

University of Nevada

Reno

The Effect of pH on the Bacterial Oxidation
of Arsenic Sulfides

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science
in Metallurgical Engineering

by

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ABSTRACT

The effect of pH on the microbiological oxidation of arsenic sulfides was studied using the chemosynthetic bacteria, Ferrobacillus ferrooxidans. Tests were conducted on ore from Getchell, Nevada, containing two arsenic sulfides, orpiment (As_2S_3) and realgar (As_2S_2). The bacteria were grown in an arsenic enriched medium. The initial tests were conducted in shaker flasks. The bacteria adapted to the orpiment substrate, but the biological oxidation of realgar was unsuccessful. It is proposed that the unsuccessful oxidation of realgar is due to the mineral's unique crystal structure.

Batch experiments were done initially using a chemostat. Utilizing continuous pH monitoring, the maximum rate of arsenic sulfide dissolution was found to be at pH 2.7. Data from the batch runs showed that the oxidation rate was not first order but rather autocatalytic. After finding the optimum pH, a continuous fermentation run was started in the chemostat. The chemostat was used as a constant stirred tank reactor.

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INTRODUCTION

The purpose of this thesis was to find the optimum pH conditions for the biooxidation of arsenic sulfides using the bacteria Ferrobacillus ferrooxidans. The arsenic sulfides studied were orpiment (As_2S_3) and realgar (As_2S_2) collected from the Getchell mine at Getchell, Nevada. Studies were conducted using both batch and continuous processes.

There are many gold and silver bearing arsenic sulfide deposits in Nevada (1). In many cases the recovery of the gold and silver from these ores is not possible because of metallurgical problems associated with the arsenic in the ore. In 1941 the Getchell mine installed roasting equipment to remove the arsenic sulfides (1). The ore at the Getchell mine is a massive arsenic sulfide with quartz and carbonate gangue. A photograph of the ore is shown in Figure 1. Gold present is associated with the sulfides and not the quartz. The orpiment and realgar were oxidized during the roast to arsenic oxide. In the oxide form the arsenic could be removed and the gold recovered. The process is now impractical because it requires large amounts of energy and endangers the environment.

The fine arsenic oxide powder, produced by the roasting process can be seen covering the area surrounding the mine and mill complex. The arsenic oxide fines plus mill tailings have damaged the areas' ecology (2) and endangered the health of the people in the area (3).

Because of these problems there is interest in finding a low energy, nonpolluting method for removal of arsenic sulfides.

Bacterial oxidation or leaching offers a possible method for arsenic sulfide removal.

The ability of the bacteria Ferrobacillus ferrooxidans to oxidize metal sulfides to sulfates is well documented (4). These bacteria are autotrophic (able to obtain self nourishment and energy for their life from inorganic compounds) and aerobic (requiring oxygen). They are found in nature in acidic mine drainages and can function over a broad range of pH values. The products produced by the bacterial oxidation of the sulfide substrate are sulfates, sulfuric acid, and in some cases elemental sulfur. Ferrobacillus ferrooxidans has a limited oxidizing potential and can not change the state of the metal cation present. The bacterial oxidation of arsenic sulfides, orpiment, realgar, and arsenopyrite has been studied by Ehrlich (5, 6). His work shows that the microbiological leaching of arsenic sulfide is possible, but his published work doesn't explain the mechanisms of the system. Ehrlich's work shows that the presence of the Ferrobacillus ferrooxidans increases the aqueous concentration of arsenic in acidic solution. Bacterial oxidation of sulfide minerals is valuable because most metal sulfides are insoluble. However, the sulfate form is soluble. Orpiment is an example of an acid insoluble sulfide, but when biooxidized by Ferrobacillus ferrooxidans a soluble sulfate is produced.

To the author's knowledge no work has been done on the system to describe the optimum pH conditions or its adaptation to a continuous process. Data have been collected to determine the optimum pH for the biooxidation of orpiment. Data were also collected from continuous



Figure 1. The sample collected from Getchell, Nevada shows the red realgar and the yellow orpiment. The black and white areas are calcium and magnesium carbonate with quartz.

runs and yielded information on the mechanism of the biooxidation of arsenic sulfides. A chemostat similar to the one used by Lacey and Lawson (7) was used for both the continuous and batch runs. A chemostat gives control of temperature, agitation, pH, aeration, foam level, and in continuous operation, flow rates. A photograph of the chemostat is shown in Figure 2.

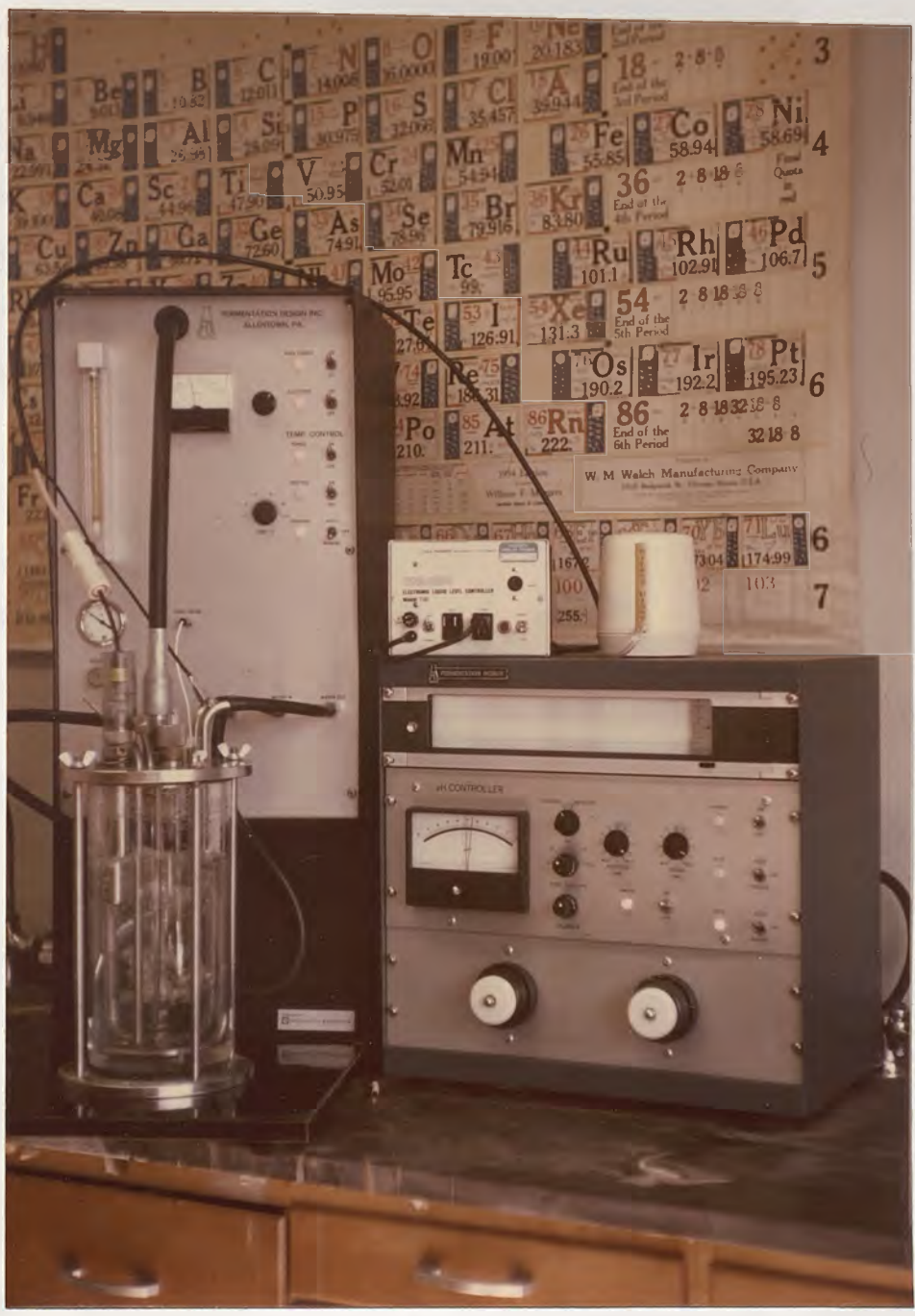
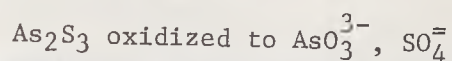


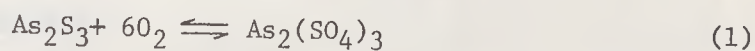
Figure 2. Chemostat with pH controller.

THEORETICAL CONSIDERATIONS

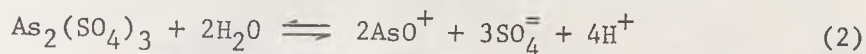
The dissolution of orpiment by bacteria in acid solution is recorded in the literature (4, 5, 6). It is known that in order to live in an inorganic medium the Ferrobacillus ferrooxidans must derive energy by oxidizing an inorganic compound in the culture medium. The bacteria is known to oxidize sulfides and in this case orpiment is the only sulfide present. The reaction is given as (9):



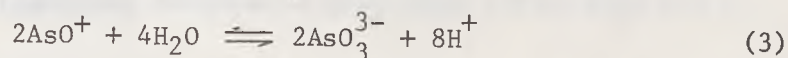
The probable reaction, knowing that the bacteria oxidized sulfides to sulfates:



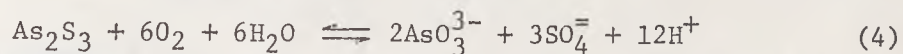
But arsenic sulfate is soluble in water so:



The AsO^+ species is unstable and hydrolyzes:



The overall equation, combining equations 1, 2, and 3 is:



Equation 4 shows the products of the biooxidation of the orpiment substrate to be soluble arsenic, sulfate, and hydrogen ion. Any change in the concentration of the soluble arsenic or the pH can be measured but the high sulfate concentration of the culture medium makes sulfate

measurement impossible. So the rate of the biooxidation can be measured by the rate of production of H^+ or AsO_3^{3-} .

A kinetic model for biooxidation of arsenic sulfide can be derived by writing a mass balance about a constant stirred tank reactor. Using accumulation = in - out + production, the equation for the substrate is written as (10).

$$V \left(\frac{dC_a}{dt} \right) = QC_{ao} - QC_a + V \left(\frac{dC_a}{dt} \right)_p \quad (5)$$

where

C_a = outgoing substrate concentration, mg/ml.

C_{ao} = incoming substrate concentration, mg/ml.

V = volume of the fermentor, ml.

Q = flow rate, ml/day

t = time

Assuming no production or biooxidation Equation 5 can be reduced to

$$V \left(\frac{dC_a}{dt} \right) = QC_{ao} - QC_a \quad (6)$$

Solving for C_a by integrating Equation 5 from time = 0 to time = t gives

$$\ln(C_{ao} - C_a) = -(Q/V)t = \ln C_{ao} \quad (7)$$

rearranging Equation 7

$$C_a = C_{ao} - C_{ao} e^{-(Q/V)t} \quad (8)$$

Equation 8 describes what happens if there is no production.

When writing a mass balance about the substrate with biooxidation taking place, the product term represents substrate consumption. Assuming perfect mixing, arsenic sulfide in the fermentor at any time is sulfide accumulation less any sulfide consumed by the bacteria or accumulation - product = in - out.

$$v \left(\frac{dCa}{dt} \right) - v \left(\frac{dCa}{dt} \right)_p = QC_{ao} - QC_a \quad (9)$$

A new equation can be written where the accumulation term includes the sulfide consumed by the bacteria.

$$\left(\frac{dCa}{dt} \right)_b = \frac{Q}{V} (C_{ao} - C_a) \quad (10)$$

Values for this accumulation term can be determined from the data collected from the inoculated runs. The new accumulation term in Equation 10 can be written as

$$\left(\frac{dCa}{dt} \right)_b = \left(\frac{dCa}{dt} \right) - \left(\frac{dCa}{dt} \right)_p \quad (11)$$

Now the sulfide consumed by the bacteria can be expressed as the difference between the accumulation term from Equation 5 and the accumulation term from the Equation 10.

$$\left(\frac{dCa}{dt} \right)_p = \left(\frac{dCa}{dt} \right) - \left(\frac{dCa}{dt} \right)_b \quad (12)$$

In a batch process there is no flow so the in and out terms are zero and the accumulation is equal to the production. Equation 5 can be written as

$$v \left(\frac{dCa}{dt} \right) = \cancel{QC_{ao}} - \cancel{QC_a} + v \left(\frac{dCa}{dt} \right)_p \quad (13)$$

Assuming first order the production term can be written as kC_a .

Rearranging Equation 13.

$$\frac{dC_a}{dt} = kC_a \quad (14)$$

or

$$\frac{dC_a}{C_a} = kdt \quad (15)$$

Integrating Equation 15 from $C_{a0} = 0$ to C_a , and time = 0 to time = t

$$C_a = C_{a0}e^{-kt} \quad (16)$$

If the biooxidation process is autocatalytic the reaction rate is not first order and Equation 16 does not hold. A simple autocatalytic reaction can be described by an equation where one of the products acts as a catalyst (11).



When a reaction is autocatalytic the production term is no longer equal to kC_a as in Equation 14. The production term in autocatalytic reactions is written as

$$\frac{dC_a}{dt} = kC_aC_r \quad (18)$$

The molar sum of C_a and C_r is constant, so at any time

$$C_0 = C_a + C_r = C_{a0} + C_{r0} \quad (19)$$

Combining Equations 18 and 19 the rate of production becomes

$$\frac{dC_a}{dt} = kC_a(C_0 - C_a) \quad (20)$$

Rearranging Equation 20 into partial fractions

$$\frac{dC_a}{C_a(C_o - C_a)} = \frac{1}{C_o} \left(\frac{dC_a}{C_a} + \frac{dC_a}{C_o - C_a} \right) = kdt \quad (21)$$

Integrating Equation 21 yields

$$\ln \frac{C_{ao}C_r}{C_a C_{ro}} = -C_o k t \quad (22)$$

Equation 22 is in the form of a straight line with a zero intercept and a slope of $C_o k$. If plotting the reaction data gives a straight line with a zero intercept, the reaction is autocatalytic and a value for k can be found from the slope.

EXPERIMENTAL

Apparatus

The following is a description of the equipment used to provide a controlled environment for biooxidation by Ferrobacillus ferrooxidans.

Chemostat

A 3 1/2 liter fermentor, purchased from Fermentation Design Inc., was equipped with automatic control for temperature, agitation, and aeration. It controlled temperature within 2°C by circulating hot or cold water. The solution was agitated by two flat blade turbines on a shaft with variable speed control. A Silent Giant diaphragm pump was used to supply air. In all runs the temperature was set at 30°C, agitation at 200 rpm, and air flow at 3 cc/sec.

An automatic pH control unit made by Fermentation Design Inc. was used with the fermentor. The pH controller would maintain a set pH by using two peristaltic pumps to add either 9N H₂SO₄ or NH₄OH from supply tanks. The pH controller was equipped with a slow speed chart recorder for recording pH changes.

Foam level was controlled by a Dyna-Sense Electronic Liquid Level Controller and a motor driven syringe. The syringe would dispense a small amount of Antifoam A Emulsion, purchased from Sigma Chemical, when the foam level was high enough to be sensed by the Dyna-Sense electrodes.

The apparatus is illustrated in Figure 3.

For the continuous runs two peristaltic pumps were used to precisely pump the substrate through the chemostat. A continuously

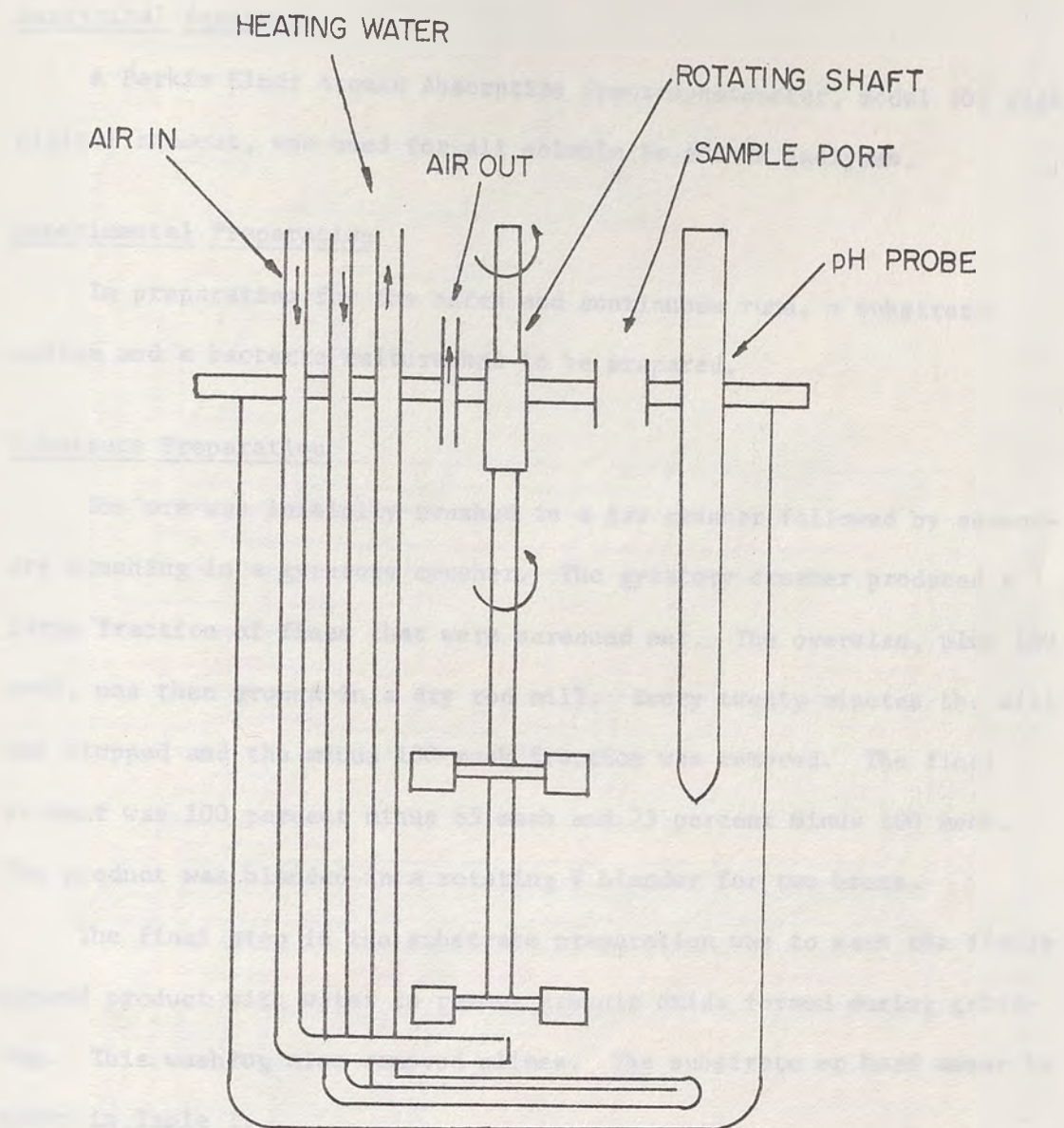


FIGURE 3. FERMENTOR USED IN BATCH TESTS

stirred tank was used to mix the substrate slurry. The apparatus is illustrated in Figure 4.

Analytical Equipment

A Perkin Elmer Atomic Absorption Spectrophotometer, model 303 with digital readout, was used for all soluble Fe and As analyses.

Experimental Preparation

In preparation for the batch and continuous runs, a substrate medium and a bacteria culture had to be prepared.

Substrate Preparation

The ore was initially crushed in a jaw crusher followed by secondary crushing in a gyratory crusher. The gyratory crusher produced a large fraction of fines that were screened out. The oversize, plus 100 mesh, was then ground in a dry rod mill. Every twenty minutes the mill was stopped and the minus 100 mesh fraction was removed. The final product was 100 percent minus 65 mesh and 73 percent minus 100 mesh. The product was blended in a rotating V blender for two hours.

The final step in the substrate preparation was to wash the finely ground product with water to remove arsenic oxide formed during grinding. This washing also removed slimes. The substrate or head assay is given in Table I.

Bacteria Preparation

A freeze dried culture of Ferrobacillus ferrooxidans was purchased from the American Type Culture Collection (ATCC). The culture was listed as Ferrobacillus ATCC #13661.

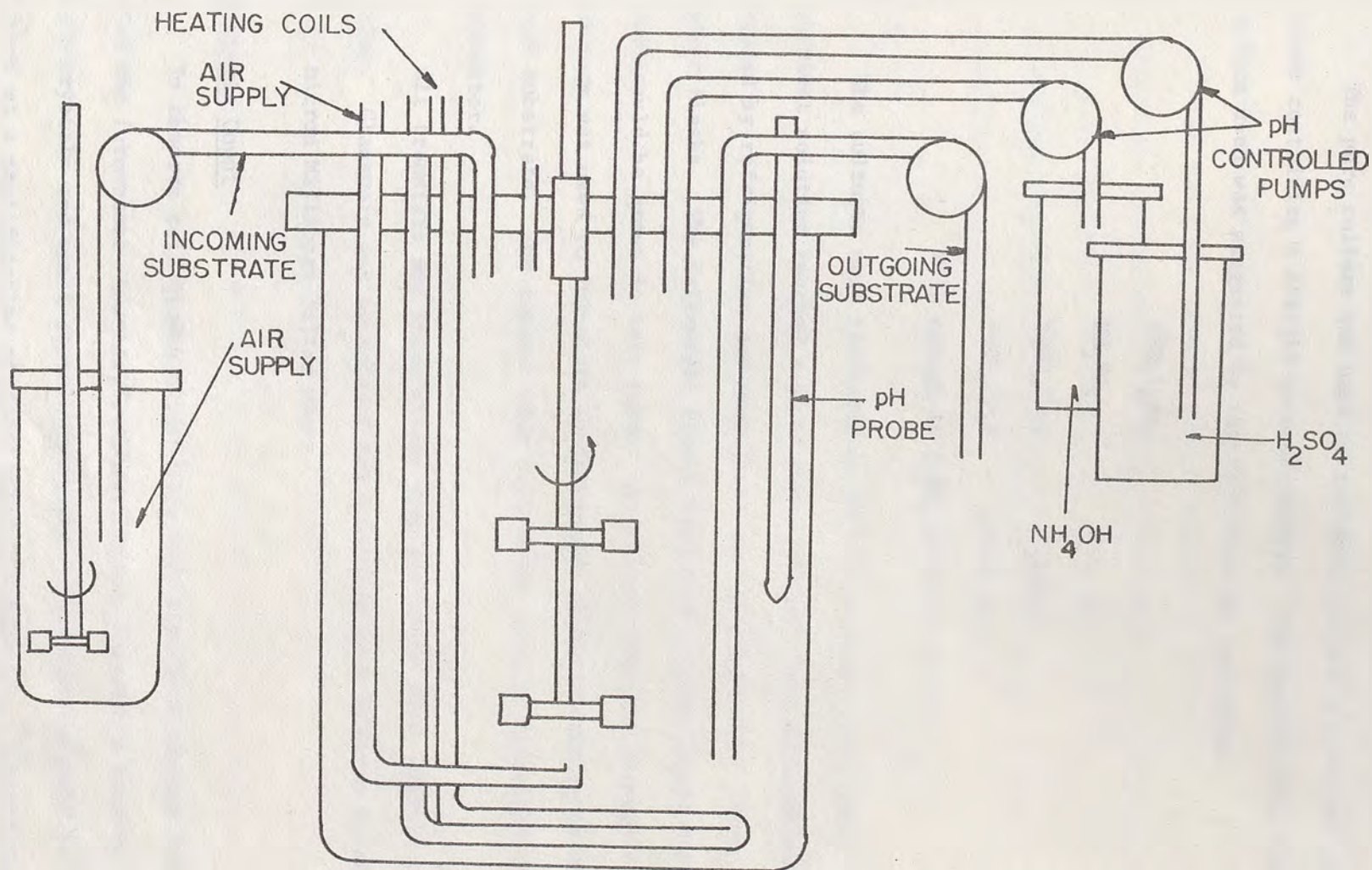


FIGURE 4. CHEMOSTAT WITH AUTOMATIC pH CONTROL AND CONTINUOUS SUBSTRATE FLOW

The pure culture was used to inoculate several sterilized test tubes containing a sterile nutrient medium. The ferrous rich nutrient medium used was suggested by the ATCC (12) and contained:

$(\text{NH}_4)_2\text{SO}_4$	0.8 g/l.
KH_2PO_4	0.4 g/l.
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.16 g/l.
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	20.00 g/l.

with enough 1N H_2SO_4 to reach pH 2.8

The cultures were incubated at 28°C on a shaker table until the nutrient solution reached a pH of 2.3. The test tube cultures were stored by refrigeration and were later used to inoculate 2 liter Erlenmeyer flasks. The Erlenmeyer flasks were used to grow larger cultures than could be grown in test tubes. An aliquot from the ferrous rich culture was used to inoculate an Erlenmeyer flask containing an arsenic rich substrate. The arsenic rich culture was used to inoculate the fermentor.

All transfers and inoculations were performed under sterile conditions. Glassware was autoclaved and solutions were filtered through 0.47 micron Millipore filter paper.

Bacteria Count

To observe cell growth slides were made from loop samples taken from the fermentor. Quantitative counting was impossible because of salt crystals and small arsenic particles. The bacteria could be observed at a magnification of 1000X using oil-emersion. Comparisons of

slides from different days clearly showed an increase in cell mass. Other researchers have reported difficulties in making quantitative cell counts (6, 7).

Care was taken to maintain sufficient ventilation around the chemostat. Arsine (AsH_3) was produced in small quantities in the fermentor. Arsine is not stable in the moist, oxygen rich air in the laboratory. It decomposes to arsenic and hydrogen or diarsenic dihydride (As_2H_2) (13).

Chemical Analysis

Arsenic Head Assay. Several methods for the volumetric analysis of arsenic were attempted. These methods give good precision and accuracy when used in pure systems. Iron was a major interference in the three methods examined. The methods were iodate, permanganate, and cerous titrations. Conductometric titrations also had an iron interference. Compleximetric titrations were tested but they were not quantitative. A gravimetric method for arsenic was selected (14). This method consisted of dissolving the rock in sodium hydroxide. The sample was diluted to a known volume with 9N HCl. The arsenic is precipitated as As_2S_3 using thioacetamide in the presence of hydroxylamine hydrochloride.

Carbonate. Samples of the rock powder were dissolved in 12N HCl on a steam bath. The samples were filtered and diluted to a known volume. An aliquot of a lanthanum carbonate solution was added. The samples were analyzed for calcium and magnesium by atomic absorption (15).

Silicates. Samples were digested in aqua regia and taken to near dryness several times with nitric acid. Added phosphoric acid and sulfuric acid were present to prevent the sample from going to complete dryness. The residue were dissolved in 6N HCl and filtered. The solids and filter paper were charred for two hours at 500°C and fired at 1100°C for one additional hour. The ash was weighed and transferred to a platinum crucible and treated with HF. The ash was reweighed and the difference was recorded as SiO₂ (14).

Arsenic and Iron in Solution. Samples were taken from the chemostat and filtered using 0.47 micron Millipore paper. The filtrate was analyzed for As and Fe by atomic absorption (15).

Gold and Silver. Samples were fire assayed by Marcel DeGuire at the Nevada Mining Analytical Laboratory at the University of Nevada.

Experimental Procedure

Three different types of experiments were conducted in the study of the biooxidation of arsenic sulfides. Shaker flask tests were conducted in Erlenmeyer flasks to determine if the biooxidation would take place. Three different batch tests or runs were conducted in the fermentor. The first run was inoculated and the other two runs were controls. The final experiment was a continuous run.

Shaker Flask Tests

Erlenmeyer flasks partly filled with the nutrient medium without ferrous sulfate were inoculated with the bacteria. Various arsenic sulfides were used as substrates. The flasks were placed on a shaker

table in a room with a constant temperature of 28°C. The pH and arsenic concentration was measured at the beginning and end of each test. Comparison of the initial and final data were used to see if the bacteria had adapted to the sulfide in the nutrient solution.

Batch Tests

Batch tests were run in the fermentor. The fermentor was filled with 3.4 liters of nutrient solution without ferrous sulfate. Twenty grams of the finely ground arsenic sulfide was added. The air flow was regulated at 3 cc/sec.; the agitation was adjusted to 200 rpm; and the temperature was brought to 30°C. The system was given time to come to these conditions before inoculating with 100 ml. of the arsenic rich culture of Ferrobacillus ferrooxidans. In the first batch run the pH was not controlled. Periodically aliquots were removed from the fermentor for analysis and the pH of the system was recorded. When samples were removed for analysis they were filtered through 0.47 micron Millipore filter paper.

A second batch run was used as a control for comparison with the inoculated batch run. In this run all conditions duplicate the earlier run except that the fermentor was not inoculated. As before periodic aliquots were removed, filtered, and pH recorded.

The third batch run duplicated the conditions of the second batch run except that sulfuric acid was added. 1N H_2SO_4 was added dropwise to duplicate the pH change in the inoculated batch test. Samples were taken for analysis and the pH was recorded.

Continuous Run

In the continuous run the environmental parameters of temperature, air flow, and agitation were the same as the batch runs. The substrate was pumped from a 2 liter stirred supply tank, using a peristaltic pump. A second peristaltic pump removed the effluent solution from the fermentor. These two pumps metered the flow at 0.85 l/day. As in the first batch run the fermentor was inoculated with 100 ml. of the arsenic rich culture. In addition pH was controlled and kept at 2.65. The effluent was sampled by filtering the stream for a known period of time. The solids were weighed to get percent solids and the filtrate was analyzed for arsenic.

RESULTS

Experimental results are shown in graphic form in Figures 5 through 12. The environmental parameters of air flow, agitation, and temperature were kept the same in all runs and are listed in the Procedure section. In the inoculated runs the fermentor was inoculated with 100 ml of the arsenic rich culture. In the continuous runs pH was held at $2.65 \pm .05$.

Batch Test

Figure 5 shows the results of a control run where the fermentor was not inoculated and acid was not added. The concentration of arsenic in solution remained the same and pH slowly increased. This test showed that the dissolution of arsenic sulfide does not take place in acid solutions with air bubbled through. The test also showed that the system tends to become more basic with time.

Figure 6 shows the effect of adding H_2SO_4 to a sterile medium in the fermentor. Note that the arsenic concentration increased with increasing H^+ . The test duplicates chemical conditions in the inoculated tests.

Figures 7, 8, and 9 show results from an inoculated batch run in the fermentor. The only source of additional H^+ was the Ferrobacillus ferrooxidans. The figures show that arsenic concentration increased as H^+ also increased. The arsenic reached a final concentration of about 40 ppm in the runs. The figures also show that the initial arsenic concentration is dependent on initial pH.

Figure 12 shows data from the inoculated batch run plotted according to Equation 21. This is a test to see if the system is autocatalytic. The straight line indicates that the system is autocatalytic. A value for k of 1.1×10^3 ml/day/mg was calculated from the slope of the line in Figure 12.

Shaker Flask Tests

In the shaker flask tests with the orpiment rich substrate, the bacteria adapted and these cultures were used to inoculate later runs. When a realgar rich substrate was inoculated, the bacteria did not change the system. The inoculated realgar substrate remained the same as the substrate in the sterile control. This is shown in Table II. In the realgar tests the arsenic concentration remained the same while pH increased.

Continuous Run

In the continuous run pH was set at 2.6. After four days there was sufficient acid production to maintain pH 2.6 even though the substrate was pumped in at pH 4. The fluid volume in the fermentor was the same as in previous tests, 3.5 liters and the flow rate, Q , was set at 0.85 l/day.

A value for C_{ao} was found by collecting samples pumped from the substrate supply tank. The value was 0.510 mg/ml. Using Equation 8, C_a values were calculated assuming no production. The experimental C_a value was determined by collecting the solids in the effluent solution. The experimental C_a increased to reach a steady state value of 0.383

mg/ml in four days. The calculated C_a reached 90 percent of C_{a0} within ten days with a value of 0.465 mg/ml. These C_a values are plotted in Figure 11. Figure 10 shows the arsenic concentration in the effluent stream of the fermentor.



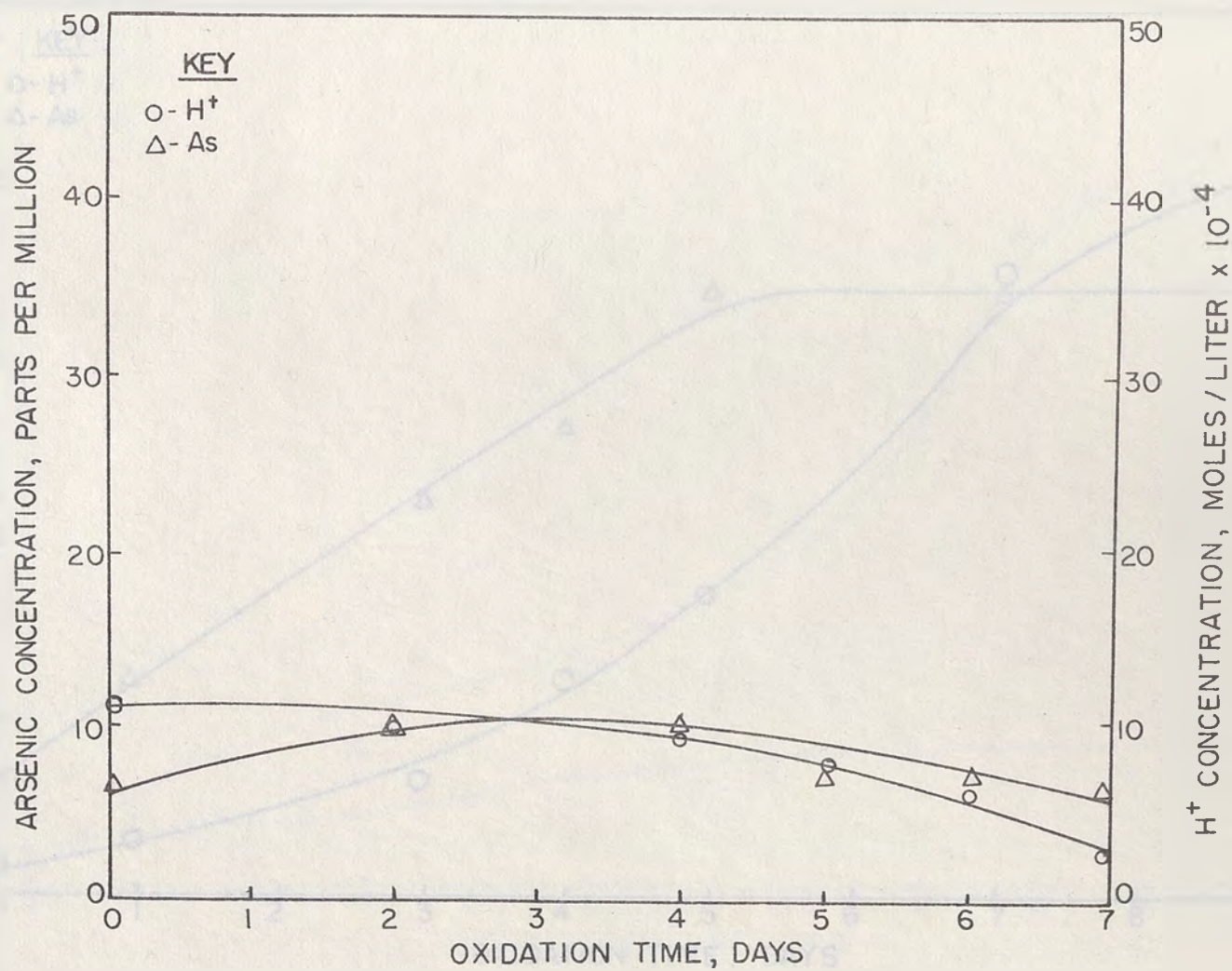


FIGURE 5. Sterile batch control with no acid added.

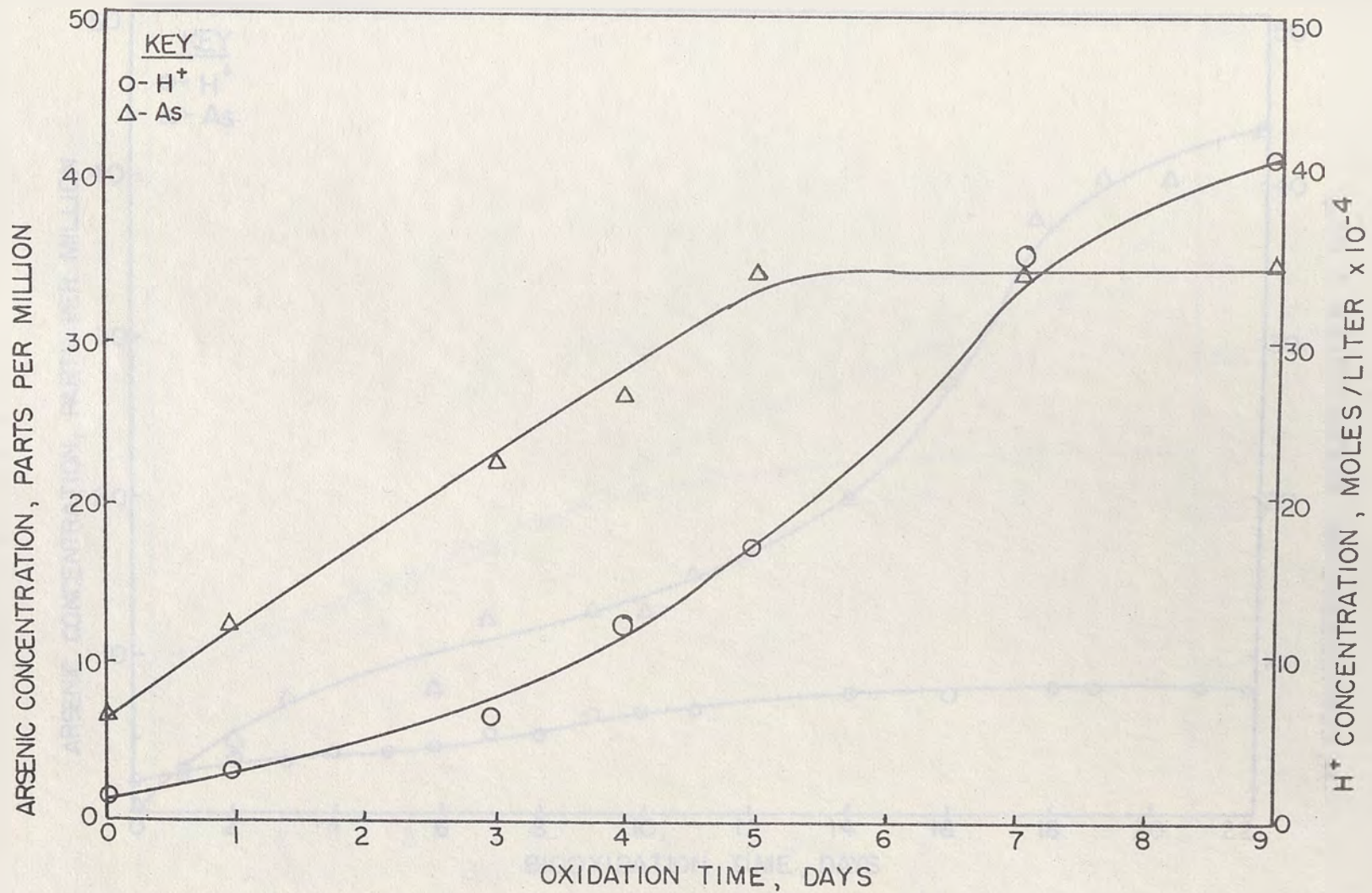


FIGURE 6. Sterile batch test with artificial acid change.

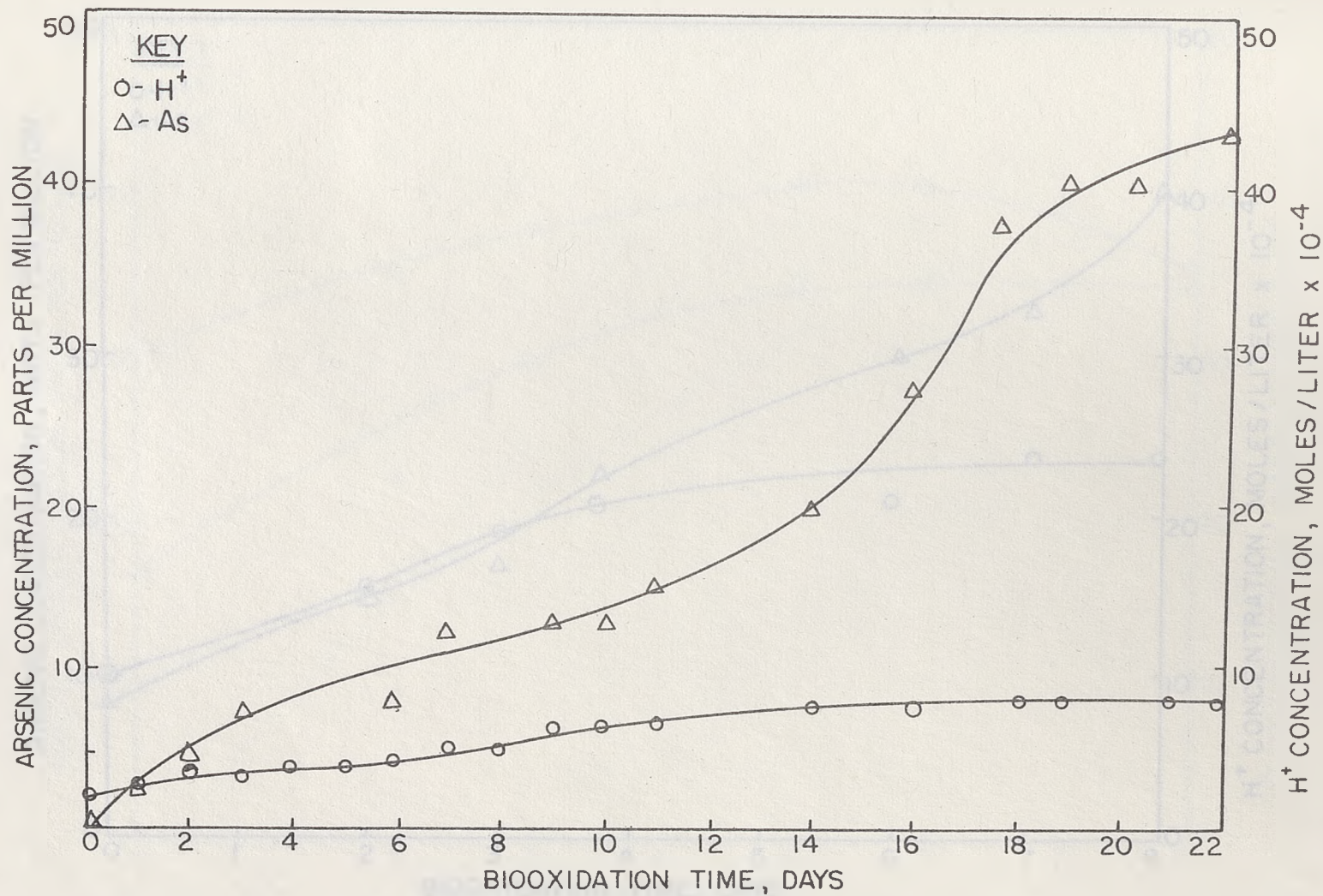


FIGURE 7. Inoculated batch run no.1. pH range 3.61 to 3.07

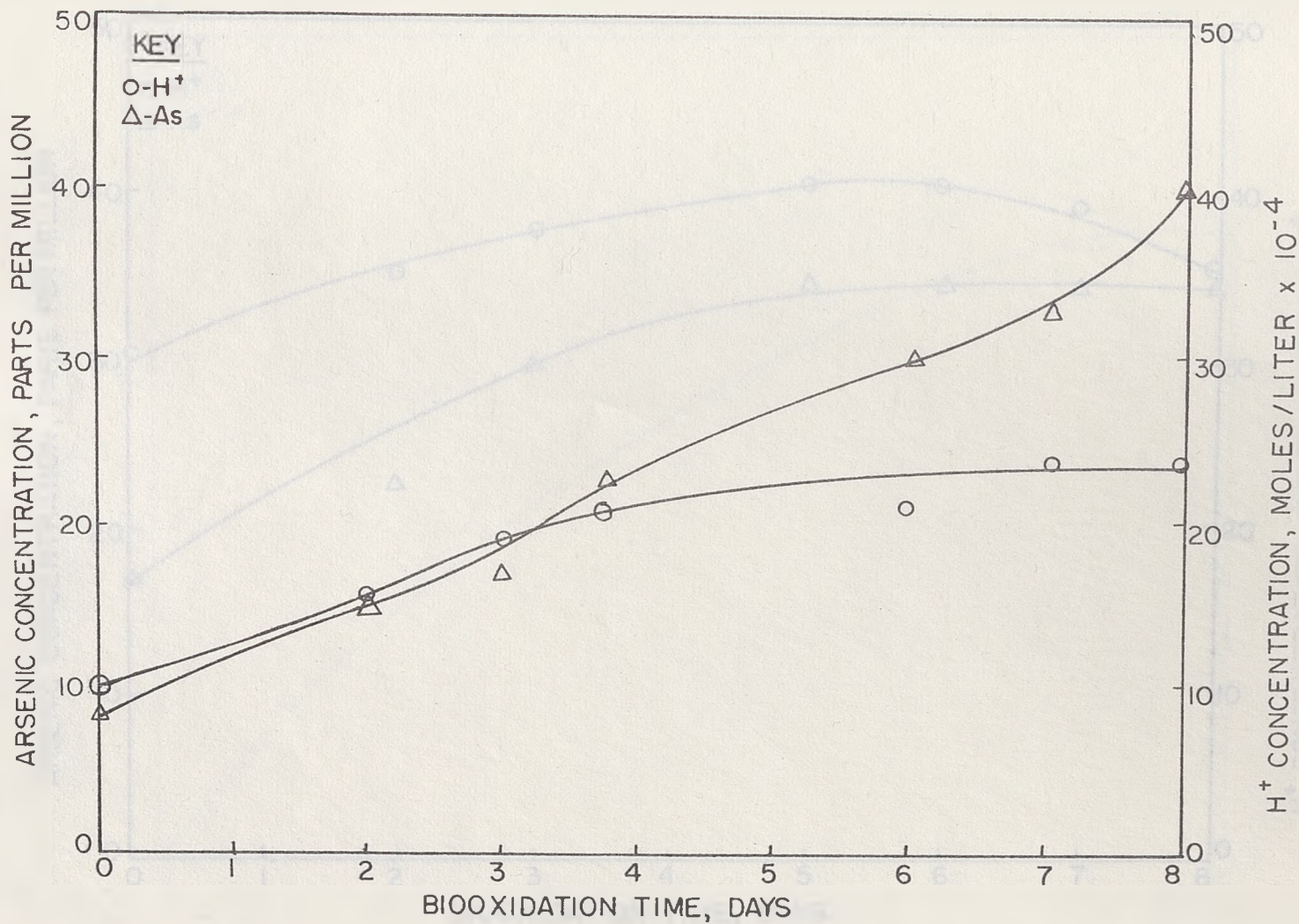


FIGURE 8. Inoculated batch run no.2. pH range 3.0 to 2.62.

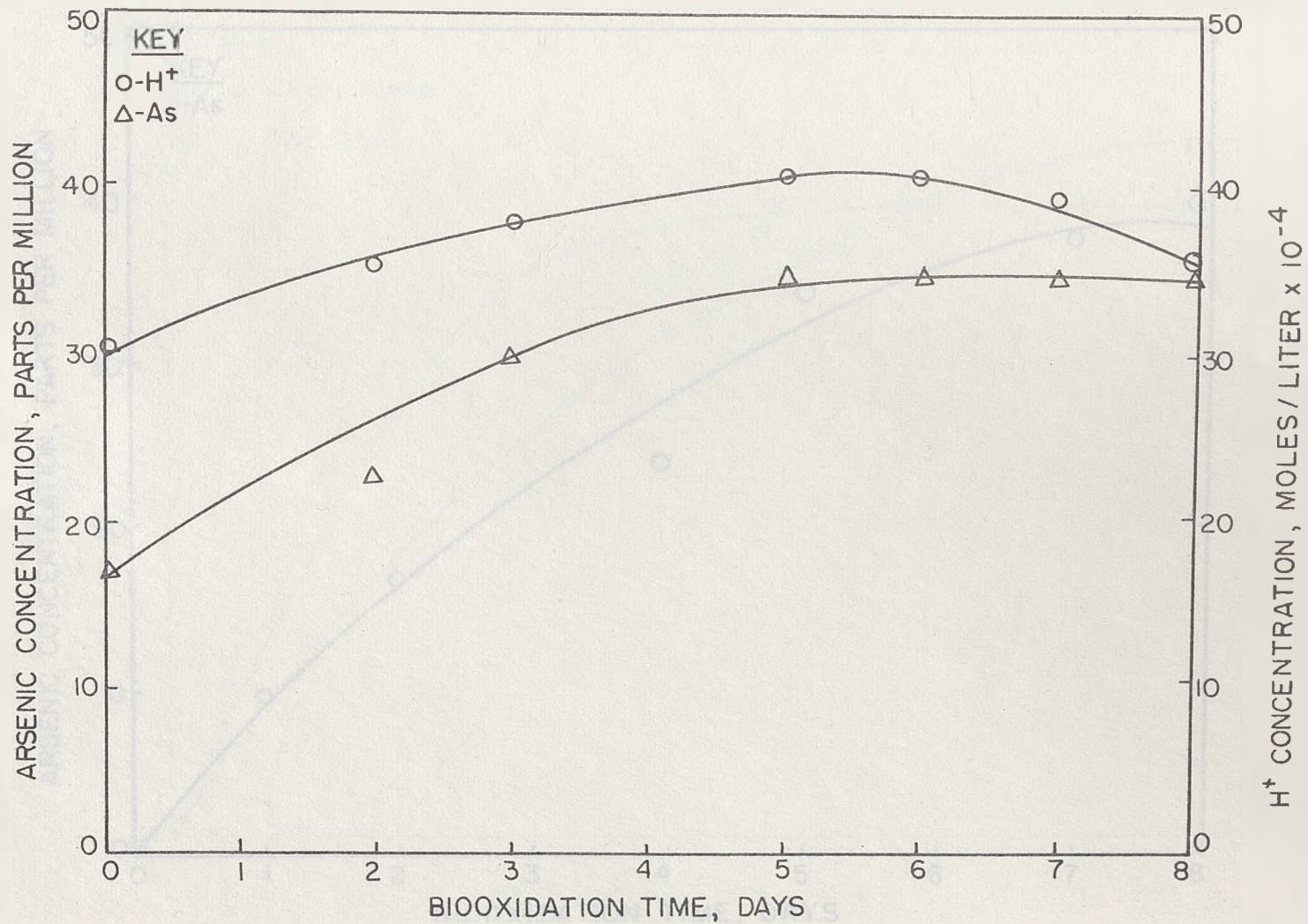


FIGURE 9. Inoculated batch run no. 3. pH range 2.52 to 2.45.

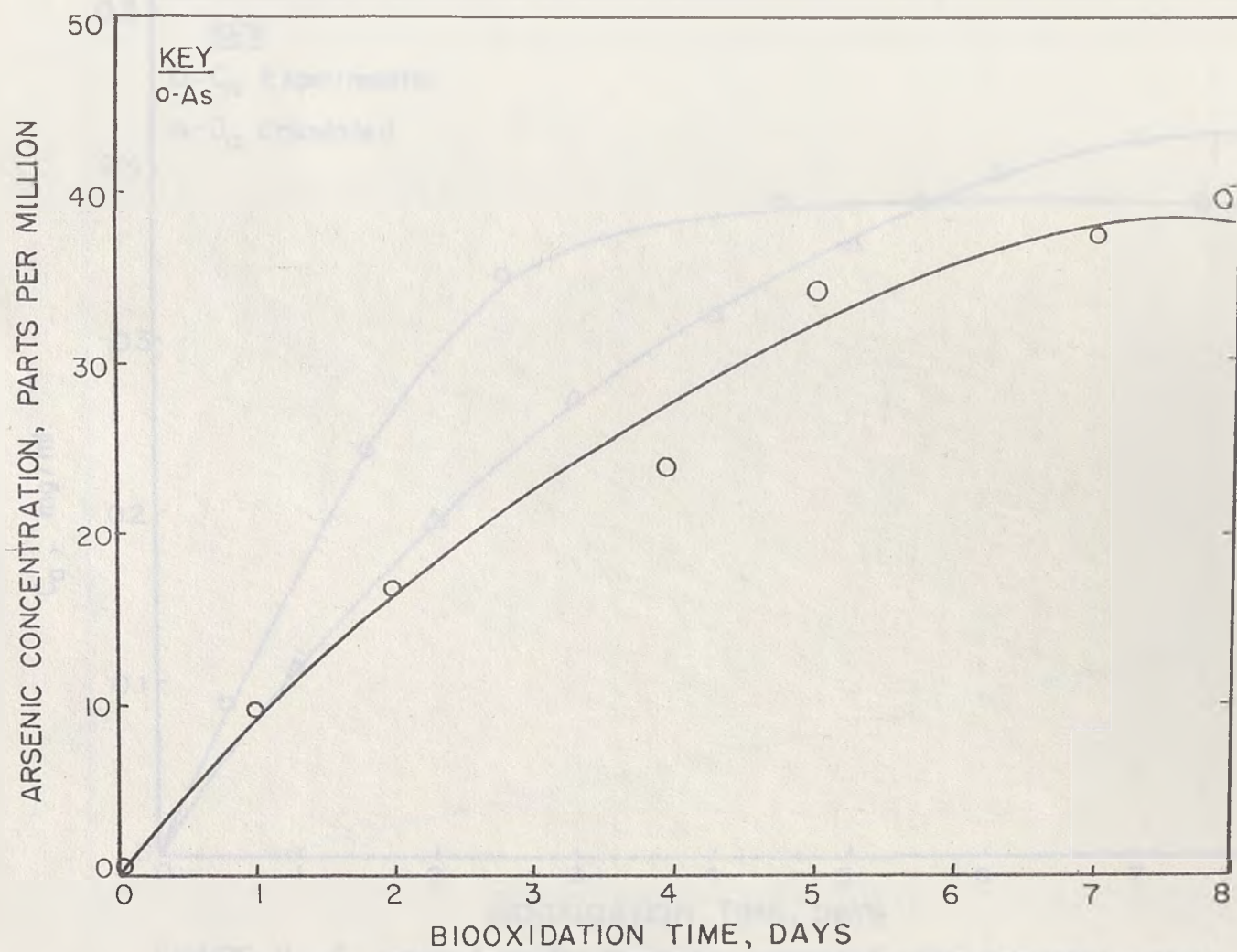


FIGURE 10. Arsenic production in a continuous fermentation run.

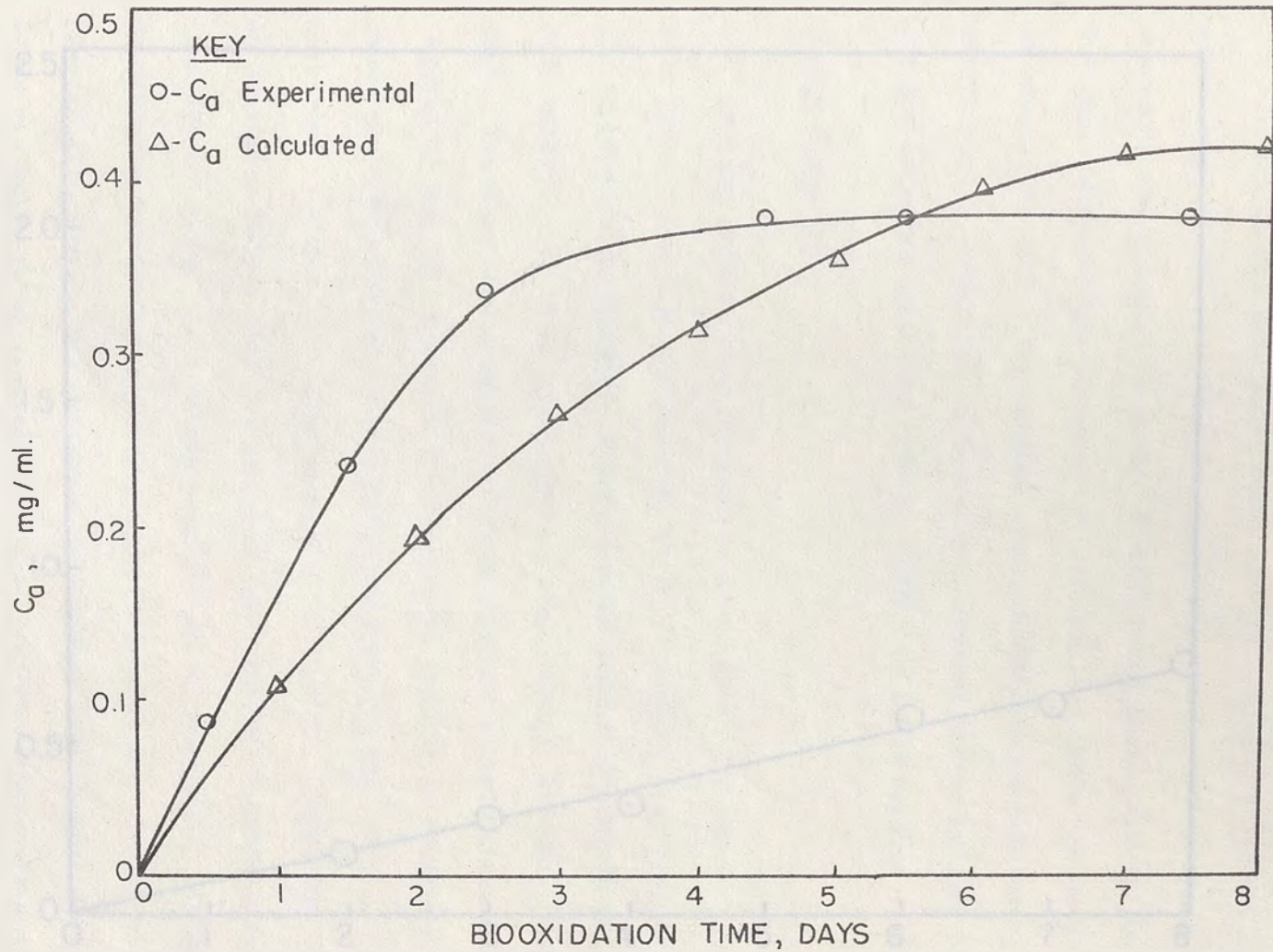


FIGURE II. C_d values from the fermentor compared with calculated values assuming no production.

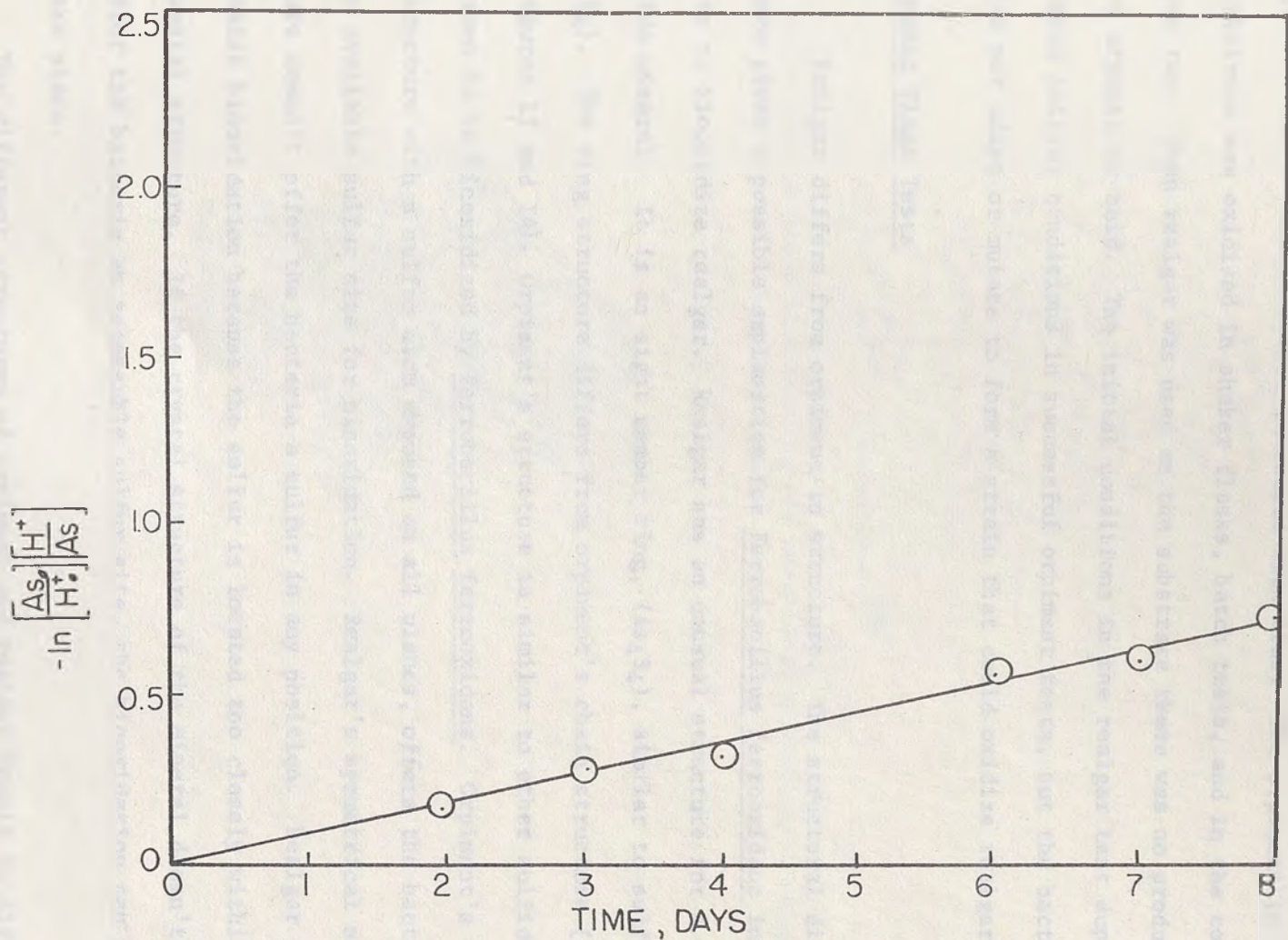


FIGURE 12. AUTOCATALYTIC TEST ON INOCULATED BATCH RUN.

DISCUSSION

In all of the inoculated tests with an orpiment substrate, the arsenic and H^+ concentration increased with time. The orpiment in the substrate was oxidized in shaker flasks, batch tests, and in the continuous run. When realgar was used as the substrate there was no production of arsenic or acid. The initial conditions in the realgar test duplicated initial conditions in successful orpiment tests, but the bacteria did not adapt or mutate to form a strain that could oxidize realgar.

Shaker Flask Tests

Realgar differs from orpiment in structure. The structural difference gives a possible explanation for Ferrobacillus ferrooxidans inability to biooxidize realgar. Realgar has an unusual structure for a sulfide mineral. It is an eight member ring, (As_4S_4), similar to sulfur (S_8). The ring structure differs from orpiment's chain structure (see Figures 13 and 14). Orpiment's structure is similar to other sulfides known to be biooxidized by Ferrobacillus ferrooxidans. Orpiment's chain structure with a sulfur atom exposed on all planes, offers the bacteria an available sulfur site for biooxidation. Realgar's symmetrical structure doesn't offer the bacteria a sulfur in any position. Realgar may resist biooxidation because the sulfur is located too closely within the crystal structure. If the crystal structure of the mineral doesn't offer the bacteria an accessible sulfur site, the biooxidation can not take place.

The different structures of orpiment and realgar result in different molecular bonds. In the realgar molecular each sulfur is bonded to

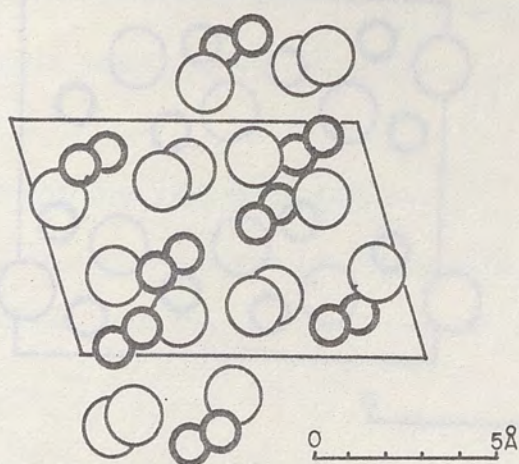


Figure 14a. A projection showing the monoclinic structure of realgar. Arsenic atoms are the larger circles.

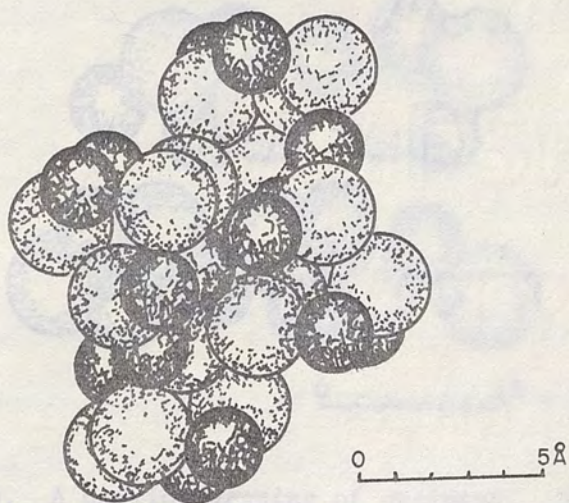


Figure 14b. A packing drawing of realgar. The sulfur atoms are the dark, smaller circles.

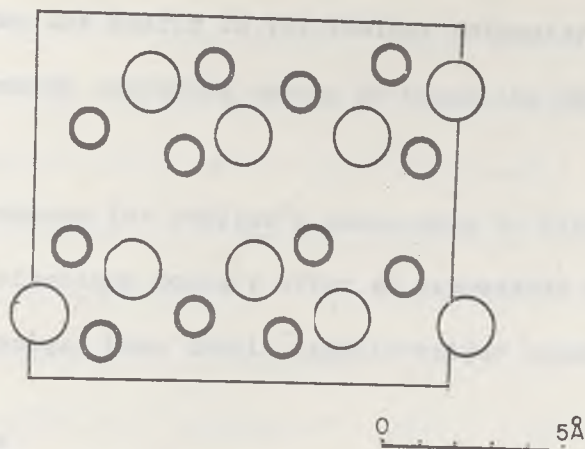


Figure 13a. A projection showing the monoclinic structure of orpiment. Arsenic atoms are the larger circles.

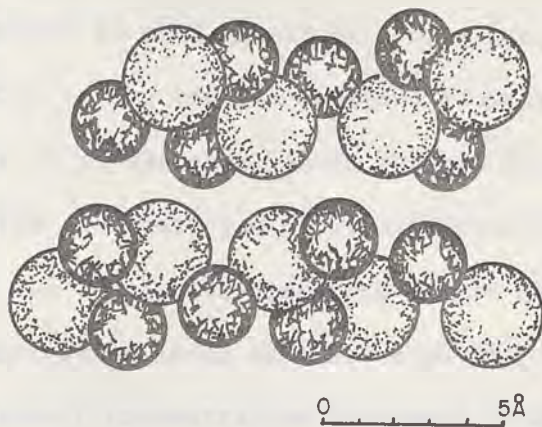


Figure 13b. A packing drawing of orpiment. The sulfur atoms are the dark, smaller circles.

two arsenic atoms. Two sulfurs in orpiment are also double bonded. The third sulfur at the end of the orpiment chain is bonded to just one arsenic. The sulfur with one arsenic-sulfur bond would be easier to biooxidize than any sulfur in the realgar molecular. The bacteria might not have enough oxidizing energy to break the double arsenic-sulfur bond.

Two reasons for realgar's resistance to biooxidation are: realgar's molecular structure doesn't offer an accessible sulfur and all sulfur atoms in realgar have double arsenic-sulfur bonds.

Batch Tests

A comparison of the inoculated batch test with the sterile control shows a significant increase in arsenic and H^+ production with the bacteria present. The sterile control demonstrates that orpiment does not oxidize unaided in acid solutions. In a period of a week the arsenic concentration in the control remained constant while the pH increased. An increase in pH was observed in systems when air was bubbled through the fermentor and there was no biooxidation producing acid. The increased pH was due to the buffering action of carbon dioxide.

The inoculated batch test had a pH drop of 3.61 to 2.39 in three runs and arsenic concentration increased to approximately 40 ppm. Comparing rates of arsenic liberation at different times in the inoculated tests gave the greatest rate at pH 2.6. This pH was taken for the continuous run.

Further comparison of the batch tests show that initial arsenic concentration increased with initial acid concentration. This lead to

an assumption that arsenic concentration was acid dependent. A second sterile control was run with sulfuric acid slowly added to the system. The test, shown in Figure 5, was an attempt to duplicate the pH conditions in an inoculated system. In the sterile, acid added batch test arsenic concentration increased. The system shows characteristics of being autocatalytic rather than first order.

The straight line through the origin in Figure 12 indicates the reaction is autocatalytic. Figure 12 shows that H^+ acts as a catalyst in the biooxidation of orpiment. The rate of biooxidation of orpiment is controlled by H^+ production. The H^+ production can be a product of the biooxidation or artificial addition as in the sterile control.

Continuous Run

By comparing the C_a values shown in Figure 11 conclusions were made about the continuous run. C_a values are a measure of the mg of orpiment per ml of substrate. There was a difference between C_a values before the fermentor reached steady-state conditions. Experimental C_a values were higher than would be calculated using Equation 8. Equation 8 assumes perfect mixing and mixing in the fermentor was not perfect because of the problem of settling solids.

As the system achieved steady-state, experimental C_a values dropped below calculated C_a values. The steady-state C_a value of 0.465 mg/ml assumed that there was no biooxidation consuming orpiment and the substrate was 100 percent orpiment. The experimental C_a reached a steady-state value of 0.383 mg/ml. The difference between these two C_a values was 0.082 mg/ml. The amount of orpiment consumed in the

fermentor can be calculated from the arsenic produced. The calculation gave 0.066 mg/ml of orpiment consumed to yield 40 ppm of arsenic. The value of 0.066 mg/ml is less than the 0.082 mg/ml found by subtracting the C_a values. Difference between these last two values can be explained by the substrate not being 100 percent orpiment and poor mixing in the fermentor.

Poor mixing in the fermentor was a primary problem in the continuous run. The biooxidation in the fermentor consumed 14.2 percent of the Getchell ore passing through.

CONCLUSIONS

1. Ferrobacillus ferrooxidans could biooxidize orpiment but not realgar.
Realgar resisted biooxidation because of its structure and bonding.
2. Optimum pH, where orpiment dissolution was the greatest was 2.7.
3. Biooxidation of orpiment was autocatalytic.
4. Orpiment was successfully used as a substrate in a continuous fermentation process with 14.2 percent of the ore consumed.

APPENDIX

TABLE I

Chemical Analysis of Getchell Ore

As (reported as As_2S_3)		95.00 %
SiO_2		8.49 %
$CaCO_3$		2.28 %
Au		0.1 oz/ton
Ag		0.2 pz/ton

TABLE II

Shaker flask test with a realgar substrate

Time, days	pH	Inoculated		Sterile	
		As ppm	pH	As ppm	
0	2.8	0*	2.8	0*	
5	2.8	0	2.8	0	
9	2.8	0	2.8	0	
16	2.85	0	2.84	0	
21	2.87	0	2.88	0	

* Arsenic not detected by AA.

TABLE III

Sterile batch test with no acid added

Time, days	pH	H ⁺ mole/l x 10 ⁻⁴	As ppm
0	2.95	11.2	10
1	--	--	--
2	3.00	10.0	10
3	--	--	--
4	3.03	9.34	10
5	3.11	7.76	10
6	3.20	6.31	10
7	3.27	2.34	10

TABLE IV

Sterile batch test with sulfuric acid added

Time, days	pH	H ⁺ mole/l x 10 ⁻⁴	As ppm
0	3.78	1.66	10
1	3.45	3.55	15
2	--	--	--
3	3.20	6.31	20
4	2.90	12.6	20 - 30
5	2.75	17.8	35
6	--	--	--
7	2.45	35.5	35
8	--	--	--
9	2.39	40.7	35
10	2.39	40.7	35

TABLE V
Inoculated Batch Run, No. 1

Time, days	pH	H ⁺ mole/l x 10 ⁻⁴	As ppm
0	3.61	2.46	0
1	3.50	3.16	5
2	3.49	3.23	10
3	3.45	3.55	10
4	3.40	3.98	--
5	3.40	3.98	--
6	3.34	4.57	10
7	3.30	5.01	10 - 15
8	--	--	--
9	3.22	6.03	10 - 15
10	3.20	6.31	10 - 15
11	3.20	6.31	15
12	--	--	--
13	--	--	--
14	3.13	7.41	20
15	--	--	--
16	3.13	7.41	25 - 30
17	--	--	--
18	3.13	7.41	35 - 40
19	3.09	8.13	40
20	--	--	--
21	3.09	8.13	40

TABLE V (cont.)
Inoculated Batch Run, No. 1

Time, days	pH	H ⁺ mole/l x 10 ⁻⁴	As ppm
22	3.09	8.13	40 - 45
23	--	--	--
24	3.07	8.15	40 - 45

TABLE VI
Inoculated Batch Run, No. 2

Time, days	pH	H^+ mole/l x 10^{-4}	As ppm
0	3.0	10.0	10
1	--	--	--
2	2.81	15.5	15
3	2.72	19.1	20
4	2.68	20.9	20
5	--	--	--
6	2.68	20.9	30
7	2.62	24.0	30 - 35
8	2.62	24.0	40

TABLE VII

Inoculated Batch Test No. 3

Time, days	pH	H^+ mole/l x 10^{-4}	As ppm
0	2.52	30.2	20
1	--	--	--
2	2.45	35.5	25 - 30
3	2.42	38.0	30
4	--	--	--
5	2.39	40.7	35
6	2.39	40.7	35
7	2.40	39.8	35
8	2.45	35.5	35

TABLE VIII
Continuous Run

Time, days	C_a mg/ml	C_a^* mg/ml	As ppm	Fe ppm
0		0.0	0	190
1	0.0876	0.108	10	164
2	0.2.40	0.195	10 - 15	123
3	0.3.45	0.264	--	---
4	--	0.317	20	82
5	0.3.83	0.344	35	54
6	0.3.83	0.391	--	---
7	--	0.417	35 - 40	21
8	0.3.83	0.437	40	21

$$* C_a = C_{ao} - C_{ao} e^{-(Q/N)t}$$

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