biomining belong to the bacterial domain of Archaea, that are acidophilic and chemolithotrophic.

In biomining, there are bacteria of *Acidiphillium* sp., *Leptospirillum* sp., *Sulphobacillus* sp., *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* sp., *Acidithiobacillus caldus*, *Acidithiobacillus ferridurans* and *Acidithiobacillus ferrivorans*, whereas Archaea includes *Acidianus* sp., *Ferroplasma* sp., *Metallosphaera* sp. and *Thermoplasma* sp. Till today, species of *Acidithiobacilli* – *At. ferrooxidans*, *At. thiooxidans*, *At. ferriphilus*, *At. albertensis*, *At. ferrivorans*, *At. ferridurans*, *At. sulfuriphilus* and *At. caldus* – are observed. The most influential metal bioleaching microbes are *At. ferrooxidans* and *At. thiooxidans*.

Microbial leaching can be direct or indirect[1]. *At. ferrooxidans* and *At. thiooxidans* are capable of catalysing oxidation of reduced sulphur compounds (sulphide, elemental sulphur, thionates, etc.), generating sulphuric acid as the final product. Both these bacteria reduce sulphite and thiosulphate as intermediate products that directly/indirectly solubilize metal-associated sulphides. This lowers the formation of passivating substances on reactive ore surfaces, oxidizing elements directly by transferring electrons from ore to biomass. *At. ferrooxidans* can catalyse oxidation of Fe^{2+} to Fe^{3+} that strongly oxidizes sulphides/other compounds whose oxidation is required. Dopson *et al.*[2] expressed doubts about the incapability of organomixotroph *Ferroplasma* strains to fix carbon dioxide. *Leptospirillum*, as member of the bioleaching consortium, is providing them with fixed carbon[3].

Bioleaching of Indian ores describes heap bioleaching in acid medium (H_2SO_4) at pH 1.4–3.5. Incorporating selected biomass with increased oxidizing property has been attempted. However, it is difficult to maintain selectivity in Indian heaps. Increasing heap temperature to 50°C to accelerate bioleaching is impractical for cost constraints. Organic carbon is further added to improve the rate of leaching; but cost nullifies implementation possibilities. Another impending factor is maintaining carbon under varying heap conditions, because of specificities of ore of a particular region.

For improvement in heap bioleaching as executed in Chile, addition of *Licananantase* and H_2SO_4 at pH 1.5–1.9 was considered. *Licananbutase* lipoprotein, an active agent of *Licanantase*, is observed in secreted protein fractions of *At. ferrooxidans* and *At. thiooxidans*. The initial fractions of 3.5–30 kDa indicate an increment in copper recovery. The reagent contains 5–99% of *Licanantase* lipoprotein along with 1–95% H_2SO_4 at pH 0.8–3.0. In India, working pH ranges between 1.4 and 1.9, depending on ore specificities. The above reagent has to be added at a concentration of 0.01–100 mg/l. For ores of four deposits worked with, the concentration was of higher order, varying from 70 to 80 mg/l, whereas *Licanantase* lipoprotein was around 87% with 90% H_2SO_4.

In chalcopyrite–pyrite-dominated low-grade ores of India, copper recovery improved by around 9–11%. The Chilean experts, however, claimed a progress of up to 20%.

Molecular genetic studies of *Acidithiobacilli* are time-consuming because of long generation time, low yields in liquid media and poor growth on solid media. S^o was earlier accumulated in low-temperature bioleaching of chalcopyrite and associated ores with psychrotolerant pure and mixed cultures, primarily causing passivation of copper surface. Studies on microbial ecology of bioleaching till date have relied primarily on standard techniques of molecular genetics, especially in the spatio-temporal distribution of microorganisms in heap bioleaching. Presently, emerging fields of comparative genomics and metagenomics have broadened our understanding of microbial ecology and metabolic processes of bioleaching.

Bioinformatics of the genome sequence of *At. ferrooxidans* has led to several metabolic regulatory models; some were validated in part through experimental studies like sulphur and iron uptake and assimilation[4,5], carbon metabolism[6], etc. Comparisons on a large scale of genomes addresses fundamental questions, such as the number of functional genes, identification of species-specific genes, distribution of genes among functional families, etc. Functional abilities of bacteria are in fact limited by genomic characters.

Recent reports[7] on *Sulfobacillus thermo-sulfidooxidans*, *At. ferrooxidans* and *At. caldus* reveal how the extent of functional abilities depends on genomic attributes. Comparative genomics helps consolidate gene identification. For example, in iron oxidation specific to *At. ferrooxidans*, the potential genetic differences between various *Acidithiobacillus* species are combined to develop evolutionary models of the respective genomes and mutual species interactions (ecophysiology). If leaching problems (which bacteria cannot handle alone) are visible, the process needs to be complemented by additional leaching abilities of bacteria and *Archaea*. The case of *Licanantase* lipoproteins, discussed earlier, is a pointer.

Bioleaching experimentation at laboratory/pilot/commercial scales in dumps/heaps have been done over three decades. In the late 1980s, the first copper dump leaching was attempted at Khetri. However, it did not work due to improper leaching pad design. In the early 1990s, copper heap/dump leaching-cementation plant was established indigenously at Malanjkhand to utilize sub-economic resources, and cement copper produced was fed into the copper converter at Khetri Flash Smelter.

The thin layer leaching of Peruvian copper mines was attempted at Malanjkhand. The ore was cured with strong acid and agglomerated before being heaped. Sliced heaps gave better results[8]. The ore in heap was cured with 15% H_2SO_4 and wetted for long as feasible, before leaching commenced. The higher the period of wetting, better the result. However, there was no consortium of bacteria integrated by *At. ferrooxidans* and *At. thiooxidans*, and pH was varying between 2 and 3. In the initial period, results were quite encouraging but soon things faded as the days passed, with a recovery of about 50% in 5 years.

There is a perceptible relationship between geological origin of ore deposits and rate of bioleaching. The latter is related to geology, genesis of deposits and mode of evolution of the respective genomes of mutually interactive microbial species in the consortium.

Fluid inclusion studies on Singhbhum copper ore[9] show that copper mineralization is of magmatic/metasomatic parentage, or evolved meteoric water at low water/rock ratio after its interaction with granitic pluton. The Malanjkhand copper deposit occurs within calc-alkaline tona-lite-granodiorite of early Proterozoic age[10]. The Amba–Mata deposit is a strata-bound exhalative sedimentary deposit, metamorphosed[11]. Copper mineralization in Khetri–Kolihan belt is sedimentary–diagenetic, later remobilized and metamorphosed[12].

Various members of the consortium, proactive in leaching, form separate clusters and may or may not be mutually interactive at the initial stage of heap bioleaching; but, as the exothermic reaction proceeds, members of the consortium become mutually interactive.

Leaching operations under the Iron Mountains (Northern California, USA) metagenomic project have, however, added more comprehensive understanding of heap bioleaching[13]. Heap bioleaching proceeds in three stages. It arises from temperature increase due to exothermic biological oxidation of (Fe II) and S°. An early stage of leaching is found at 30–40°C by mesophilic organisms such as *At. ferrooxidans*, *At. thiooxidans* and *Sulfurisphaera*. As temperature begins to rise and soars to 40–55°C, *Leptospirillum*, *At. caldus* and *Ferroplasma* groups are found prevalent. At the final stage at 55–65°C or higher, dominantly active *Sulpholobus*, *Alicyclobacillus* and proactive *Ferroplasma* (Archaea) flourish.

Consortium of *At. ferrooxidans* and *At. thiooxidans* with addition of Licanantase protein can remove ~40% copper from well-organized heaps in 45 days. The figure is significantly less if a different consortium is used. The data are relevant when copper has igneous associations. With sedimentary metamorphosed ores, recovery could drop to ~30–35% copper in 50 days, with other additional reagents remaining the same. In India, where heap leaching is set up for bioleaching of low, marginal-grade, sub-economic resources of chalcopyrite, the final stage of leaching at 55–65°C is not achievable. Therefore, recovery of metal is always in the lower range. The absence of third/final stage of bioleaching is characteristic of Indian copper biomining. Low mineral surface availability to bacterial mass, primarily on account of pervasive rock matrix, is probably another cause of less metal recovery.

At. ferridurans[14] is located only in Singhbhum because of the influence of uranium around Jaduguda. *At. caldus* is more prevalent than *At. ferridurans*. The latter probably indicates radioactive con-tamination in the copper belt of Ghatsila. *At. ferridurans* has similarities with the gene sequence of *At. ferrooxidans*; hence it was earlier incorrectly identified as *At. ferrooxidans*.

The whole genome sequencing of different *Acidithiobacilli* species has provided new insights into variegated functions. Until now nine *At. ferrooxidans* strain genomes are available[15]. *At. ferrooxidans* observed in Indian deposits has not been genetically analysed. Hence it is unknown whether it is a variant strain. *At. ferrooxidans* IO-2C genome[16] has shown significant genetic variants of over 75,000 variants. In Indian mines, it is not known which variant and gene functions (like iron and sulphur metabolism, nitrogen fixation, heavy metal resistance, etc.) we are dealing with. The rate of leaching is dependent on gene functions. Comparison of genomes addresses basic questions such as the number of functional genes, identification of species-specific genes, distribution of genes among functional families, mechanisms of reshuffling of genomes, rate of divergence of sequence, etc.[17]. Genomic analysis can confirm whether bacteria in heap can oxidize Fe^{2+} and sulphur, say at pH 1.0, and cope with the extreme environment. Comparative genomic study can identify genetic differences between mutually interactive species in leaching heaps and allow prediction of mutual interactions between species[18]. Microbes must be analysed through bioinformatics to unravel the genomic structure. This is a prerequisite for successful biomining. Once the genomic structure of proactive microbes is available, capabilities of functional genes in leaching operations may be understood. The said data presently are, however, non-existent in India.

In this study, geological factor appears to be the key component in explaining the rate of solubilization of metal in bioleaching operations. The key component is the presence or absence of igneous activity during ore formation.

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The re-emerging Karnal bunt disease of wheat and preparedness of the global wheat sector

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Karnal bunt caused by smut fungus *Tilletia indica* (syn. *Neovossia indica* (Mitra) Mundkur), is one of the major fungal diseases of wheat that is considered to have a high potential of re-emergence, particularly in the North Western Plains Zone (NWPZ) of India[1]. It is named after the Karnal district of Haryana, where it was first discovered in 1931 by Manoranjan Mitra[2]. Although in the NWPZ Karnal bunt has been historically present in non-epidemic proportions, precipitation or high humidity at the flowering stage (February–March) may increase the infection percentage as high as up to 40%. During the post-harvest surveys conducted under the All India Coordinated Research Project on Wheat and Barley from the grain markets in 2019, 32.02% samples from a total of 7321 were found infected by *T. indica*. Maximum infected samples were reported from Haryana (56.69%), followed by Jammu and Punjab with 54.85% and 45.18% infected samples respectively. Fortunately, none of the samples collected from Madhya Pradesh, Gujarat, Maharashtra and Karnataka was found to carry the Karnal bunt disease[3]. Table 1 presents details of post-harvest sample survey for Karnal bunt for ten states in India, including the range of grain infection for each state. The percentage of infected samples is high; this should be a matter of concern for the Indian wheat sector and calls for stricter internal quarantine.

The rise in infection percentage in recent years has been primarily attributed to the adaptability of the pathogen to the prevailing weather fluctuations and absence of immunity in wheat megavarieties. The extensive use of urea (nitrogen fertilizers) for higher yield and more irrigation events are also responsible for an increase in the intensity and incidence of Karnal bunt disease outbreaks. Karnal bunt is also reported to have a high propensity of becoming endemic in new geographies across Europe and Australia, which are at present free from this disease[4,5]. The associated risk becomes exceedingly important as *T. indica* is a fungus of high quarantine importance across the world with more than 70 countries having quarantine regulations imposed against it[6]. Therefore, the economic losses caused by the fungus should be understood in the form of a non-tariff barrier to the global wheat trade, rather than the direct yield losses which are minor only. The loss of yield due to Karnal bunt has been quantified to range between 0.01% and 1% in India and Mexico[4,7,8]. Owing to this, in the beginning, the disease was assumed to be of intermediate economic significance only[9]. The indirect losses caused by Karnal bunt also pertain to the rejection of wheat lots containing more than 3% of infected grains for human use[10]. Therefore, it is a peculiar wheat fungal disease that, unlike others (rusts, mildews, etc.), causes monetary losses to the growers more by affecting the quality and less by reduction in the quantity of the produce. Nonetheless, the studies of Murray and Brennan[11] and Stansbury and McKirdy[12] have shown that we should not be misled by the quantum of direct yield losses, as the former reported that an economic loss to the tune of AUD 490,900,000 per annum (17% of wheat economy) will accrue to the wheat commerce if *T. indica* gets an entry into Australia. Likewise, the latter study expected an even higher economic loss that could increase up to a significant 25%. The losses estimated included those due to reduction in yield, quality and the expected quarantine regulations that will be in place in case of such a scenario. The expected percentage of losses are enough