Lecture 4

Microorganisms In Biohydrometallurgy

Keywords: Mining Microorganisms, Acidithiobacillus, Thermophiles

Some chemolithotrophic bacteria useful in biohydrometallurgy are listed below:

- *Acidithiobacillus ferrooxidans*
- *Acidithiobacillus thiooxidans*
- *T. caldus*
- *L. ferrooxidans*
- *Arthrobacter sp.*
- *Acidianus brierleyi*
- *Sulfobacillus thermosulfidooxidans*
- *Sulfolobus rivotincti*
- *Sulfolobus metallicus*
- *Metallosphaera sedula*

For microbiological and experimental discussions on *Acidithiobacillus* bacteria, refer to lectures 43 and 44.

*Acidithiobacillus*

*Acidithiobacillus* is known for its ability to oxidize elemental sulphur and sulphur containing compounds, but the conditions (such as pH, temperature) may vary depending on the physiology. Bacteria of the genera *Acidithiobacillus* are aerobes and are obligate or facultative chemolithotrophs. They grow at pH values between 0.5 – 9 as acidophiles and neutrophiles. They are mesophiles having optimum temperatures for growth at 25- 30°C. Some are moderately thermophilic such as *At. caldus*, oxidising sulphur above 40°C and have been used in bioliberation of gold from pyrite and arsenopyrite.
Chemolithotrophic bacteria including the genus *Acidithiobacillus*, can oxidise various sulphur compounds (i.e. \( S^- \), \( S^0 \), \( S_2O_3^- \), \( S_2O_4^- \), \( SO_4^- \)).

\[
2S + 3O_2 + 2H_2O = 2H_2SO_4 \\
Na_2S_2O_3 + 2O_2 + H_2O = Na_2SO_4 + H_2SO_4 \\
2H_2S + O_2 = 2S^0 + 2H_2O \\
H_2S + 2O_2 = H_2SO_4
\]

Moreover, some species derive energy from oxidation of ferrous ions. (*At.ferrooxidans* and *L. ferrooxidans*)

\[
2FeS_2 + 7O_2 + 2H_2O = 2FeSO_4 + 2H_2SO_4 \\
4FeSO_4 + O_2 + 2H_2SO_4 = 2Fe_2(SO_4)_3 + 2H_2O
\]

Main species of *Acidithiobacillus* include *At. thioparus*, *At.dentrificans*, *At.thiooxidans*, *At. intermedius* and *At.ferrooxidans*.

**Leptospirillum**

*Leptospirillum ferrooxidans* (*L.ferrooxidans*) is a moderately thermophilic iron oxidizer which can oxidize only ferrous ions, and can grow at higher temperatures and also at stronger acidity levels. *L.ferrooxidans* has a higher affinity for \( Fe^{++} \) than *At.ferrooxidans* and a lower affinity for \( Fe^{+++} \), a competitive inhibitor.

*L.ferrooxidans* can tolerate higher concentrations of toxic metals such as uranium, molybdenum and silver than *At.ferrooxidans*.

**Thermophiles**

Thermophilic iron-oxidising bacteria can be classified into moderate and extreme thermophiles. Optimum temperature for growth and leaching are in the range between 65\(^0\) and 85\(^0\)C for
extreme thermophiles and about 40° to 60°C for moderate thermophiles. A number of thermophilic microorganisms, such as *Sulfolobus* have been enriched and isolated from bioleaching environment. The bacteria grow chemolithotrophically at the expense of iron. Some are facultative autotrophs.

A moderately thermophilic bacterium, *Sulfobacillus thermosulfidooxidans*, is a Gram-positive, nonmotile, sporeforming, rod-shaped eubacterium, with optimum growth at 28-60°C. They are facultative autotrophs. They can also grow autotrophically on Fe (II), S, or mineral sulphide as energy source.

Examples of the extreme thermophiles are *Sulfolobus acidocaldarius* and *Acidianus brierleyi*, both in the genera of *Archaeabacteria*. There are four of these, namely, *Sulfolobus*, *Acidanus*, *Metallosphaera*, and *Sulfurococcus*. All are aerobic, extremely thermophilic and acidophilic bacteria oxidising ferrous ions, elemental sulphur and sulphide minerals between 55 and 90°C and pH 1 – 5.

*Acidithiobacillus ferrooxidans* is the most studied bioleaching bacterium.

They are Gram-negative rods (0.5 x 2 µm) and occur singly or in pairs. Cell mobility is achieved by means of a single polar flagellum. They are generally characterized by the following properties.

- Chemolithotrophic – growth and maintenance energy is derived from the oxidation of ferrous ions or reduced valence sulphur compounds.
- Autotrophic – carbon dioxide is used as a cellular carbon source and nitrogen and phosphorus needed as nutrients for growth and synthesis along with the trace minerals, K, Mg, Na, Ca and Co.
- Aerobic-oxygen is the electron acceptor and in contact with air derive oxygen and carbon dioxide.
- Mesophilic-bacterial growth and iron oxidation occurs at temperatures 20°C and 30°C.
Acidophilic – bacterial growth at a pH 1.5 to 2.5. They are regarded as obligate chemolithotrophs which derive energy from the oxidation of ferrous ions and reduced sulphur compounds. Carbon in the form of carbon dioxide is assimilated through the Calvin-Benson cycle. They can also grow on elemental sulphur or mineral sulphides under anaerobic conditions using ferric ions as the electron acceptor.

Mesophilic and thermophilic bioleaching bacteria

- *T.ferrooxidans* first isolated from mine drainage in 1947 and in 1951 Temple and Colmer isolated and described the acidophile.
- Microorganisms cultured from leach systems in waste rock dumps at the Bingham mine.
- Numerous investigations on *At.ferrooxidans* followed. *Leptospirillum ferrooxidans* described in 1972.
- *At.thiooxidans* and *At.Caldus* were used in leaching.
- Importance of *L.ferrooxidans* and use of mixed cultures containing *At.ferrooxidans*, *At.thiooxidans* and *L.ferrooxidans* was later established.
- Molecular biology techniques indicated dominant presence of *L.ferrooxidans* in stirred tank reactors for gold at pH 1.6 and temperature of 40°C. In many columns, also *L.ferrooxidans* was found to dominate.
- New species such as *Leptospirillum ferrophilum* isolated which were moderate thermophiles (2002).
- Another organism *Ferroplasma*, an iron oxidizing archaeon identified (2000).
- *At.Caldus* and *L.ferrooxidans* were dominant iron oxidizers functioning at 45°C, acidic conditions.
- Use of thermophiles in bioreactor and bioheap leaching was found to be useful since exothermic biooxidation of sulfides generates heat.
- There is considerable interest to use thermophiles in the leaching of chalcopyrite.
- Moderate and extreme thermophiles were identified and isolated.
- In actual situations, bioleaching processes involve consortia of microorganisms.
Typical microorganisms inhabiting waste copper ore dumps and heaps are given in table 4.1.

**Table 4.1: Microorganisms present in waste ore dumps**

<table>
<thead>
<tr>
<th>Acidithiobacilli</th>
<th>Heterotrophic bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acidithiobacillus ferrooxidans</em></td>
<td><em>Bacillus</em></td>
</tr>
<tr>
<td><em>Acidithiobacillus thiooxidans</em></td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td><em>T. denitrificans</em></td>
<td></td>
</tr>
<tr>
<td>Iron-oxidisers</td>
<td>Fungi and yeasts</td>
</tr>
<tr>
<td><em>Gallionella</em></td>
<td><em>Aspergillus</em></td>
</tr>
<tr>
<td><em>Lepotothrix</em></td>
<td><em>Penicillium</em></td>
</tr>
<tr>
<td>Sulphate reducing</td>
<td><em>Cladosporium</em></td>
</tr>
<tr>
<td><em>Desulfovibrio spp.</em></td>
<td><em>Saccharomycae</em></td>
</tr>
<tr>
<td>Thermophiles</td>
<td>such as</td>
</tr>
<tr>
<td>such as <em>sulfolobus</em></td>
<td></td>
</tr>
</tbody>
</table>
Microbiological advancements with potential benefits to heap bioleaching are given in table 4.2.

**Table 4.2: Microbiological aspects in heaps with their potential benefits**  

<table>
<thead>
<tr>
<th>Microbiological developments</th>
<th>Potential benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of bacteria that can initiate oxidation at higher pH values and condition the ore for conventional bioleaching bacteria</td>
<td>Effective bioheap leaching of high acid consuming ores.</td>
</tr>
<tr>
<td>Comprehensive recognition of adaptation or succession process of the bacteria to the changing character of the leach solutions.</td>
<td>Use of low quality water for make-up of leach solutions and operation of the bioheaps – lower capital and operating costs.</td>
</tr>
<tr>
<td>Realization of succession of thermophiles in bioheaps, interpreting their function and effects on minerals and precipitates.</td>
<td>Bioheap leaching of more refractory ores-increase metal recoveries with shorter leach time, at reduced costs.</td>
</tr>
<tr>
<td>Understanding of heterotrophic and Chemolithotrophic microorganisms in bioheaps related to function and nutrients.</td>
<td>Better understanding of conditions that could optimize bioheap performance.</td>
</tr>
</tbody>
</table>