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Potential impact of microbial consortia in biomining and bioleaching of commercial metals

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Abstract

Biomining is the use of microorganisms for the commercial extraction of lavish metals from ores and mines with least effect on environment. Microbes play vital role in bioleaching procedures in commercial mining. The bacterial cells are used to detoxify/replace waste cyanide, marginal biomass and activated carbon. These methods are preferred over conventional techniques due to energy efficient, low cost, environment friendly and production of useful by-products. At industrial scale, different microbial strains (*Acidophilic, Sulphobacillus, Rhodococcus, Ferrimicrobium* & *chemolithotrophic*) are deployed to boost the processes of copper and uranium bioleaching. About 20% of the world's copper is extracted by using this technique. These extraction procedures involve oxidation of insoluble metal sulphides to soluble sulphates. The isolation of thermophilic microbes for mineral biooxidation increase the commercial extraction of minerals at industrial scale. The conventional pyrometallurgical techniques have environmental concerns as they result in depletion of high grade ores and release harmful gaseous. The microbe-assisted gold mining is expected to double the yield of gold and needs to be fully explored using diverse array of microbes. Bioleaching is simple and low cost method for the developing countries with large ore deposits. About 30 strains of microbes have been discovered so for with potential impact on bioleaching. With advances in molecular genetics, physiology and microbial genomics, more promising strains with increased bioactivities are possible. Further efforts are underway to culture diverse range of *archaea* and improving its genetic potential to be used as industrial tool for commercial bioleaching. The currents review enlightens the recent trends in biomining/bioleaching and implementation of modern biological approaches to engineer target microbes for commercial use.

Introduction

The use of microorganisms is an old process first introduced in Roman times and perhaps before that. In earlier times, the microbial activity was used by the miners for copper extraction but they were unknown of their activity. Different microbes have been used for the extraction of metals (copper, uranium & gold) from low grade mines and metal soluble deposits [1]. *Acidophillic*, *chemolithotrophic*, *hetrotrophic* bacteria and fungi are mostly used in bioleaching. Some *thermophillic* bacteria are also deployed to extract metals which can bear the temperature from 15^{0}C to 80^{0}C. The most common genera of bacteria reported for this process are *Thermobacillus* and *Leptosprillium* [1]. Traditional extraction methods involve complex steps like roasting, smelting, bassimerization and electrolytic refining with adverse effects on environment. Biomining simply ignore the waste in the surroundings and give 90% concentration of metal from ores [2]. Therefore, industries prefer biomining than other mechanical processes. The bacteria and some fungi serve as biocatalyst in the leaching processes by providing secondary raw material (metals extracting from its ore and converting them into more or less water soluble compounds). These techniques are used to recover expensive metals like gold, copper, uranium and iron with increased industrial values [3]. Sulphides ores are oxidized to the soluble sulphates and sulphuric acid during bacterial activity [3].

The metals are extracted from the leaching solution by using different bacterial strains. Moreover, *acidophilic* bacteria are used in the above procedures for the extraction of toxic metals from mine wastes under the process of bio-hydrometallurgy [2]. *Thiobacillus* bacteria have the ability to accelerate the solubility of heavy metals from minerals and to increase the leaching process aerobically about 104 folds or more [4]. About 20-25% copper production in USA and 5% of the world copper production is carried out by bioleaching [4]. Chile is the world's biggest copper exporter. About 20% copper, 5%gold & other metals in small quantities are obtained by bioprocessing [5].

Bioleaching is the leaching of heavy metals using microbial consortia with least effects on environment. When it rains the sulphur and iron, dumps are exposed to rain producing sulphuric acid resulting an open field for microbes to act upon [6]. Phytominning and mycominning are also being used for metal recovery but these are not as much popular as bacterial minning. Bacteria used in biomining occur naturally but are also being produced by genetic engineering and conjugation, which bear desired characteristic genes to increase the rate of bioleaching operations [7]. Major bacterial and fungal strains used in biomining process includes *Chemolethotrophes*, *Thiobacillus, Thiooxidans, Aspergillus niger* and *Penicillium simplicissimum* [2].

Methods

Literature Search and Selection Criteria

Data for this paper has been retrieved from Google Web Browser and Google Scholar by providing the key terms 'Biomining', 'Bioremediation', 'Bioleaching', 'Industrial Biotechnology', 'Microbial consortia' in different combinations. Research papers published and data related to biomining selected for this review.

Discussion

Characteristics and bioactivity of bacteria used in Biomining

Chemolithophillic

The most important living organisms used in bioleaching are *chemolithotrophic* (rock eating). These bacteria get their energy from the oxidation of the inorganic substances specially sulphur containing compounds [4]. Most of the bacteria among them are autotrophic and get the carbon element from atmosphere in the form of CO_2 for the synthesis of cellular components, rather to obtain carbon from organic material. The leaching bacteria use to live in such environment which is not suitable for the growth and survival of the other microbes [2, 3]. They can live in high concentrated sulphuric acid with high soluble metals. Some *thermophillic* bacteria live at the temperatures above 50^{0}C and other strains are found at temperature near the boiling point of water. Almost all *lithotrophic* bacteria have the ability to survive and flourish in the completely aqueous inorganic medium or environment where oxidized substrate is in excess amount and CO2 is available [3].

Thiobacillus ferrooxidans

Thiobacillus ferrooxidans bacteria are rod shaped and first strain to be discovered and used for commercial metal extraction [3]. These species were discovered in the acidic water a draining coal mines. *Ferroxidan* is an acidophillic and prefer to live in the acidic environment, volcanic fissures, hot spring and sulphide ore deposits where sulphuric acid is in excess amount. This organism is also moderately thermophillic in nature so can also live at 20-35^{0}C. This bacteria can easily grow at the temperature ranges between 45-48°C while the optimum temperature for this bacteria is 70-75°C. Similarly, the pH ranges for the high growth is 1-6 while optimum pH is 2 [4]. These bacteria either oxidize sulphur or iron to obtain energy. The iron which is in the form of ferrous, bivalent form (Fe^{++}) is oxidized to ferric a trivalent form (Fe^{+++}) by bacterial action. The bacteria can attack different sulphur compounds. These compounds may include elemental sulphur, soluble and insoluble sulphides, and soluble compound as thiosulphate or tetrathiosulphate ions. The transformation substance in any case is that substance which contains sulphur having few valence electrons, responsible for the formation of sulphates ion.

Thiobacillus ferroxidan and _Thiooxidans_

Besides *T. ferrooxidans*, some other types and strains of bacteria are being used for the extraction of metals. Some rod shaped bacteria; *Thiooxidans,* can be grown on the elemental sulphur and other soluble sulphur compounds [8]. The culture of two bacteria (*T. ferrooxidans and T. thooxidans*) is actively more effective than used alone. Similarly, the mixture of *Leptospirillium ferrooxidans* and *T. Organoparus* degrade pyrite (FeS2) and chalcopyrite ($CuFeS_2$). It is not accomplished by organism alone. *T. ferroxidans* are more tolerant of sulphates and convert sulphur into sulphuric acid.

Acidophillus and _Thermophillic_ genus of _Sulpholobus_

Obligate acidophilus especially eukaryotic bacteria form colonies with prokaryotic bacteria at lower temperature ranges<35°C. These bacteria involve *Sulpholobus* and *Acidianus* [2,3]. *Sulpholobus*, an archaea bacterium has rigid cell wall, round shaped cell and are about 0.8-1.0 mm in diameter [9]. The extraction of metals through thermo-*acidophillus* bacteria in geo-biotechnology is an ideal source especially for mining industries. These microbes are sufficiently being used for the extraction of zinc, copper, molebdinum, aluminum, nickel, iron and many other metals. A large amount of temperature resistive bacterial strains have been collected from hot regions, which can carry out oxidation reaction very efficiently. Due to their ability to bear highly concentrated and toxic environment, these bacteria are widely used as mining tools. *Thiobacillus ferrooxidans* have the capability to oxidize ferrous ions aerobically and anaerobically [10]. Sulphur in hot areas is found in elemental form and is oxidized by sulphur oxidizing bacteria to from sulphuric acid. Chemical reaction between hydrogen sulphide and sulfur dioxide results in the formation of elemental sulphur. A large number of bacteria (acidic) are isolated from the acidic environment. Other microbes and living organisms like fungi algae and plants can also be found there which either decompose the deposit or survive the other metabolites of host. Sulphur ore deposits and all other sulfur containing regions are full of *Thiobacillus* bacteria because they use sulphides and other sulfur containing compounds as a substrate. The chemical reactions carried by acidic bacteria results the production of acids which increase the acidity of the environment leaving behind a suitable media for the growth of other acidic bacteria. Under neutral condition and temperature range of 50-70^{0}C, *Thermothrix thiopara* readily oxidizes sulphyhydral ions (HS), sulphite ions (SO_3), elemental sulphur and thiosulphate ions (S_2O_3) to the sulphate ions (SO_4^{2-}). *Thermothrix thiopara* and other filamentous bacteria are widely found in volcanic fissure, hot springs and other thermal regions. Among these bacteria, *acidophillic* and *thermophillic* bacteria are well known. These bacteria show maximum growth on the bivalent iron deposits and minerals such as chalcopyrites, pentlandite, covellite and pyrite.

Use of fungi and plants in biomining

Fungal species are also being used for biomining. Many experiments have been conducted by using two fungal strains *Aspergillus niger* and *Penicillium simplicissimum* [11]. These have the ability to assemble copper and tin up to 65% while zinc, lead and aluminum up to 90-95% [3]. Similarly, phytomininig is also working in which some plant species are used for metal extraction. According to Albaniac researchers a plant reported is *Alyssum murale*, which grows at ultramafic soils found in Albania. This is considered as hyper accumulated plant specially nickel accumulator. Ultramafic soil is highly enriched with nickel, zinc, cobalt and magnesium. *Alyssum murale* has the tendency to accumulate nickel about 100kg/hector [12]. The selection of such plants is based upon their ability of metal accumulation (bioaccumulation), storage of metals in plant cells in large amount. Such plants species are called hyper accumulators. These plants are grown in those areas which are highly enriched with heavy metal in high concentration. These plants are first grown to such areas and then allowed to burn resulting the production of metal containing ash which are then separated by electrolysis process but as this process is too long and time consuming so is no used widely. Plants also help to remove toxicity of the soil by up taking the toxic metals from the soil and are harvested six times more a year [13]. *Helianthus annuus and Nicotiana tabacum* are also in practice as metal accumulators from problem soils [14]. Plants having *mycorhizal* association have antioxidant defense system which provide resistance to the plants against toxic metallic stress. They secrete antioxidant enzymes which detoxify the effects of heavy metals. Moreover, *myccorhizal* fungi increase the phyto-stabilization and solubilization as these fungi secrete specific enzyme to solubilize the heavy metals like cadmium, zinc and other. These mycorhizal fungi accumulate the pollutant metals into their vesicle and fungal hyphae roots which prevent the inhibition of the growth and uptake of nutrients [15].

Techniques used in mining processes

Two main techniques applicable in mining process are Agitation and Percolation. The later technique includes the percolation of soluble and insoluble particles via a static bed hole agitation includes the agitation of fine sized particles in a lixiviate. Bacterial mining used in large scale operations therefore percolation is most preferred technique at commercial level. The principal methods used commercially are In situ leaching, Vat leaching, Heap leaching and Dump leaching.

In situ method technique involves the pumping of air inside and forced under pressure into mines and ore deposits making it permeable through explosive charging. As a result of this process the metals enriched solutions are obtained into the wells, which are drilled below and recovered. Three different kinds of ores body are considered for in situ leaching includes (a) Deep deposits below water tables (b) Surface deposits above water tables (c) Surface deposits below water tables.

Dump and Heap leaching

Dump leaching includes the piles of uncrushed waste ores or rocks. In these dumps, the metals like copper are too low to be recovered conventionally and profitably. Some of these dumps are too huge consisting of 10 million of waste deposits. Heap leaching involves the preparation of the ore, firstly size is reduced so as to increase the mineral lixiviate interactions and completely lying on the impermeable base to prevent the loss of lixiviant and water pollution. Lixiviants are those chemical solutions which are used to accelerate the dissolution of ores in mining processes. More frequently used lixiviant used are cyanide solution and sulphuric acid solution [16]. The metals are recovered by solvent extraction and electro-winning-methods. Heap leaching is carried out by pilling the ore material upon impermeable liner. Acidic solution especially cyanide solution is sprinkled from the top of the pile which percolates down into the heap and leach the metals [17]. Both of these methods include the usage of lixiviant from the top of the heap or dump surface and metal is recovered in the laden solution. This solution seeps to the bottom due to the gravity flow. From the top, diluted sulphuric acid is sprinkled which percolates through the dump. As a result, the pH of the solution lowers which enhances the growth of the *acidophilus spp*. The acid is collected from the bottom and shifted to the recovery station by using solvent extraction and electrolysis methods. The wet leaching involves the crushing of material and its dilution in confined tank. By using bioreactor, the recovery of metal is increased, but this requires high cost. This method is currently applied to the oxides of ores. However, the precious metals are actively extracted by this method.

Factors affecting the biomining

Temperature and pH of the environment are the major factors affecting biomining. Either the growth of the organism is interrupted or the activity (biooxidation) is interrupted. It is the inherent property of microbes to respond the temperature and pH.

Effect of pH

The ranges of temperature and pH vary among microorganisms. Although the mostly used bacteria are *T. ferrooxidans* and *leptosporillium ferrooxidans*. The major reason for their usage in industries is due to their oxidation potential; certainly Fe^{3+}/Fe^{2+} ratio. The pH value ranges from 1.8-2.5 is considered the optimum pH for the growth and activity of *T. ferrooxidans* while *L. feroxidans* have more acidic resistant ability than *T. ferooxidans* and grow at the pH of 1-2.

Effect of temperature

With the temperature changes the most preferred one is *Leptosporillium ferooxidans*, *T. ferooxidans* cannot tolerate the high temperature. The optimal temperature considered for the *T. ferooxidans* is 30-35^{0}C but strains can be activate at 10^{0}C.While *leptosporillium ferrooxidans* show their activity at maximum of 45^{0}C and minimum of 20^{0}C. Most of the biooxidation of gold recovery methods and concentrates operations are carried out at 40^{0}C and 1-6 pH value [6].

Biomining of copper

Biomining of copper involves the conversion of water insoluble copper sulfides to water soluble copper sulphates. Copper minerals and ores for example: covellite and chalcopyrite are first crushed then allow to acidify with sulphuric acid. For homogenization, the whole material is converted to a rotating drum before piling in heaps and dumps. The heaps are then allowed to come in contact with the iron containing solution which penetrates into the heap and provides media for the growth of the bacteria and other microbes which catalyze the ore to release the copper. The ferric ion thus produced react with the copper to give copper sulphate. The charged solution contains 0.5 g/l to 2.0 g/l copper and 20g/l iron which is collected and is sent to the recovery plant [18]. Precipitation is one of the most common method used for copper extraction with large cementation units, electro winning and solvent extraction.

Bio-discharge of gold

Solubilization of ore with cyanide solution recovers gold in the solution. The small gold particles are covered by the insoluble sulphides. In the first stage, the ore arsenopyrite is oxidized by bacterial breakdown to the sulphur which is then oxidized to high oxidizing states reducing dioxygen by H_2 and Fe_{3+} all the soluble components are dissolve in this process. The whole process takes place at the cell membrane of the bacterial cell where electrons pass into the cell to provide energy to the cell which is used in the reduction of oxygen to water.

Now the metal is oxidized to high oxidizing state by bacteria. The electrons obtained from that Fe^{3+} is oxidized to Fe^{2+}. The gold is separated from the ore particles of solution. Biooxidation increase the gold extraction from 15-30% to 85-95%. Moreover, gravity concentration method is also in practice for gold extraction because in elemental form Au has a gravity of 19.3 and in ore form has 2.6 [18].

Uranium recovery

The recovery of uranium from ore particles is followed by the same step as of copper extraction.

First the insoluble uranium oxides are converted to the soluble uranium sulphates by the activity of ferric iron and sulphuric acids produced by the bacteria. The activity of the bacteria has limitation to the oxidation of ferric ion and pyrite. Biomining is applicable successfully for uranium extraction from waste gold ores. Most of the other metals present in the form of insoluble sulphur are converted to form sulphate and sulphite which are soluble. The metals are then recovered from these soluble solutions by using different bacterial strains. The metals extracted includes the minerals of zinc sulphides, nickel sulphides and cobalt containing pyrites. Lead is recovered from the solution containing toxic lead acetate. The solution is further recycled for leaching of the lead sulfidic minerals and solution containing lead ore particles.

Conclusion

In conclusion, the future of biomining is so promising because of its advantages over conventional extraction methods. The techniques of biomining are cost effective, environment friendly and not time consuming because bacterial activity is only involved. Moreover, environmental factors do not affect a lot. If industry will use temperature resistive equipment's for the microbial growth, then bio-oxidation will also increases to facilitate the metal extraction at commercial level. As most of the bacteria are sensitive to the temperature, hence most preferably those bacteria are selected which can bear a temperature of up to 80°C. Genetic engineering of targeted microbes would also increase their ability to be used as extraction tools at commercial scale.

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