Technical Annual Report for the Project

ELECTROCHEMISTRY OF Thiobacillus ferrooxidans
REACTIONS WITH PYRITE

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1. STATEMENT AND OBJECTIVES

The objective of this project is to provide the fundamental information on the mechanisms of bacterial leaching of pyrite. The knowledge of how bacterial leaching of pyrite functions is essential for design and development of a technology for coal cleaning with bacteria. The features of major electrochemical techniques will be examined to find out if any of them can provide a diagnostic information on the mechanisms of related reactions.

2. SCIENTIFIC AND TECHNICAL PROGRESS

2.1 GENERAL

The research in this reporting period was focused on one key parameter, which was how to improve the chemical activity of bacteria. Conceptually, the bacterial leaching of pyrite is dependent on chemical activity and concentration of bacterium cells. The activity of the bacterium cells can be modulated by modulating the conditions of the environment where the cells reside. The environmental conditions imply the solution composition with respect to pH, heavy metals concentration, composition and concentration of extracellular products, temperature, and composition and concentrations of nutrient salts. In the same time, by modulating the chemical activity of bacteria via the environmental conditions their multiplication and therefore their

concentrations can be also modulated.

Two major approaches were undertaken to increase the activity and concentration of bacteria.

1. One approach was to provide more nutrient salts. It was anticipated that by providing higher amounts of nutrients the concentration and the activity of bacteria would increase.

2. The other approach was to provide fresh environment to bacteria for the growth. This was an important concept. According to this concept, the bacterium during its activity produces extracellular products among which some are waste. During the growth of bacteria the waste amount also accumulates and becomes toxic to the cells above certain concentration level. If the bacteria cells were taken out from the environment saturated with toxic waste products and placed into a fresh solution environment then the bacteria cells should continue their growth, multiplication, and therefore the concentration.

Before the experiments on the improvement of T. ferrooxidans activity were started, it was decided to first examine their activity with time, i.e. at what fermentation time the bacteria become the most active and what is the fate of bacteria after longer time intervals when the concentration of waste products is very high and the nutrient is depleted. There was no literature

information available on this subject.

3. The effect of the key parameter, the solution pH, was studied. It was found that pH had an effect on the chemical activity of *T. ferrooxidans* with respect to oxidation of ferrous ion, and with respect to their growth. With respect to the growth rate of bacteria, pH of the solution was most influential in the region pH=1-1.5. With respect to the oxidation rate of ferrous ion (chemical activity) pH had two distinct regions. In the region pH=1-2.0, the increase of pH increased the activity of bacteria, and in the region pH=2-3.5 the activity of bacteria sharply declined.

2.2 EXPERIMENTAL WORK

The experimental apparatus was the same as explained in the previous reports. It consisted of a four liter fermenter, electrodes, and computerized potentiostat-galvanostat.

The activity of bacteria was monitored during the growth stages (fermentation) by cyclic voltametry. After the fermentation was finished, the bacterium cells were removed from the solution by filtration and its activity tested by the rate of redox potential change, the method previously developed by the author.

2.3 RESULTS

Activity of T. ferrooxidans with time

There is no literature information on the activity of T. ferrooxidans with time. However, this information is needed and is very important for many reasons. In the fermentation reactor design area, it would be important to know at which stage of fermentation the bacterium reached its maximum activity and concentration. In the bioleaching area, it would be important to know about the fate of bacterium with respect to its activity at the prolonged leaching times.

For the above reasons it was decided to perform very lengthy and time consuming, but very important, experiments to study the activity of T. ferrooxidans with time. This study was performed by running fermentation experiments for different time lengths, extracting all the cells (harvesting) produced during that time interval, and determining their activity by calculating the rate constant of ferrous iron oxidation (redox reaction). The experimental procedure is described below.

A four liter fermenter was prepared and all medium (described in the previous reports) and nutrient salts (100 grams of ferrous sulfate) were added. Then a stock of T. ferrooxidans culture (250 ml) was transferred from the incubator into the fermenter. After

exact number of days of fermentation, the fermentation part of experiment was ceased, the filtration performed to remove the precipitates and the filtrate solution transferred to membrane filtration to remove the cells. The cells were also washed from the iron salts on the same membrane filter. After washing, the cells were removed from the filter membrane into a container by maintaining a constant volume of the cells (34 ml). The prepared batch of concentrated cells was used to determine their activity with respect to the rate of ferrous ion conversion to ferric. To check the activity, a 0.5 ml of concentrated cells was added into a separate electrochemical cell to measure the rate of ferrous ion oxidation. From the redox rate data, which represented the activity of T. ferrooxidans at the particular number of fermentation days, the iron oxidation rate constant was calculated and plotted. The results are presented in Figure 1.

The analysis of data presented in Figure 1 show three distinct ranges of activities, one range of increasing activity and two ranges of decreasing activities. In the first range (2, 4, and 5 days) the activity of T. ferrooxidans was increasing with time. This was expected and it is directly related to the exponential stage of bacteria growth. At 5 days of growth (fermentation) the population density of bacteria reached maximum and therefore the activity of bacteria reached the maximum.

During fermentation for longer periods than 5 days, the activity of

T. ferrooxidans showed two exponential ranges of decreasing activities, 5-12 days (second stage), and 12-24 days (third stage). The activity decrease in the 5-12 day time interval was not dramatic and one can safely assume that activity of T. ferrooxidans in this time interval is high enough for practical leaching purposes. The most dramatic activity decrease occurred in the 12-24 days range. At the final studied time, 24 days, the activity of bacteria was very low almost non-existent.

The decrease of bacteria activity can be related to the exhaustion of the nutrient, ferrous sulfate. Ferrous sulfate became mostly oxidized to ferric sulfate during the growth stage (stage I). The lack of food therefore could be the primary reason for the first stage of decreasing activity. During the oxidation of ferrous to ferric ion, which is necessary for bacteria growth, jarosite is also formed as the reaction byproduct. The jarosite formation initiates on the surface of bacterium cells. With time, the cells become embedded into the jarosite product and the cells die. The lack of food and the formation of jarosite product are the main reasons for the fast decay of bacteria activity in the second decreasing stage.

Effect of Fresh Environment on the Activity of T. ferrooxidans

Because bacteria produce extracellular products during the active stage (growth and multiplication) some of these products may become toxic and detrimental to the activity of bacteria if accumulated in their environment. With time, the concentration of extracellular products (waste) can become so high, therefore toxic, that the bacteria have to die. If the bacteria cells had to continue their growth then they would not be able to do so in the existing environment where they originally grew. Instead, they must be harvested and moved into the next cycle with fresh and clean environmental conditions. Under the fresh conditions, provided that all other nutrient salts are present, the bacteria will vigorously grow until the food is exhausted and the waste byproducts accumulated to the toxic levels. Because all of the cells from the first cycle were transferred into the second cycle the number of cells will continue to increase and the cells concentration will be much higher at the end of the second cycle than at the end of the first cycle. The bacteria can be removed from the solution in the second cycle (harvesting) and transferred into the fresh solution to start the third cycle of growth. The concentration of bacteria at the end of this cycle should be still higher than the produced concentration in the second cycle. The cycles involving growth, harvesting at the end of growth, and transferring into the fresh environmental conditions, can continue until satisfactory population density of T. ferrooxidans is achieved.

From practical point of view, the above described concept can be very significant because T. ferrooxidans can be produced in very high quantities (high population density) in separate biofermenters and then transferred into the reactors for bioleaching. The leaching with concentrated bacteria will be much faster because of the large amount of cells present in the reactor right from the beginning of leaching.

Under the normally practiced bioleaching conditions the bioleaching reactors are used for simultaneous inoculation, growth of bacteria and leaching. However, this approach has some serious disadvantages.

1. Because the incubation stage of growth can take long times the bioleaching reactor is not efficiently used and its capacity is severely reduced.

2. Fast bioleaching requires a high concentration of bacteria, which can be achieved by the growth. However, the growth of bacteria in the bioleaching reactor can be hindered by the presence of toxic solution components produced by the cells. Or, the bioleaching of minerals can release toxic heavy metals which may also hinder the bacteria growth. Therefore, it can happen that the bacteria never reaches the satisfactory population density for bioleaching at a satisfactory rate.

A much better approach is to grow T. ferrooxidans in separate fermenters to obtain as high concentration of the cells as

possible, to harvest the cells, and to transfer them into the bioleaching reactors for leaching. According to this concept, a very high density of bacterium cells can be produced because the bacterium growth was done under controlled fermentation conditions. In addition, because of the absence of toxic solution constituents the growth of bacteria is much faster.

For the above described reasons, it was decided to prove the above concept that bacteria can be produced at a very high population density by providing fresh and clean (waste free) environmental conditions. For that reasons many experiments were performed and each experiment was consisted of the above described cycles: growth, harvesting, transfer into fresh environment (fresh medium+nutrient solution).

Experimentally, a starting batch of concentrated T. ferrooxidans had to be produced first. This took five days. After the first batch of concentrated bacteria was produced, it was transferred into the fresh environment and from that point on the growth of bacteria was done for only one day, i.e. each following cycle lasted one day, see Figure 2. One day duration for a cycle was chosen because it was found that this time interval was sufficient to completely convert the nutrient ferrous sulfate to ferric sulfate. It can be noticed from Figure 2 that the activity of bacteria did not change much in the first 7 one-day-cycles. The activity increased somewhat in the first 2 one-day-cycles and then

stayed constant. From the results in the first 7 one-day-cycles appears that the fresh environment did not produce the anticipated positive effect. The explanation lies in the amount of available food in each cycle. The harvested bacteria was being transferred into the next cycles with the fresh environment under identical experimental conditions for all cycles. The identical conditions also mean the same amount of nutrient, ferrous sulfate. Because the harvested bacteria from the previous cycle is using the same amount of food in the following cycle then the concentration of bacteria cannot grow beyond the point that the amount of nutrient allows. Therefore, the activity of bacteria in the first seven cycles was about the same because constant amount of food was available, 100 grams of ferrous sulfate for each cycle.

However, when harvested T. ferrooxidans was transferred into the 8th one-day-cycle but with higher amount of food, 150 grams of ferrous sulfate instead of 100 grams, the activity of bacteria immediately increased. In the 9th one-day-cycle the harvested bacteria from the 8th one-day-cycle was transferred into fresh environment with higher amount of food, 200 grams of ferrous sulfate. The activity of bacteria dramatically increased because the concentration of bacteria dramatically increased. One more experiment was performed. The T. ferrooxidans cells harvested in the 9th one-day-cycle were transferred into the fresh solution (environment) with 250 grams of ferrous sulfate. In this case the concentration of T. ferrooxidans was so high that it could not be

harvested by filtration (the membrane filter was completely plugged up with the bacteria cells).

To prove that high concentrations of T. ferrooxidans cells cannot be produced by a simple higher amount of food addition the separate set of experiments was performed. In these experiments the effect of food on bacteria activity was studied. Five different concentrations of ferrous sulfate were examined: 50, 100, 150, 200, 250, and 300 grams. (Total volume in all above and these experiments was 4 liters). Basically these experiments were 5 days fermentation experiments each with different amount of food. (5 day fermentation period is optimum, see Figure 1). The results are presented in Figure 3. It can be seen that the increased addition of food could not produce increased activity of bacteria. The obtained activities (or concentrations of T. ferrooxidans) even with the highest amount of food were not much different from the activities corresponding to the horizontal portion of the graph in Figure 2. Only when the cells are moved into the fresh environment with abundant supply of food they can grow and reach high population density. The results presented in Figure 2 and Figure 3 therefore represent a direct proof on the importance of a fresh environment, or conversely the importance of the accumulated extracellular products, on the growth of bacteria.

Effect of pH:

The research continued in the area of exploring the effect of fundamental solution conditions on the biochemical activity of *T. ferrooxidans*. The new parameter studied was the effect of solution pH on the activity of bacteria. The activity of bacteria was monitored by the same method discussed above, the combination of cyclic voltametry technique and redox potential measurements.

pH Variation During the Growth: The activity of *T. ferrooxidans* was examined in the pH range 1-3.5. The experiments were initiated at a constant initial pH. The performance of *T. ferrooxidans* was monitored with time by monitoring the solution pH, the redox potential of the solution, and by recording the cyclic voltamograms. The cyclic voltamograms were recorded daily by using two pyrite electrodes, one conditioned, and one control electrode. To study the effect of pH another pyrite electrode was built. This is an indicator electrode which was used to record the redox potential of the solution during the growth stage of bacteria.

Variation of pH during Growth: The pH variation during bacteria growth is presented in Figure 4. Very interesting results were obtained. At lower pH range, such as pH=1, there was no pH change during the growth stage. This was somewhat expected for two reasons: 1) At this pH, bacteria activity was low that the no solution conditions were changed, including the pH. 2) The initial pH itself was too low, i.e. the initial concentration of hydrogen ions was very high and could not be changed by the consumption or

generation of hydrogen ions on a smaller scale. In other words, the background concentration of hydrogen ions was so high enough that the possible hydrogen ion concentration changes could not be detected.

At initial pH=1.5, the pH of the solution increased during the five day bacteria growth. The same happened when the experiment was initiated at the initial pH=2.0. The increase of pH represents the removal of hydrogen ions from solutions. The reaction which could be responsible for the observed phenomenon is:

According to reaction (1), hydrogen ion is consumed during the reaction of oxidation of ferrous ion with oxygen. But this reaction is too slow at these low pH values, Mathews and Robins (1), Huffman and Davidson (2), and for the studied initial pH values (pH=1.5, and pH=2.0) it should take many months to achieve just 50% of reaction (half life). The role of *T. ferrooxidans* is then to catalyze the rate of the given reaction, Pesic, Oliver and Wichlacz (3).

In the higher pH range, pH=2.5-3.0, the pH of the solution decreased with time during the growth of bacteria. This phenomenon can be explained by the acid production caused by the acid generating iron precipitation reactions.

According to reactions (2) and (3) the ferric ions generated by the oxidation of ferrous sulfate is being precipitated as a solid reaction product simultaneously generating sulfuric acid. The solid reaction product can be either a basic iron sulfate, $\text{Fe}(\text{OH})\text{SO}_4$, or jarosite, $(\text{H}_3\text{O})\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6$. The formation of jarosite was documented by the authors in our previous report (4). According to our findings, the *T. ferrooxidans* cells serve as the nucleation sites for generation of jarosite precipitates.

Effect of pH on the Chemical Activity of Bacteria: The bacteria grown at each individual initial pH, the experiments related to Figure 4, were extracted from the solution at the end of each experiment. The separate study was subsequently performed with the extracted cells. The objective of the following study was to examine the cells' ability to oxidize ferrous ion as a function of the pH of the solution pH at which they were initially grown. The rate of ferrous ion oxidation, and the associated calculated rate constant, would represent the activity of bacteria. The results from the ferrous ion oxidation rates are presented in Figure 5. In this figure, the rate constants, representing the rate of $\text{Fe}(\text{II})$ oxidation, are plotted versus the initial pH of solution from which the bacteria was extracted. It can be seen that in the studied pH range, $\text{pH}=1-3.5$, the only pH range that had an importance was

between pH=1 and pH=1.5. In this pH range, the bacteria activity went dramatically up when the bacteria was grown at pH=1.5 instead at pH=1.0. In the initial pH range, pH=1.5-3.5, there was not much influence of the solution pH on the chemical activity of bacteria, but it can be noticed that the activity of bacteria began to decline toward the higher pH values. Higher than pH=3.5 values were reported at the present time because of the experiments being in progress.

The activity of bacteria, represented by Figure 5, may mean the real bioactivity of the cells, or it may simply reflect the concentration of the extracted cells. If certain solution conditions, such as the studied initial solution pH, the concentration of the grown cells is higher then the higher number of cells will be extracted during harvesting. If the concentration of the harvested cells is higher, then their rate of iron oxidation will be higher. Therefore, the results in Figure 5 really represent the effect of the initial solution pH on the growth rate of *T. ferrooxidans*. From practical point of view, the bioleaching of pyrite, the highest concentration of the cells in the leaching solution may always be desirable because it would mean the highest leaching rates. According to Figure 5, the highest bioleaching rates, if these are controlled by the concentration of cells, could be achieved in the pH region pH=1.5-3.0.

Chemical Activity of Bacteria Based on Redox Potentials: The objective of this study was to examine the chemical activity of *T. ferrooxidans* by measuring the redox potential of the medium solution during the growth stage. For this reason a separate indicator pyrite electrode was built. This electrode was used only periodically to measure the redox potential of the solution, and it was polished before each use. The redox potentials were measured with time for each experiment initiated at a particular pH. The results are presented in Figure 6. During the fermentation, bacteria is oxidizing continuously its nutrient source, ferrous ion. The conversion of ferrous ion to ferric results in a redox potential increase. The faster conversion of ferrous to ferric the faster increase of the redox potential will be.

Very little redox potential change was obtained for the experiment performed at initial pH=1. For the rest of the experiments, the redox potential curves had the expected three characteristic regions: induction, autocatalytic, and post-autocatalytic. Each redox region interprets the corresponding bacteria growing stage. Notice also that when the bacteria growth is finished, which means that all the food is consumed and all ferrous is converted to ferric, then in each pH case the final redox potential was the same, about 550mV.

The redox potential measurements from Figure 6 were used to calculate the reaction rate constants. The reaction rate constants

were calculated by using the first order reaction equation. The reaction rate constants as a function of initial pH are plotted in Figure 7. Note two very distinct regions of reaction. According to this plot the ability of *T. ferrooxidans* to grow and oxidize ferrous ion to ferric is extremely dependent of pH. The rate of oxidation sharply increased in the region pH=1-2.5, and sharply declined above pH=2.5.

If the results from Figure 7 were used to interpret the performance of bacteria as a function of the concentration of the cells, then one may conclude that the lower reaction rates at pH=3 and pH=3.5 were obtained because of the lower cell concentrations at these two pHs. However, when the cells were harvested, at these two individual initial pHs, and the performance of the harvested cells examined, then it was found that their ability to oxidize ferrous ion to ferric did not change much, Figure 5. Therefore the results obtained in Figure 5 indicate that the declined reaction rate constants obtained in Figure 7 were not due to the concentration of the cells (growth at particular pH conditions) but due to their chemical activity toward the oxidation of ferrous ions.

Lowered reaction rate constants, but not the concentration of the cells produced at initial individual pH values, was probably caused by the solution conditions which impaired the chemical ability of the cells to oxidize iron. One of the reasons could be the amount of jarosite produced as a function of the initial pH. In the above

experiments, after fermentation, harvesting, and chemical activity of the cells testing, the residues produced in the fermentation solutions were also collected and their weights measured. It was found that the weight of the residues increased with pH, as presented in Figure 8. It is anticipated that the produced residues are jarosites. The residues were not analyzed to confirm their composition, and the analysis will be performed in the near future.

3.0 SUMMARY

1. The activity of T. ferrooxidans with duration of fermentation was examined. It was found that there were three distinct stages of fermentation. The activity of T. ferrooxidans increased in the first stage because of the growth of the cells (multiplication). The activity of T. ferrooxidans decreased in the second and third stage of fermentation.
2. The optimum fermentation time was 5 days. This time interval was well defined, and the activity of T. ferrooxidans rapidly decreased before and after this time interval.
3. Decreased activity of T. ferrooxidans beyond five days of fermentation is directly related to the lack of food and jarosite precipitation, i.e. the cells die.

4. A conceptual method to increase the concentration of T. ferrooxidans (activity) during fermentation was developed. The method is consisted of fermentation, harvesting and transfer of T. ferrooxidans cells into the fresh environment. This concept has a great potential for design of bioreactors for production of large quantities of T. ferrooxidans to be used for bioleaching of pyrite.

5. The role of fresh environment was proved by performing separate set of experiments in which the large amount of food (ferrous sulfate as nutrient) was added. The concentration (activity) of T. ferrooxidans could not be increased by food addition alone without transferring the cells to the fresh environment.

6. The effect of pH on the growth and chemical activity of T. ferrooxidans was examined.

7. The pH has a very important role. In the low pH range, pH=1-1.5, pH increase has a positive effect on the activity and concentration of T. ferrooxidans. Above pH=1.5, pH has a negative role on the activity (ability to oxidize ferrous), and a small effect on the growth (concentration of the cells).

8. The amount of produced residue continuously increased with pH increase.

4.0 FUTURE WORK

The future work will be related to the use of some other electrochemical techniques to study the role of T. ferrooxidans with pyrite.

Some of the previously done cyclic voltametry experiments will be repeated in order to find what exactly happens on the pyrite surface between 1.5 and 2.0 days of fermentation (See the annual report, 1989). A separate SEM study in this time region will be also performed.

The effect of solution conditions on the chemical activity and growth of T. ferrooxidans will continue.

References

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Fig. 1 Bacteria activity variation with time.

Fig. 2 Activity of bacteria after recycling into a fresh nutrient medium. Bacteria was harvested after 1-day of fermentation and recycled into the next 1-day fermentation cycle.

Fig. 3 Effect of nutrient salt addition on activity of *T. ferrooxidans*.

Figure 4. Variation of pH with fermentation time.

Figure 5. Effect of initial fermentation pH on the activity of harvested *T. ferrooxidans*.

Figure 6 Redox potential variation during fermentation of *T. ferrooxidans* at different initial solution pH.

Figure 7. Rate constants variation as a function of fermentation pH. Rate constants calculated from the redox potentials of the fermentation solutions.

Figure 8 The weight of residue produced during fermentation at various Initial pH values.

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