Chapter - VII
Study of role of iron bacteria - Thiobacillus ferroxidan
in iron ore leachates and its effect on waterbodies and environment

Summary

The present work aims to determine the beneficial part of thiobacillus ferrooxidan and also its adverse effect on water bodies and environment. Not only is the bacterium autotrophic, but also it is capable of fixing atmospheric nitrogen which enhances the growth of some plants. Thiobacillous ferroxidan can grow via the direct oxidation of UO$_2$ in the presence of iron which facilitates the production of nuclear fuel. The study of iron bacteria thiobacillus ferroxidans gives an overview of the role played by this chemolithotrophic bacterium in the extent of contamination level of iron ore leachate. Thiobacillous ferroxidan grows at low pH and can obtain energy by oxidizing ferrous iron. In presence of oxygen, Thiobacillous ferroxidan creates food for itself by catalyzing, or speeding up, the oxidation of the iron and sulfur, creating acid mine drainage. Pyrite in iron ore mines when exposed to air and water, oxidizes, producing iron and sulfuric acid. Ferric iron, when discharged to surface water, hydrolyzes to produce hydrated iron oxide which causes more acidity. The acid lowers the pH of the water, making it corrosive and unable to support many forms of aquatic life.
INTRODUCTION

Bacteria are powerhouses in the living world, surviving in adverse condition, generate more complexity in the living system. Some bacteria produce toxins that are dangerous to humans, while others are absolutely vital for our survival and the survival of “higher” life forms in the stream. In streams, an enormous variety of bacterial life coats rocks, leaves, and woody debris. Several aquatic macro invertebrates survive on these bacterial films, often “grazing” on the bacteria as if goats in the pasture. From near-boiling hot springs on the surface to geologic strata buried deep in the earth over thousands of years, bacteria can make a home just about anywhere. Some of the most inhospitable places for life are the flooded mine pools that underlie minerals and the mine tailings on the surface. Often draining a large amount of dissolved metals and extreme acidity, this water is toxic to most aquatic life. Yet, bacteria survive and can even flourish in these harsh conditions [1-2].

Drainage from open cast iron ore mines and mining waste piles are the pollution problem in iron ore mining region. In mineral processing iron bacteria such as Thiobacillus ferroxidans, Thiobacillus thioxidan, leptospirillum ferroxidans have greater application in bioleaching of minerals, especially thiobacillus ferroxidan have greater interest due to its use in metal extraction, to attack sulfide containing mineral to convert the insoluble sulfides to soluble metal sulfate. Bacterial catalysis of the dissolution of sulfide minerals has the direct mechanism which is able to interact with the mineral and enhance the rate of dissolution of the mineral above the rate achieved during chemical leaching by ferric ions under the same conditions [3].

These group of bacteria are also able to attack a variety of ores such as Cu, Pb, Zn, Ni etc. Thiobacillous ferroxidan is a acidophilic, chemolithotrophic, gram negative, rod-shaped autotrophic bacteria, suited for growth in inorganic mining environment[4-5]. All T. ferroxidans strains examined have the genes for nitrogen fixation. Diazotrophy is therefore likely to be the general property of these bacteria [6-7]. Energy is obtained by the oxidation of ferrous iron to ferric ions. It grows best in an aerobic environment with oxygen as an electron acceptor. T. ferroxidans can grow via the direct oxidation of UO$_2$ in presence of iron and that it possesses a molybdenum –oxidising enzyme[8]. In addition to its unique physiology, T. ferroxidans has other features that makes it suitable for use in biomining operations [9]. One of this is its
inherent resistance to high concentration of metallic ions. It has been applied to trace acid mine drainage, bio-leach metal sulfide and remove H$_2$S from sour gases [10-14]. It can also be adapted to chemolithotrophic growth on a number of inorganic sulfur compounds, including thiosulfate, tetrathionate, trithionate, elemental sulfur, and metal sulfides. Bounds and Colmer examined the kinetics of thiosulfate and tetrathionate oxidation by T. ferrooxidans, Ferrobacillus ferrooxidans, and F. sulfooxidans as a means of differentiating between these bacteria, and although some differences were apparent in their affinity for thiosulfate, all three are now regarded as strains of T. ferrooxidans. T. ferrooxidans plays a major role in the bacterial leaching of sulfide minerals so that its basic physiology and biochemistry have been much studied. More recently, continuous culture studies have been used to provide additional fundamental information on the kinetics of growth and biochemical energy yield on iron. The kinetics of tetrathionate and thiosulfate oxidation by cell suspensions and the behavior of Thiobacillus ferrooxidans on growth-limiting supplies of tetrathionate or thiosulfate in continuous-flow chemostats, to define the limits of bacterial growth, substrate turnover, and biochemical energy yields during growth on inorganic sulfur compounds. The biuret method was used to determine protein content. The capability of the heterotrophic bacterium for molybdenum reduction was established. Molybdenum blue was oxidized enzymatically by aerobically growing cells of Thiobacillus ferrooxidans [15-20].

Thiobacillus ferrooxidans, is a bacterium that can actually contribute to acid mine drainage (AMD) formation. In the presence of oxygen, T. ferrooxidans creates food for itself by catalyzing, or speeding up, the oxidation of the iron and sulfur, creating AMD. This bacterium, as part of its metabolic process, creates acidity.

**Methodology**

To the study the presence and effect of Thiobacillus ferroxidan in iron ore leachate, three reported methods were used to check for the culture preparation:

**Method (A) [21]**

The sample was cultured in a medium containing 2 g of (NH$_4$)$_2$SO$_4$, 0.1 g of KCl, 0.25 g of K$_2$HPO$_4$, 0.25 g of MgSO$_4$ 7H$_2$O, 0.01 g of Ca(NO$_3$)$_2$, and 167 g of FeSO$_4$ H$_2$O in 1 liter of deionized, distilled H$_2$O, adjusted to pH 2.4. The FeSO$_4$ solution was filter sterilized and then added to the remaining autoclaved medium components. The concentration of Fe$^{2+}$ in the
complete medium was 0.1 M. Cultures were grown in Fernbach-type flasks, each containing 1 liter of medium (10% inoculum) at 25°C in a rotary shaker at 200 rpm. Cells were harvested in mid-log phase by centrifugation at 5,000 x g for 15 min at 4°C after removal of insoluble ferric ion deposits. Ferric ion was removed by allowing the cultures to sit for 1 h at 4°C, carefully pipetting the liquid phase, and then passing the remainder through Whatman no. 1, filter paper and washing with sterile, deionized, distilled H₂O, adjusted to pH 2.4. The cell pellets were pooled and then washed three times with deionized, distilled H₂O, pH 2.4. The final cell suspension was 5.4 x 10⁹ cells/ml (direct microscopic count) or 4.5 mg/ml (dry weight). Acid washed glassware was used in all experiments.

Method (B) [22]

A rapid and sensitive spectrophotometric procedure was used for monitoring the growth of thiobacillus ferrooxidans in liquid culture. Values determined for the optical densities at 490 nm of washed. The utility of this procedure was demonstrated by conducting physiological studies on the influence of CO₂ and FeSO₄ availability on the growth of T. ferrooxidans. 1 g of soil sample suspended in 100 ml of sterile distilled water to make percentage solution. From the above solution 1 ml of sample was transferred to 9 ml sterile distilled water to make 10⁻¹ dilution and like wise made different dilutions 10⁻², 10⁻³. Took 1 ml of 10⁻¹ diluted sample and pour it to 9K medium (pH 1.5) like wise other dilutions and incubated four sets at 30°C for one week. Colourimetric examination of orange colored solution of the medium indicates the bacterial leaching of ferrous ion to ferric, fig -2 shows the growth of bacterium in different dilution. Medium formulations, designated 100:10 and 10:10, were developed for the growth of Thiobacillus ferrooxidans.

Method (C) [23]

The medium used for the growth of *T. ferrooxidans* was a modified 9K medium (M9K): 0.4 g (NH₄)₂SO₄, 0.1 g K₂HPO₄, 0.4 g MgSO₄, 7H₂O and 33.3 g FeSO₄, 7H₂O per liter and adjusted to pH 2.3 with H₂SO₄.

Sample Analysis

During the study of the leaching experiments, 10 - mL samples of slurry were collected from the shake flasks and immediately frozen in order to arrest bacterial activity. The samples
were thawed and filtered through a Whatman No. 1 filter paper to remove ore particles and then centrifuged at 5,000 g for 5 min in order to remove any bacterial cells. The resulting clear samples were then analyzed for Fe, Zn and Cr by atomic absorption spectrophotometry at the Agricultural research lab Jagdalpur.

**RESULTS AND DISCUSSION**

The goals of the present work were twofold: to determine beneficial part of *T*. *ferroxidan* and its adverse effect on water bodies and environment. In order to establish the criteria experimental technique and wide-ranging literature survey was used.

Procedure one and three were not gave an appreciable growth. With the procedure two it was able to identify the bio leaching property of the bacterium. It shown in figure 1 and figure 2. Acid formation by the thiobacillous ferroxidan is shown in the figure 3. *Thiobacillus* ferrooxidans isolates grown on ferrous iron were not able to leach metals from ore leachates. Rise in pH stopped the growth of the organism.

Pyrite in iron ore mines when exposed to air and water, oxidizes, producing iron and sulfuric acid. Ferric iron, when discharged to surface water, hydrolizes to produce hydrated iron oxide and more acidity. The acid lowers the pH of the water, making it corrosive and unable to support many forms of aquatic life. Acid formation is most serious in areas of moderate rainfall where rapid oxidation and solution of exposed minerals can occur. Various impacts range in severity from isolated nuisance type problems to severe water quality impacts affecting large volumes of groundwater and miles of watercourse. Impacted uses include agricultural (irrigation and livestock), industrial, and potability of water supplies along with recreational uses, scenic resource appreciation, and aquatic organism habitat [45]. The aggressive nature of mine drainage may also result in corrosion and incrustation problems with respect to such man-made structures as pipes, well screens, dams, bridges, water intakes, and pumps. The compromising of well casings (water supply or oil and gas wells) can be extremely troublesome because it can then allow the migration and co-mingling of water from one aquifer with another, often leading to inter- and intra-aquifer contamination. Acidic mine drainage in particular can also be toxic to vegetation when recharging to the shallow groundwater system and soil water zones.
results for the leaching of hematite with and without bacteria and with bacteria, kinetic rate of leaching much faster in the sets with bacteria. The presence of bacteria can significantly increase the rate of the overall leaching process. In addition to the above reactions,

The outflow of untreated acid mine drainage into streams can severely degrade both habitat and water quality often producing an environment devoid of most aquatic life and unfit for desired uses. The severity and extent of damage depends upon a variety of factors including the frequency, volume, and chemistry of the drainage, and the size and buffering capacity of the receiving stream". Figure 4 predicting the Fe(II) oxidation rate, the Fe(II) oxidation rate and T. ferrooxidans cell growth are sensitive by both the reaction product [Fe(III)] and also, above a certain Fe(II) concentration, by the substrate itself. Heavy metal concentrations on the rate of Fe(II) oxidation (or the corresponding dissolved oxygen concentration reduction) and bacterial growth clear from the figure.

CONCLUSION

T. ferrooxidans, which love oxygen, reduce sulfate to sulfide in a process that causes dissolved metals in the water to drop out and also increases the water pH. Iron generally does not interfere with the daily lives of aquatic organisms. However, in high concentrations dissolved in AMD, iron has a devastating impact. Besides harming fish it has further implications for the terrestrial environment: reduced hatching success and other toxic effects have been reported in birds that feed on freshwater invertebrates that have bioaccumulated iron. It also impair growth in plants around acidic mine spoils. Scanning electron microscopic picture of Thiobacillus ferrooxidans given in figure 5 obtained from google images.

Greater conversion of the mineral in a bacterial leaching experiment than in the corresponding chemical leaching experiment without bacteria would have indicated that the bacteria contributed to the dissolution of the mineral beyond their ability to oxidize ferrous ions.

Increased growth rate of thiobacillus ferrooxidans at low pH indicates the impact of mining on adjoining water bodies by iron bacteria, which flourish in iron orewash water. The study of iron bacteria thiobacillus ferrooxidans gives an overview of the role played by this chemolithotrophic bacterium in the extent of contamination level of iron ore leachate and the harmful effect in waterbodies.
Figure 1. Leaching of sulfide minerals by bacterium

\[
\text{FeS}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{Fe}^{3+} + 2\text{H}^+ + \text{SO}_4^{2-}
\]

Figure 2. Thiobacillus Ferroxidan in 9k Media/0.02g yeast; Temp 30°C, pH = 1.5
Figure 3. Effect of iron concentration in bioleaching

Figure 4. Oxidation of ferrous ion (in g/L) to ferric iron
Table 1. Effect of pH on bacterial Growth by M9K medium

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Figure 5. scanning microscopic picture of thiobacillus ferroxidan
References