



Review

Development of bioleaching: proteomics and genomics approach in metals extraction process

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Abstract: Microbes are key components of the structure and function of bioleaching process. Increasing consciousness of the role of microbes has led to a quick growth of descriptive and investigational studies of their abundance and activities. However, the detail information of complex functional molecules contain in promising microbes which are very important for understanding microbial processes in bioleaching, are lacking. Therefore, molecular functions of microbes in the bioleaching process are very essential to understand about the microbial activities, especially in the process of the extraction of metals in mineral industries. In this review, the current state of proteomics and genomics of bioleaching in metals extraction processes and the major developments of these analytical methods at industrial scales are highlighted.

Keywords: bioleaching; biomineralization, microbes; proteomics; genomics

1. Introduction

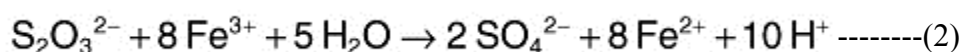
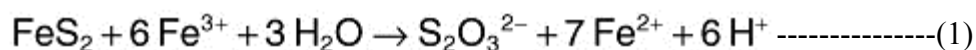
There is increasing interest in the use of microbes as alternatives to chemicals in mineral industries. Identification of dedicated microbes to bioleaching is an efficient mechanism for metals extraction. However, despite the long history of bioleaching and many laboratory demonstrations of the ability of a wide range of microbes to improve the extraction of metals, there are still very few molecular approach in the bioleaching process are established. The immediate study of the full set of

genomics and proteomics of living microbes will perhaps lead this field to extract metals perfectly. Because genomics is not the only accountable for life's complexity, proteomics are also required to establish a complete biological tool in bioleaching field [1,2].

In bioleaching, conversions of insoluble metal sulfides into soluble forms are occurred by the microbial-catalyzed process. The phenomenon of metal sulfide dissolution in an acidic medium was first indorsed to the action of certain microbes [3] in 1940s. After this time, application of microbiology to mining and research in acidophilic microbes in this field were started. Recovery of metals from sulfide ores engages several sorts of extremophiles, including bacterial and archaeal species. Therefore, the study of proteomics could be the ways to discovery of biomarkers of existing or past extra-terrestrial life [4]. The microbes naturally present in the native flora of minerals have a vital role in the bio-hydrometallurgical leaching processes. The presence of microbes has been associated to enhanced extraction of metals including gold, nickel, copper, zinc, cobalt, and uranium. In this review, I summarize the very important approaches of genomics and proteomics and their current and future status in the development of bioleaching and the major developments of these analytical methods at industrial scales.

2. Bioleaching/Biomining Processes

Microbes play a key role in leaching metals from ores, rocks, and metal bearing wastes. Metal leaching is a chemical process and it mainly occurs by the reactions of ferric iron and protons. These reactions occur in the extracellular polysaccharide by cells growing in biofilms and these biofilms formation significantly speed up the reactions of metal leaching. There are two main reactions. A thiosulfate mechanism which is proposed for the oxidation of acid - insoluble metals sulfides and a polysulfide mechanism for acid-soluble metal sulfides.



Therefore, a vital role of the microbes is to produce sulfuric acid for proton attack and to possess the iron in the oxidized ferric state for oxidative attack on the metal [5,6] reported an overview of acidophilic microbes and their chemistry of carbon, iron and sulfur metabolism.

There are some toxic chemicals uses in conventional metals extraction process. However, the release of toxic chemicals are much lower in bioleaching, thus making it an environmentally friendly process. With its important phenomenon, bioleaching has proceeded to recovery of metals from industrial wastes and secondary ores. To complete the chemical processes of bioleaching, however, molecular tools such as proteomics and genomics are significantly necessary. Identification of proteins, genes and enzymes using proteomics and genomics approach will greatly help to identify dedicated microbes to extract metals in bioleaching process. In addition, purified proteins from microbes might have some similarity with many other proteins from public database. The information of close homology of proteins from bioleach microbes will provide us functional and evolutionary clues about the structure and functions of their sequences. These homologous proteins

could be used to identify candidate microbes for metals extraction and a potential resource for bioleaching research.

3. Genomics of Microbes on Bioleaching Process

Recent development of genomics has made a major impact on our knowledge of bioleaching processes [7–13]. After the first genome sequence of *Acidithiobacillus ferrooxidans* [14], the knowledge of bioleaching processes has surprisingly increased. There are 55 bacterial and 36 archaeal whole genomes from bioleaching microbes are widely accessible and they are available to the NCBI database [2]. The knowledge gained from the genomics of leaching microbes has a great contribution to improvements in this field, which would be the major future impacts at industrial scale.

The species *Acidithiobacillus ferrooxidans* is the most significant microorganism for metal sulfide bioleaching. The advances in genomics knowledge has driven the exploration of extreme mineral leaching environments for new microbes with potential commercial applications, resulting in a more inclusive understanding of the biodiversity of acidophilic environments. Very recently, Zhang et al., [15] also reported about metabolic diversity and adaptive mechanisms of iron and sulfur-oxidizing autotrophic acidophils in extremely acidic environments. Researchers used genomics information as the base for the development of patented techniques that allow an accurate monitoring of the microbial populations during the bioleaching process [16]. The changes of population dynamics have been described during bioleaching phases [17]. The population dynamics also depend on the oxidation state of the minerals. In my point of view, the spatio-temporal analysis of acidophilic microbial populations during bioleaching is a major impact that meta-genomics will generate in the coming future. Future development of genomics tools including next-generation sequencing technologies will lead to faster and reliable bioleaching operational design and control [18,19]. Allen et al.[20] reported that *Ferroplasma acidarmanus* related population exhibits a mosaic genome structure in which a small number of sequence types dominate, suggesting that such structure may be a common feature of natural archaeal populations. According to the report of Orellana and Jerez [21], *Acidithiobacillus ferrooxidans* has ATCC 23270 and ATCC 53993, 2397 genes in common, which represent between 78 and 90% of their genomes.

Carbon and nitrogen fixation are very important pathways for bioleaching bacteria since they live in extreme environments. A study [22] with a comparative genomic analysis of three bioleaching bacteria isolated from mine sites in Chile demonstrated that there are important differences in the carbon dioxide and nitrogen fixation mechanisms among bioleaching bacteria that coexist in mining environments. They applied genomic and molecular evidence to verify carbon and nitrogen fixation mechanisms for three bioleaching bacteria and provided an analysis of the potential regulatory pathways and functional networks that control carbon and nitrogen fixation in these microorganisms. The evidences showed that both *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* incorporate CO₂ via the Calvin-Benson-Bassham cycle, the former bacterium has two copies of the Rubisco type I gene whereas the latter has only one copy. They demonstrated that *Leptospirillum ferriphilum* utilizes the reductive tricarboxylic acid cycle for carbon fixation. This genomic study also showed an important phenomenon that all the species they analyzed can incorporate ammonia by an ammonia transporter, *Acidithiobacillus thiooxidans* could also assimilate nitrate and nitrite but only *Acidithiobacillus ferrooxidans* could fix nitrogen directly from the air. Another important

benefit of genomics study is to understand the extraction process of gold from the low-grade gold ores [23]. These reports point out that new and existing species in the bioleaching process will have a great contribution to improve with further modifications of genomics data generation and analysis. Future studies in the direction of metabolic functional models could be able to predict the behavior of microbial leaching communities in order to design new strategies to enhance and optimize productivity in bioleaching processes.

4. Proteomics of Microbes on Bioleaching Process

Proteomics is an important tool to understand the microbe-mineral interaction, characterization of microbes and microbial biodiversity. Several proteomics studies demonstrated that protein associated molecules present in the extracellular polymeric substance (EPS) layers are able to accumulate sulfur and enhance the bioleaching of metal sulphides [24]. Important functions of microbes in bioleaching processes are the oxidation of iron and reduced inorganic sulfur compounds, which occur mainly in the extracellular space. A high-throughput proteomic study revealed the presence of 131 proteins in the periplasmic fraction of thiosulfate-grown cells. A very important feature of proteomics is to understand on heavy metal resistance in bioleaching. *Acidithiobacillus ferrooxidans* ATCC 23270 is known to tolerate high concentrations of copper (> 100 mM copper sulfate). Two high-throughput proteomics studies have described the global proteomic responses of this bacterium against copper stress.

Biofilm formation is a central phase of bioleaching. Biofilm cells are embedded in a matrix of EPS which plays an important role in attachment to solid surfaces and their further deterioration [25,26]. The first high-throughput proteomic study of the biofilm formation in *Acidithiobacillus ferrooxidans* ATCC 23270 was done in order to map the changes during its early biofilm formation process. Proteomes from planktonic and sessile cells upon 24h of biofilm formation on pyrite were compared using semi-quantitative shotgun proteomics. Several molecular adaptations for growing on pyrite surfaces were observed to occur in the biofilm subpopulation. Proteomics analyses of these biofilms have been also performed with laboratory-cultivated biofilms, inoculated with these environmental samples [27,28]. Interestingly, laboratory-grown biofilms were also dominated by *Leptospirillum* group II, with a lower abundance of *Leptospirillum* group III. A proteomic comparison was done between field and laboratory biofilms. Proteins related to functional categories such as energy production and conversion, cell motility, cell wall/membrane/envelope biogenesis, and intracellular trafficking, as well as secretion and vesicular transport were found to be more abundant in field biofilm samples while transcriptional proteins as well as proteins probably involved in defense mechanisms were more abundant in laboratory-grown biofilms [28]. Further studies determined that *Leptospirillum* group II proteins involved in amino acid and nucleotide metabolism, as well as cell membrane/envelope biogenesis were overrepresented at high pH. In addition, a pH-specific niche partitioning was shown to occur for some low-abundance bacteria and archaea, since *Leptospirillum* group III was more abundant in biofilms grown at higher pH values, whereas archaeal species were more abundant at lower pH values [27]. In this context, the use of proteomics as a tool to analyze the microbial biodiversity and their specific adaptations within field operations will strongly impact the monitoring and control of industrial bioleaching processes in the near future [29].

Future proteomics studies focused on new species isolated from bioleaching processes, their biofilm formation and functional characterization of microbial new proteins will provide new knowledge for the optimization of bioleaching and new biotechnological essences for industrial applications.

5. Future Development of Bioleaching Microbes and Challenges

Because of precious metals extraction, biomining probably to be hot in the near future. A lot of mineral ores around the world and they are full of metals, some very precious. But a big question—how to extract them? There is huge challenge to control the millions of microbes in the biomining process since microbes have diverse functions. However, molecular analyses such as proteomics and genomics could be the best analytical tools in this field. Particularly proteomic approach be more effective than other techniques. Since the biomineralization mechanisms of bioleaching calcifying microbes and other calcifying organisms, especially marine calcifiers [30–32] have some similarity so the same sort of instruments such as MALDI-TOF-TOF, Raman, FTIR, XRD, AFM could be used for their analyses towards identification and characterization of precious metals. Therefore, after a few modification of methods (e.g., sample preparation), similar techniques as used for marine calcifiers can be applied for bioleaching microbes such as crystal formation, proteins, enzyme and genes identification.

Besides analytical methods, there are some issues in bioleaching process and they should have to give an attention. As bioleaching heaps get hotter they get more efficient, but when the temperature gets too high then the moderate thermophiles cannot function and the need for seeding with extreme thermophiles becomes necessary. The search is necessary for microbes which will have a broader temperature range of activity. New isolates from hot environments may help solve these problems. Extreme thermo-acidophile genomes [33] can be examined for pathways responsible for conferring desirable biomining traits. Emerging molecular genetics tools for extreme thermoacidophiles, metabolic engineering of biomining organisms could be a solution.

Future attempts to understand biofilm formation and development of relevant industrial strains, and their interaction with mineral surfaces in mixed species cultures, as well as development of biomarkers to analyze the microbial biodiversity within field operations are major challenges which will enhance our knowledge for future bioleaching process development and monitoring.

A committee of the United States National Research Council (2002) states “the application of biotechnology to the extraction and recovery of metals, is becoming an increasingly important hydrometallurgical processing tool.” The mining industries also recognize that biotechnology offers another tool for economic recovery of metal values. However, future developments will expand the role of biotechnology in the extraction and recovery of many metal values.

6. Concluding Remarks

In this brief review, recent advances and future trends in applications of molecular approaches of microbes in metals extraction and the major developments of these analytical methods at industrial-scale bioleaching have been discussed. The past results demonstrated that bioleaching microbes possess significant potential to generate precious metals, however, application of proteomics and genomic tools which still have lacking, could introduce a new insight in this field.

Understanding the proteinaceous components from bioleaching microbes is an important step toward advancing the science of bioleaching chemistry. As a discovery-driven science, the techniques discussed here allow researcher to identify specific type of microbes for metal extraction and identify unknowns' species without missing unanticipated interactions.

Conflict of Interest

The authors declare no conflict of interest.

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