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INVESTIGATION OF MECHANICS OF MINE ACID FORMATION
VOLUME 1

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FOREWORD

This investigation was performed under contract with the U.S. Department of Energy, Contract No. ET-78-C-01-8977. The contract was administered under the technical direction of the Carbondale Mining Technology Center, Environment Branch, with Mr. Robert J. Wenzel as the Technical Project Officer and Mr. Eugene F. Callaghan of the Office of Procurement Operations, Washington, D.C., as the Contracting Officer.

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In view of the analytical nature of the work performed, no inventions were made under this program.

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1. ABSTRACT

The objective of the contract was to determine, by the combination of laboratory experiments and mine samplings, the kinetics and mechanisms of the various reactions that produce mine acid drainage.

To achieve this goal, primary investigations were performed utilizing the minerals deemed responsible for acid formation, namely pyrite and marcasite, in pure form, free from coal. The effects of temperature, concentration, surface area, media nature, oxygen, additional ion presence, and bacterial action with respect to dissolution rates were measured. Two bacteria were included in this study, T. ferrooxidans and T. thiooxidans, both purchased and freshly mine isolated.

The mine samplings were performed to determine: a) the type and quantity of bacteria present, b) the effect of weathering upon coals' propensity to produce acid and other drainage, c) the effect of presence and concentration of minerals upon ion liberation, d) the effect of the nature of the mine water upon ion liberation, e) the effect of the different kinds of bacteria upon the above process. The data thus generated was correlated with the pure mineral studies. Based on these, mechanisms were developed and clarified and the influence of specific factors upon these was assessed.

To summarize, it was established that both pyrite and marcasite, regardless of crystal nature, provided sufficient surface area is exposed, will produce hydrogen, sulfate, and iron ions. This process is greatly accelerated in the case of pyrite and marcasite by the presence of T. ferrooxidans and ferric ions. T. thiooxidans was found to accelerate marcasite solubilization and the dissolution of iron disulfide present in coal, but had no effect on museum grade pyrite. The behavior of the field samples in regard to acid formation and soluble iron production was found to vary with

their location within the mine, e.g. the pyrite in the coal from a freshly cut face was solubilized at a different rate than that present in coal from a long ago mined sump area. These rate differences were explainable by taking into account the content of pyrite, the degree of weathering, and the quantities of calcium carbonate present. The utilization of the latter findings in mine acid abatement has been delineated.

2. INTRODUCTION

Acid mine drainage pollution is a major environmental constraint on exploitation of coal resources[1,2]. At least 75 percent of the mine drainage pollution is produced in the traditional coal mining states of West Virginia, Pennsylvania, Ohio, Kentucky, and Maryland. Mine drainage pollution comes from both abandoned mines and active mines. Production of acidic drainage can continue and actually increase, in particular from underground mines, long after active mining has ceased. Prevention of acid drainage, however, is difficult, if not impossible, in the absence of detailed knowledge in regard to the processes and their mechanisms and rates which produce this type of drainage.

Coal mine acid drainage results from the "transformation" of inorganic sulfides and polysulfides, present in virtually all coal deposits, into oxidized products which form strongly acidic aqueous solutions. The details of this overall "transformation", e.g., the mechanisms and the rates of the reaction steps involved, have so far not been elucidated, based on the information available in the literature, despite the existence of numerous reports and publications dealing with isolated, readily identifiable and controllable processes under specific conditions. As a matter of fact, the problem of acid mine drainage formation in its entirety has not been addressed at all when compared to the efforts made to neutralize its adverse effects.

Over 40 years ago, the basic reactions,

$$2\ FeS_2 + 7\ O_2 + 2\ H_2O \longrightarrow 2\ FeSO_4 + 2\ H_2SO_4$$

$$2\ FeSO_4 + H_2SO_4 + 1/2\ O_2 \longrightarrow Fe_2(SO_4)_3 + H_2O$$

$$Fe_2(SO_4)_3 + 6\ H_2O \longrightarrow 2\ Fe(OH)_3 + 3\ H_2SO_4$$

$$2\ FeS_2 + 7.5\ O_2 + 7\ H_2O \longrightarrow 2\ Fe(OH)_3 + 4\ H_2SO_4$$

were postulated by Carpenter and Herndon[3] and later expanded by Weiss[4]. The possibility of bacterial involvement in iron precipitation was mentioned as early as 1919[5]; however, it was only in the late 1940's[6] that bacteria became definitely identified as being present in mine waters and affecting FeS_2 transformation. Since that time, a tremendous amount of work has been carried out on the various aspects of mine acid production as attested by the publications cited in the various reviews[2,7-9].

There are several reasons why a comprehensive and consistent description of the mechanisms and rates of all processes leading from inorganic sulfides to acid drainage has not been developed. The number of interdependent variables affecting acid formation is huge - to name just a few: the type and mix of minerals present, their degree of dispersion, the large number of sulfur compounds most likely involved in the overall process, the permeability of the coal bed, the nature and activity of the overburden, the pH and hardness of incoming water, the type and quantity of other water borne or leached species including catalysts or nutrients, the variety of microorganisms not only capable of survival, but able to adapt and even change via mutation. In establishing the kinetics and mechanisms of the acid forming reactions, all these variables need to be considered in a systematic manner utilizing the findings from other investigations whenever possible, and generating reliable experimental data to supplement, prove or disprove the existing theories. This has not been done to date. Furthermore, some of the reviews and recommendations are based entirely upon surveys of literature without any "experimental" understanding of the subject involved, consequently leading to erroneous deductions.

It was the aim of the present program to elucidate the mechanisms of acid production in the underground mine environment based upon data obtained by actual experimental investigations supplemented and confirmed by the findings reported in the literature. Thus the program required a thorough familiarity with past efforts in addition to an experimental investigative approach designed to generate the data needed for basic or general mechanism elucidations inclusive of accommodating the variations inherent in the field conditions and capable of predicting their effects upon the overall mine acid formation process. To arrive at that goal, experimental studies of "pure" minerals known to be responsible for mine acid production were performed in the laboratory. As a second prong of the attack, investigations of carefully selected samples from mines were carried out, utilizing in part the actual mine environment as a long term testing facility.

3. RESULTS AND DISCUSSION

Iron disulfide is the species responsible for the various effects described as coal mine acid drainage. Iron disulfide exists in two major forms: pyrite, a cubic crystalline arrangement, and marcasite, a rhombic structure. Based on recent crystallographic examinations, pyrite is the most prevalent form present in U.S. coals[11-15]. This is not the case with, e.g., Australian coals wherein marcasite is the predominant form of iron disulfide[7]. In U.S. coals, certain investigators report to have found marcasite[11,12,16], others claim that none is present[13]. A portion of the pyrite present in coal is in the form of conglomerates of small spherical crystallites denoted as framboidal pyrite. According to Caruccio[17], it is the quantity of framboidal pyrite, not the total pyrite present in coal, which determines to a large extent the mine acid production from a given formation. The pyrite in some mines is evenly distributed, in others it is concentrated in e.g. upper layers. The other minerals present in coal are calcite ($CaCO_3$, trigonal), minor amounts of gypsite ($CaSO_4 \cdot 2\ H_2O$, monoclinic), quartz (SiO_2), and at least in Iowa coals, trace quantities of sphalerite (ZnS, cubic) and galena (PbS_2, cubic)[11].

The nature and quantity of minerals occurring in coal is of utmost importance in predicting the acid production potential of a given bed both during actual mining and after a long time lapse, i.e. after abandonment. Of course, the availability of water and its mineral content does affect to a large extent the acid production of a given mine; consequently, hydrology considerations must enter into these assessments. The various aspects of all the minerals' interactions will become more clear in the latter sections of this report, specifically in the discussion of the actual experimental results.

Since pyrite and, to a degree, marcasite are the "precursors" of mine acid drainage, the mechanism and kinetics of pure or bulk iron disulfide "dissolution" and the effect of various factors such as surface area, concentration, pH, bacterial action, etc. upon this reaction need to be known to understand the equivalent processes in coal and to devise control procedures. Consequently, the discussion was separated into sections dealing with these specific aspects under the headings: a) Pyrite Studies, b) Marcasite Studies, and c) Coal Investigations. The last section includes the description of the sites sampled in addition to investigations of the mine waters and solid samples obtained.

3.1 Pyrite Studies

3.1.1 Inorganic Reactions

Museum grade pyrite represents the cubic form of pyrite in its most unreactive or pure form as compared to the pyrite present in coal. Conflicting claims exist in the literature regarding the reactivity of this mineral. Caruccio[18] for example, reported that "stable" or museum grade pyrite pulverized to 2-5μ particles will not oxidize. On the other hand, Baas Becking, et al[19] found pyrite to produce a pH of 3.1 within 24 hr when suspended in water. It is clear from the data given in Table I that suspension of finely ground pyrite in water lowers the pH down to 2.8 in less than 504 hr; in 48 hr the pH reached was 3.3-3.2. The lower value found by Baas Becking, et al[19] could be due to oxidation of pyrite during grinding[20]. The grinding under this program (see experimental section) was performed in dry nitrogen. The results listed in Table I prove the reproducibility of the tests. The quantity of iron and acid liberated was found to be directly proportional to the quantity of pyrite present as evident from the compilation given in Table II and Figures 1 and 2. These results can be in turn interpreted with respect to the available surface area of pyrite, since pyrite is suspended, not dissolved in water. Accordingly, using the same quantity of different particle size pyrite should produce a higher solubilization rate for the smaller particle size sample. The data given in Table III and Figure 3 fully support this stipulation.

A number of investigators[20-22] claim that the rate of ferrous iron oxidation is increased by the presence of cupric ion. Assuming that the ferric ion attack upon pyrite constitutes one of the potential mechanisms of pyrite solubilization[23], one would expect the presence of cupric ion to accelerate this process. Comparison of tests carried out in the presence of cupric ion (at concentrations corresponding to those of the soluble iron) with those wherein no cupric ion was added fails to show this effect (Table III, Figure 4). Thus, it can be concluded that the presence of cupric ion does not catalyze pyrite solubilization.

It is an accepted fact that no dissolution of pyrite occurs in the absence of oxygen. Based on this premise and the studies performed at Ultrasystems, it is believed that the solubility value of 0.49 mg/100 ml given in the Handbook of Chemistry and Physics for pyrite in water is meaningless, since it most likely corresponds to the surface oxidation prior to immersion. For instance, a 500 mg portion of pyrite, $< 38\mu$ diameter (ground in an inert atmosphere enclosure), when washed quickly in air with 100 ml of deionized water, liberated 1.2 mg of soluble iron per 100 ml of water. An equivalent sample exposed under nitrogen to de-aerated water for 24 hr produced 0.8 mg of soluble iron per 100 ml of water. The latter number is close to the 0.49 mg/100 ml solubility value given in the Handbook. As noted in the previous paragraphs, the quantity of iron solubilized depends on pyrite surface area; thus, not knowing the particle size of the sample on which the dubious solubility figure is based and since apparently no attempt was made to recover unchanged pyrite from the aqueous solution, no validity can be attached to the solubility value quoted.

Irrespective of the mechanism of pyrite solubilization, i.e. whether the oxidation proceeds via direct oxygen attack upon pyrite or whether oxygen first oxidizes the ferrous ion and this action is then followed by the attack of the ferric ion upon pyrite, the stochiometry of the equation,

$$FeS_2 + 3.5\ O_2 + H_2O \longrightarrow FeSO_4 + H_2SO_4 \qquad [1]$$

must be followed provided the only products formed are ferrous sulfate and sulfuric acid. To confirm the validity of this equation, to prove that at lower temperatures the same mechanism is operative but the process occurs at a slower rate, and to show that the rate of oxygen uptake is directly proportional to the quantity of pyrite present, the three test series presented in Tables IV-VI were carried out using a Gilson IGR-14 respirometer. In a typical experiment, a denoted quantity of pyrite was suspended in 5 ml of water in each of a series of 14 flasks. Using a duplicate sample, after a certain interval of time, ~0.5 hr, the pH, soluble iron, and sulfate ion were determined (0 hr values). In the meantime, the remaining flasks were lowered into the water bath of the respirometer and allowed to equilibrate both with respect to pressure and temperature. After equilibration, ~0.5 hr, the measurement of oxygen uptake was initiated. After specific periods of exposure, successive duplicate samples were withdrawn and analyzed as above. Only in Table IV are the sulfate values included; these were omitted from the other experiments due to the initial difficulties in obtaining reproducible sulfate analyses. These aspects are discussed fully in the experimental section. It is evident from these compilations that in all three test series, the ratio of iron solubilized to oxygen consumed is fairly close to the theoretically required ratio of 1:3.5. Actually, the average values for Table IV through VI are 3.4, 3.1, and 3.1, respectively; whether the ~10% discrepancy from theory is due to an inherent experimental error or whether the oxidation of all the sulfur atoms liberated from pyrite does not proceed fully to the sulfate state, has not been unequivocally established. On the other hand, it should be noted that latter studies conducted in this laboratory (see e.g. Table XIX) show consistently a $SO_4^{=} : Fe^{t}$ ratio of ~2±0.2, which would tend to indicate that the low oxygen or high iron values found in Respirometer Series 4R and 5R are due to experimental errors. Comparing the results of tests 4R and 5R, it is obvious that lowering the temperature from 25°C to 15°C decreases the rate of pyrite oxidation to roughly 50% in agreement with the common rule that for each 10°C temperature decrease the reaction rate is approximately halved. The linearity in the rate of oxygen uptake and soluble iron production is shown by the graphical presentation given in Figure 5.

Examining closely the rate of pyrite solubilization, it is apparent that the initial rate, at least under the experimental conditions employed, is significantly higher than the rate established after several days of exposure to water and air (see Figure 6). According to Singer and Stumm[24], the rates of ferrous ion oxidation above 3.5 pH are strongly pH dependent; assuming ferric ion production to be the rate determining step in pyrite degradation, the initial high rates observed could be attributed to the pH factor. However, this is not the case since pyrite recovered from the experiments listed in Table I (after washing) failed to exhibit the initial high rate of dissolution as evident from Table VII data. It is believed that the originally observed behavior is due to surface irregularities of the freshly ground pyrite, resulting in a high surface area and in particular points of high surface energy. The tumbling associated with sample shaking acts like pebble polishing giving a smooth surface. Once this is achieved, the points of high surface energy have been eliminated and the surface area and consequently the rate stay constant. To further explore the effect of initial pH, the test series summarized in Table VIII was conducted. Based on these experiments, the initial pH does not affect significantly the rate of pyrite solubilization. Of course, it must be stressed that the neutral carbonate solution was not buffered, thus, once pyrite solubilization products were liberated, the sodium carbonate was transformed into neutral sodium sulfate.

In the absence of air or oxygen, pyrite dissolution in the presence of dissolved trivalent iron occurs supposedly according to:

$$FeS_2 + Fe_2(SO_4)_3 \longrightarrow 3\ FeSO_4 + 2\ S^o \qquad [2]$$

i.e. for every mole of Fe^{+3} introduced, 1.5 mole of Fe^{+2} is produced, as proven by Silverman[23]. The investigations of Sato[25], based on Eh considerations, indicate that the initial dissolution of pyrite affords ferrous ion and diatomic non-crystalline sulfur, i.e.,

$$FeS_2 \longrightarrow Fe^{+2} + S_2 + 2\ e \qquad [3]$$

On the other hand, Garrels and Thompson[26] found that at pH 0 to 2, the oxidation of pyrite by ferric sulfate, in the absence of oxygen, was in an overall agreement with:

$$FeS_2 + 7\ Fe_2(SO_4)_3 + 8\ H_2O \longrightarrow 15\ FeSO_4 + 8\ H_2SO_4 \qquad [4]$$

To verify Silverman's[23] postulate and to prove that this process takes place also in the presence of air, i.e. that Fe^{+3} is involved only in the oxidation of the disulfide ion to zero valent sulfur, i.e.,

$$S_2^{=} + 2\ Fe^{+3} \longrightarrow S_2^{o} + 2\ Fe^{+2} \qquad [5]$$

and that the subsequent oxidation of the "nascent" diatomic sulfur is due to direct oxygen action, the tests presented in Table IX were performed. These results support Silverman's conclusions as shown (at 191 hr) by the 0.008 mg/ml and 0.044 mg/ml increase in total iron produced in Runs H-3, H-4 and Runs H-5, H-6, respectively, in excess of the iron added (as compared to the control experiments, Runs H-1, H-2). In both instances, the increase corresponds to 50% of the Fe^{+3} added in agreement with equation [2].

It is apparent from the plots given in Figures 7 and 8 that after the immediate increase of soluble iron production following Fe^{+3} addition, the dissolution rate, as measured by Fe^{t}, drops down to that exhibited by "controls". The dissolution rate drop in agreement with theory is accompanied by a drop in the concentration of Fe^{+3} ion.

Tests listed in Table X were performed in 1/10 m9K utilizing significantly higher ferric ion to pyrite ratio to approximate the ferric ion buildup in bacteria-containing experiments to be discussed later. The rates of Fe^t production, at least up to 102 hr, were found to be comparable to those attained in bacteria-assisted reactions. However, the extent of pyrite solubilization attributed to the action of ferric ion does not agree with the mechanism postulated by Silverman[23]. This is more evident from the data presented in Table XI. The Fe^{+2} liberated varied between 46 and 29% of the value calculated according to the equation [2]. Yet, even in the worst case, the iron solubilized is higher by a factor of two than predicted by the equation [4].

It would thus appear that at these high ferric ion concentrations, the reduction of Fe^{+3} to Fe^{+2} is not only due to $S^=$ oxidation to S^o, but possibly also to oxidation of other sulfur species, thereby lowering the rate of pyrite dissolution. It should be stressed that Silverman[23] conducted his tests with rigid exclusion of air, thus it is possible that ferric ion does not oxidize zero valent sulfur, but that it will oxidize sulfur in the higher intermediate oxidation states.

3.1.2 Pyrite-Bacterial Reactions

Prior to any bacterial studies, the sterilization procedure had to be evaluated to assure that such a treatment would not affect the reactivity of the pyrite. The five techniques investigated were: a) steam, b) dry heat, c) ethylene oxide atmosphere, d) vacuum, and e) addition of 50μg/ml of chloramphenicol into the aqueous pyrite suspension. The effect of the steam treatment on solubilization of pyrite is shown by the data given in Table XII and by the graphical presentation in Figure 9. It should be noted that the initial concentration of Fe^t found corresponds to that produced by untreated pyrite after ~ 240 hr (see Table I, Series D), yet the subsequent rate of dissolution was significantly lower than that observed for the untreated pyrite. Consequently, it would seem that the steam sterilization does alter the pyrite nature sufficiently to make any comparison studies between "virgin" and steam sterilized pyrite unacceptable. The effect of the other four treatments upon pyrite's iron production and pH change are summarized in Figures 10 and 11. Based on the more sensitive iron data, the dry heat appeared to affect least, if any, the chemical behavior of the pyrite.

To test the effectiveness of a given sterilization procedure, a weighed sample of pyrite together with a measured quantity of T. ferrooxidans suspension was subjected to a specific sterilization method.

In the case of chloramphenicol, the pyrite together with the cell suspension was incubated for 2 hr; subsequently, the pyrite-cell mixture was centrifuged and washed with deionized water to remove the chloramphenicol.

In each instance, following approximately three weeks of incubation at 28°C, the inoculated 9K medium[27] was examined microscopically to determine growth of the bacteria. The results of these experiments are summarized in Table XIII.

TABLE XIII

EFFECTIVENESS OF STERILIZATION PROCEDURES
USING T. FERROOXIDANS

Procedures	T. ferrooxidans growth[a]
dry heat (160°C, 2 hr)	-
ethylene oxide (ambient, 2 hr, 315 mm Hg)	-
vacuum (ambient, 2 hr)	+
chloramphenicol (ambient, 2 hr)	+
uninoculated control	+

a) + = growth and - = no growth after 3 weeks incubation at 28°C.

Based on the results discussed above, the two procedures found effective in bacteria sterilization, without altering the chemical behavior of pyrite, are ethylene oxide and dry heat treatments.

Media approximating the composition of "mine waters"[27-29] were found to be optimum for propagation of <u>Thiobacillus ferrooxidans</u> and <u>Thiobacillus thiooxidans</u>. However, it has to be understood that, depending on the nature of the overburden and the quantity of rain water percolating through it, the type and quantity of salts present in solution washing over pyrite would be expected to vary widely. A bad case for survival of bacteria and their ability to affect pyrite degradation would be a medium free of salts, i.e. deionized water, possible in a heavy rain water ingress situation. To verify the above postulate, the tests summarized in Table XIV were performed utilizing two different concentrations of the iron oxidizing bacteria, <u>T. ferrooxidans</u>.

The higher bacterial concentration, which corresponded approximately to 1.8×10^7 bacteria per ml of solution[27], definitely accelerated pyrite dissolution as measured by the increase in soluble iron concentration and the pH drop. The effect of bacterial action is shown in particular by the high concentration of ferric ion. At the lower bacterial concentration, no accelerated solubilization, as compared to the controls, was observed up to 120 hr. It is quite possible that given sufficient time definite rate increases could have developed. These experiments definitely prove that <u>T. ferrooxidans</u>, under adverse conditions, will survive and even prosper providing the initial concentration is sufficiently high to ensure survival.

The majority of tests involving bacteria were performed utilizing either 1/10 9K[27] or 1/10 m9K media. The compositions of these media are given in Table XV. The salt content of 1/10 9K was 1/10 that usually employed by other investigators in the field. The reason for selecting the lower concentration was to minimize the effect of ion interference in subsequent analysis. The substitution of 1/10 m9K (wherein all the sulfates were replaced by equimolar amounts of the corresponding chlorides) for 1/10 9K was done to permit sulfate analysis in the pyrite tests without any interference from the sulfate contained in the nutrient solution. Based on the data given in Table XVI, it is apparent that insofar as iron oxidation efficiency is concerned, there is virtually no difference between the 1/10 9K and 1/10 m9K media. To evaluate bacterial activity with respect to iron oxidation and the viability, as determined by the rate increase of Fe^{+3} production with time, the experiments listed in Table XVII were performed. It should be noted that due to the partial precipitation of trivalent iron, the rate employed in this assessment was that of the consumption of divalent iron. Apparently, the ATCC-strain starts acting after an incubation period of ~ 69 hr. The increase in the rate between 93 and 117 hr points to an ~ 13 hr generation (cell division) period. The values quoted by Silverman and Lundgren[27] for T. ferrooxidans are 5.3-9.6 hr, whereas the values given by Tuovinen and Kelly[30] are of the order of 6.5-15 hr. Based on the data obtained for the two concentrations of bacteria, the initial concentration does not seem to affect the rate of iron oxidation which is inconsistent since one would expect significant rate differences after the incubation period.

The effect of T. ferrooxidans upon pyrite solubilization was much more pronounced in 1/10 9K, Table XVIII, than in deionized water (see Figure 12). After the incubation period of 24 hr, the rate of iron production rose over the subsequent period of 144 hr to 5.9×10^{-3} mg/ml/hr as compared to 1×10^{-5} exhibited by the bacteria-free control, a factor of ~600. Conducting an equivalent test in 1/10 m9K, see Table XIX, resulted in an attainment of a comparable rate after ~ 192 hr of exposure (5.8×10^{-3} mg/ml/hr); the maximum rate of 6.5×10^{-3} mg/ml/hr was reached after 216 hr. It should be noted that the incubation period in 1/10 m9K was between 71-143 hr as compared with a 48 hr time interval in 1/10 9K medium. The observed delay is most likely due to the initial absence of the sulfate ion believed to be necessary for the functioning of the bacterial oxidation system[31]. The leveling in the rate of oxidation observed in the CC-series and in the RR-series, see Tables XIX and XX, after ~ 400-500 hr at pH ~ 1.9 could be due to several factors: a) the achievement of maximum bacterial concentrations, b) attainment of pH not conducive to growth, c) saturation of the sites on pyrite by either the Fe^{+2} or Fe^{+3} moieties, d) depletion of the nutrients.

Early studies of Silverman and Lundgren[27] indicate that at lower pH growth does cease. The best pH for oxidation according to these authors is 2.5-4.2; on the other hand, Landesman et al[32] claim the value to be 1.75. This discrepancy is most likely due to different strains involved. The site saturation aspect was mentioned by Silverman[23] in his investigations of ferric ion action and the work discussed earlier in this report (see Table X) tends to support this stipulation. It is noteworthy that the pyrite dissolution rate achieved by the added ferric ion is lower than that measured in the bacteria-assisted tests, although the total level of iron in solution is comparable which, in a way, weakens the site saturation theory. The more effective solubilizing action of T. ferrooxidans as compared to ferric iron alone would tend to imply the existence of the direct action[23], possibly supplemented by the involvement of the bacteria in sulfur oxidation which was implied by Norris and Kelly[33]. Bacterial leaching patterns were observed by Bennett and Tributsch[34] on pyrite, others[35] found up to 90% of bacteria attached to the solid substrates further supporting the direct action concept. It should be noted that in the RR-series, the concentrations of bacterial protein initially added differed from those employed in the CC-series (Table XIX) and MM-series (Table XVIII) by factors of 10 and 100. In the RR-series (Table XX), maximum rates and, surprisingly, the incubation period and the rate attainment times were comparable for the two concentrations. However, the incubation period, ~ 167 hr, was longer than that found for the higher bacterial concentration utilized in the CC-series. The media in these two series were identical. The lowest concentration employed, 3.2×10^{-4} mg protein/ml, corresponded to 3×10^{6} bacteria/ml.

The effect of introduction of additional pyrite upon the iron and sulfate liberation rates is summarized in Table XXI. The additional pyrite was introduced at 216 hr; a 23 hr exposure resulted in an average increase of 3.4×10^{-2} mg/ml of Fe^{t}. The initial rate increase would appear disproportionately high, yet this is to be expected based on the previously observed high initial pyrite dissolution, see Figure 6. However, after 72 hr, the rate of total iron production in CC-9 and CC-10 runs approached twice the rate of tests CC-1 and CC-2 in agreement with the findings discussed earlier (see Table II).

Comparison of tests wherein chloramphenicol was introduced (Table XXII, Runs CC-7 and CC-8) with those performed in its absence, Runs CC-1 and CC-2, shows clearly that this substance does not interfere with pyrite dissolution in 1/10 m9K. Insofar as the suppression of bacteria is concerned (Runs CC-5 and CC-6), the additive seemed to be truly effective only up to 143 hr. In the case of Run CC-5, the suppression lasted up to 359 hr; however, in CC-6 at 143 hr, a definite rate increase was evident. On the other hand, even this rate (5.4×10^{-4}) is lower by a factor of ~ 5 as compared to the rate registered in the absence of chloramphenicol (see Table XIX, Runs CC-4 and CC-12 at 143 hr).

Examining the data given in Table XXIII for the CC-4 and CC-12 Runs, it is apparent that after 311 hr a drop or leveling in the pyrite solubilization does occur. Introduction of additional bacteria at 216 hr into the duplicate Run CC-3 (after correcting for the dilution by the 7 ml bacteria-containing solution) seemed to raise somewhat the rate of iron production and the Fe^{+3} concentration. This effect is more evident in the graphical presentation given in Figure 13 than in the listing compiled in Table XXIII. On the other hand, considering the quantity of bacteria added, one would expect in analogy with the initial bacterial action to see a significant rate increase within ~ 140 hr (i.e. at 389 hr) in Run CC-3 as compared to Runs CC-4 and CC-12. This did not materialize. Introduction of further quantities of the growth media (at 389 hr) failed to increase the rate in the case of Run CC-3; although in the Runs CC-4 and CC-12 a limited increase in the rate of iron production was noted. This finding tends to indicate that at pH ~ 1.9 the growth of bacteria is considerably slowed. Actually, the increase in ferrous ion concentration after 719 hr points also to a drop in bacterial oxidizing ability.

Addition of pyrite to the bacteria-free tests, as noted above, increased the rate of iron production in accordance with the increase in pyrite concentration. In Run CC-11, wherein bacteria were present, the additional pyrite failed to affect significantly the rate of total iron production (beyond that calculated for the increased concentration), yet there was a definite increase in the concentration of ferrous iron (Fe^{+2}) at the expense of ferric iron (Fe^{+3}). This finding would indicate that at this stage the ferric ion present in solution attacked the available sites in the newly introduced pyrite. One would expect the resultant increase in divalent iron to provide more energy source for T. ferrooxidans and to be translated finally into an increase in total solubilized iron; but this apparently is not the case, nor does it appear that any extra solubilized iron was precipitated as $Fe(OH)_3$ or related species since the $SO_4^{=}/Fe^{t}$ ratio stayed reasonably constant. Thus, at this relatively low pH, the oxidizing efficiency of T. ferrooxidans is either strongly impaired, which is not indicated by the control runs CC-4 and CC-12, or the additional pyrite in the small volume of solution (considering the sample withdrawals for analysis) provides detrimental grinding action upon bacteria.

Up to this point, all the studies were performed using the ATCC bacterial strain. To compare the action of T. ferrooxidans freshly isolated from mine water, the investigations summarized in Tables XXIV-XXVI were conducted. The origin of the bacteria will be discussed in the section dealing with mine sampling. From the comparison of the activity of the two bacterial strains (see Tables XVII and XXIV), it is obvious that the incubation period is significantly longer, 119 versus 69 hr, for the mine-isolated T. ferrooxidans. At the low concentration 0.26μg/ml (2×10^6 bacteria/ml) only after 288 hr an evidence of bacterial action was noted. Based on the rate increase, a generation period of ~ 24 hr could be computed. In the pyrite solubilization studies (see Table XXV), utilizing the intermediate bacterial concentration (3.2μg protein/ml), the onset of activity was found to occur only after 427 hr; yet the maximum rate attained, 5.3×10^{-3} mg Fe^t/ml/hr, was directly comparable with that recorded for ATCC strain. It should be noted that only one of the duplicate runs exhibited the bacterial action. The long incubation period could be due to the inhibitory action of the chloride ions present in the 1/10 m9K medium; although in the case of the ATCC strain this effect was relatively insignificant (see Table XIX). Repeat of the above test runs (see Table XXVI) showed that employing a higher bacterial concentration (Runs VV-5 and VV-6) resulted in the activity onset at ~ 239 hr. Again the maximum rate attained, 6.2×10^{-3} mg Fe^t/ml/hr was closely comparable to that found in all the previous tests. It has to be noted that up to 575 hr no activity was registered for the experiments having the initial low 3.2μg protein/ml (2.5×10^7 bacteria/ml) concentration.

T. thiooxidans was isolated from mine waters by a number of workers[7,36] This microorganism was found to produce acid from marcasite but not museum grade pyrite[7,36,37]. With respect to its action upon sulfur balls, the reports are contradictory[7,36]; this is not surprising since the composition of sulfur balls varies widely[7]. T. thiooxidans apparently functions equally well in 1/10 9K and 1/10 m9K media as shown by the values given in Table XXVII. The results obtained at Ultrasystems fully confirm the findings by the other investigators[7,36], since neither in deionized water, Table XXVIII, nor in the 1/10 9K and 1/10 m9K media, Tables XXIX and XXX, was an acceleration in acid production from museum grade pyrite observed in the presence of T. thiooxidans.

3.2 Marcasite Studies

As noted in the earlier portions of this report, discrepancies exist regarding the occurrence of marcasite in Appalachian coals. The investigations carried out here on this mineral were significantly reduced in scope as compared to the pyrite studies. Marcasite, as shown by the results presented in Table XXXI and Figure 14, behaves similar to pyrite insofar as its solubilization in water is concerned, although the dissolution rate is higher than that of pyrite of an equivalent mesh-size. The latter behavior could be possibly due to a catalytic action of the impurities present. The rate of solubilization, as would be expected,was found to be directly proportional to the quantity of the marcasite in a given volume.

The oxygen uptake pattern of marcasite deviates considerably from that established for pyrite. Inspection of Table XXXII clearly reveals two distinctly different oxygen uptake rates. During the first two days, the ratio of oxygen consumed to total soluble iron produced varied from 17 : 1 to 51 : 1 (as compared to the theoretically expected 3.5 : 1), whereas from day No. 6 to 9 this ratio was on average 2.2 : 1, well below that needed for transformation of all the disulfide ions into sulfate. This initially higher than theoretically possible O_2 : Fe ratio is not due to the presence of lower oxidation state sulfur species on the marcasite surface since based on results given in Table XXXI, the $SO_4^=$: Fe^t ratio of 1.9±0.2 : 1 is in reasonable agreement with a ratio of 2 : 1 predicted (if all sulfur had been oxidized to an oxidation state of +6). Inasmuch as the deionized water used in these measurements was saturated with oxygen, it would appear that the impurities present in marcasite are most likely responsible for the initially observed anomalous behavior.

To assess the effect of T. ferrooxidans on marcasite dissolution, the series summarized in Tables XXXIII and XXXIV and illustrated in Figure 15 were carried out. Utilizing water and bacteria, the noticeable increase in rates, as shown by comparison with a test wherein no bacteria were present, took place only after 190 hr. The increase in the rate of total iron production was accompanied by a large increase in the ferric ion concentration. Even in the test where no bacteria were added, after 287 hr the ferric ion content rose to ~ 17% of the total iron, which is higher than the ~ 5% observed in the corresponding pyrite studies (see e.g. Table IX, Tests H-1, H-2; Table X, Tests QQ-1, QQ-2). This action cannot be due to bacterial contamination since microscopic examinations and culture tests failed to show bacterial presence. Comparing the quantities of total and ferrous iron in the compilation given in Table XXXI reveals relatively high ferric ion content. These findings, together with the results of respirometer investigations, indicate that this unexpected behavior is probably due to some catalysis by the impurities present in the marcasite.

To eliminate, at least partly, the interference of the initial high dissolution rates from the T. ferrooxidans action in the runs carried in 1/10 9K (see Table XXXIV), the bacteria were added only after 67 hr. The rate increase to 2.5×10^{-3} mg iron/ml/hr as compared to 0.7×10^{-3} mg iron/ml/hr in the controls (after 168 hr of exposure to T. ferrooxidans), and in particular the high content of ferric ion, demonstrate clearly the presence of bacterial action.

T. thiooxidans was found to have no effect, in the case of pyrite, upon the pH, the production of soluble iron and sulfate ion. Examining Table XXXV and Figure 16, it is apparent that in the presence of T. thiooxidans, marcasite dissolution, based on soluble iron production, occurs at an increased rate in the first 119 hr. More significant than the acceleration in dissolution rate is the marked lowering in pH. Unexplained however is the lower than two $SO_4^=/Fe^t$ ratio found for the bacteria-free tests (Runs UU-1 and UU-2). There could be some interference with the medium itself since in water (see Table XXXI) the ratio was close to 2. On the other hand, one would have expected a similar result for the bacteria-assisted UU-3 experiment and this is not the case. Again, as previously noted also in these runs, a high ferric ion content was measured (up to 41% of the total iron in the T. thiooxidans assisted test), but it should be stressed that in none of these runs was there T. ferrooxidans contamination present.

To summarize, marcasite of comparable mesh size as the museum grade pyrite undergoes dissolution at a higher rate. Furthermore, in the total soluble iron, the ferric ion is present in excess of 20%. Whether the relatively high reactivity of marcasite is inherent to the mineral itself, or whether it is caused by weathering or impurities of the specimen tested has not been determined, although it would seem that the latter accounts for a large part of it. Regarding the response to bacterial presence, T. ferrooxidans has a pronounced effect on marcasite dissolution, but the magnitude of rate increase is lesser than in the case of pyrite. The presence of T. thiooxidans definitely results in acid production from marcasite shown by the lowering of pH, as compared to bacteria-free controls, in agreement with other investigations[36,37].

3.3 Coal Investigations

During this program, two underground mines were sampled and characterized. The first was an abandoned Anthracite mine which, according to personnel of the Pennsylvania Department of Environmental Resources and other cognizant sources, was the only accessible abandoned underground mine in Pennsylvania and most likely in all of Appalachia. Since this mine was the site of a backfill operation study (for the conduct of which an entry shaft had been drilled and the mined-out workings of several overlaying coal beds had been prepared to allow safe entry) which was to be completed within a few months, it was decided to use the opportunity of sampling an abandoned underground mine even though it was an anthracite and not a bituminous coal mine. It was reasoned that the pyrite degradation process should be similar, if not identical, in both types of coals and that the long time since closure, during which flooding occurred, should have allowed the development of all acid producing processes, in particular those involving bacteria, specifically the "mysterious" Metallogenium, to the fullest extent in an environment undistrubed by common mining operations, including rock dusting. The results from this mine are described in Section 3.3.1.

In dealing with acid production from underground coal mines, the possibility of back-reactions, e.g. the re-formation of pyrite from the components of acid drainage, cannot be ignored. The occurrence of such back-reactions, although not necessarily at the location of the acid production, was shown by a study of rice paddies in South Carolina[17] which became unusable because of pyrite deposition in the subsoil. If such a reversal of the acid production process could be initiated at the acid producing site, a lowering of acid, iron, and sulfate concentrations in the effluent could be achieved and, theoretically, if a complete equilibrium could be established, no net acid production should occur at all. This, in effect, would constitute an abatement method. It was therefore of interest to the present investigations when on one of the sampling trips the existence of three water overflow bore holes became known, in the immediate surroundings of which the odor of hydrogen sulfide and/or mercaptans was unmistakable. To investigate the possibility of backward reactions taking place under conditions normally associated with acid production, samples from the pond surrounding the bore holes were consequently taken for analysis. The results of these analyses are also described in Section 3.3.1.

The second extensively investigated site was an active underground mine operating in the Pittsburgh seam. The major reasons for choosing this site were the relatively high present production volume from the Pittsburgh seam, its location in an extensively mined geological area, the "dryness" of the mine, and the availability of extensive core bore hole data. The data from this investigation are given below in Section 3.3.2.

As discussed later in greater detail, Metallogenium was neither found in the samples of the two mining sites mentioned above, nor in the overflow bore hole water samples. According to Walsh and Mitchell[45], Metallogenium is an important link in a succession of acid producing microorganisms, being most effective in the intermediate pH range and actually responsible for creating optimal growth conditions for T. ferrooxidans. In all of the above cases high pH as well as low pH active species were identified (T. thioparus, T. thiooxidans, and T. ferrooxidans), but not the intermediate pH active Metallogenium. In the case of the Lower Kittanning seam, generally considered to be a high acid producer, an effort was made to obtain samples from the gob areas of a mine. This was unsuccessful mainly because no safe access to such an area could reasonably be secured. Therefore, as a compromise, water samples high in suspended matter were collected from a naturally, not intentionally, flooded mine. The analysis of these samples is described in the Section 3.3.3.

3.3.1 Abandoned Anthracite Mine and Overflow Boreholes

This mine, when in operation, produced from eight overlaying, dipping seams simultaneously. It was closed in ~ 1930 for economical reasons. Because of subsidance problems and the desire to dispose of unsightly and acid producing refuse piles, it was partially reopened in 1977/78 to evaluate the feasibility, procedures, and effectiveness of backfilling with coal refuse. In the time between abandonment and partial reopening, all beds had been flooded and at least the upper ones drained and reflooded several times by natural changes in the water table. At the time of sampling, only the two uppermost beds, ~ 80 and ~ 100 feet below the surface, respectively, were still safely accessible. Two water, one coal, one rock, and two yellowboy samples were taken. One of the yellowboy samples was from the floor, the other from the roof, the latter having the appearance of a stalactite, yet it was soft and otherwise had the texture of muddy yellowboy.

The three ~ 30 inch diameter water overflow bore holes were drilled in the general area of the abandoned anthracite mine to relieve the hydrostatic pressure in the extensively mined-out workings in this anthracite region which got flooded because of naturally existing conditions. These conditions caused seepage, leakage, and outright drainage at various undesirable and uncontrollable locations so that the overflow holes were drilled primarily for the same reasons as in the case of the Lower Kittanning seam mine to be described in Section 3.3.3, namely to direct all overflow caused by hydrostatic pressure to one location for possible treatment and safe discharge. The water flow from the three boreholes created a shallow pond, over which the odor of hydrogen sulfide and/or mercaptans was clearly discernible. The fact that a hydrogen sulfide odor was recognizable points to a low hydrogen sulfide concentration since high concentrations cannot be detected in this manner. At the same time, the recognition of this odor almost unequivocally proves the presence of dissolved hydrogen sulfide in the exiting water, possibly even in considerable quantities. The sites, at which the samples were taken relative to the bore hole locations, the pond circumference, and the water flow pattern within the pond are given in Figure 17.

Inspection of Table XXXVI and XLII reveals that all attempts at isolating Metallogenium from any of the samples were unsuccessful. This result is unexpected since it is believed that the conditions existing at these sampling sites, including pH and dissolved ferrous iron, would be more favorable for the establishment of Metallogenium populations[39,42,43,45] than the conditions expected to be prevailing in active underground mines where rock dusting is employed. Another unexpected finding was the simultaneous presence of sulfate reducing bacteria along with the thiobacilli in all but one of the samples collected. On the other hand, the presence of sulfate reducers could have been anticipated since prior to visiting the bore hole overflow pond, it was learned that at this location the smell of hydrogen sulfide was clearly recognizable. This information was actually the reason for adding the sulfate reducers substrate to the test matrix.

The enumeration of microorganisms in the water samples was not performed by the "most probable number" (MPN) technique[44], but the numbers obtained by colony count per ml can be used as a very rough estimate of the actual populations; these are given in Table XXXVII.

TABLE XXXVII

ENUMERATION OF MICROORGANISMS IN WATER SAMPLES FROM AN ABANDONED MINE AND BORE HOLE OVERFLOW

	Microorganisms/ml		
No.[a]	$SO_4^=$ reducers	Heterotrophic Population	Yeast Population
1	7	>300	43
2	3.5	170	86
3	5.5	45	25
4	2.5	73	36
5	0.5	30	23
6	1.0	3	1

a) The number given corresponds to sample number as denoted in Table XXXVI.

3.3.2 Active Pittsburgh Seam Mine

This mine working the Pittsburgh seam on a relatively large scale, including long wall production, was opened as a shaft and drift mine in the late thirties and has been in continuous operation since then. It extends over a large area and is quite dry despite several surface water bodies and regularly occurring precipitation. Although the seam is, of course, not completely horizontal, inclines are gentle.

In Figure 18 are given the locations of the sampling sites relative to bore holes drilled to obtain core samples. Based on these data, the type and variation in overburden at the given sample site can be deduced. Site No. 1 was a freshly (less than 10 hours before sample taking) cut active face. Site No. 2 was mined in the order of months before sampling, Site No. 3 had been opened several years ago and since then had been flooded at least once, since that time it is the lowest point of the mine. In Figures 19 and 20 are presented the vertical geological cross sections for the drill holes of interest along the horizontal cross sections A-A' and B-B', respectively, corresponding to the locations given in Figure 18. As can be seen from the diagrams, the immediate overburden of the mined seam is sandstone admixed with sandy shale. Since sandstone usually is capable of retaining respectable amounts of water and since no appreciable moisture entering naturally could be observed, it must be concluded that other overlaying strata in this region are impervious to water seepage thus preventing the sandstone from getting "saturated" with water. Although at present of no immediate importance to this study, Figure 21 depicts vertical geological cross sections below the Pittsburgh seam at two bore hole locations in the vicinity of the bore holes identified in Figure 18. These data are included for complete presentation of all available information and in the belief that an advanced understanding of the effects and the (positive or negative) contributions of surrounding parameters may add further to these data.

A total of twelve coal and water samples were taken from this mine in addition to four "surface" water samples. All these are listed in Table XXXVIII together with their locations. The samples have been separated into groups differentiated by the materials' in-mine location. None of the freshly mined areas were rock-dusted. Thus, the interference of mineral introduction could be avoided in these samples. A sample of rock dust used at this mine was also obtained; however, its investigation in regard to composition and effect on acid formation could not be accomplished because of lack of time and funds.

All the specimens were subjected to a number of microbiological and chemical analyses. Thus, all the solids were analyzed for pyritic content, ash, and calcium. A portion of each of the solid samples was washed with a measured quantity of water, and on the aqueous solutions, pH, soluble iron, and sulfate were determined. The results of these analyses are presented in Table XXXIX. The pyrite content was obtained indirectly based on the pyritic iron. The independent sulfate analysis, in the case of all the coals, was in good agreement with the iron determinations; this was not the case with the shales. The low $SO_4^=/Fe^t$ ratios found for VV-6 and VV-8 shales are most likely caused by the high calcium content of these samples, however this does not appear to be the case with VV-3. It is possible that in this instance the lowered $SO_4^=/Fe^t$ ratio is due to solubility of the iron species other than FeS_2 in the dilute acid employed in pyritic iron determinations. On cursory examination, there seems to be no connection between the soluble iron and the sulfate ion content of the coal washings. But if one assumes calcium to be present in the coal predominantly in the form of calcite, $CaCO_3$, then the observed results become meaningful. At the high pH, due to the presence of calcium carbonate, the following would be expected to occur:

$$FeS_2 + H_2O \xrightarrow[pH\sim7]{CaCO_3} Fe(OH)_3\downarrow + Ca^{++} + SO_4^= + CO_2\uparrow \qquad [6]$$

In agreement with the following factors: a) pyrite in the presence of water and air is transformed into ferrous, sulfate, and hydrogen ions (see the studies described in Section 3.1), b) at pH > 6, the air oxidation of ferrous ion to ferric ion is rapid[24] and under these conditions, ferric ion tends to precipitate as ferric hydroxide, hydrated ferric oxide, and/or ferric hydroxy sulfate, c) the initially co-produced hydrogen ion attacks solid calcium carbonate liberating soluble calcium ion and gaseous carbon dioxide, the major soluble species expected to be present would be calcium and sulfate ions. The ultimate concentrations of these ions would in turn depend upon the solubility of calcium sulfate in the "mine water".

All the waters collected from the VV-mine had high pH. The lowest, pH 7, was that exhibited by VV-10 sample obtained from a sump of a very old acid region (see Table XXXVIII). The relatively high pH even of this sample can be best explained by the relatively high calcium content of the coal and shale of the VV-mine in conjunction with low water ingress into this mine. Under these circumstances, one can visualize the "residual" mine water acting as a buffer. This has been verified by studies discussed later (see Table XLVIII).

Only two of the water samples, VV-9 and VV-10, were subjected to complete inorganic analysis by an independent laboratory. The results of these determinations are presented in Table XL. The pH and sulfate values are close to those found by Ultrasystems, however, the reported iron concentrations are higher by a factor of ~ 2,000 in the case of VV-9 and by a factor of ~ 3 in the case of VV-10. To test the reliability of these laboratories, a simulated mine water sample was submitted for analysis. Based on the results given in Table XLI, it is apparent that anion analysis is reliable; the values for cations are meaningless.

On all the samples collected from the mine, the bacterial populations were determined. The particular species analyzed for were Thiobacillus thioparus, Thiobacillus thiooxidans, Thiobacillus ferrooxidans, Metallogenium, sulfate reducers (e.g., Desulfotomaculum and Desulfovibrio species), heterotrophs (e.g., Pseudomonas and Bacillus species), and yeast. The results of these determinations are given in Table XLII. Examining these compilations, it is evident that in none of the solid samples, which were subjected to testing both as solid suspensions and washings, were thiobacilli unequivocally identified. Inasmuch as significant bacterial populations were found in the waters adjacent to the location from where the coal and shale specimens were obtained, it is reasonable to assume that these bacteria were most likely also present on the solid samples and the failure to propagate these is most likely due to their relatively low concentrations.

It is noteworthy that although the pH of the water samples VV-9, VV-10, and VV-11 was high (see Table XXXIX), Thiobacillus ferrooxidans was observed in these environments; but the population of the Thiobacillus thioparus (sulfur oxidizer, pH ~ 7) was higher than that of T. ferrooxidans in the basic waters (VV-9 and VV-11). It has been claimed[41] that in the mine waters sulfur oxidizers predominated over iron oxidizers; however, in VV-mine waters the quantities were comparable. The presence of sulfate reducers together with the sulfur and iron oxidizers is in agreement with the findings reported for the abandoned mine in Section 3.3.1 and with the observation of these bacteria in mine waters by others[41,46,47]

The well water (see W-1 and W-2 in Tables XXXVIII and XXXIX) represents in a broad sense the incoming water into the mine; the presence of sulfate would tend to indicate some pyrite dissolution from the solid formations. The absence of iron can be explained by its precipitation at these high pH values. The W-3 and W-4 samples are the actual mine waters used in the recirculation water spraying scheme employed in this particular mine. Thus, the presence of bacterial populations (see Table XLII) in these waters is to be expected. It should be stressed that in none of the mine waters, solids, and solids washings was Metallogenium observed; the procedures employed in the attempted enrichment and isolation of these bacteria followed directly the recommendations of Walsh and Mitchell[39]. No additional information could be obtained from Dr. Walsh; attempts of procuring an inoculum were equally unsuccessful.

To assess the drainage potential of coals and shales obtained from the VV-mine and to hopefully correlate a) the pyritic content of the specimens, and b) the exposure of given samples in the mine to air and water with the ease of liberation of the sulfate, hydrogen, and soluble iron species, the tests summarized in Tables XLIII-XLIX and Figures 22-26 were performed.

In the case of the coals obtained from the freshly mined face, the pH initially stayed neutral and only a trace of iron was detected, yet the production of sulfate ion was found to proceed at a measurable rate. However, as time progressed, pH started to drop and the concentration of soluble iron started to increase. This is more evident in the VV-2 coal than in the VV-1 sample. The difference in behavior between the two coals is most likely due to the difference in their respective calcium contents (see Table XXXIX). The variation in coal composition depending on location has been amply documented[7]. For the coals obtained from old areas, namely samples VV-4, VV-5, and VV-7 (see Tables XLIV - XLVI and Figures 22 and 23), the pH drop occurred within a relatively short time, ~ 24 hr, with concurrent appearance of soluble iron. The only exception to this behavior was the washed sample of VV-7. It should be noted that in the case of VV-5, no significant difference was observed between the washed and unwashed sample. Inasmuch as the washing was performed on unground (large coal chunks) samples, the effect of washing upon the specimens' solubilization characteristics would be expected to be minimal. The observed difference in the VV-7 behavior is most likely due to the particle size since the washed material consisted of: 82% of particles $< 250\mu$, $> 125\mu$ and 17% of particles $< 125\mu$; whereas the unwashed material contained a significantly higher content of the finer particles, 69% $< 250\mu$, $> 125\mu$ and 27% $< 125\mu$.

As briefly mentioned before, the difference in the behavior of the specimens obtained from the freshly mined face (VV-1 and VV-2) as compared to the old coals is best explained by the presence of a neutralizing ingredient in the coal samples. This constituent, after exposure to water and pyrite dissolution products under laboratory conditions or equivalent weathering treatment in the mine environment, is finally depleted allowing the observation of the "normal" pyrite dissolution products.

Based on the calcium content of VV-1 (assuming it to be in the form of $CaCO_3$ in agreement with the reported presence of calcite in coal), the following series of reactions would account best for the observed variations in the soluble Fe : S ratio as exemplified by the initially low concentration of soluble iron (as compared to sulfur in the form of sulfate) and the subsequent approaching of $SO_4^{=}/Fe^{t}$ ratio of 2:1.

$$2\ FeS_2 + 2\ H_2O + 7\ O_2 \longrightarrow 2\ FeSO_4 + 2\ H_2SO_4 \tag*{[7]}$$

$$CaCO_3 + H_2SO_4 \longrightarrow CaSO_4 + H_2O + CO_2 \tag*{[8]}$$

accompanied at a pH $\sim$ 7 by:

$$2\ FeSO_4 + 1/2\ O_2 + H_2O \longrightarrow 2\ Fe(OH)SO_4 \tag*{[9]}$$

$$2\ Fe(OH)SO_4 + 4\ H_2O \longrightarrow 2\ Fe(OH)_3 + 2\ H_2SO_4 \tag*{[10]}$$

Thus, the complete transformation of pyrite to the hydrated oxide can be represented by the following equation:

$$2\ FeS_2 + 7\ H_2O + 7.5\ O_2 \longrightarrow 2\ Fe(OH)_3 + 4\ H_2SO_4 \tag*{[11]}$$

Thus, for every mole of pyrite dissolved, four equivalents of acid are formed and these in turn can neutralize two moles of calcium carbonate forming the insoluble hydrated ferric oxide and the initially soluble calcium sulfate. However, once the concentration of calcium sulfate reaches the saturation point (2.09 mg/ml at room temperature), precipitation of calcium sulfate will take place. This action is responsible for the $SO_4^{=}/Fe^t$ ratio of 1.4 after 961 hr in the case of VV-5 (data from Table XLV).

Based on the solid analysis, VV-1 contains 2.25% of FeS_2 which corresponds to 0.0188 mol of FeS_2 per 100 g of coal. The respective values for $CaCO_3$ are 2.8% (assuming all the calcium in VV-1 to be present as calcite) or 0.028 mol. Now every mole of FeS_2 can theoretically neutralize 2.0 moles of $CaCO_3$; consequently (assuming even distribution of FeS_2 and $CaCO_3$) the pyrite present can neutralize 0.0376 moles of $CaCO_3$; since only 0.028 moles of calcium carbonate are present, 0.0048 moles of FeS_2 are left over. Thus, after sufficient lapse of time, providing water and air are also available, acid production from this coal would be expected. The results of the test summarized in Table XLVII and expressed graphically in Figure 24 confirm fully the above postulations.

The buffering action of the mine waters cannot be ignored, as evident by the data presented in Table XLVIII wherein utilizing sterile mine water, VV-9, the production of soluble iron and pH drop were significantly delayed.

Several aspects must be emphasized at this point, namely: the experiments conducted in the laboratory in which relatively fine crushed coal and agitation were utilized represent an accelerated test as compared to conditions existing in the mine. Yet, the ultimate results are comparable showing the validity of these approaches. The quantity of pyrite per a given volume of water present in the coal runs as compared to the pyrite runs, discussed in Section 3.1 of this report, was lower by a factor of 10. In addition, the mesh-size of the coal was significantly higher than that of the pyrite employed in the above-discussed experiments. The amount of pyrite per a given volume and its particle size are the governing surface area factors found to be of major importance with respect to the rate of pyrite dissolution. Based on these premises, one would expect the solubilization of the coal pyrite to occur at a much lower rate than that observed for the museum grade pyrite $< 38\mu$, 500 mg/100 ml. There are several reasons for this apparent discrepancy: the pyrite in coal is present definitely as finer crystals than a chunk of museum grade pyrite (disregarding even the claimed activity of framboidal pyrite[17]) and the grinding of coal must result in further subdivision. In addition, the presence of coal itself would be expected to facilitate pyrite dissolution due to its catalytic action[48] in oxidizing $Fe^{+2} \longrightarrow Fe^{+3}$. The solubilizing action of Fe^{+3} ions upon pyrite has been amply proven by the work described in Section 3.1.1.

Inasmuch as the shales and binders are inherently associated with coal seams, their potential contribution to mine drainage production is of equal if not greater importance than that of coal itself. The results of dissolution studies performed on the shales, VV-3, VV-6, and VV-8, are summarized in Table XLIX and presented graphically in Figures 25 and 26. Again, here after prolonged exposure to water and air, a drop in the pH and the appearance of soluble iron was noted. From the pyrite and calcium contents (assuming the latter to be present as calcium carbonate), the drop in pH accompanied by soluble iron liberation, with the exception of the VV-3 shale, is unexplained. Consequently, either the calcium value is in error or calcium is not present as a carbonate.

The presence of <u>T. ferrooxidans</u> in coal mines has been definitely established in the late 1940's[6] and since then a large number of publications dealing with bacterial involvement in mine acid formation have been published[1,9].

To evaluate the "susceptibility" of coals exposed to the mine environment for different periods of time to bacterial attack, the tests summarized in Tables L and LI were performed. It is evident that the coal from the freshly mined face did not provide a habitable environment for the bacteria even though the medium used, as proven by the pyrite studies, was suitable for propagation. This finding is in agreement with the published data[49] regarding the prolonged survival of T. ferrooxidans at pH above 6.5. The presence of carbonate acts also as a bacteria inhibiting factor as reported by Silverman, Rogoff and Wender[50]. These authors found that it was necessary to remove calcium carbonate from coal by washing with hydrochloric acid for T. ferrooxidans to act upon pyrite. The weathering of coal (exposure to mine environment) in a way serves the same function as hydrochloric acid treatment, except in this case it is the incipient sulfuric acid which in effect removes the carbonate. Once this is accomplished, the bacteria can function as attested by the experiments utilizing the coal from old areas where the presence of bacteria was responsible for the highly increased rate of soluble iron, ferric ion and sulfate ion production and the marked drop in the pH (see Table LI, Figure 27). It should be noted that the increase in the rate of soluble iron production between 479 and 358 hr corresponded to a factor of 500 as compared to the bacteria-free controls.

In none of the tests was sterilized coal employed since, based on the propagation investigations discussed earlier, the bacteria (if any) present on the coal were in insufficient concentration to allow enrichment. It should be mentioned at this point that, based on the behavior of the mine isolated strain in the activity evaluation tests, discussed in Section 3.1.2, this strain is apparently much harder to propagate under the conditions optimal for the ATCC received strain.

To assess the effect of sterilization upon the coal chemical activity, i.e. pyrite dissolution, the experiments listed in Table LII were conducted. Based on these data, it would appear that dry heat sterilization decreased the activity of the coal, most likely due to rendering the coal particles more resistant to wetting. The "drying up" of coal is probably also responsible for the observed decreased dissolution rate of coal samples after several months storage as evidenced by the comparison of the values given for VV-5 coal in Tables XLV and LII (LL-3 and LL-4 Runs).

To ascertain that the different batches of the VV-5 coal exhibited comparable activity, the tests listed in Table LIII were performed. Comparison of these values with those listed for the runs LL-3 and LL-4 (see Table LII) shows clearly the equivalency of the two batches.

The reproducible effectiveness of the mine isolated T. ferrooxidans in increasing pyrite dissolution from coal is clearly evidenced by the data given in Tables LIV and LV and by the plots depicted in the Figure 28. It is surprising, however, that the higher initial bacterial concentration (compare Runs YY-3, YY-4 with Runs YY-5, YY-6; Table LV) failed to affect the incubation period and the initial and the maximum rates of dissolution attained. The nature of the media, 1/10 m9K or 1/10 9K, had no apparent effect upon the behavior of the bacteria. The action of the mine isolated strain upon VV-5 coal was directly comparable to that exhibited by the ATCC procured strain.

As noted in Section 3.1.2, conflicting reports exist regarding the effectiveness of T. thiooxidans in catalyzing the production of acid from sulfur balls[7,36]. T. thiooxidans was found to be inactive on all pyrites tested by some workers[50], including pyritic coals. As evident from the data given in Table LVI and even more so from the plot presented in Figure 29, T. thiooxidans does definitely accelerate the dissolution of the pyrite in the VV-5 coal. The relative ratio of Fe^{+3} to Fe^{t} in these tests is somewhat higher than that encountered in the bacteria-free tests in which significant rate of dissolution was encountered (see Tables XLIV and XLVI). Thus, from the limited investigations performed here, it would appear that T. thiooxidans is indeed effective in transforming minerals present in the Appalachian coals into hydrogen, sulfate, and iron ions. This is not totally unexpected if one considers the results obtained for the sulfur balls[7].

As noted earlier, the presence of T. thiooxidans was reported in all instances where T. ferrooxidans was observed. Our data confirm these findings (see Table XLII). To exist in the mines in populations comparable to those of T. ferrooxidans, T. thiooxidans must be able to obtain the energy for propagation. Two of the possible sources are the minerals present in coals or symbiosis with T. ferrooxidans wherein T. ferrooxidans liberates S^o and the latter or its intermediate oxidation products are then oxidized further by T. thiooxidans. The presence of sulfate reducers offers another donor of a possible energy source for T. thiooxidans. However, the results obtained here to date would tend to indicate the direct involvement of T. thiooxidans in coal minerals dissolution.

3.3.3 Water from Flooded Lower Kittanning Seam Mine

Since this mine was abandoned and flooded, no solid samples could be retrieved. Flooding of this otherwise sealed mine occurred by natural processes and, because of prevailing hydrostatic conditions, with time resulted in leakage and seepage. To prevent this seepage from occurring in an uncontrollable manner at numerous undesirable locations, active removal of water by pumping into a pond where treatment is feasible was initiated.

The sample listed in Table LVII was taken at this pumping station before treatment. The observed lower than 7 pH in conjunction with the relatively high content of trivalent iron would tend to indicate the possible presence of iron oxidizing bacteria, yet for all practical purposes, none were detected. It should be noted that both sulfur oxidizers and sulfate reducers were present in this sample.

3.4 Summary and Recommendations

The investigations performed established that museum grade pyrite will undergo dissolution on exposure to air and water. The rate of dissolution is dependent on the available surface area, i.e. particle size and quantity of pyrite in a given volume of water. Below pH ~ 4, the rate of dissolution is unaffected by further lowering of pH. The dissolution of pyrite follows directly the rule which states that the reaction rate doubles for each 10°C temperature rise. Cupric ion was found to exert no influence upon the dissolution rate. The dissolution products and oxygen consumption were in agreement with:

$$FeS_2 + H_2O + 3.5\ O_2 \longrightarrow FeSO_4 + H_2SO_4$$

Ferric ion in low concentration was found to accelerate the dissolution rate according to:

$$FeS_2 + 2\ Fe^{+3} \longrightarrow 3\ Fe^{+2} + 2\ S^{o}$$

At high ferric ion concentration, the ratio of iron solubilized to ferric ion consumed was lower than 0.5:1; however, it definitely was higher than the 1:14 ratio indicated by the following equation:

$$FeS_2 + 7\ Fe_2(SO_4)_3 + 8\ H_2O \longrightarrow 15\ FeSO_4 + 8\ H_2SO_4$$

T. ferrooxidans, both the ATCC purchased and mine isolated strains, were found to accelerate the dissolution rate by up to a factor of ~ 600. T. thiooxidans had no effect upon pyrite dissolution.

Marcasite exhibited a behavior parallel to that of pyrite, although its rate of dissolution in water was significantly higher; T. thiooxidans was effective in increasing its dissolution rate.

Solid and water samples were obtained from three mines; from a number of these specimens sulfur oxidizers, sulfate reducers, iron oxidizers (T. ferrooxidans), heterotrophs, and yeast were isolated and enumerated. In no instance was Metallogenium detected.

Exposure of coal to air and water resulted in pyrite dissolution. Depending on the relative calcium carbonate and pyrite content in the coals and shales, and the time of exposure to air and water, the nature of soluble ions varied. Providing the calcium carbonate to pyrite ratio in the coal is lower than 2 after a certain lapse of time, the initial alkaline drainage, containing Ca^{++} and $SO_4^{=}$ as major ions (excluding the ions present in the ingress water), will change into acidic drainage containing hydrogen and iron ions in addition to the calcium and sulfate. If the calcium carbonate to pyrite ratio is higher than 2, the drainage will remain alkaline. All the iron liberated will be precipitated within the mine.

T. ferrooxidans did not flourish in the alkaline environment provided by the calcium carbonate rich coals. After removal of the carbonate, the bacteria was found to propagate and to catalyze strongly (by a factor of ~600) the pyrite dissolution. T. thiooxidans was also found to exert an accelerating action.

From the data collected to date, no specific inhibitors to mine acid production can be recommended. On the other hand, a predictive system based on the mineral content of the coal and possibly the shale formations could be developed, thus governing the mine site selection and mining design. It is also believed that in special situations, when hydrofracturing is performed to release methane, this operation could be modified to include calcium carbonate slurry, thereby impregnating the seam with finely dispersed acid neutralizing agent. It should be emphasized that the presence of calcium carbonate not only prevents the acid liberation into mine waters, but it also hinders the growth of acid producing bacteria.

4. EXPERIMENTAL

4.1 Materials

The museum grade pure pyrite utilized in these investigations was from the Rico District, Colorado, and was procured from Burminco. Marcasite was obtained from the Siglo XX Mine, Liallagua, Bolivia (found: Fe, 36.3; S, 44.1; theory: Fe, 46.6; S, 53.4). No purer sample could be obtained. The origin of all the other water and solid samples investigated is given in Tables referenced in the Technical Discussion.

4.2 Microbiology Aspects

The growth media employed for enumeration, enrichment, isolation, and maintenance of bacterial cultures were as follows: *Thiobacillus thioparus* was grown in the medium of Vishniac and Santer[38]; the 9K medium of Silverman and Lundgren[27] with added ferrous sulfate was employed for growing *T. ferrooxidans*; the same 9K medium with added elemental sulfur was utilized to grow *T. thiooxidans;* to enrich *Metallogenium,* the medium recommended by Walsh and Mitchell[39] was used; sulfate reducing bacteria were propagated on the P3 medium of Postgate[40]; for the heterotrophs, the Tryptone Glucose Extract Agar (Difco) was selected; the yeast were grown on Sabouraud Dextrose Agar (Difco).

Three cultures of thiobacilli were purchased from the American Type Culture Collection (Rockville, Maryland): *Thiobacillus thioparus* ATCC 23648, *Thiobacillus thiooxidans* ATCC 15494, and *Thiobacillus ferrooxidans* ATCC 23270. The mine-isolated strain of *T. ferrooxidans* was obtained from the water sample VV-10 (see Table XXXVIII).

Enumerations of mine samples were performed following the procedures of Tuttle, et al.[41]. The tables listed in Standard Methods[44] were utilized to calculate bacterial numbers from the three tube most probable number technique employed during enumeration. For these tests, measured amounts of untreated mine samples, either water or solid, were added directly to the solutions used for bacterial enumeration and enrichment.

The bacteria used in the dissolution studies were grown in 1.5 liter batch cultures. Batch cultures of T. ferrooxidans were harvested following the methods of Silverman and Lundgren[27]. Batch cultures of T. thiooxidans were harvested following the methods of Silverman, et al[50]. The quantity of protein in the resulting bacterial suspension was determined using the portein assay procedure of Lowry, et al[51].

4.3 General Procedures

The grinding of all minerals was performed in a nitrogen atmosphere. Pyrite and marcasite were ground in an inert atmosphere enclosure using pestle and mortar, sieving was also done in the inert atmosphere enclosure. Coals and shales were ground using a ball-mill.

All the dissolution experiments were performed at a minimum in duplicate; the denoted quantity of solid was agitated, using a reciprocating shaker, in a 250 ml conical flask in 100 ml of the specified sterilized medium. The shaking speed utilized for pyrite and marcasite was 120 excursions/min and for coal was 88 excursions/min. At time intervals given in the tables summarizing a specific experiment, a total of 6 ml of solution was taken out asceptically, filtered and subjected to pH measurement, $SO_4^{=}$, Fe^t, Fe^{+2}, and Fe^{+3} analyses.

pH determinations were performed using a microbe combination electrode (Vanlab, cat. #34106-079) in conjunction with a Beckman Model 76 pH meter.

For sulfate analysis a somewhat modified turbidimetric method of Hach[52] was adopted with the modification consisting of using 10 ml solution and 100 mg of Sulfaver IV reagent; the absorption measurements were performed in 1 cm cells at 450 nm employing a Beckman spectrophotometer Model 35.

The accuracy of the method is best presented by the graph given in Figure 30; the standard used was sodium sulfate. In the analyses performed the concentration range covered was 8-25 mg/l of $SO_4^=$ since both at higher and lower concentrations, the reproducibility was unacceptable. Other potential methods investigated involved the barium chloranilate procedure[53-56]; here the accuracy was poor and the routine tedious.

Ferrous ion, Fe^{+2}, was determined using the general procedure of Stookey[57], based on the absorption at 562 nm of the $Fe^{+2}\cdot$ ferrozine complex; the modification of Gibbs[58] (which eliminates the heating regime) was included in the adopted method. To obtain total iron concentration, reduction with hydroxylamine was carried out. Ferric ion was measured by colorimetry, independently of Fe^t and Fe^{+2} as the thiocyanate complex[59].

Coal analyses were performed following standard procedures[60]. These included ash[61], pyritic sulfur by the indirect method[62] (with the modification that Fe^t was determined as ferrozine complex colorimetrically). Iron in the ash was again measured as the ferrozine complex after removal of the silicates[63]. Calcium was determined gravimetrically as calcium carbonate using a combination of methods[63,64], which included removal of silicates, iron and aluminum and initial precipitation as calcium oxalate.

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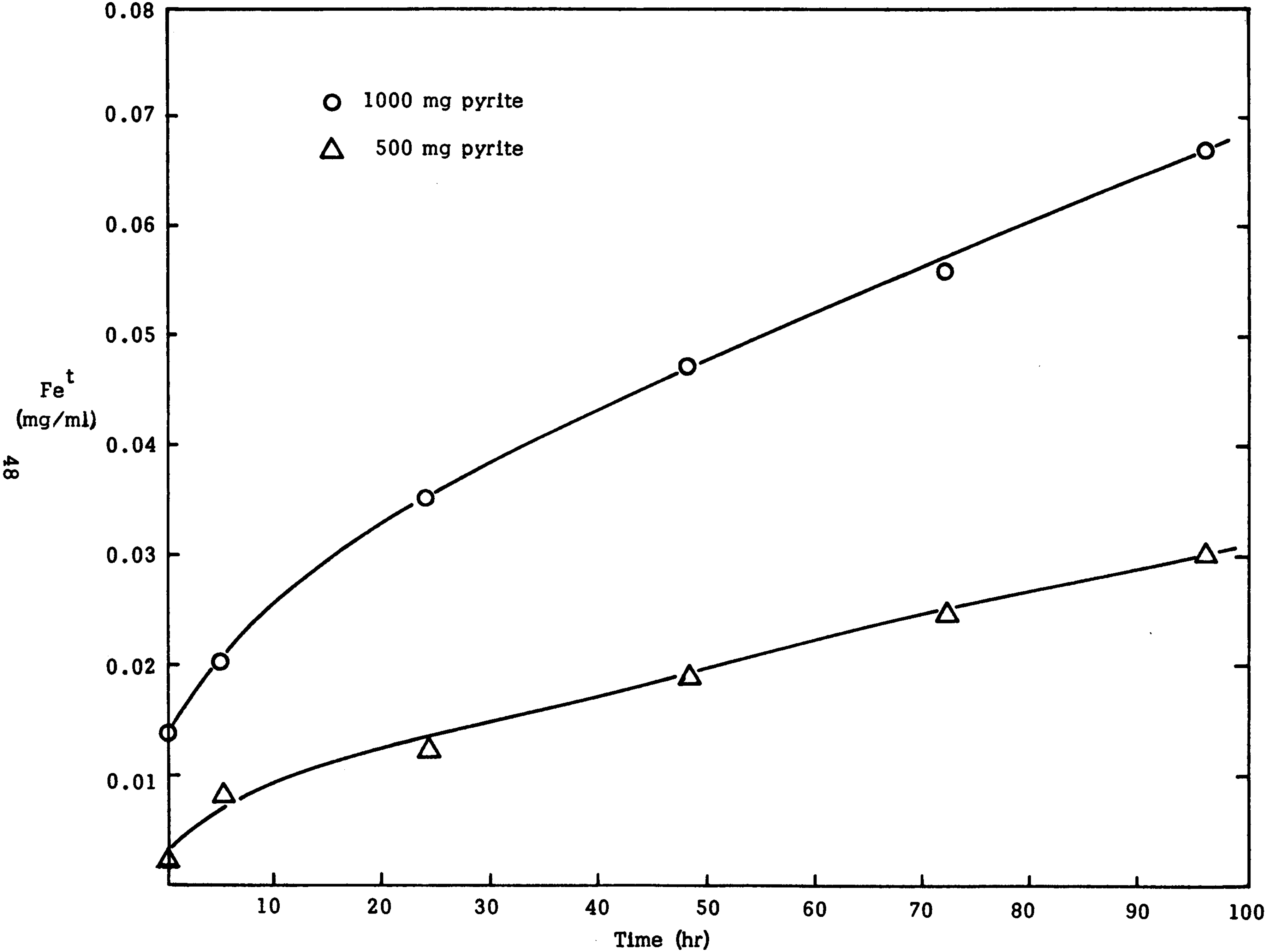


Figure 1: Soluble iron production as a function of time for different quantities of pyrite suspended in 100 ml of water.

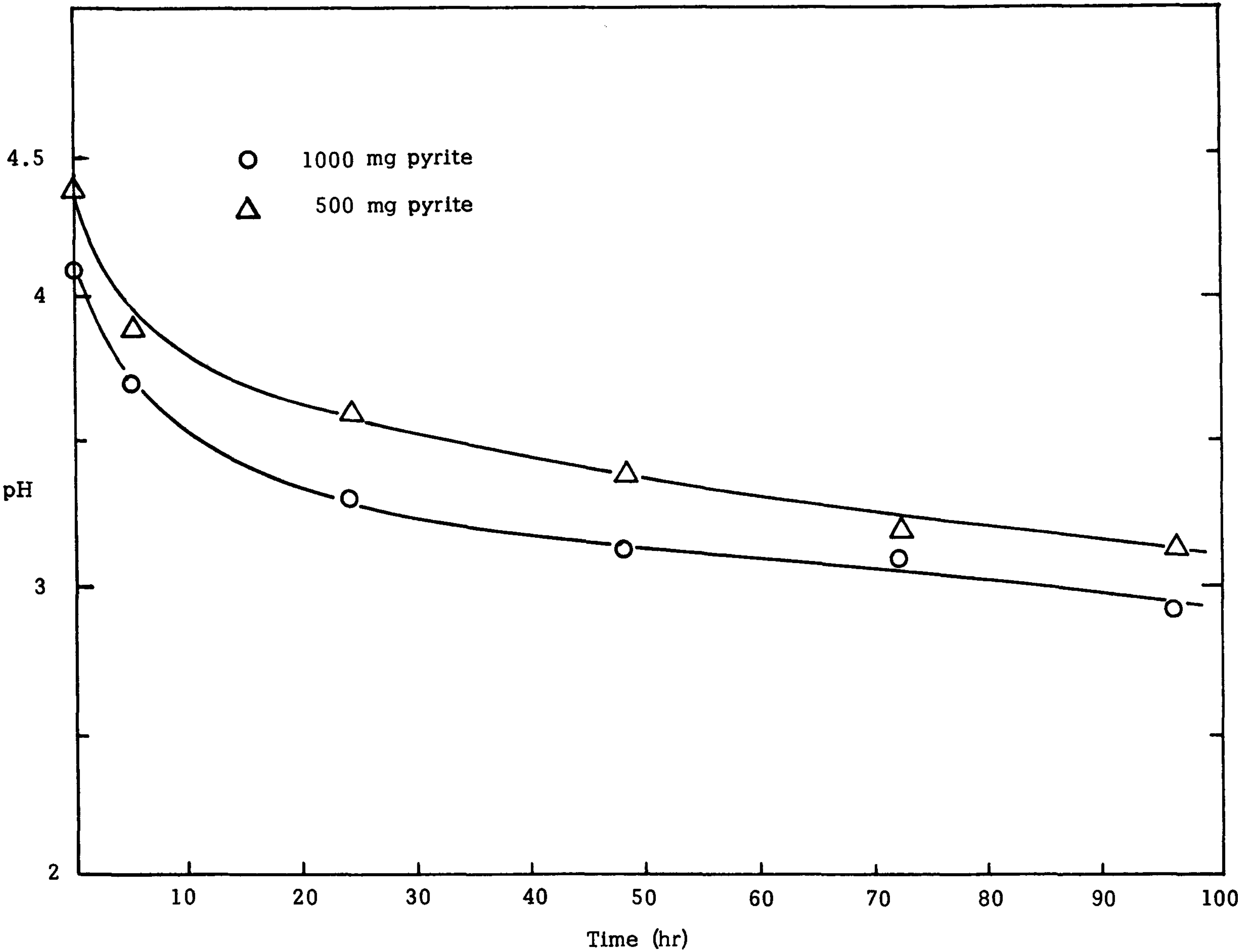


Figure 2: pH drop as a function of time for different quantities of pyrite suspended in 100 ml of water.

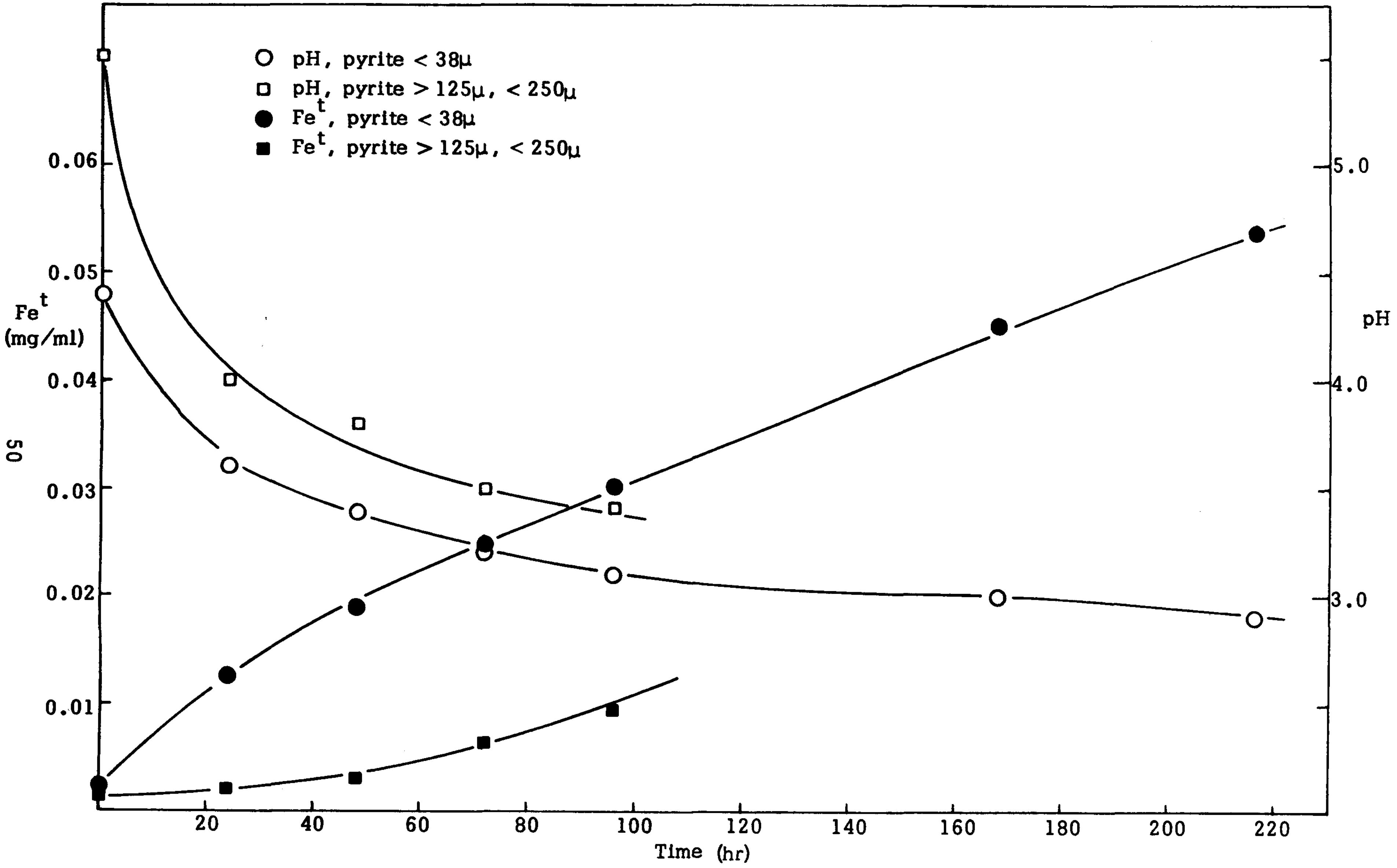


Figure 3: Effect of particle size upon pyrite solubilization.

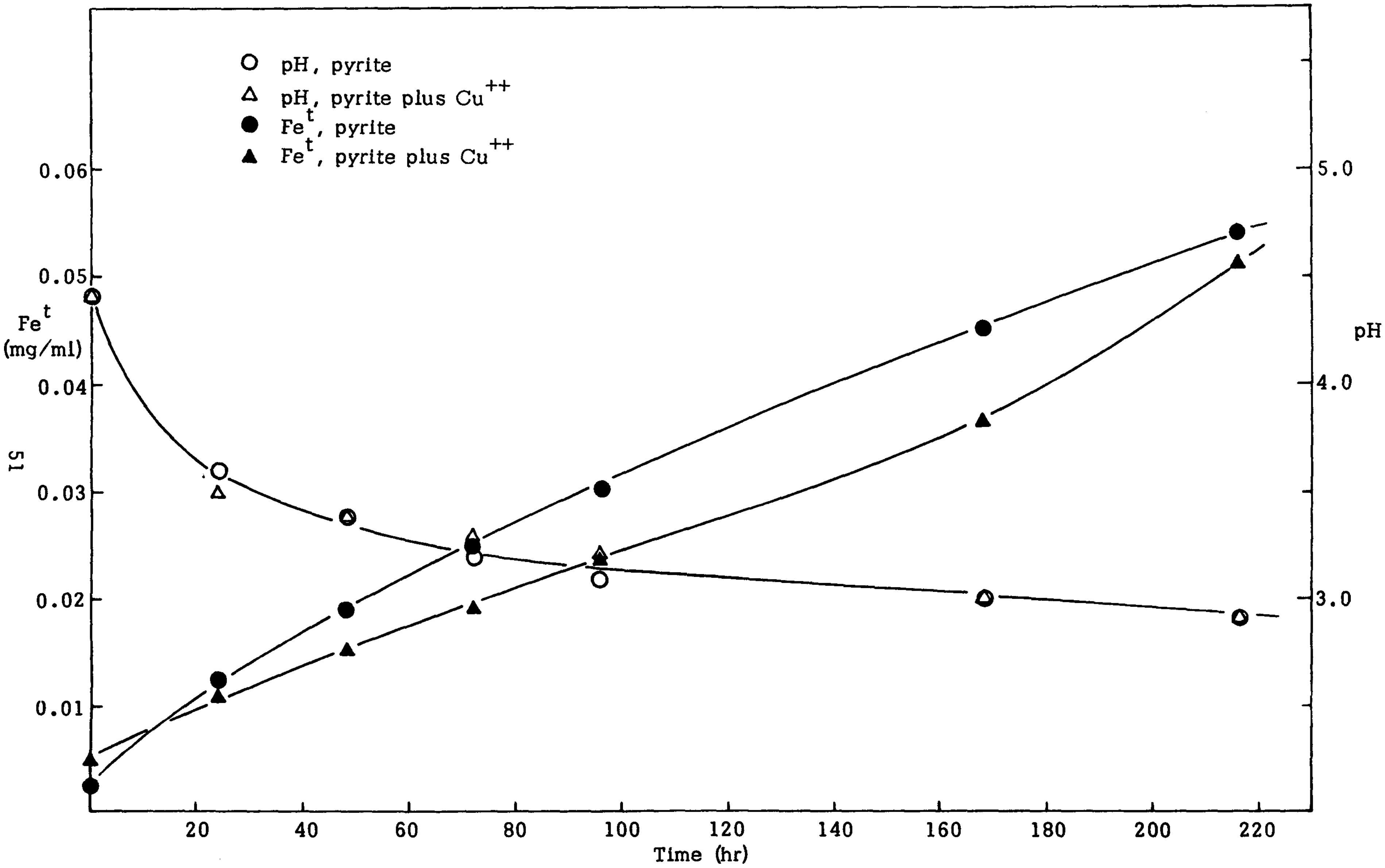


Figure 4: Effect of cupric ion presence upon pyrite solubilization.

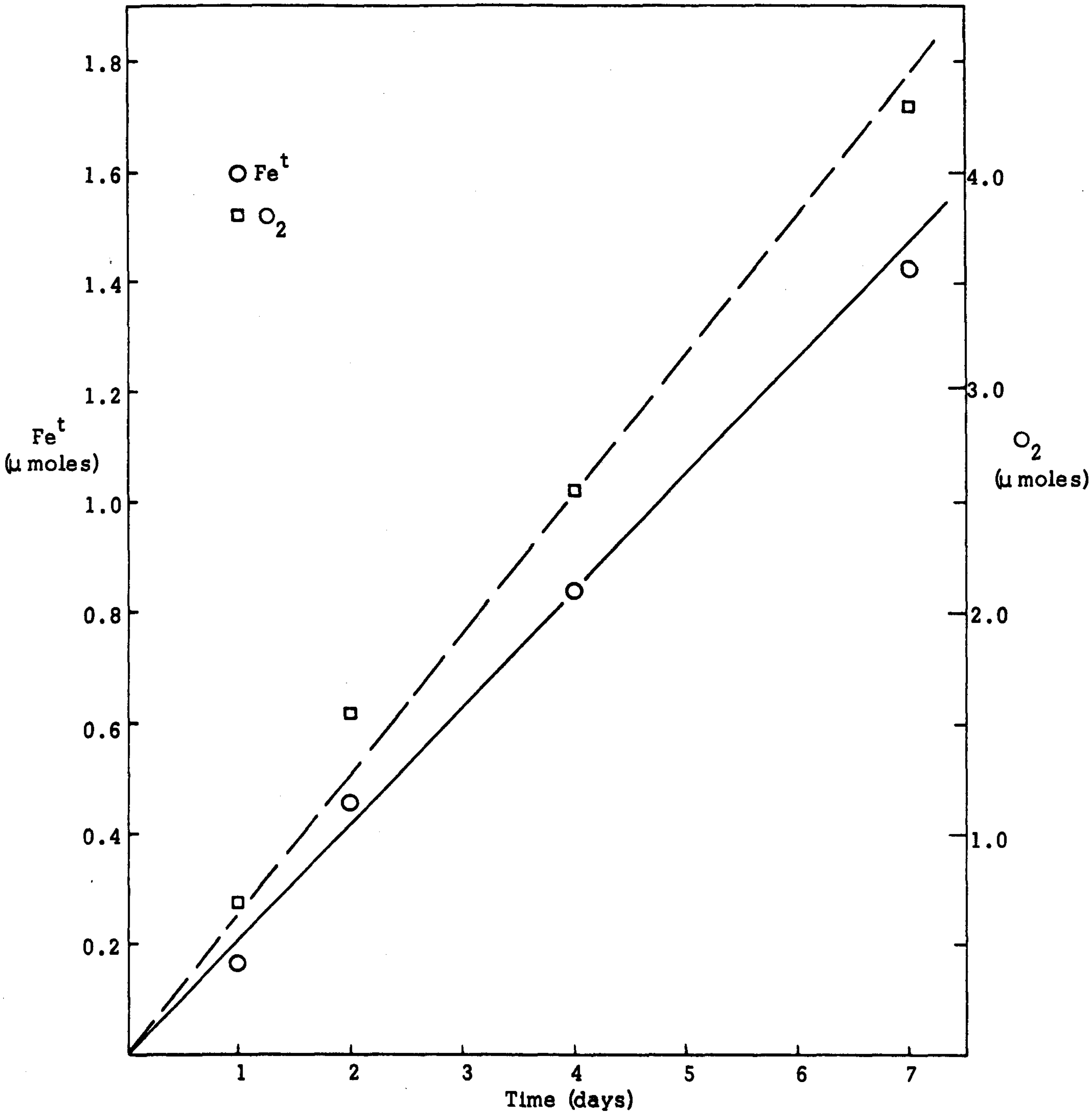


Figure 5: Plot of oxygen uptake and soluble iron production versus time for 25 mg FeS_2/5 ml H_2O at 14°C.

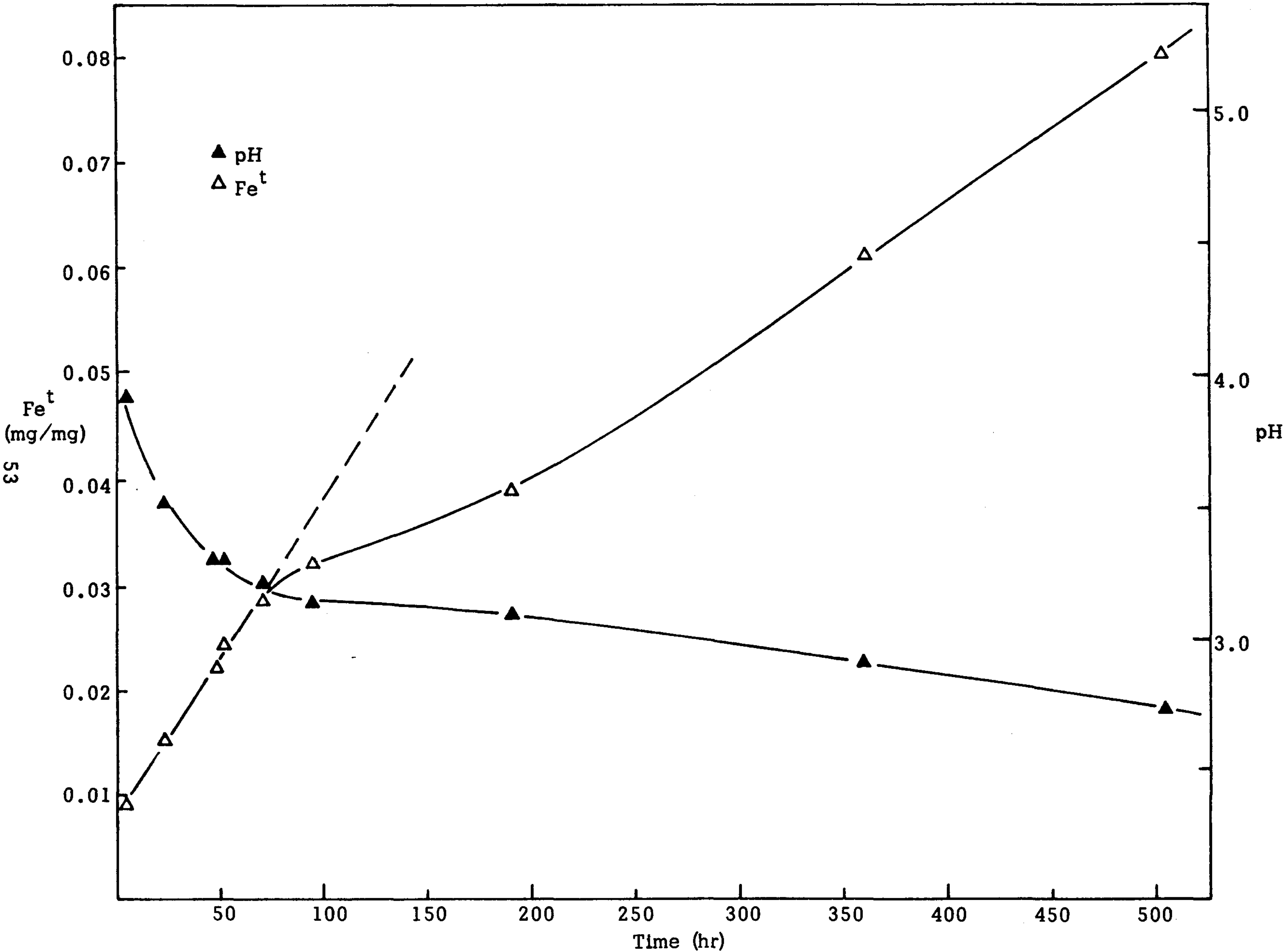


Figure 6: Plot of Fe^t and pH versus time for ~ 500 mg FeS_2/100 ml H_2O.

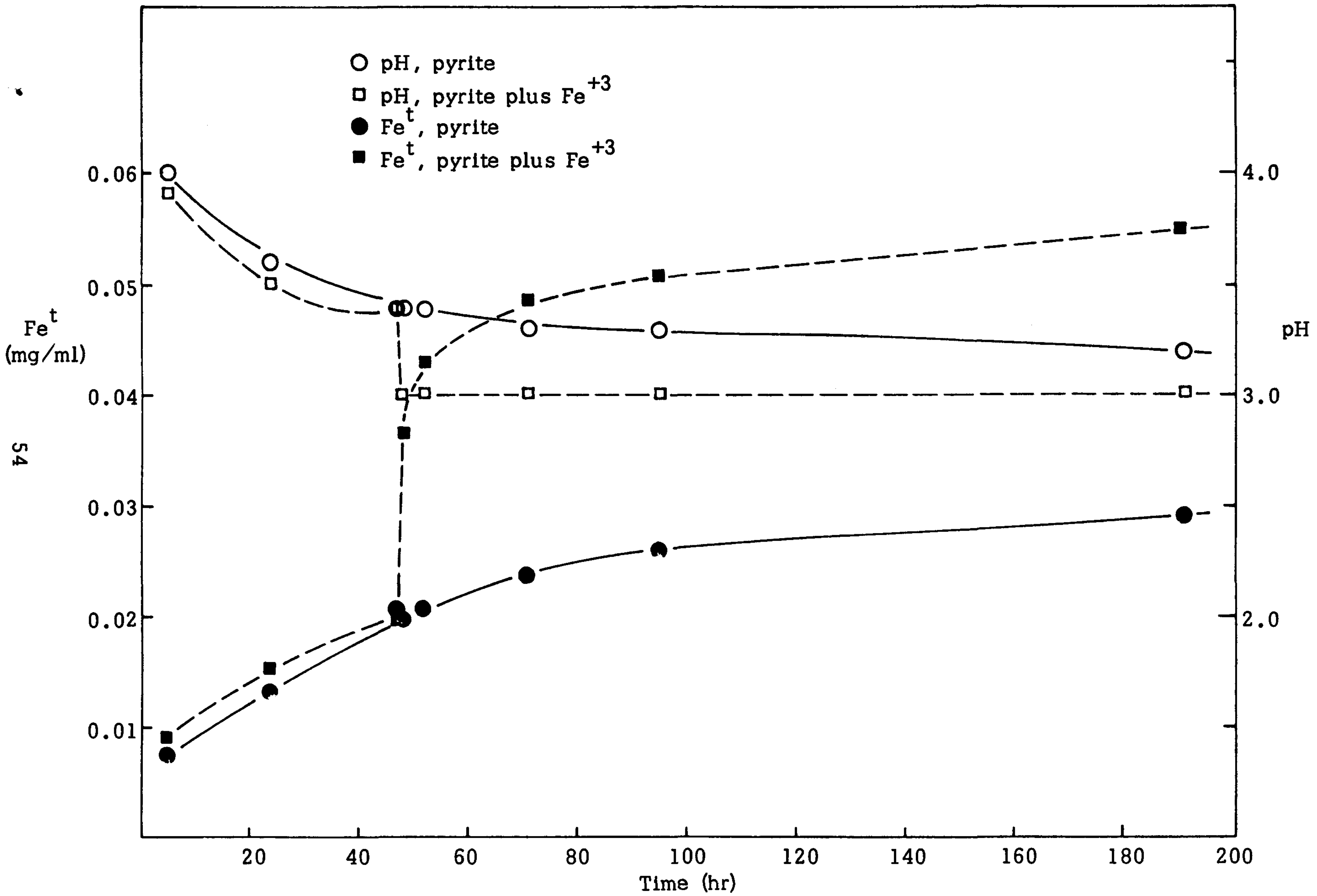


Figure 7: Effect of ferric ion upon pyrite dissolution (low ferric ion concentration).

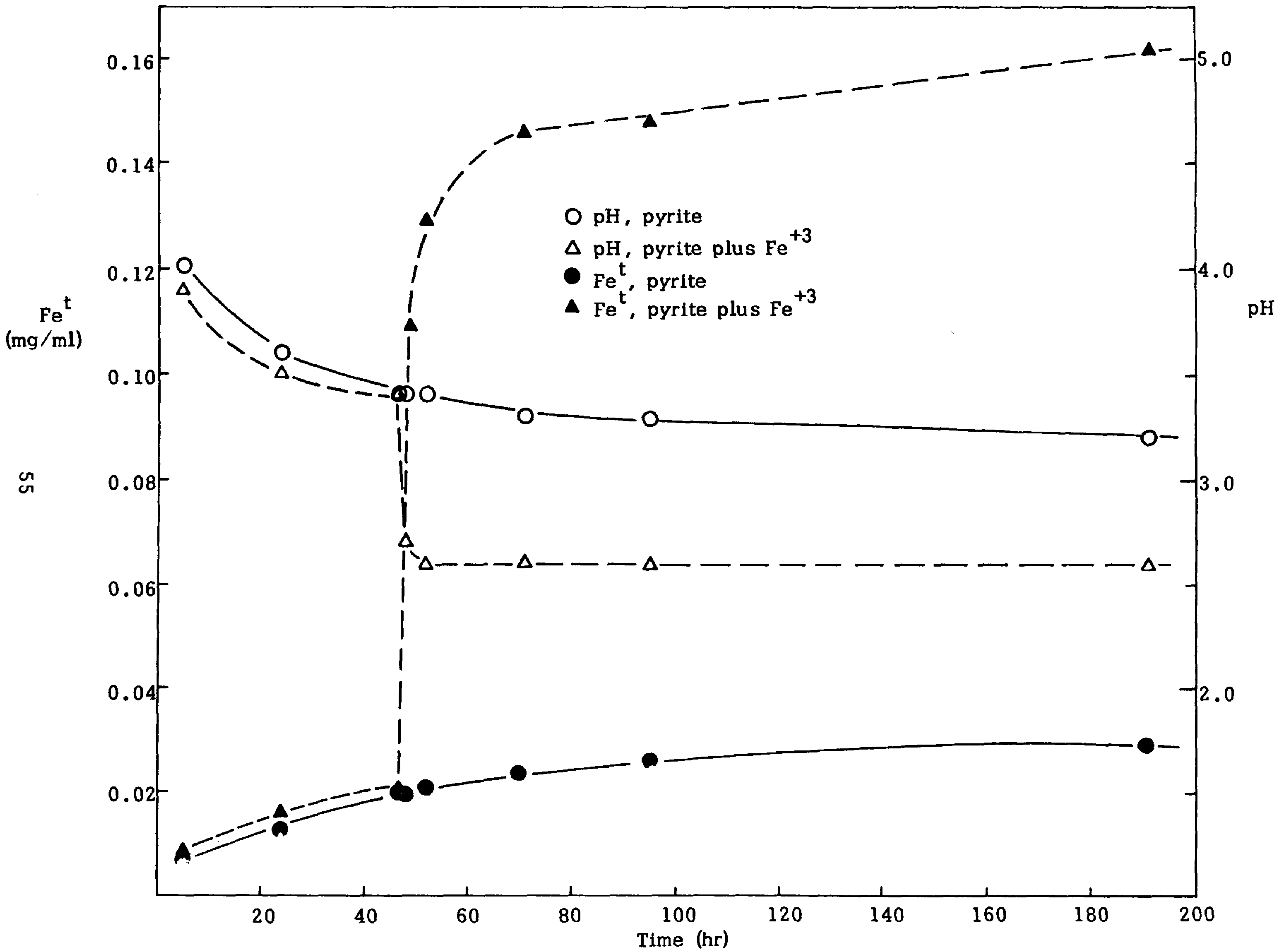


Figure 8: Effect of ferric ion upon pyrite dissolution (high ferric ion concentration).

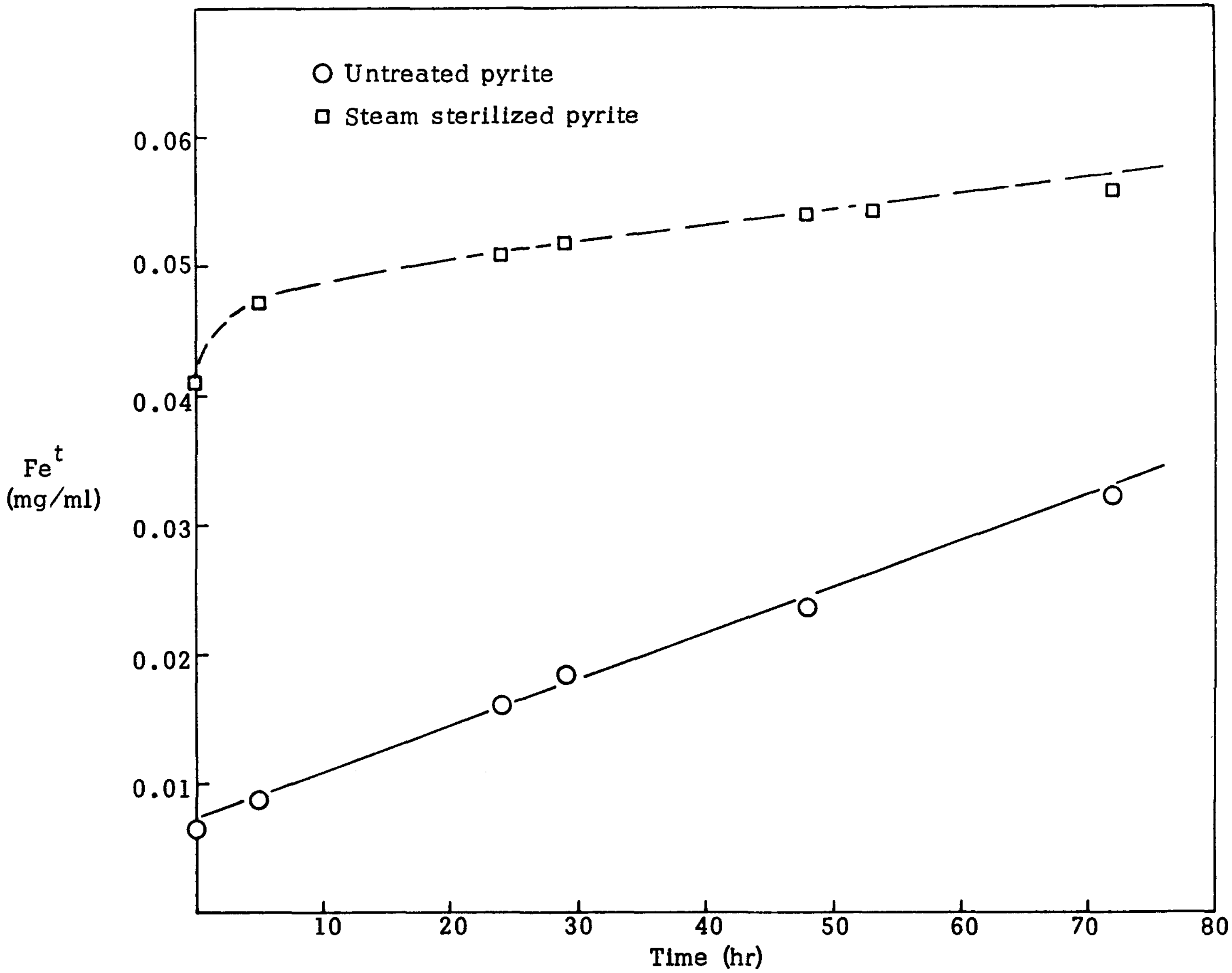


Figure 9: Effect of steam sterilization upon pyrite solubilization.

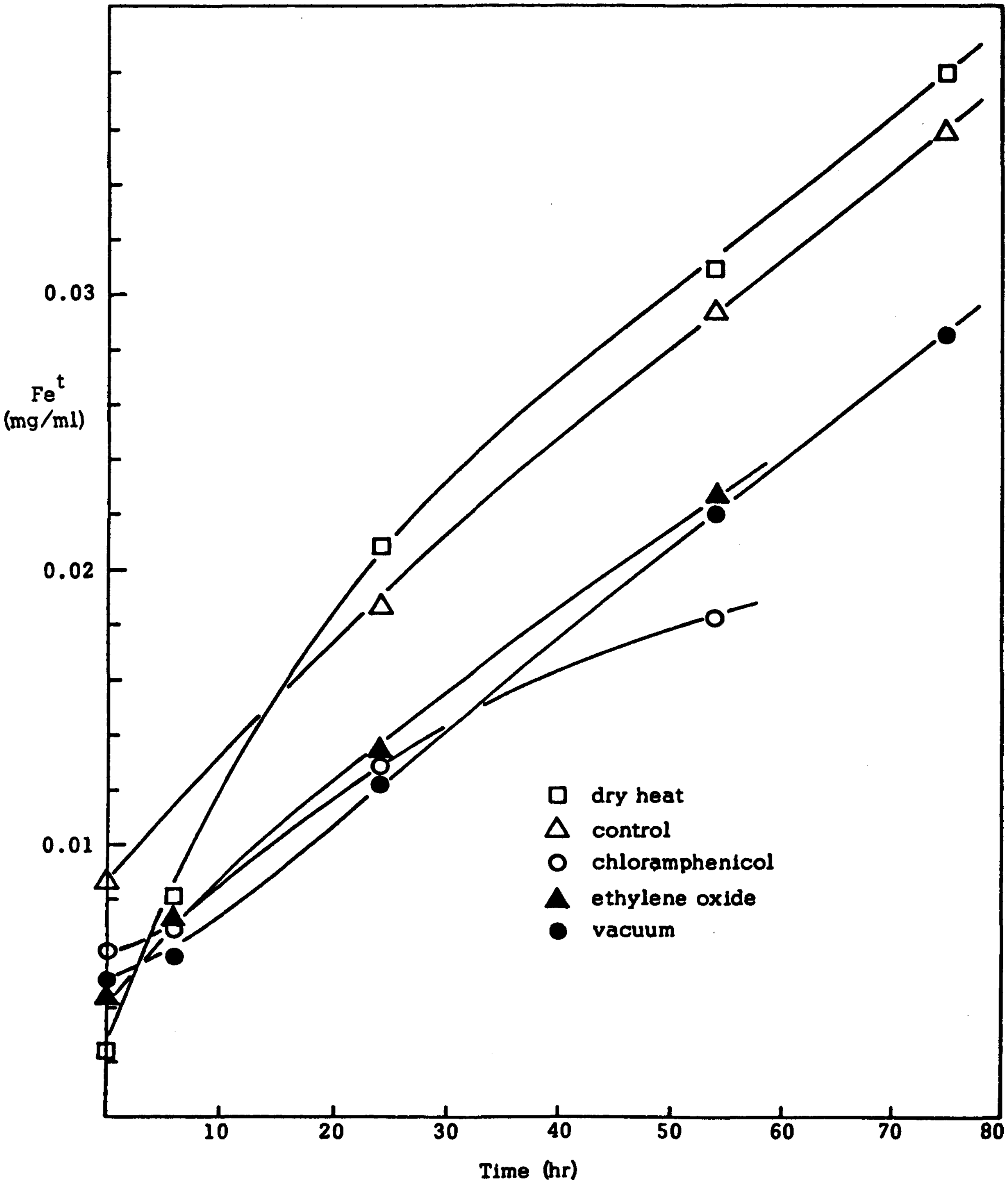


Figure 10: Effect of sterilization procedures upon pyrite dissolution, total iron versus time.

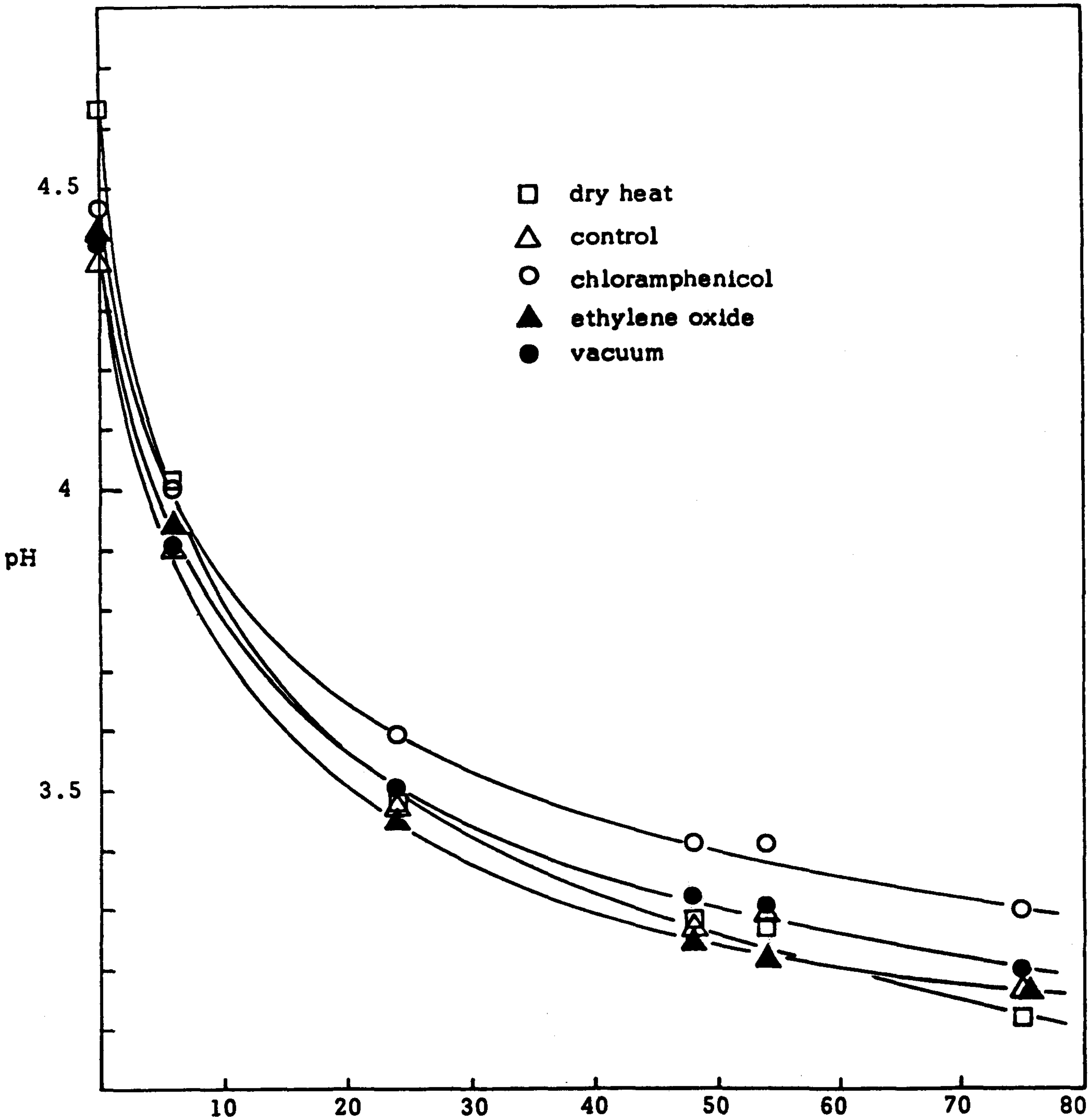


Figure 11: Effect of sterilization procedures upon pyrite dissolution, pH versus time.

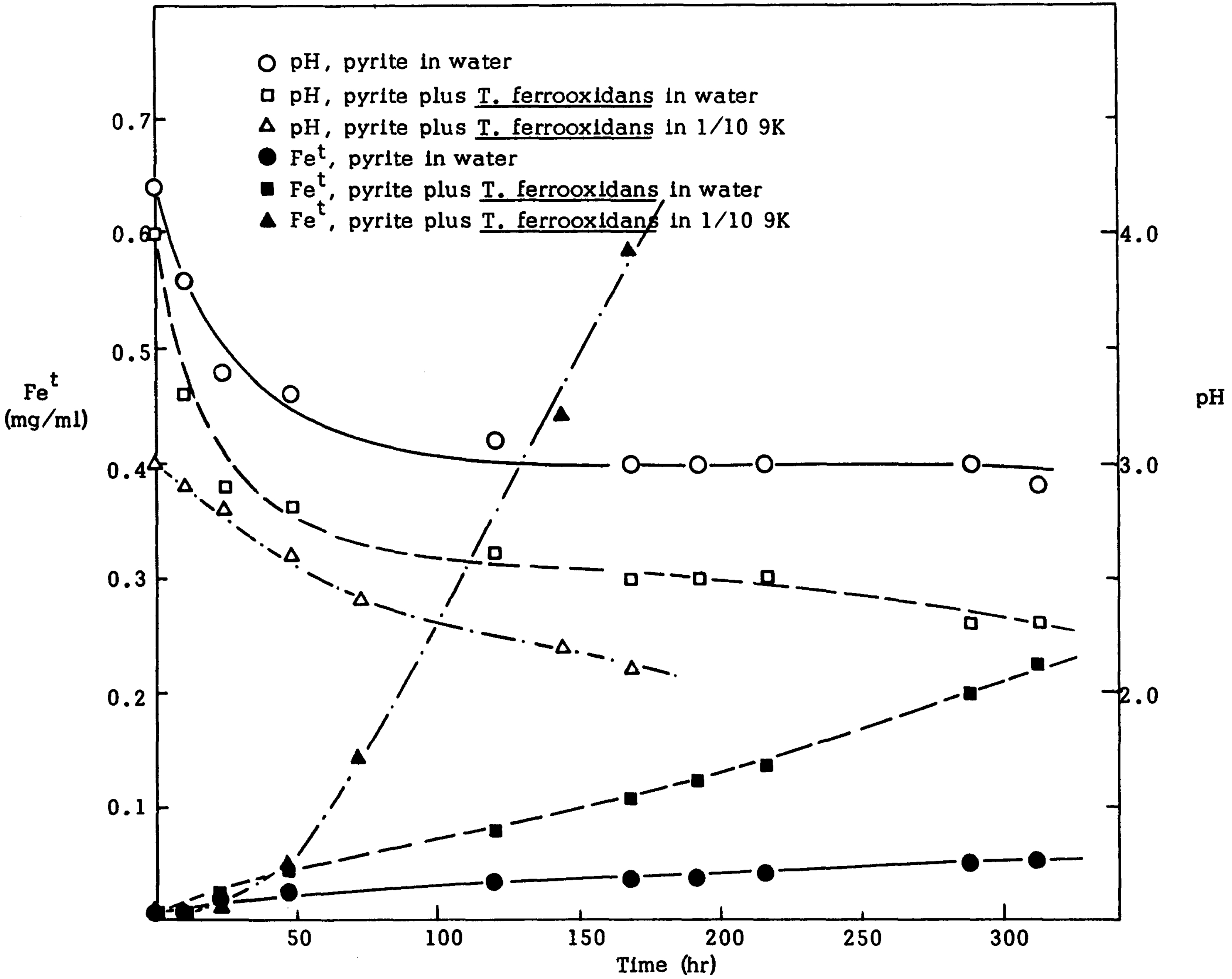


Figure 12: Effect of <u>T. ferrooxidans</u>, ATCC strain, upon pyrite dissolution in various media.

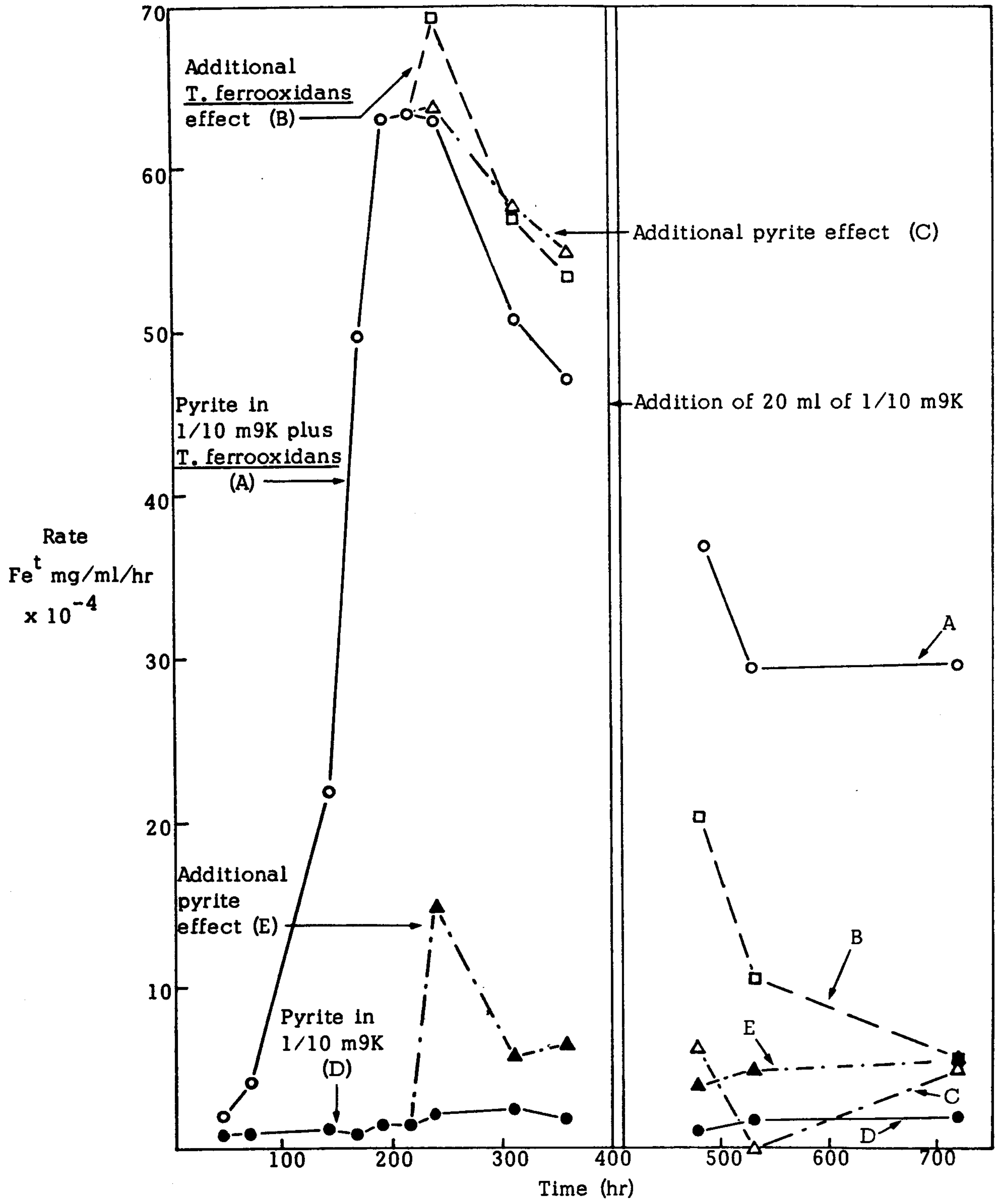


Figure 13: Pyrite solubilization as affected by the presence of T. ferrooxidans, changes in pyrite, and T. ferrooxidans, ATCC strain, concentrations.

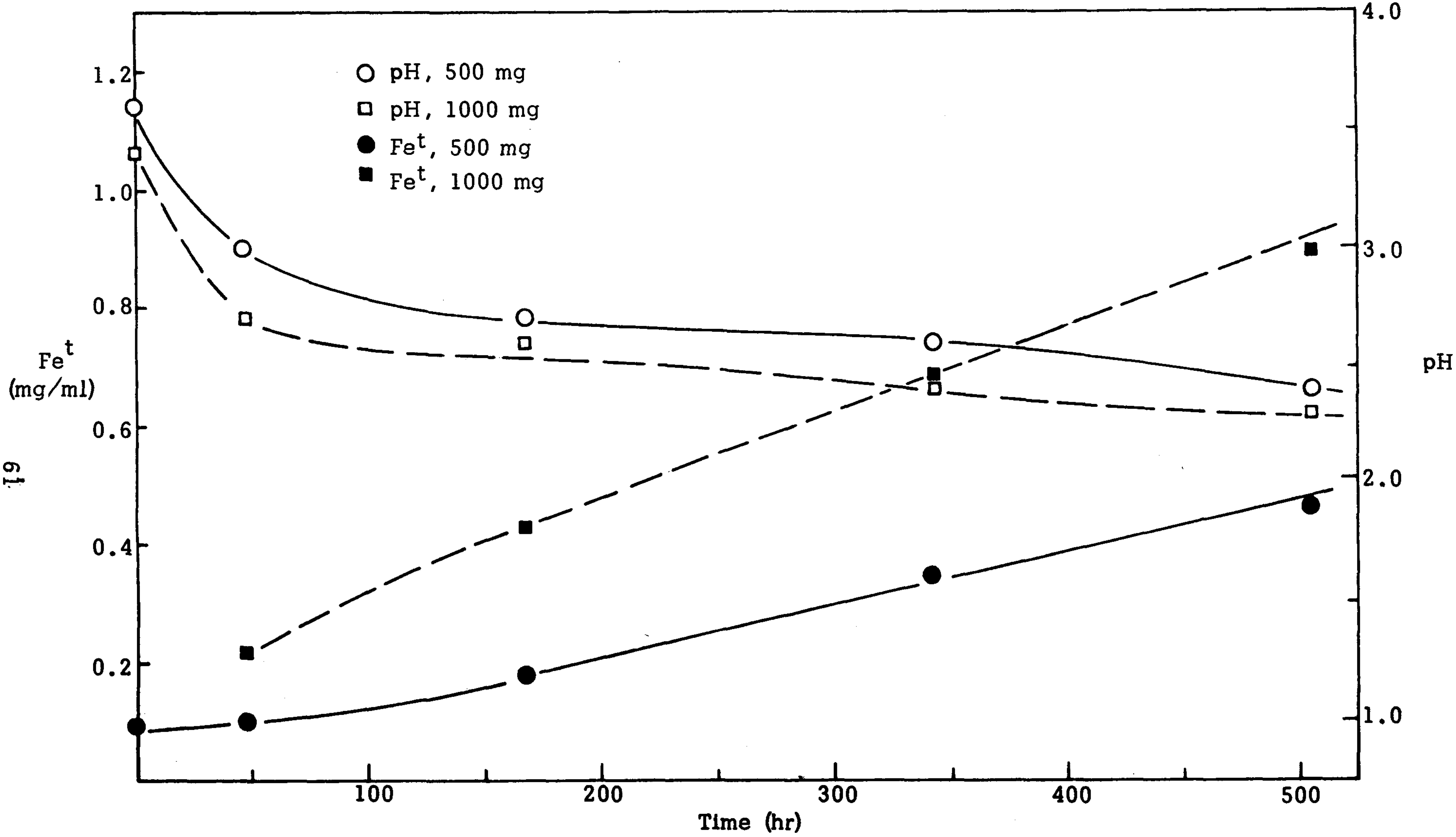


Figure 14: Changes in pH and soluble iron production as a function of time for different quantities of marcasite suspended in 100 ml of water.

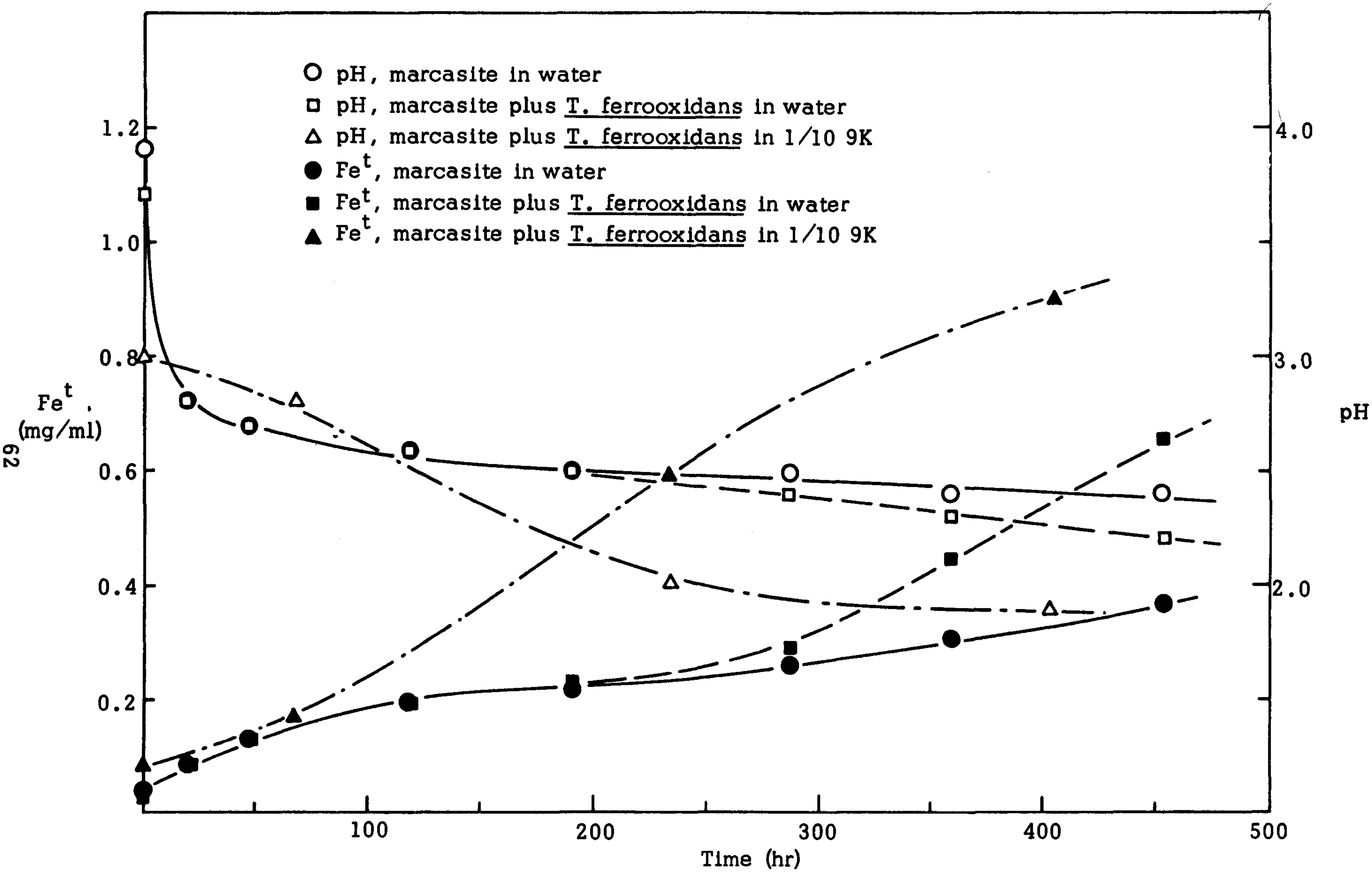


Figure 15: Effect of <u>T. ferrooxidans</u>, ATCC strain, upon marcasite dissolution in different media.

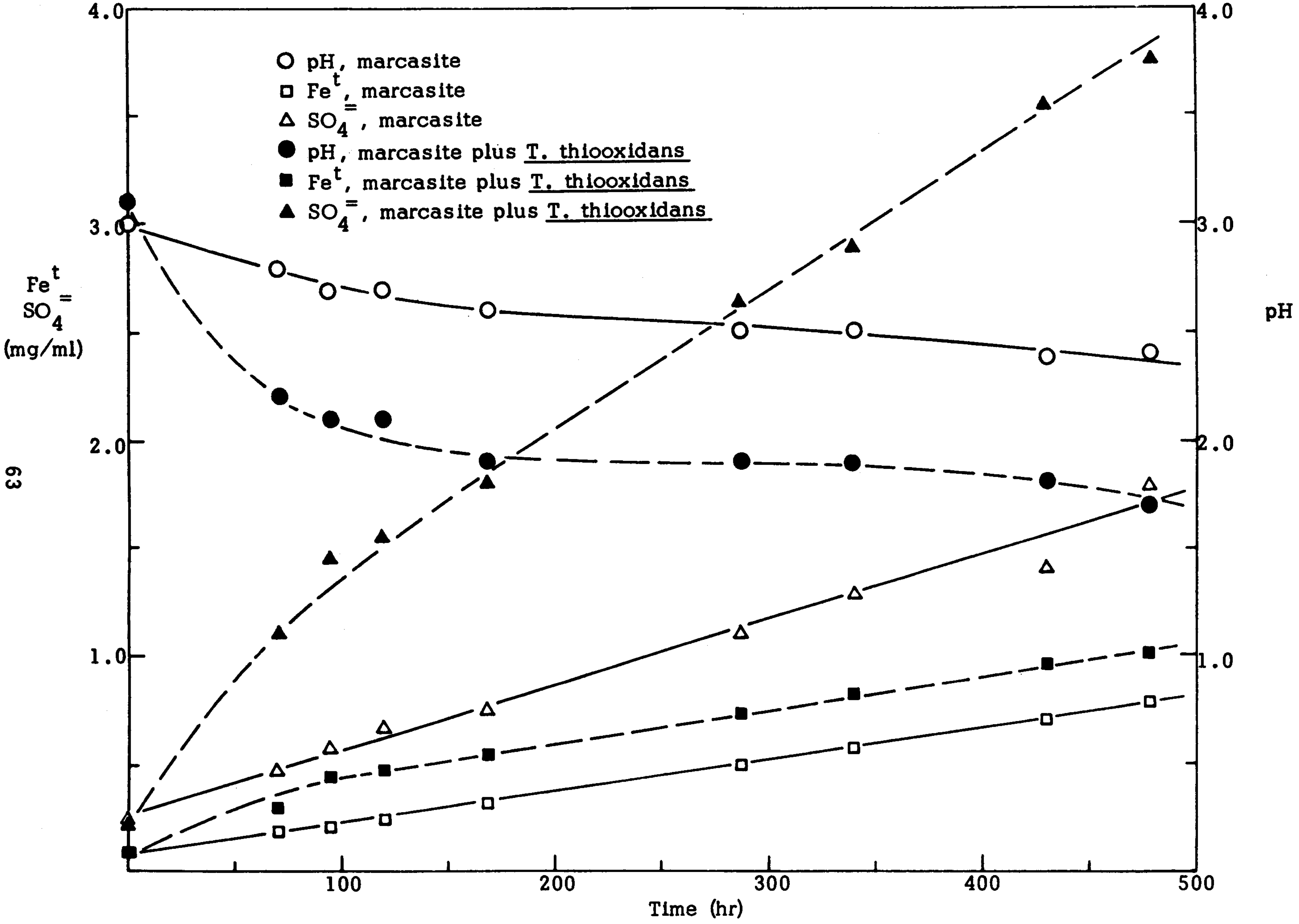


Figure 16: Effect of T. thiooxidans, ATCC strain, upon marcasite dissolution.

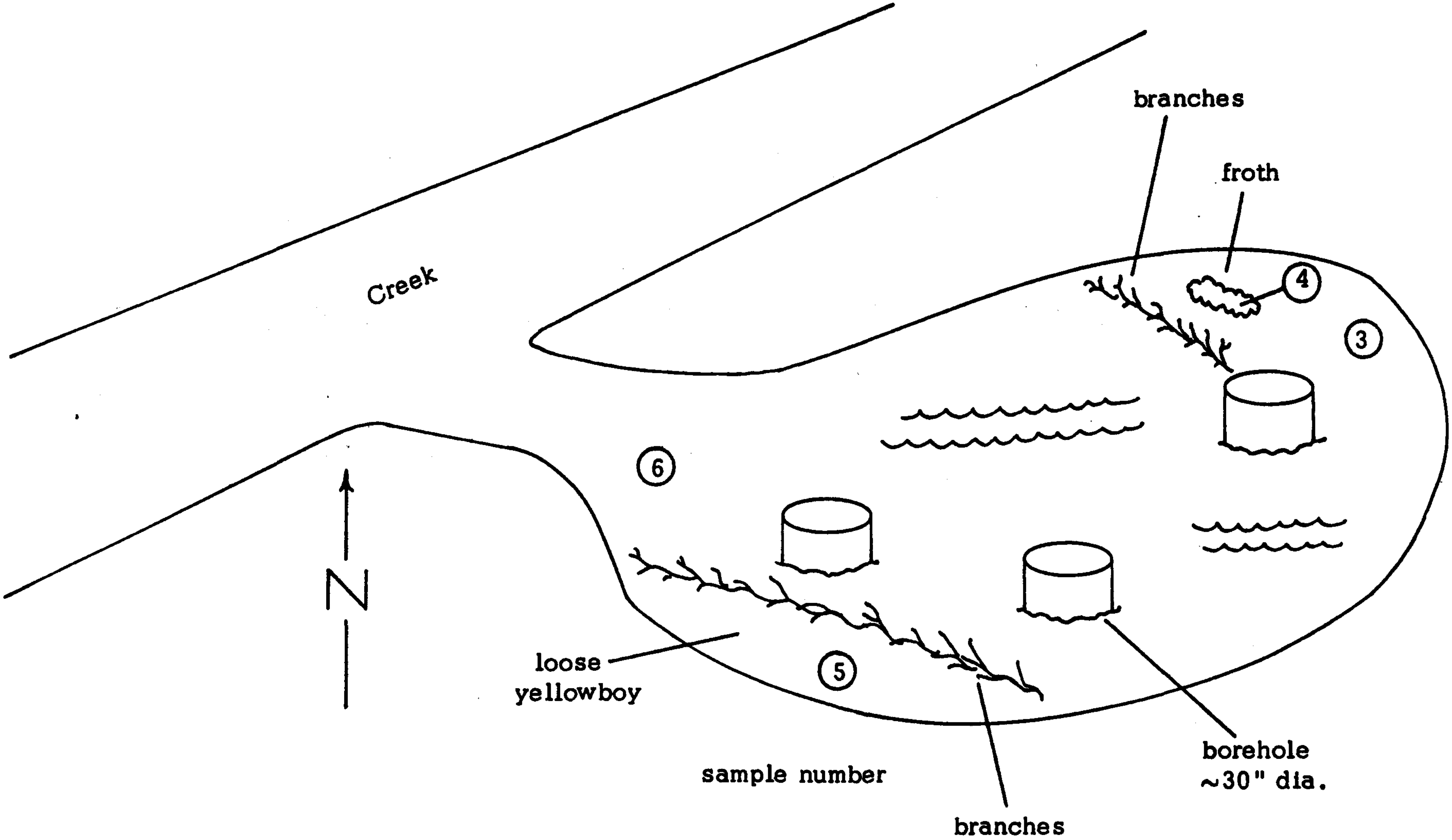


Figure 17: Diagram of sampling spots from bore hole overflow pond.

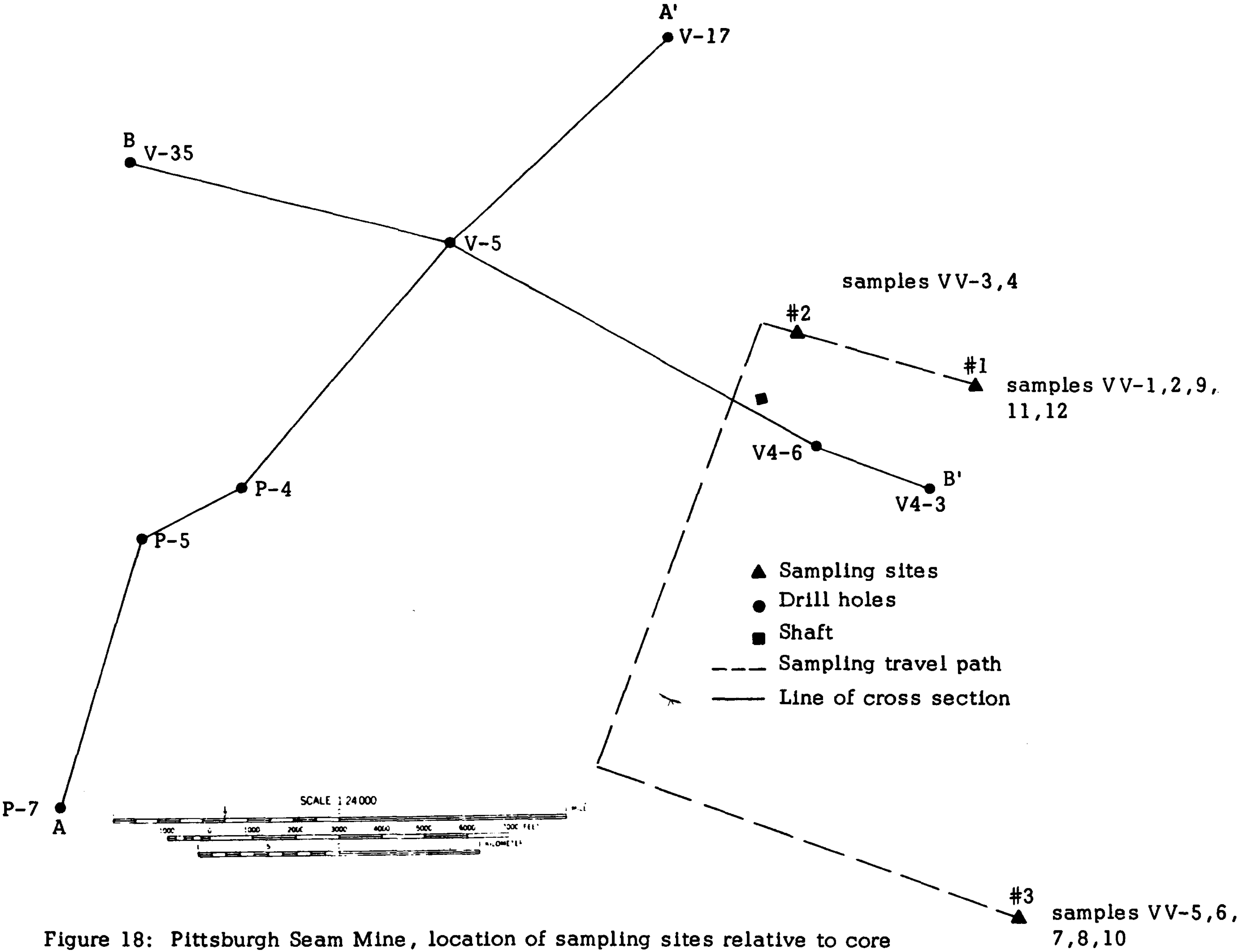


Figure 18: Pittsburgh Seam Mine, location of sampling sites relative to core bore holes.

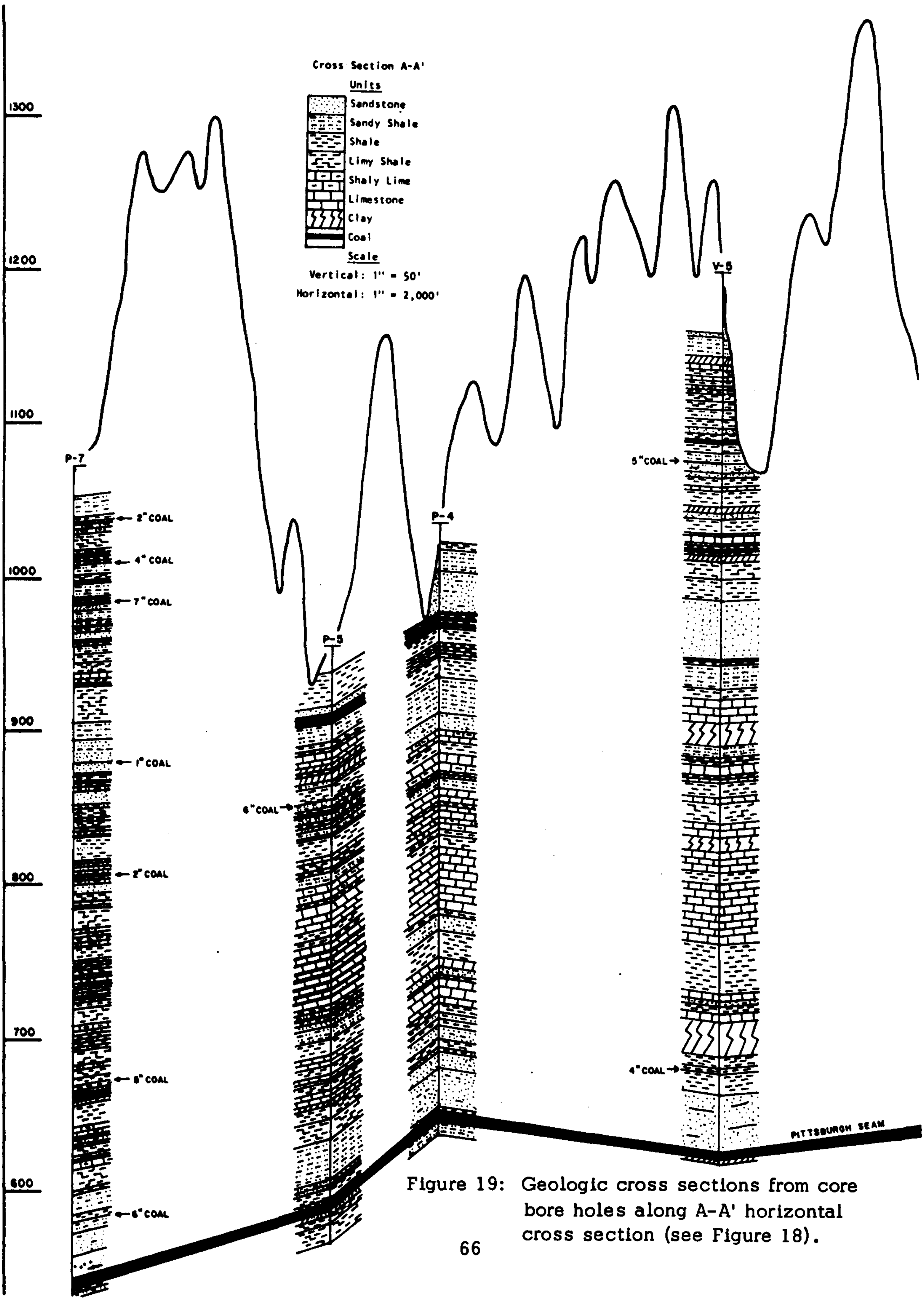


Figure 19: Geologic cross sections from core bore holes along A-A' horizontal cross section (see Figure 18).

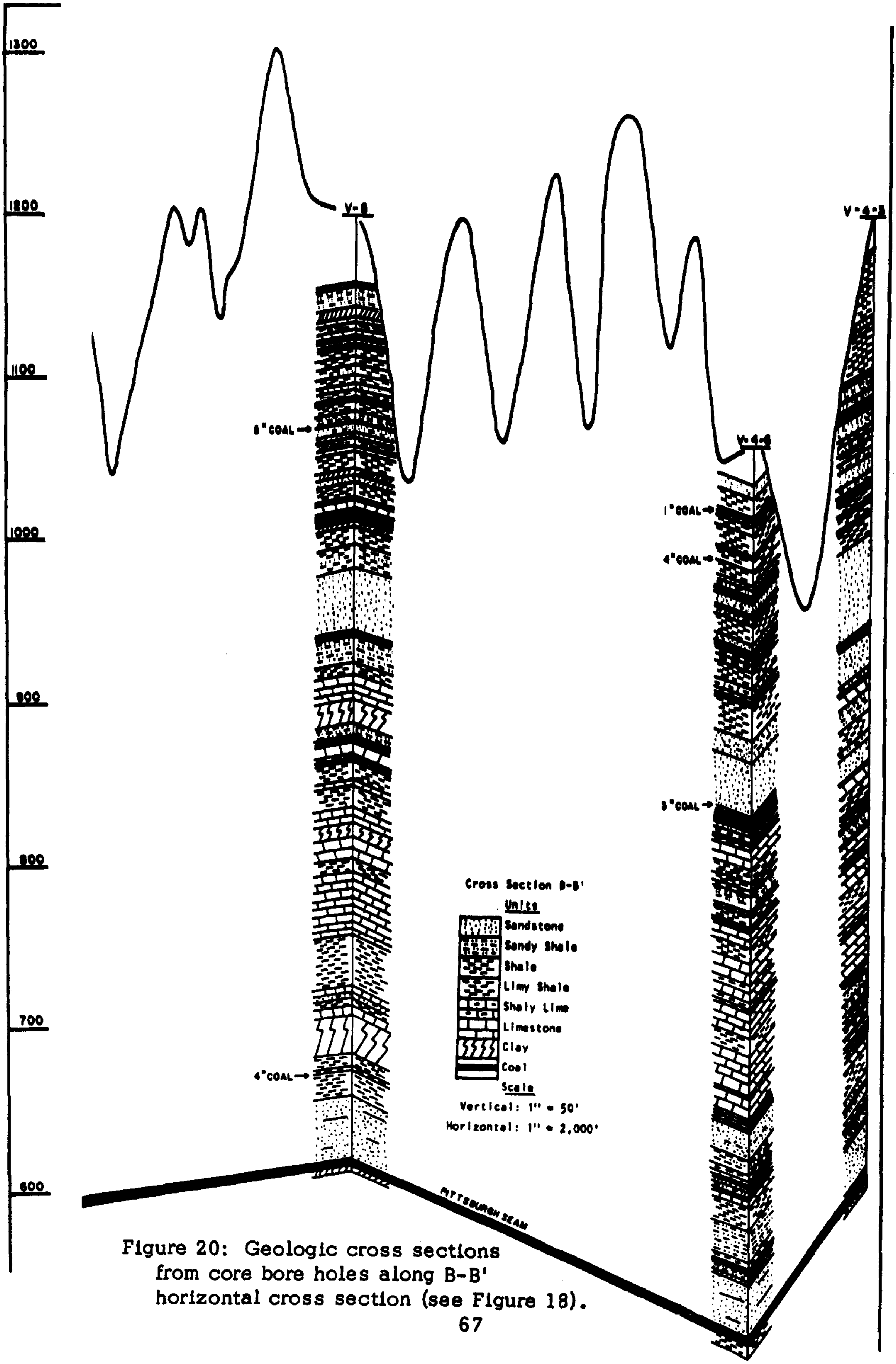
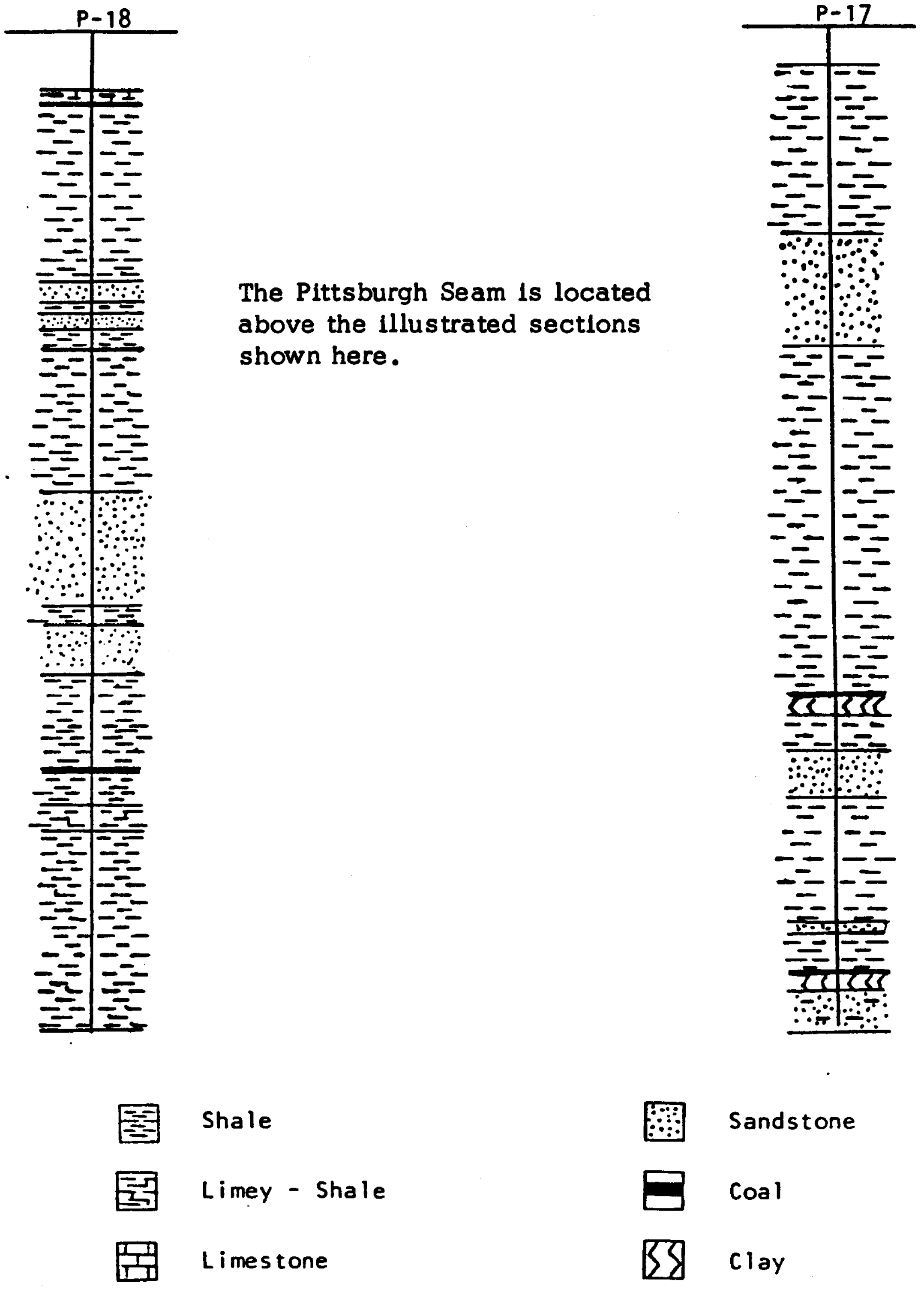


Figure 20: Geologic cross sections from core bore holes along B-B' horizontal cross section (see Figure 18).



The Pittsburgh Seam is located above the illustrated sections shown here.

Figure 21: Core bore hole logs below Pittsburgh seam in the vicinity of the area shown in Figure 18.

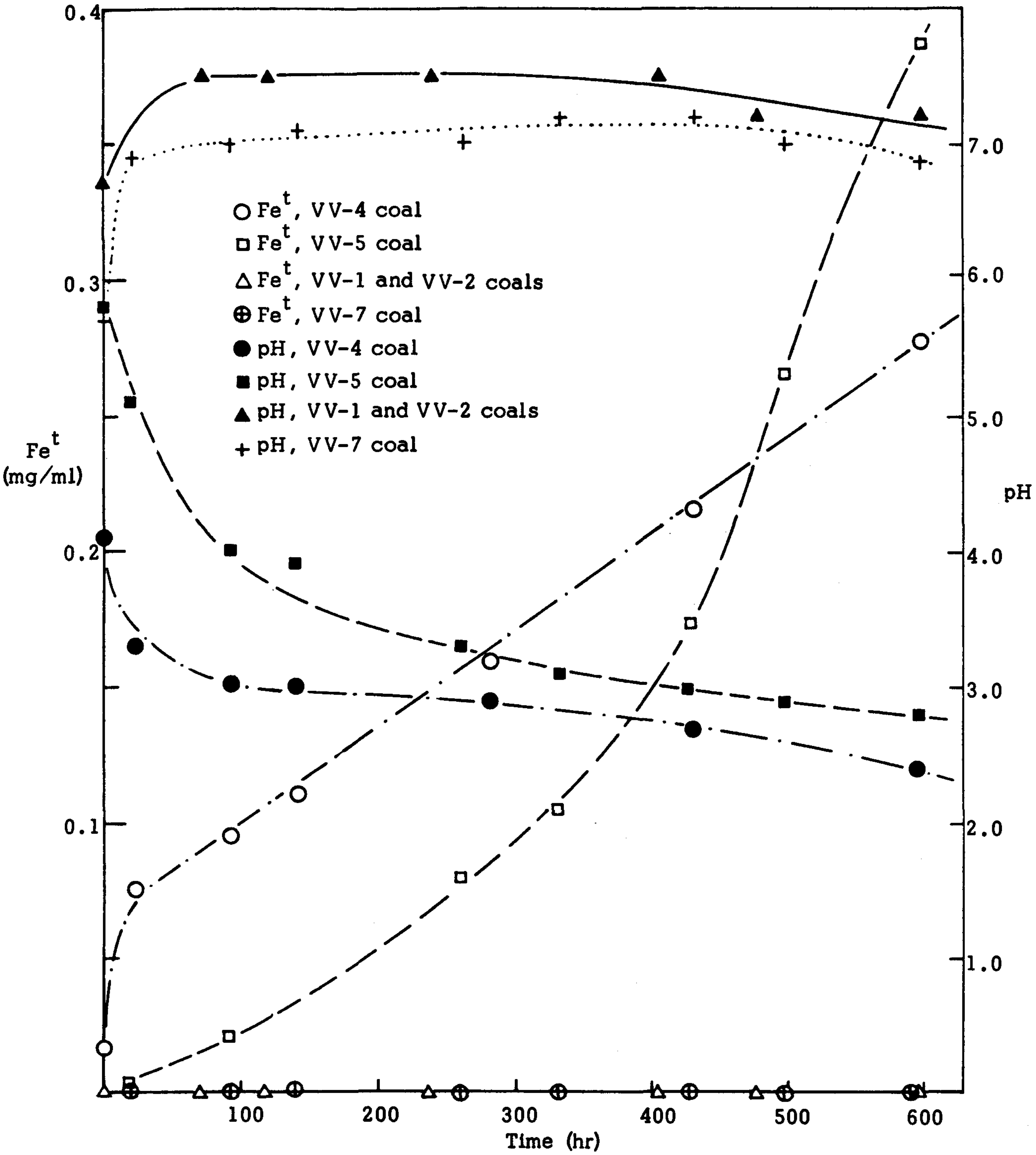


Figure 22: pH drop and soluble iron production as a function of time for coals obtained from different locations of VV-mine.

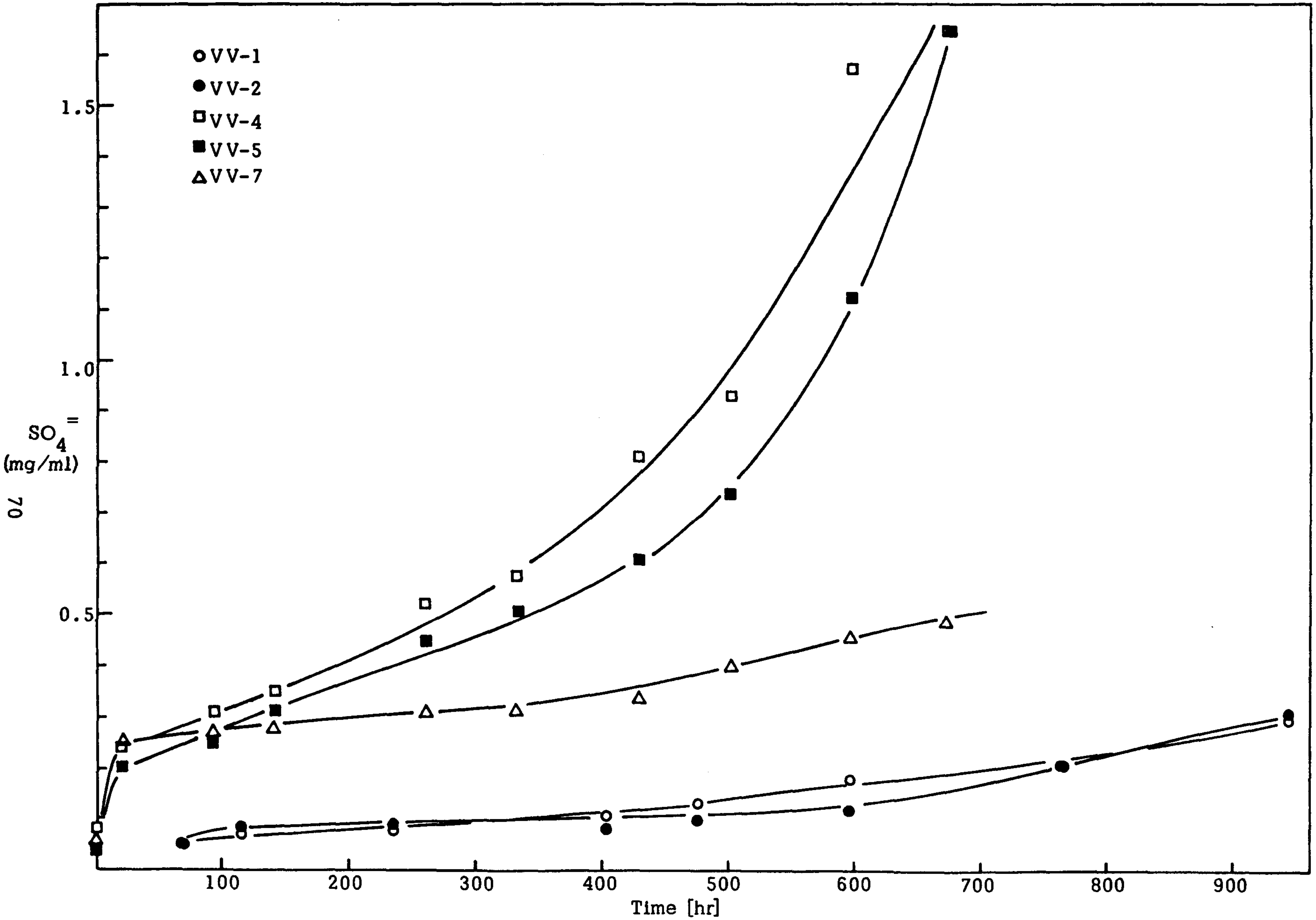


Figure 23: Soluble sulfate production from coals obtained from different locations of VV-mine.

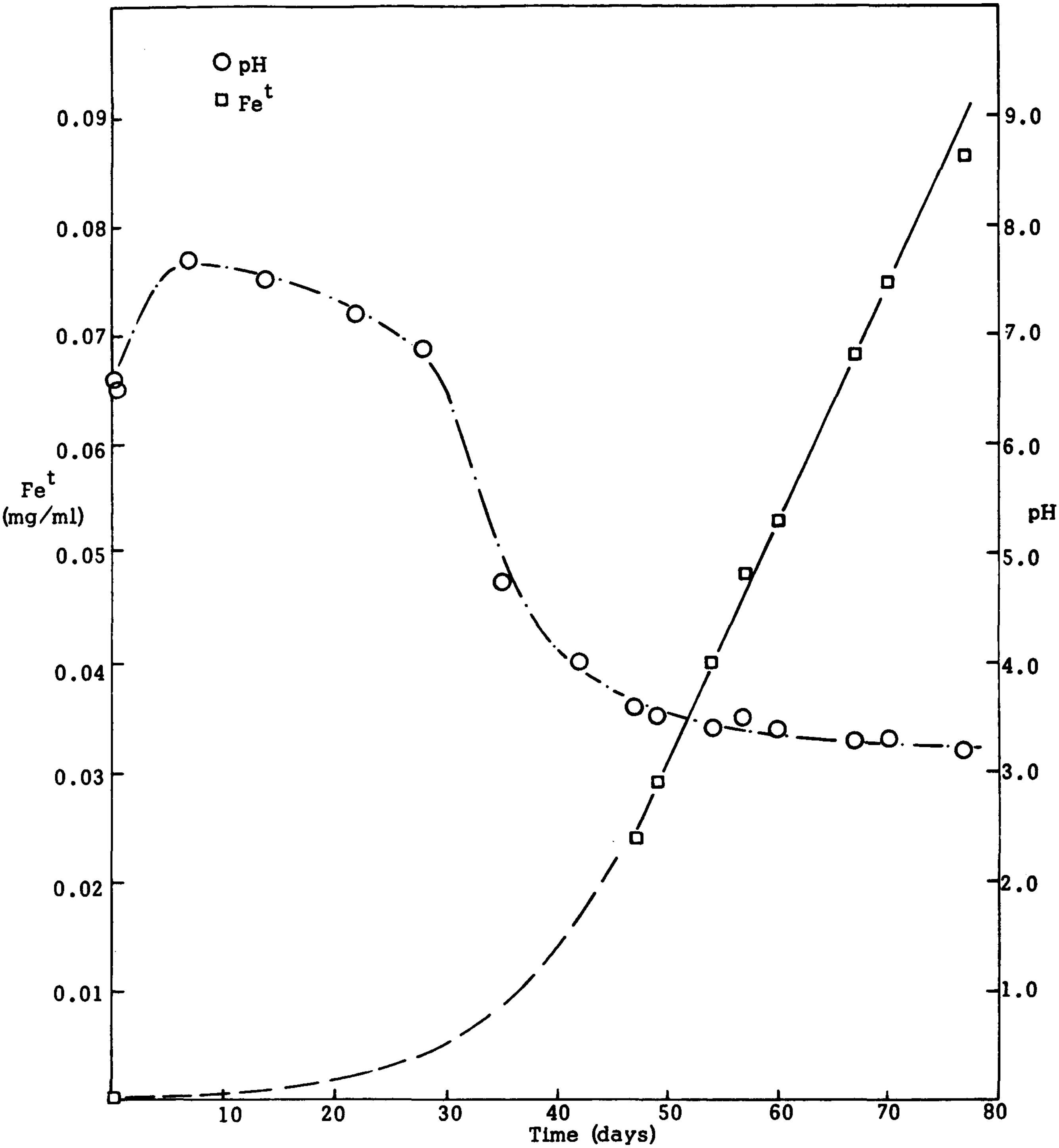


Figure 24: Effect of prolonged exposure of VV-1 coal to water and air.

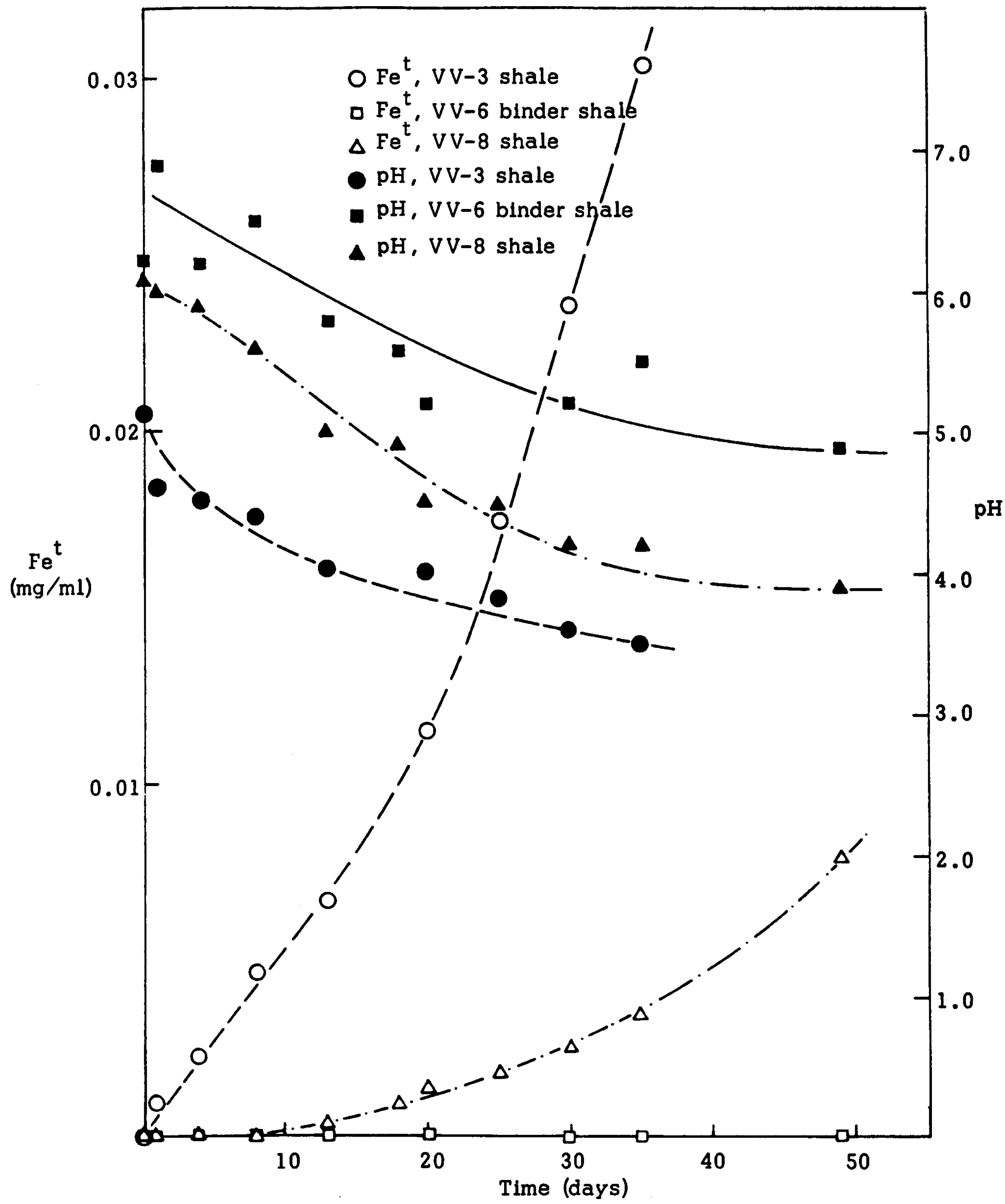


Figure 25: pH drop and soluble iron production as a function of time for shales and binders obtained from different locations of VV-mine.

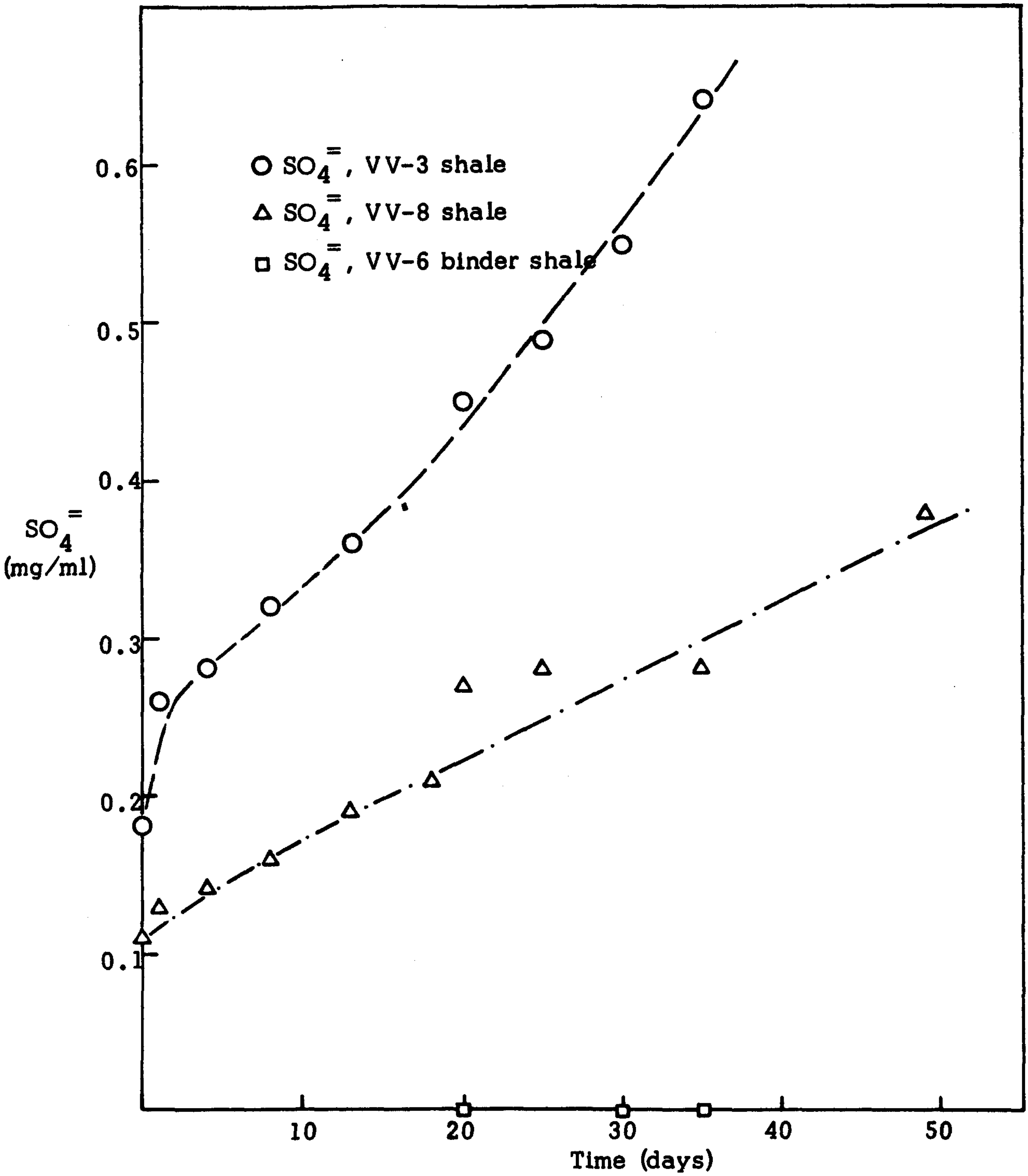


Figure 26: Sulfate ion production as a function of time for shale and binders obtained from different locations of VV-mine.

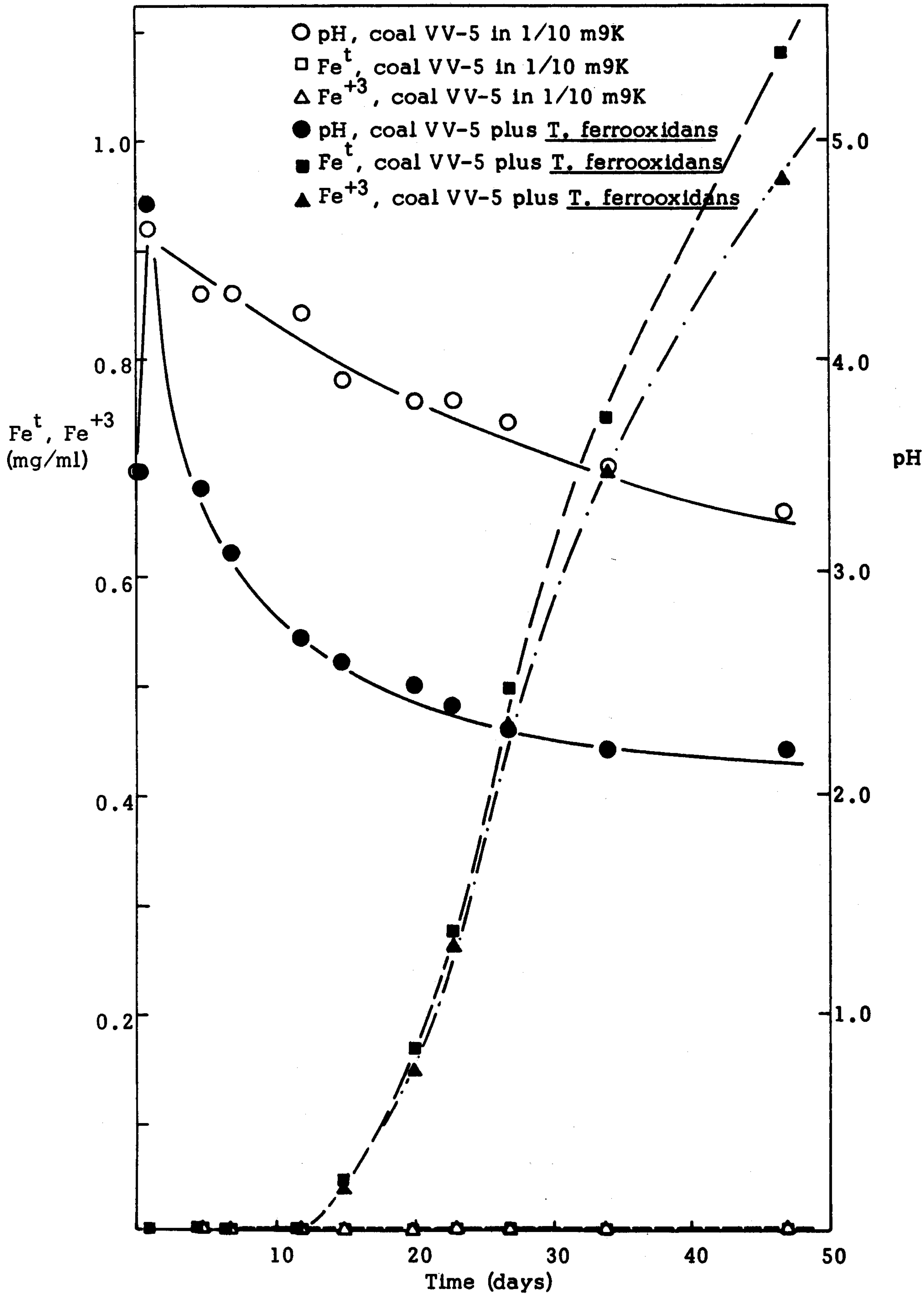


Figure 27: Effect of *T. ferrooxidans*, ATCC strain, upon coal solubilization.

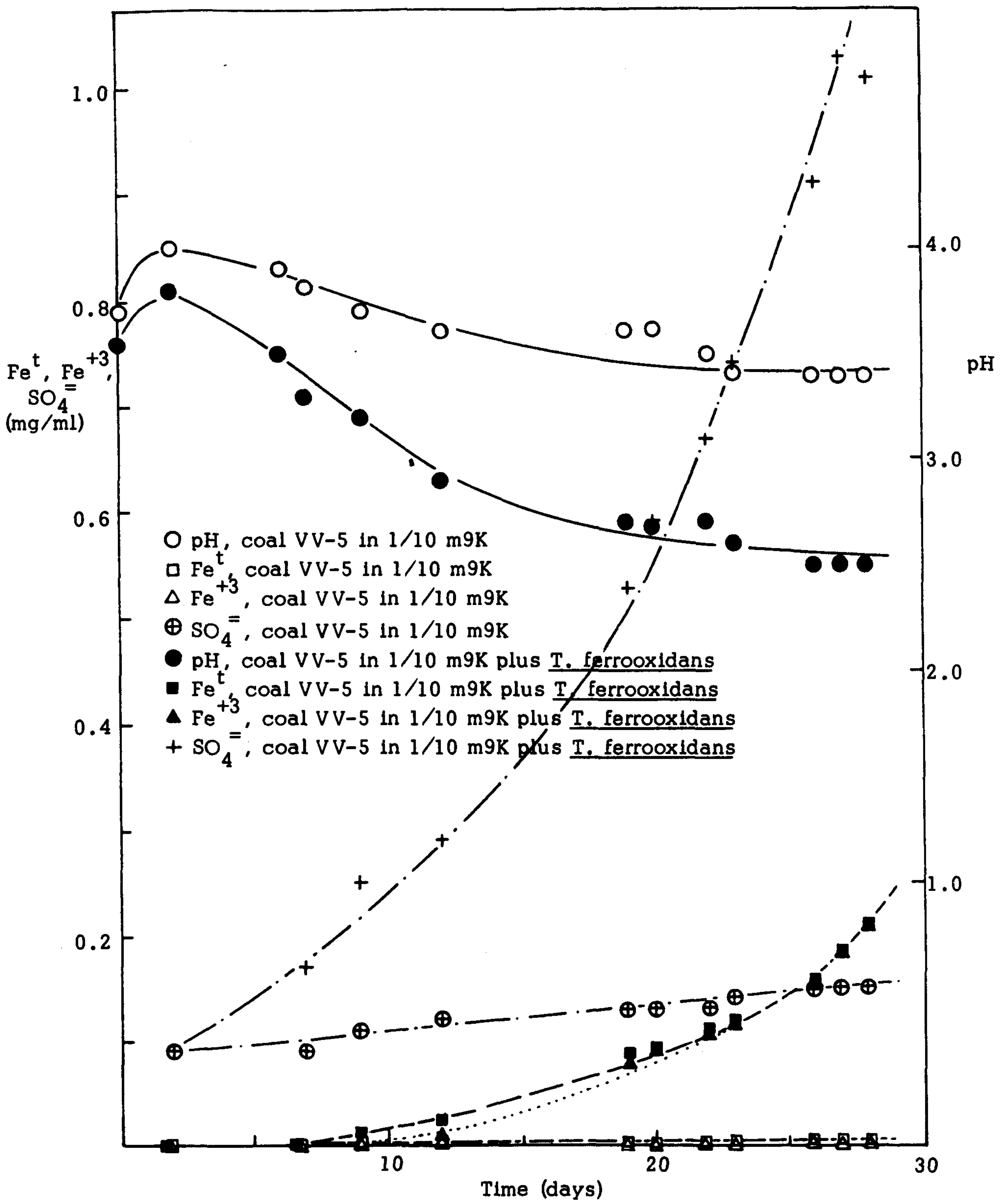


Figure 28: Effect of T. ferrooxidans, mine isolated strain, upon coal solubilization.

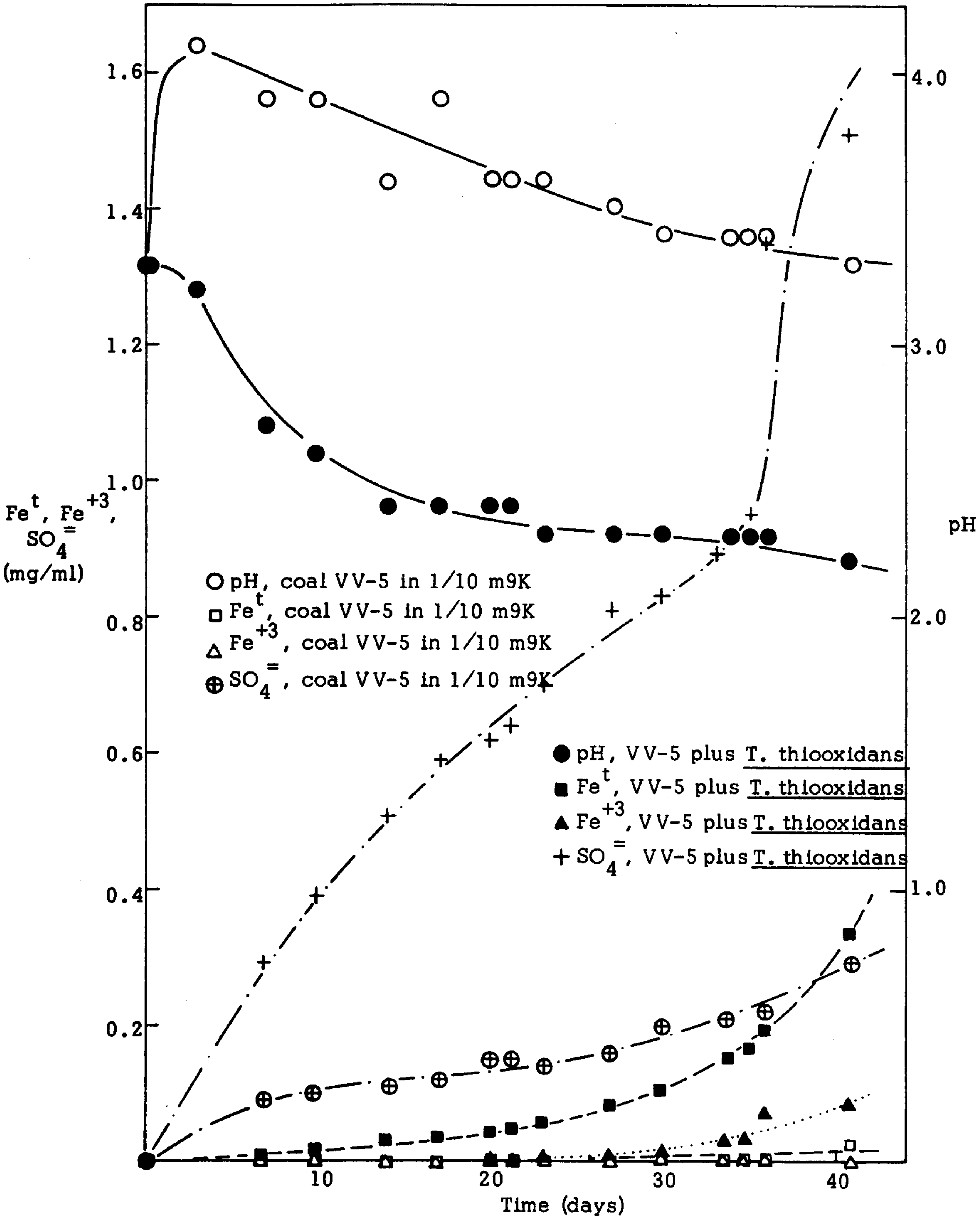


Figure 29: Effect of <u>T. thiooxidans</u>, ATCC strain, upon coal solubilization.

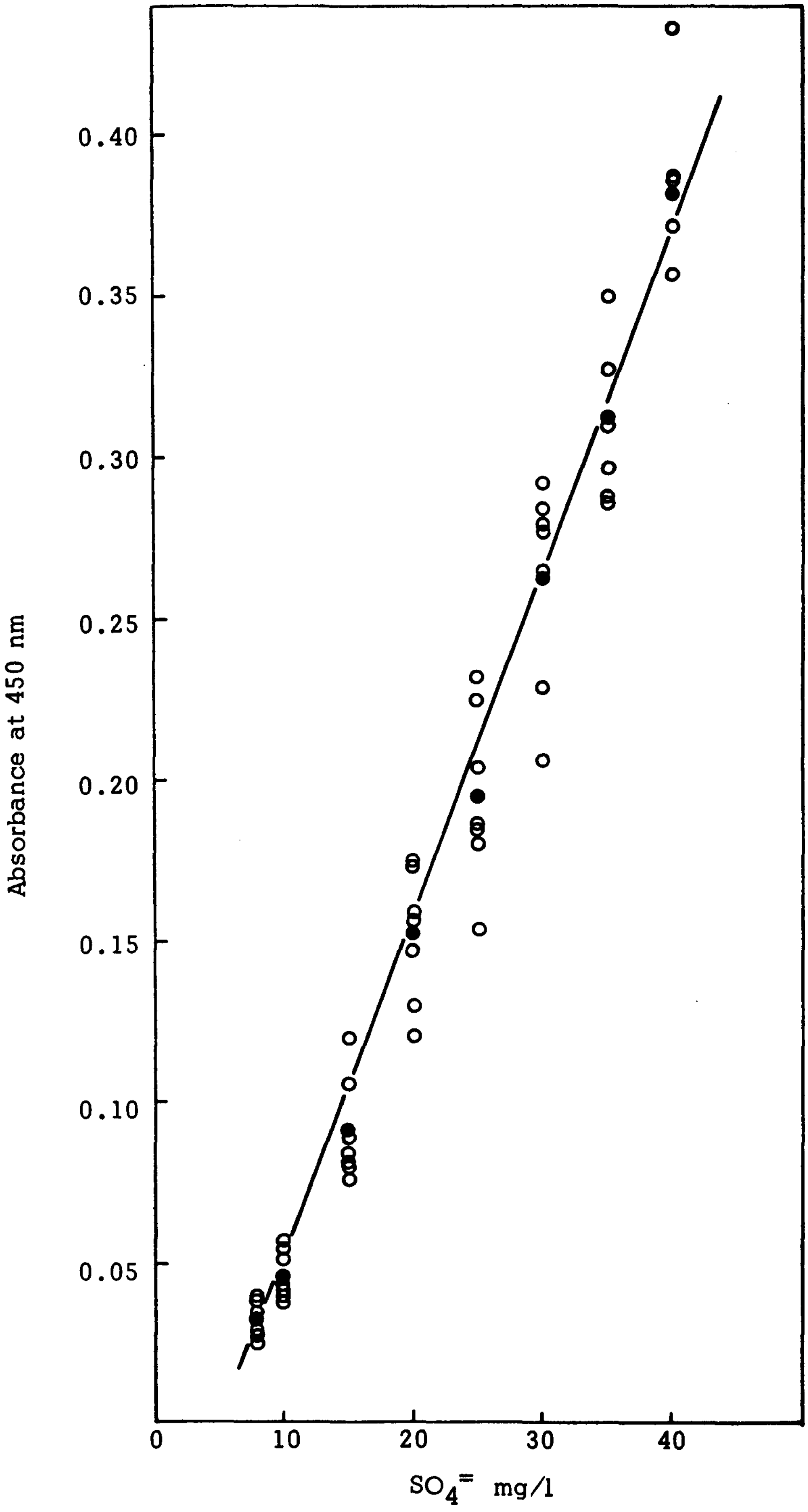


Figure 30: Calibration curve for sulfate determinations, turbidity method.

TABLE I

CHANGES IN pH, TOTAL SOLUBLE IRON CONCENTRATION AND RATES OF SOLUBLE IRON PRODUCTION AS A FUNCTION OF TIME USING A CONSTANT PYRITE TO WATER RATIO[a]

(Pyrite, water)

Time Interval hr	Series D			Series E			Series G			Series H			Series K			Series L		
	pH	$Fe^{t,b}$ 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	pH	Fe^t 10^{-3}	Rate Fe^t 10^{-4}	pH	Fe^t 10^{-3}	Rate Fe^t 10^{-4}	pH	Fe^t 10^{-3}	Rate Fe^t 10^{-4}	pH	Fe^t 10^{-3}	Rate Fe^t 10^{-4}	pH	Fe^t 10^{-3}	Rate Fe^t 10^{-4}
0	4.2	6.8	-	4.1	6.2	-	4.2	5.9	-	-	-	-	4.3	5.3	-	4.2	4.4	-
5-0	3.9	8.6	-	3.7	8.8	5.2	3.9	8.0	4.2	3.9	8.1	-	3.9	9.3	8.0	3.8	7.0	5.2
24-5	3.5	14.6	3.1	3.4	16.0	3.8	3.7	11.7	1.9	3.6	14.2	3.2	3.5	15.3	3.3	3.4	19.1	6.3
48-24	3.3	23.7	3.8	3.2	23.7	3.2	n.d.[d]	n.d.	-	3.4	21.2	2.9	n.d.	n.d.	-	3.3	26.3	3.0
72-48	3.3	24.8	0.5	3.1	32.1	3.5	3.7	15.0	-	3.3	26.2	2.1	n.d.	n.d.	-	n.d.	n.d.	-
96-72	3.2	26.4	0.7	n.d.	n.d.	-	3.5	16.9	0.8	3.2	29.2	1.2	3.1	26.3	1.5	n.d.	n.d.	-
120-48	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	3.2	32.3	0.8
120-96	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	3.1	28.7	0.9	n.d.	n.d.	-
168-96	3.1	35.9	1.3	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	3.1	36.5	0.8
173-96	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	3.1	35.5	1.3	n.d.	n.d.	-
191-96	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	3.2	34.2	0.5	n.d.	n.d.	-	n.d.	n.d.	-
216-168	2.9	53.9	3.8	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-
336-216	2.7	80.3	2.2	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-
360-191	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	3.0	52.1	1.0	n.d.	n.d.	-	n.d.	n.d.	-
504-360	n.d.	n.d.	-	n.d.	n.d.	-	n.d.	n.d.	-	2.8	69.0	1.1	n.d.	n.d.	-	n.d.	n.d.	-

a) In this test series, museum grade pyrite, $< 38\mu$, $\sim$500 mg in 100 ml of deionized water was agitated at room temperature; all the values given are averages of duplicate runs.
b) Fe^t is the total soluble iron in mg/ml.
c) The rate is the total soluble iron produced per ml of solution per hr during the time interval specified.
d) Not determined.

TABLE II

EFFECT OF PYRITE TO WATER RATIO UPON PYRITE SOLUBILIZATION[a]

(Pyrite, water)

Time Interval hr	1000 mg			500 mg[b]		
	pH	$Fe^{t,c}$ 10^{-3}	Rate $Fe^{t,d}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Rate Fe^{t} 10^{-4}
0	4.1	14.2	-	4.2	6.8	-
5-0	3.7	20.4	12.4	3.8	8.6	3.6
24-5	3.3	35.1	7.7	3.5	14.6	3.2
48-24	3.1	47.2	5.0	3.3	23.7	3.8
72-48	3.0	56.0	3.7	3.3	24.8	0.5
96-72	2.9	67.1	4.6	3.2	26.4	0.7
168-96	2.8	99.2	4.5	3.1	35.9	1.3
216-168	2.7	117.6	3.8	2.9	53.9	3.8
336-216	2.5	172.7	4.6	2.7	80.3	2.2

a) In these tests, the denoted quantity of museum grade pyrite, < 38u, in 100 ml of deionized water was agitated at room temperature.

b) The values given are the averages for the specific time intervals.

c) Fe^{t} is the total soluble iron in mg/ml.

d) This rate is the total soluble iron produced per ml of solution per hr during the time interval specified.

TABLE III

EFFECT OF PARTICLE SIZE AND THE PRESENCE OF CUPRIC ION UPON PYRITE SOLUBILIZATION[a]

Time Interval hr	$< 38\mu$			$> 125\mu < 250\mu$			$< 38\mu$, Cu^{++}, 0.013 mg/ml		
	pH	$Fe^{t,b}$ 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Rate Fe^{t} 10^{-4}	pH	Fe^{t} 10^{-3}	Rate Fe^{t} 10^{-4}
0	4.4	2.3	-	5.5	1.5	-	4.4	4.7	-
24-0	3.6	12.6	4.3	4.0	2.0	0.2	3.5	10.7	2.5
48-24	3.4	19.0	2.7	3.8	3.0	0.4	3.4	15.2	1.9
72-48	3.2	24.7	2.4	3.5	6.5	1.5	3.3	19.2	1.7
96-72	3.1	30.3	2.3	3.4	9.5	1.2	3.2	23.7	1.9
168-96	3.0	45.2	2.1	-	-	-	3.0	36.6	1.8
216-168	2.9	53.9	1.8	-	-	-	2.9	46.3	2.0

a) In this test series, ~ 500 mg of pyrite in 100 ml of deionized water was agitated at room temperature.
b) Fe^{t} is the total soluble iron in mg/ml.
c) The rate is the total soluble iron produced per ml of solution per hr during the time interval specified.

TABLE IV

RESPIROMETER TEST SERIES 4R

pH, OXYGEN UPTAKE, IRON AND SULFATE PRODUCTION DATA FOR PYRITE IN WATER AT 23°C[a]

Sampling Period day	pH[b]	$Fe^{t,b}$ µmoles	$SO_4^{=,b}$ µmoles	O_2^{b} µmoles	Mole Ratio of Species Produced during a given time interval Fe	:	$SO_4^=$	:	O_2
1	3.6	0.600	0.25	2.45 (2.45_6)	1		0.4		4.1
2	3.4	1.151	1.14	3.76 (3.77_4)	1		1.6		2.4
3	3.4	1.401	1.70	4.67 (4.67_2)	1		2.2		3.7

a) All the tests were performed at 23°C using ~ 25 mg of pyrite per 5 ml of deionized water. The values given for the specific time periods are corrected for the zero time sampling, i.e., the values obtained at zero time sampling are subtracted out.

b) The value given is the average for two samples, while the value given in parenthesis is the average value for this time period and the subscript value is the number of samples averaged.

TABLE V

RESPIROMETER TEST SERIES 5R

pH, OXYGEN UPTAKE AND IRON PRODUCTION DATA FOR PYRITE IN WATER AT 14°C [a]

Sampling Period, day	pH[b]	$Fe^{t,b}$ µmoles	O_2[b] µmoles	Mole Ratio of Species Produced during a given time interval Fe	:	O_2
1	3.8	0.184	$0.68\ (0.73_8)$	1		3.7
2	3.7	0.452	$1.53\ (1.46_5)$	1		3.2
4	3.4	0.842	$2.55\ (2.81_3)$	1		2.6
7	3.3	1.424	$4.29\ (4.42_2)$	1		3.0

a) All the tests were performed at 14°C using 25 mg of pyrite per 5 ml of deionized water. The values given for the specific time periods are corrected for the zero time sampling, i.e., the values obtained at zero time sampling are subtracted out.

b) The value given is the real value for the sample, while the value given in the parenthesis is the average value for this time period and the subscript value is the number of samples averaged.

TABLE VI

RESPIROMETER TEST SERIES 4R

pH, OXYGEN UPTAKE AND IRON PRODUCTION DATA FOR PYRITE IN WATER AT 23°C[a]

Sampling Period day	pH[b]	$Fe^{t,b}$ μmoles	O_2[b] μmoles	Mole Ratio of Species Produced during a given time interval Fe	: O_2
1	3.5	1.150	$4.03\ (4.19_6)$	1	3.5
2	3.3	2.296	$6.55\ (6.68_4)$	1	2.2
3	3.2	2.932	$8.79\ (8.79_2)$	1	3.5

a) All the tests were performed at 23°C using 50 mg of pyrite per 5 ml of deionized water. The values given for the specific time periods are corrected for the zero time sampling, i.e., the values obtained at zero time sampling are subtracted out.

b) The value given is the average for two samples, while the value given in parenthesis is the average value for this time period and the subscript value is the number of samples averaged.

TABLE VII

EFFECTS OF PREVIOUS TREATMENTS UPON PYRITE SOLUBILIZATION[a]

(Pyrite, water)

Time Interval hr	pH	$Fe^{t,b}$ 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}
0	5.1	+	-
24-0	3.9	0.9	0.3
48-24	3.8	1.3	0.2
120-48	3.6	3.8	0.3
168-120	3.5	6.3	0.5
216-168	3.3	7.9	0.3
288-216	3.2	11.2	0.4

a) In these tests, museum grade pyrite, < 38μ, recovered from the test series listed in Table I was used. The test conditions (~ 500 mg in 100 ml deionized water, room temperature agitation) were the same as for previous series.

b) Fe^t is the total soluble iron in mg/ml (values below 0.0005 mg/ml are denoted by +).

c) The rate is the total soluble iron produced per ml of solution per hr during the time interval specified.

TABLE VIII

EFFECT OF INITIAL pH UPON PYRITE SOLUBILIZATION[a]

(Pyrite, water)

Time Interval hr	Neutral[b]			Acid[c], Series K			Basic[d], Series J		
	pH	$Fe^{t,e}$ 10^{-3}	Rate $Fe^{t,f}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Rate Fe^{t} 10^{-4}	pH	Fe^{t} 10^{-3}	Rate Fe^{t} 10^{-4}
0	4.2	6.8	-	3.1	9.9	-	7.3	6.2	-
5-0	3.8	8.6	3.6	3.1	12.7	5.7	4.0	7.3	2.2
24-5	3.5	14.6	3.1	3.0	18.5	3.1	3.6	14.6	3.8
96-24	3.2	26.2	1.6	2.9	30.6	1.6	n.d.[g]	n.d.	-
120-24	3.1	28.7	1.4	n.d.	n.d.	-	3.5	16.9	0.2
120-96	3.1	28.7	1.0	2.8	33.8	1.3	n.d.	n.d.	-
173-120	3.1	35.5	1.3	2.8	41.3	1.4	n.d.	n.d.	-

---

a) In this test series, museum grade pyrite, < 38µ, ~ 500 mg in 100 ml of deionized water was agitated at room temperature.

b) The pH, Fe^{t} and rate values given for the "neutral", i.e. aqueous solution, containing only deionized water and pyrite, are the averages for the specific time intervals based on the experiments listed in Table I.

c) The "acidic" medium was prepared by adding to the deionized water a quantity of sulfuric acid required to bring the pH to that denoted at the zero hr.

d) The "basic" medium was prepared by adding to the deionized water a quantity of sodium carbonate required to bring the pH to that denoted at the zero hr.

e) Fe^{t} is the total soluble iron in mg/ml.

f) The rate is the total soluble iron produced per ml of solution per hr during the time interval specified.

g) Not determined.

TABLE IX

EFFECT OF FERRIC ION ADDITION UPON PYRITE SOLUBILIZATION[+]

(Pyrite, water, $Fe_2(SO_4)_3$)

Time Period hr	Average H-1 and H-2 Runs Control, no Fe^{+3} added				Average H-3 and H-4 Runs 0.017 mg/ml Fe^{+3} added						Average H-5 and H-6 Runs 0.089 mg/ml Fe^{+3} added					
	pH	$Fe^{t,*}$ 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	$Fe^{t}-Fe^{a}$ 10^{-3}	$Fe^{t}-Fe^{a}-Fe^{c}$ 10^{-3}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	$Fe^{t}-Fe^{a}$ 10^{-3}	$Fe^{t}-Fe^{a}-Fe^{c}$ 10^{-3}
5	4.0	7.2	7.1	n.d.["]	3.9	8.8	8.7	n.d.	-	-	3.9	8.5	8.1	n.d.	-	-
24	3.6	13.1	13.3	n.d.	3.5	15.2	14.6	n.d.	-	-	3.5	16.0	14.1	n.d.	-	-
47[#]	3.4	20.7	18.3	n.d.	3.4	19.3	18.7	n.d.	-	-	3.4	20.2	18.8	n.d.	-	-
48	3.4	19.6	18.1	n.d.	3.0	36.5	34.1	n.d.	19.4	-	2.7	109.1	70.7	47.7	20.1	0.5
52	3.4	20.8	19.8	0.7	3.0	43.1	41.3	1.6	26.0	5.2	2.6	129.1	120.6	9.6	40.1	19.3
71	3.3	23.7	22.1	n.d.	3.0	48.6	46.8	n.d.	31.5	7.8	2.6	146.0	135.1	n.d.	57.0	33.3
95	3.3	25.9	24.3	1.0	3.0	50.8	49.6	1.1	33.2	7.8	2.6	148.1	143.8	6.8	59.1	33.2
191	3.2	29.1	30.0	+	3.0	55.0	55.8	n.d.	37.4	8.0	2.6	162.3	159.1	2.5	73.3	44.2

[+] In this test series, museum grade pyrite, < 38u, ~ 500 mg in 100 ml of deionized water was agitated at room temperature.

[*] Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml (values below 0.0005 mg/ml are denoted by +); Fe^a is the equivalent value for the extra added iron; Fe^c is the equivalent value of total iron at the specific time interval in the sample wherein no extra iron was added (control).

[#] At 47 hr a sample of the solution was taken for analysis. Subsequently, to runs H-1 and H-2, 1 ml of water was added; to runs H-3 and H-4, Fe^{+3} (1.46 mg in 1 ml of water) was added; and to runs H-5 and H-6, Fe^{+3} (8.00 mg in 5 ml of water) was added.

["] Not determined.

TABLE X

EFFECT OF FERRIC ION ADDITION UPON PYRITE SOLUBILIZATION[a]

(Pyrite, 1/10 m9K, $Fe_2(SO_4)_3$)

Time Interval hr	Average QQ-1 and QQ-2 Runs				Average QQ-3 and QQ-4 Runs				
	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}
0	3.2	13.0	12.3	-	3.2	12.7	12.2	n.d.[f]	-
24-0	3.0	12.9	12.2	-	3.0	12.2	12.1	n.d.	-
24-24[d]	3.1	11.3	11.0	-	2.8	18.7	15.8	n.d.	-
29-24	3.1	11.8	11.4	1.0	2.8	23.9	22.9	n.d.	10.4
48-29[e]	3.0	13.8	12.2	1.1	2.8	31.2	29.2	n.d.	3.8
53-48	3.2	11.3	10.6	-	2.2	1385.8	n.d.	1359.0	-
71-53	3.0	13.3	11.7	1.1	2.1	1441.5	635.9	845.5	30.9
77-71	3.1	13.3	12.1	-	2.1	1447.5	652.2	821.0	10.0
102-77	3.1	17.1	16.3	1.5	2.1	1548.9	917.1	575.0	40.6
191-102	2.9	41.3	38.7	2.7	2.0	1574.4	1365.5	248.5	2.9

a) In this test series, museum grade pyrite, $<$ 38u, ~500 mg in 100 ml of sterile 1/10 m9K was agitated at room temperature.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml.

c) These rates are the soluble iron produced per ml of solution per hr during the time interval specified.

d) After 24 hr in QQ-1 and QQ-2 runs, 10 ml of sterile deionized water, and in QQ-3 and QQ-4 runs, 10 ml of $Fe_2(SO_4)_3$ solution containing 1.9 mg of iron were added. This theoretically increased the Fe^t by 0.019 mg/ml.

e) After 48 hr in QQ-1 and QQ-2 runs, 20 ml of sterile deionized water, and in QQ-3 and QQ-4 runs, 10 ml of $Fe_2(SO_4)_3$ solution containing 133.4 mg of iron were added. This theoretically increased the Fe^t by 1.334 mg/ml.

f) Not determined.

TABLE XI

QUANTITATIVE PRESENTATION OF Fe^{+3} EFFECT UPON PYRITE SOLUBILIZATION[a]

(Series QQ, pyrite, 1/10 m9K)

Time hr	Fe^{t} 10^{-3}	Fe^{+3} 10^{-3}	$Fe^{+3,b}_{reduced}$ 10^{-3}	$Fe^{+2,c}_{liberated}$ found 10^{-3}	Ratio $\frac{Fe^{+3}_{reduced}}{Fe^{+2}_{liberated}}$
71	1441	846	507	75	6.8
77	1478	821	532	112	4.8
102	1549	575	778	179	4.3
191	1574	249	1104	180	6.1

a) The experimental data are given in Table X. All the species' concentrations are given in mg/ml and all are averages of QQ-3 and QQ-4 Runs. The total Fe^{+3}_{added} was 1.353 mg/ml.

b) $Fe^{+3}_{reduced} = Fe^{+3}_{added} - Fe^{+3}$.

c) $Fe^{+2}_{liberated}$ found = $Fe^{t} - Fe^{+3}_{added} - Fe_{control}$; $Fe_{control}$ = average Fe^{t} of QQ-1 and QQ-2 Runs.

TABLE XII

EFFECT OF STEAM STERILIZATION UPON PYRITE SOLUBILIZATION[a]

(Pyrite, water)

Time Interval hr	E-1 Run			E-2 Run		
	pH	$Fe^{t,b}$ 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Rate Fe^{t} 10^{-4}
0	4.1	6.2	-	3.3	40.9	-
5-0	3.7	8.8	5.2	3.1	47.0	12.2
24-5	3.4	16.0	3.8	3.1	50.9	2.1
29-24	3.3	18.5	5.0	3.1	51.8	1.8
48-29	3.2	23.7	2.7	3.1	54.0	1.2
53-48	3.2	n.d.[d]	-	3.1	54.2	0.4
72-53	3.1	32.1	3.5	3.1	55.8	0.8

a) In this test series, museum grade pyrite, < 38μ, ~ 500 mg in 100 ml of sterile deionized water was agitated at room temperature. For the test E-2, the pyrite was autoclaved at 120°C, 15 psi for 15 min.

b) Fe^{t} is the total soluble iron in mg/ml

c) These rates are the total soluble iron produced per ml of solution per hr during the time interval specified.

d) Not determined.

TABLE XIV

EFFECT OF DIFFERENT INITIAL CONCENTRATION OF *T. FERROOXIDANS* UPON PYRITE SOLUBILIZATION[a]

(Pyrite, water, *T. ferrooxidans*, ATCC strain)

Time	Average L-5 and L-6 Runs					Average L-3 and L-4 Runs[b]					Average L-1 and L-2 Runs[c]				
Interval hr	pH	$Fe^{t,d}$ 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate $Fe^{t,e}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}
0	4.2	4.4	3.9	0.8	-	4.2	5.0	3.4	+	-	4.0	4.6	3.3	0.8	-
5-0	3.8	7.0	5.7	1.0	5.2	3.8	6.5	5.7	1.2	3.0	3.3	5.9	5.7	n.d.[f]	2.6
24-5	3.4	19.1	14.5	1.4	6.4	3.5	14.6	12.9	1.1	4.3	2.9	25.7	24.6	2.3	10.4
48-24	3.3	26.3	25.7	2.6	3.0	3.4	22.5	21.2	1.9	3.3	2.8	46.2	43.5	2.7	8.5
120-48	3.1	32.4	31.4	1.4	0.8	3.2	30.2	29.4	1.7	1.1	2.6	79.7	67.9	13.3	4.7
168-120	3.0	36.6	34.0	1.4	0.9	-	-	-	-	-	2.5	109.3	86.3	20.0	6.2
192-168	3.0	37.9	37.2	0.8	0.5	-	-	-	-	-	2.5	123.3	95.6	25.4	5.8
216-192	3.0	42.3	40.6	1.5	1.8	-	-	-	-	-	2.5	136.3	99.1	38.2	5.4
288-216	3.0	50.7	48.3	2.3	1.2	-	-	-	-	-	2.3	200.0	136.1	67.6	8.8
312-288	2.9	53.3	53.2	1.7	1.1	-	-	-	-	-	2.3	226.1	157.6	71.6	10.9
456-312	2.8	74.4	74.9	2.1	1.5	-	-	-	-	-	2.3	347.2	235.4	116.4	8.4

a) In this test series, museum grade pyrite, < 38µ, ~ 500 mg in 100 ml of sterile deionized water was agitated at room temperature.
b) 0.45 mg of *T. ferrooxidans* protein was added.
c) 2.25 mg of *T. ferrooxidans* protein was added.
d) Fe^{t} is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml (values below 0.0005 mg/ml are denoted by +).
e) The rate is the total soluble iron produced per ml of solution per hr during the time interval specified.
f) Not determined.

TABLE XV

COMPOSITION OF MEDIA USED FOR STUDIES OF *T. FERROOXIDANS* AND *T. THIOOXIDANS*

1/10 9K		1/10 m9K (modified)	
Salts	g/l	Salts	g/l
$(NH_4)_2SO_4$	0.30	NH_4Cl	0.25
KCl	0.01	KCl	0.01
K_2HPO_4	0.05	K_2HPO_4	0.05
$MgSO_4 \cdot 7\,H_2O$	0.05	$MgCl_2 \cdot 6\,H_2O$	0.04
$Ca(NO_3)_2 \cdot n\,H_2O$	0.0014	$Ca(NO_3)_2 \cdot n\,H_2O$	0.0014
10N H_2SO_4	0.1 ml	conc. HCl	0.1 ml

TABLE XVI

EFFECT OF MEDIUM UPON THE EFFICIENCY OF FERROUS ION OXIDATION BY *T. FERROOXIDANS* (ATCC STRAIN)[a]

Medium[b]	Inoculum	Fe^t mg/ml	Fe^{+2} mg/ml	Fe^{+3} mg/ml	$-d(Fe^{+2})/dt$
1/10 9K	none	2.77	2.62	0.15	-
1/10 9K	2.56μg/ml (5-78-68)	2.37	1.51	0.86	15.4×10^{-3}
1/10 m9K	none	2.86	2.75	0.11	-
1/10 m9K	2.56μg/ml (5-78-68)	2.30	1.38	0.92	19.0×10^{-3}

a) The tests were carried over a 72 hr period at 26°C using 50 ml samples.

b) The media compositions are given in Table XV; each medium contained full strength (44.2 g/l) of $FeSO_4 \cdot 7H_2O$.

TABLE XVII

EFFECT OF <u>T. FERROOXIDANS</u>, ATCC STRAIN, CONCENTRATION UPON ITS ACTIVITY[a]

Inoculum		Time, hr					
		0	45	69	93	117	141
none	pH	3.2	2.9	2.8	2.8	2.4	2.4
	$Fe^{t,b}$	10.14	10.26	9.92	9.83	10.20	10.10
	$Fe^{+2,b}$	9.25	9.36	9.60	9.64	9.14	8.89
	$Fe^{+3,b}$	n.d.[d]	0.18	0.22	0.24	0.30	0.37
	$-d(Fe^{+2})/dt^{c}$	-	-	-	-	-	-
0.32μg protein/ml (5-78-106-1)	pH	3.2	2.9	2.8	2.5	2.1	2.0
	Fe^{t}	10.17	10.24	9.86	9.53	8.06	7.54
	Fe^{+2}	9.25	9.50	9.29	7.41	1.44	0.06
	Fe^{+3}	n.d.	0.19	0.43	1.88	6.28	7.52
	$-d(Fe^{+2})/dt$	-	-	0.21	1.88	5.97	1.38
3.2μg protein/ml (5-78-106-1)	pH	3.2	2.9	2.8	2.5	2.1	2.0
	Fe^{t}	10.20	10.07	9.69	9.26	8.73	7.41
	Fe^{+2}	9.27	9.63	9.21	7.92	3.51	0.05
	Fe^{+3}	n.d.	0.18	0.58	1.31	4.92	7.23
	$-d(Fe^{+2})/dt$	-	-	0.42	1.29	4.41	3.46

a) The tests were performed in duplicates utilizing 1/10 9K medium with full strength (44.2 g/l) of $FeSO_4 \cdot 7H_2O$. The tests were carried out without agitation in an incubator at 26°C.

b) The concentrations are given as mg/ml.

c) The rate of disappearance of Fe^{+2} is given in mg/ml/24 hr.

d) Not determined.

TABLE XVIII

EFFECT OF <u>T. FERROOXIDANS</u> UPON PYRITE SOLUBILIZATION[a]

(Pyrite, 1/10 9K, <u>T. ferrooxidans</u>, ATCC strain)

Time Interval hr	Average M-3 and M-4 Runs					Average M-1 and M-2 Runs[b]				
	pH	$Fe^{t,c}$ 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate $Fe^{t,d}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}
0	3.0	10.0	9.6	n.d.[e]	-	3.0	5.2	4.5	n.d.	-
5-0	3.0	9.7	9.2	n.d.	-	2.9	n.d.	n.d.	n.d.	-
24-5	2.9	10.1	8.6	1.0	0.2	2.8	9.8	7.1	2.9	-
48-24	2.9	10.9	11.0	1.0	0.3	2.6	53.0	18.1	36.2	18.0
72-48	2.9	13.8	12.6	0.8	1.2	2.4	143.7	27.8	125.7	37.8
144-72	2.8	19.6	18.9	1.5	0.8	2.2	444.2	n.d.	n.d.	41.7
168-144	2.8	19.9	19.9	n.d.	0.1	2.1	585.8	n.d.	552.7	59.0

a) In this test series, museum grade pyrite, $< 38\mu$, $\sim$500 mg in 100 ml of 1/10 9K solution was agitated at room temperature.
b) 2.25 mg of <u>T. ferrooxidans</u> protein was added.
c) Fe^{t} is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml.
d) This rate is the total soluble iron produced per ml of solution per hr during the time interval specified.
e) Not determined.

TABLE XIX

EFFECT OF T. FERROOXIDANS UPON PYRITE SOLUBILIZATION

(Pyrite, 1/10 m9K, T. ferrooxidans, ATCC strain)

Time Interval hr	Average CC-1 and CC-2 Runs pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^=$ 10^{-1}	$SO_4^=/Fe^t$ mole ratio	Average CC-4 and CC-12 Runs pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	$SO_4^=/Fe^t$ mole ratio
0	3.3	11.2	10.8	-	n.d.[d]	-	3.3	10.3	9.6	n.d.	-	n.d.	-
23-0	3.5	10.6	10.3	-	n.d.	-	3.2	8.4	8.0	n.d.	-	n.d.	-
47-23	3.2	12.3	12.0	0.7	n.d.	-	3.1	13.9	13.2	n.d.	2.3	n.d.	-
71-47	3.2	14.5	13.8	0.9	n.d.	-	3.1	20.0	19.1	n.d.	2.6	n.d.	-
143-71	3.0	23.4	22.1	1.2	n.d.	-	2.5	195.0	13.3	177.7	24.2	7.8	2.3
169-143	3.0	26.2	25.6	1.1	n.d.	-	2.4	315.4	8.4	272.3	46.2	14.6	2.7
192-169	2.8	29.4	28.6	1.4	n.d.	-	2.3	448.7	17.8	402.0	57.8	19.4	2.5
216-192	2.9	32.9	31.9	1.5	1.4	2.5	2.2	604.8	19.7	569.2	65.0	26.0	2.5
239-216	2.8	38.0	36.3	2.2	1.4	2.1	2.1	749.0	26.0	627.4	62.9	29.8	2.3
311-239	2.7	54.8	52.9	2.3	2.1	2.2	2.0	1112.8	55.8	1041.3	50.5	50.2	2.6
359-311	2.7	63.9	60.3	1.9	2.3	2.1	1.9	1340.0	94.3	1234.4	47.3	56.7	2.5
389-359[e]	3.0	48.4	n.d.	-	n.d.	-	2.0	960.4	n.d.	n.d.	-	n.d.	-
479-389	2.7	58.6	53.3	1.1	2.3	2.3	1.9	1291.6	52.0	1156.0	58.4[f]	57.3	2.6
528-479	2.7	65.8	62.9	1.5	2.6	2.3	1.9	1436.8	61.2	1314.6	46.7[f]	59.6	2.4
719-528	2.6	105.3	100.9	2.1	3.5	1.9	1.8	2004.9	124.3	1843.0	47.2[f]	83.1	2.4

a) In this test series, museum grade pyrite, $< 38\mu$, ~500 mg in 100 ml of sterile 1/10 m9K was agitated at room temperature. To runs CC-4 and CC-12, 2.38 mg of T. ferrooxidans (ATCC strain) protein was added.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml.

c) These rates are the total soluble iron produced per ml of solution per hr during the time interval specified.

d) Not determined.

e) At 389 hr, prior to testing, 20 ml of 1/10 m9K solution was added to each test.

f) All these rates are corrected for the dilution due to the addition of 20 ml of 1/10 m9K at 389 hr.

TABLE XX

EFFECT OF DIFFERENT INITIAL CONCENTRATIONS OF <u>T. FERROOXIDANS</u> UPON PYRITE SOLUBILIZATION[a]

(Pyrite, 1/10 m9K, <u>T. ferrooxidans</u>, ATCC strain)

Time	Average RR-1 and RR-2 Runs					Average RR-3 and RR-4 Runs[b]						RR-5 and RR-6 Runs[c]					
Interval hr	pH	$Fe^{t,d}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,e}$ 10^{-4}	$SO_4^=$ 10^{-1}	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}
0	3.0	n.d.[f]	n.d.	-	n.d.	3.0	n.d.	n.d.	n.d.	-	n.d.	3.0, 3.0	n.d., n.d.	n.d., n.d.	n.d., n.d.	- -	n.d., n.d.
71-0	2.9	19.1	17.4	-	n.d.	2.9	n.d.	17.4	n.d.	-	n.d.	2.9, 2.9	25.2, 21.4	19.6, 17.0	n.d., n.d.	- -	n.d., n.d.
94-71	2.9	19.9	18.9	0.3	0.9	2.9	18.5	18.0	n.d.	-	0.9	2.9, 2.8	27.5, 21.1	20.8, 16.7	n.d., n.d.	- -	1.0, 1.0
119-94	2.9	21.7	20.4	0.7	1.0	2.9	20.7	18.8	n.d.	0.9	1.1	2.9, 2.8	27.3, n.d.	22.2, 16.7	n.d., n.d.	- -	1.2, 1.2
143-119	2.8	25.2	22.8	1.5	1.1	2.7	22.5	20.4	n.d.	0.8	1.2	2.9, 2.7	26.9, 45.7	27.7, 42.2	n.d., n.d.	- -	1.1, 1.9
167-143	2.8	27.8	27.1	1.1	n.d	2.8	27.4	25.7	n.d.	2.0	n.d.	2.9, 2.5	29.8, 64.4	29.6, 31.4	n.d., n.d.	1.2, 7.8	n.d., n.d.
239-167	2.7	40.8	39.7	1.7	1.9	2.3	294.5	72.6	218.6	37.1	12.5	2.7, 2.1	36.9, 585.9	34.6, 57.1	n.d., 507.0	1.0, 72.4	1.9, 24.6
262-239	2.7	43.4	41.1	1.2	n.d.	2.1	427.0	76.6	327.3	57.6	15.0	2.7, 2.1	50.5, 697.0	42.6, 75.4	n.d., 610.0	5.9, 48.3	2.3, 25.4
286-262	2.7	49.9	45.1	2.7	2.1	2.1	539.1	112.2	383.7	46.7	19.0	2.4, 2.0	158.9, 851.9	57.6, 93.7	102.2, 717.1	45.2, 64.5	7.2, 32.7
311-286	2.7	55.7	51.2	2.4	2.2	2.1	660.3	128.9	459.2	48.5	23.5	2.2, 2.0	451.4, 906.9	45.7, 125.3	371.7, 748.5	117.0, 22.0	17.7, 33.8
335-311	2.7	61.0	54.5	2.2	2.3	2.1	727.6	121.8	536.2	28.0	25.1	2.1, 2.0	705.1, 983.3	53.8, 124.3	617.7, 849.1	105.7, 31.8	25.0, 35.6
407-335	2.6	82.5	78.6	3.0	2.7	2.0	927.3	355.9	563.0	27.7	30.6	1.9, 1.9	1334.8, 1090.3	107.4, 435.1	1120.0, 629.0	84.7, 14.8	51.4, 38.6
431-407	2.6	88.5	83.7	2.5	2.8	1.9	975.5	404.0	547.2	20.2	31.7	1.9, 1.9	1553.9, 1136.2	160.0, 517.6	1402.6, 584.9	91.3, 13.5	52.7, 40.8
456-431	2.6	100.2	96.4	4.7	3.1	1.9	1029.2	478.4	585.0	21.4	31.1	1.8, 1.8	1711.9, 1161.7	177.3, 589.0	1509.6, 572.4	63.2, 10.2	60.3, 40.0

a) In this test series, museum grade pyrite, < 38µ, ~ 500 mg in 100 ml of sterile 1/10 m9K was agitated at room temperature.
b) To runs RR-3 and RR-4, 0.032 mg of <u>T. ferrooxidans</u> protein was added.
c) To runs RR-5 and RR-6, 0.324 mg of <u>T. ferrooxidans</u> protein was added.
d) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml.
e) These rates are the total soluble iron produced per ml of solution per hr during the time interval specified.
f) Not determined.

TABLE XXI

PYRITE SOLUBILIZATION AS AFFECTED BY CHANGES IN RELATIVE CONCENTRATIONS OF PYRITE[a]

(Pyrite, 1/10 m9K)

Time	Average CC-1 and CC-2 Runs						Average CC-9 and CC-10 Runs[e]					
Interval		$Fe^{t,b}$	Fe^{+2}	Rate $Fe^{t,c}$	$SO_4^=$	$SO_4^=/Fe^t$		Fe^t	Fe^{+2}	Rate Fe^t	$SO_4^=$	$SO_4^=/Fe^t$
hr	pH	10^{-3}	10^{-3}	10^{-4}	10^{-1}	mole ratio	pH	10^{-3}	10^{-3}	10^{-4}	10^{-1}	mole ratio
0	3.3	11.2	10.8	-	n.d.[d]	-	3.5	11.7	11.3	-	n.d.	-
23-0	3.5	10.6	10.3	-	n.d.	-	3.3	10.8	10.4	-	n.d.	-
47-23	3.2	12.3	12.0	0.7	n.d.	-	3.2	13.0	12.7	0.9	n.d.	-
71-47	3.2	14.5	13.8	0.9	n.d.	-	3.2	15.3	14.5	1.0	n.d.	-
143-71	3.0	23.4	22.1	1.2	n.d.	-	3.0	25.5	23.4	1.4	n.d.	-
169-143	3.0	26.2	25.6	1.1	n.d.	-	3.0	27.2	26.8	0.7	n.d.	-
192-169	2.8	29.4	28.6	1.4	n.d.	-	2.9	30.8	29.9	1.6	n.d.	-
216-192[e]	2.9	32.9	31.9	1.5	1.4	2.5	2.9	34.4	33.3	1.5	1.4	2.4
239-216	2.8	38.0	36.3	2.2	1.4	2.1	2.9	68.6	65.8	14.9	2.3	2.0
311-239	2.7	54.8	52.9	2.3	2.1	2.2	2.7	109.6	109.1	5.7	3.9	2.1
359-311	2.7	63.9	60.3	1.9	2.3	2.1	2.7	141.3	135.3	6.6	4.9	2.0
389-359[f]	3.0	48.4	n.d.	-	n.d.	-	2.8	107.5	n.d.	-	n.d.	-
479-389	2.7	58.6	53.3	1.1	2.3	2.3	2.6	142.7	138.9	3.9	5.2	2.1
528-479	2.7	65.8	62.9	1.5	2.6	2.3	2.6	166.6	158.7	4.9	5.6	2.0
719-528	2.6	105.3	100.9	2.1	3.5	1.9	2.4	270.6	259.3	5.4	8.3	1.8

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a) In this test series, museum grade pyrite, $< 38\mu$, ~ 500 mg in 100 ml of sterile 1/10 m9K was agitated at room temperature.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml.

c) These rates are the total soluble iron produced per ml of solution per hr during the time interval specified.

d) Not determined.

e) At 216 hr to Runs CC-9 and CC-10, 500 mg of pyrite was added.

f) At 389 hr, prior to testing, 20 ml of 1/10 m9K was added to each test.

TABLE XXII

EFFECT OF CHLORAMPHENICOL UPON PYRITE DISSOLUTION
AND T. FERROOXIDANS SUPPRESSION[a]

(Pyrite, 1/10 m9K, T. ferrooxidans, ATCC strain)

Time Interval hr	Average CC-1 and CC-2 Runs					Average CC-7 and CC-8 Runs[b]					Average CC-5 and CC-6 Runs[c]					
	pH	$Fe^{t,d}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,e}$ 10^{-4}	$SO_4^=$ 10^{-1}	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}
0	3.3	11.2	10.8	-	n.d.[f]	3.3	12.0	11.6	-	n.d.	3.4	9.1	8.7	n.d.	-	n.d.
23-0	3.5	10.6	10.3	-	n.d.	3.3	11.2	10.8	-	n.d.	3.4	8.8	8.0	n.d.	-	n.d.
47-23	3.2	12.3	12.0	0.8	n.d.	3.2	13.8	12.2	1.1	n.d.	3.0	15.1	13.5	n.d.	2.6	n.d.
71-47	3.2	14.5	13.8	0.9	n.d.	3.2	15.1	14.2	0.5	n.d.	3.1	18.9	17.4	n.d.	1.6	n.d.
143-71	3.0	23.4	22.1	1.2	n.d.	3.1	22.9	21.1	1.1	n.d.	3.0	36.1	34.2	n.d.	2.4	n.d.
169-143	3.0	26.2	25.6	1.1	n.d.	3.0	24.5	24.1	0.6	n.d.	2.9	44.5	41.5	n.d.	3.2	n.d.
192-169	2.8	29.4	28.6	1.4	n.d.	3.0	29.1	28.4	2.0	n.d.	2.8	55.8	50.5	n.d.	4.9	n.d.
216-192	2.9	32.9	31.9	1.5	1.4	2.9	29.8	29.4	0.3	1.4	2.6	74.4	59.2	n.d.	7.8	2.7
239-216	2.8	38.0	36.3	2.2	1.4	2.9	32.8	31.7	1.3	1.2	2.6	102.1	64.5	31.3	12.0	3.9
311-239	2.7	54.8	52.9	2.3	2.1	2.8	43.6	43.2	1.5	2.0	2.5	175.6	131.0	35.9	10.2	7.1
359-311	2.7	63.9	60.3	1.9	2.3	2.7	52.0	50.1	1.8	2.2	2.5	212.2	118.2	86.8	7.6	8.5
389-359[g]	3.0	48.4	n.d.	-	n.d.	3.0	39.5	n.d.	-	n.d.	2.6	160.4	n.d.	n.d.	-	n.d.
479-389	2.7	58.6	53.3	1.1	2.3	2.7	50.1	48.5	1.2	2.0	2.2	373.4	85.8	n.d.	23.7	15.3
528-479	2.7	65.8	62.9	1.5	2.6	2.7	57.4	55.4	1.5	2.2	2.1	613.9	108.5	465.1	49.1	25.5
719-528	2.6	105.3	100.9	2.1	3.5	2.6	91.1	89.4	1.8	3.5	1.9	1080.2	328.7	704.4	24.4	43.6

a) In this test series, museum grade pyrite, < 38u, ~ 500 mg in 100 ml of sterile 1/10 m9K was agitated at room temperature.
b) To runs CC-7 and CC-8, 4.98 mg of chloramphenicol was added.
c) To runs CC-5 and CC-6, 4.98 mg of chloramphenicol and 2.38 mg of T. ferrooxidans protein (ATCC strain) was added.
d) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml.
e) These rates are the total soluble iron produced per ml of solution per hr during the time interval specified.
f) Not determined.
g) At 389 hr, 20 ml of 1/10 m9K solution was added to each test.

TABLE XXIII

EFFECT OF CHANGES IN RELATIVE CONCENTRATIONS OF
T. FERROOXIDANS AND PYRITE UPON PYRITE SOLUBILIZATION[a]
(Pyrite, 1/10 m9K, *T. ferrooxidans*, ATCC strain)

Time Interval hr	Average CC-4 and CC-12 Runs						CC-3 Run[b]						CC-11 Run[c]					
	pH	Fe^{t}[d] 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t}[e] 10^{-4}	$SO_4^{=}$ 10^{-1}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}	$SO_4^{=}$ 10^{-1}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}	$SO_4^{=}$ 10^{-1}
0	3.3	10.3	9.6	n.d.[f]	-	n.d.	3.2	9.4	9.0	n.d.	-	n.d.	3.3	9.7	9.3	n.d.	-	n.d.
23-0	3.2	8.4	8.0	n.d.	-	n.d.	3.1	8.8	8.2	n.d.	-	n.d.	3.2	9.4	8.5	n.d.	-	n.d.
47-23	3.1	13.9	13.2	n.d.	2.3	n.d.	3.1	12.2	12.2	n.d.	1.7	n.d.	3.2	13.0	13.0	n.d.	1.5	n.d.
71-47	3.1	20.0	19.1	n.d.	2.6	n.d.	3.0	20.7	18.9	n.d.	3.3	n.d.	3.0	34.9	21.9	n.d.	9.1	n.d.
143-71	2.5	195.0	13.3	177.7	24.2	7.8	2.4	157.9	34.2	127.1	19.1	7.8	2.5	169.2	20.5	n.d.	18.7	7.3
169-143	2.4	315.4	8.4	272.3	46.2	14.6	2.3	300.6	14.1	260.4	54.9	12.9	2.3	301.6	11.8	270.5	50.9	17.8
192-169	2.3	448.7	17.8	402.0	57.8	19.4	2.2	464.7	27.9	408.9	71.3	20.2	2.2	450.4	25.2	410.1	64.7	16.9
216-192[b,c]	2.2	604.3	19.7	569.2	65.0	26.0	2.1	607.3	32.6	n.d.	59.4	27.1	2.1	603.2	40.4	540.9	63.7	22.6
239-216	2.1	749.0	26.0	627.4	62.9	29.8	2.1	672.5	31.6	727.7[h]	69.3[h]	26.4	2.1	750.0	206.5	512.6	63.8	30.2
311-239	2.0	1112.8	55.8	1041.3	50.5	50.2	2.0	1034.2	46.3	1068.4[h]	57.2[h]	47.7	2.0	1166.8	140.4	990.7	57.9	49.7
359-311	1.9	1340.0	94.3	1234.4	47.3	56.7	1.9	1263.6	59.1	1319.3[h]	53.5[h]	54.8	1.9	1432.0	205.8	1223.4	55.3	56.0
389-359[g]	2.0	960.4	n.d.	n.d.	-	n.d.	2.0	993.5	n.d.	n.d.	-	n.d.	2.0	1095.4	n.d.	n.d.	-	n.d.
479-389	1.9	1291.6	52.0	1156.0	58.4[i]	57.3	1.9	1156.6	168.1	965.5	32.5[i]	53.6	1.9	1151.4	680.7	474.9	9.8[i]	52.2
528-479	1.9	1436.8	61.2	1314.6	46.7[i]	59.6	1.9	1202.4	275.1	965.5	16.8[i]	52.2	1.9	n.d.	754.1	361.7	-	50.9
719-528	1.8	2004.9	124.3	1843.0	47.2[i]	83.1	1.8	1299.2	403.5	905.7	9.1[i]	55.9	1.8	1248.3	912.0	349.0	7.9[i]	50.4

[a] In this test series, museum grade pyrite, < 38μ, ~ 500 mg in 100 ml of sterile 1/10 m9K was agitated at room temperature. To each solution, 2.38 mg of *T. ferrooxidans* protein (ATCC strain) was added.

[b] At 216 hr to run CC-3, 2.38 mg of *T. ferrooxidans* protein in 7 ml of solution was added.

[c] At 216 hr to run CC-11, 500 mg of pyrite was added.

[d] Fe^{t} is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml; $SO_4^{=}$ is the sulfate ion in mg/ml.

[e] These rates are the total soluble iron produced per ml of solution per hr during the time interval specified.

[f] Not determined.

[g] At 389 hr, 20 ml of 1/10 m9K solution was added to each test.

[h] The Fe^{+3} and the rate given is corrected for the dilution due to the addition of 7 ml of solution after the analysis at 216 hr.

[i] All these rates are corrected for the dilution due to the addition of 20 ml of 1/10 m9K at 389 hr.

TABLE XXIV

EFFECT OF *T. FERROOXIDANS*, MINE ISOLATED STRAIN VV-10, CONCENTRATION UPON ITS ACTIVITY[a]

Inoculum		Time, hr									
		0	46	119	143	167	190	216	288	310	334
Uninoculated	pH	3.3	3.0	2.8	2.8	2.8	2.7	2.7	2.6	2.6	2.6
	$Fe^{t,b}$	9.86	9.30	9.96	9.00	8.73	8.60	8.76	8.64	8.88	8.57
	Fe^{+2}	9.00	8.66	8.31	8.74	8.37	8.02	8.18	7.72	8.18	7.91
	Fe^{+3}	-	0.22	0.26	0.28	0.31	0.45	0.41	0.53	0.59	0.64
0.259µg protein/ml (5-78-106-4)	pH	3.3	3.0	2.8	2.8	2.7	2.7	2.7	2.5	2.4	2.2
	Fe^{t}	9.30	8.87	9.33	8.84	8.74	8.54	8.62	8.08	7.44	6.78
	Fe^{+2}	8.97	8.66	8.10	8.51	8.31	7.99	8.01	5.68	3.87	1.58
	Fe^{+3}	-	0.20	0.27	0.28	0.32	0.47	0.46	2.18	3.49	5.35
	$-d(Fe^{+2})/dt^{c}$	-	-	-	-	-	-	-	0.78	1.81	2.29
2.59µg protein/ml (5-78-106-4)	pH	3.3	2.8	2.7	2.6	2.6	2.4	2.2			
	Fe^{t}	9.38	9.96	9.10	8.38	8.00	7.31	6.27			
	Fe^{+2}	8.71	9.12	7.03	-	6.55	3.20	0.10			
	Fe^{+3}	-	0.26	0.71	1.25	1.51	4.80	5.98			
	$-d(Fe^{+2})/dt$	-	-	0.69	-	-	3.35	3.10			
25.9µg protein/ml (5-78-106-4)	pH	3.2	3.0	2.7	2.6	2.5	2.3	2.2			
	Fe^{t}	9.07	8.87	8.25	7.44	7.16	6.52	5.81			
	Fe^{+2}	8.05	7.72	6.11	6.00	4.25	0.72	0.10			
	Fe^{+3}	-	0.25	0.64	1.24	2.70	5.79	5.50			
	$-d(Fe^{+2})/dt$	-	0.17	0.54	0.11	1.75	3.53	0.62			

a) The tests were performed in duplicates utilizing 1/10 concentration of m9K medium with full strength (44.22 g/l) of $FeSO_4 \cdot 7H_2O$. The tests were carried out without agitation in an incubator at 28°C.

b) The concentrations are given as mg/ml.

c) The rate of disappearance of Fe^{+2} is given in mg/ml/24 hr.

TABLE XXV

EFFECT OF MINE *T. FERROOXIDANS* UPON PYRITE SOLUBILIZATION[a]

(Pyrite, 1/10 m9K, *T. ferrooxidans*, mine isolated strain VV-10)

Time	Average SS-1 and SS-2 Runs							SS-3 and SS-4 Runs							
Interval hr	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	$SO_4^=/Fe^t$ mole ratio	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	$SO_4^=/Fe^t$ mole ratio
0	3.2	12.8	12.5	-	n.d.[d]	-	-	3.2, 3.4	11.6, 11.7	11.1, 11.4	n.d., n.d.	- -	n.d., n.d.	- -	- -
67-0	3.0	17.7	17.0	0.7	0.9	-	3.0	3.0, 3.1	17.9, 17.1	17.1, 16.3	n.d., n.d.	0.9, 0.8	0.9, 1.0	- -	2.9, 3.4
90-67	3.0	19.7	18.9	0.9	0.9	0	2.7	3.0, 3.0	19.9, 20.9	19.2, 18.0	n.d., n.d.	0.9, 1.6	1.0, 1.0	4.3, 0	2.9, 2.8
114-90	3.0	n.d.	n.d.	-	n.d.	-	-	3.0, 3.0	n.d., n.d.	n.d., n.d.	n.d., n.d.	- -	n.d., n.d.	- -	- -
139-114	3.0	27.2	25.3	1.5	1.2	6.1	2.5	3.0, 3.0	27.8, 25.7	24.8, 23.8	n.d., n.d.	1.6, 1.0	1.1, 1.2	2.0, 4.1	2.3, 2.7
163-139	2.9	30.7	27.5	1.5	1.2	0	2.3	3.0, 2.9	30.0, 27.6	26.6, 25.8	n.d., n.d.	0.9, 0.8	1.2, 1.2	4.2, 0	2.3, 2.5
234-163	2.8	45.3	44.3	2.1	1.7	7.0	2.2	2.8, 2.9	42.8, 40.2	41.6, 39.5	n.d., n.d.	1.8, 1.7	1.7, 1.5	7.0, 4.2	2.3, 2.2
258-234	2.8	50.5	47.9	2.2	1.7	0	2.0	2.8, 2.8	47.5, 44.3	46.1, 44.0	n.d., n.d.	2.0, 1.7	1.8, 1.5	4.2, 0	2.2, 2.0
284-258	2.8	56.3	52.2	2.2	1.8	3.8	1.9	2.8, 2.8	52.4, 49.5	49.5, 47.9	n.d., n.d.	1.9, 2.0	2.0, 1.7	7.7, 7.7	2.2, 2.0
306-284	2.7	n.d.	n.d.	-	n.d.	-	-	2.7, 2.8	n.d., n.d.	n.d., n.d.	n.d., n.d.	- -	n.d., n.d.	- -	- -
402-306	2.7	84.2	78.8	2.4	2.8	8.5	2.0	2.7, 2.6	84.6, 95.2	80.5, 89.5	n.d., n.d.	2.7, 3.9	2.6, 4.0	5.1, 19.5	1.8, 2.4
427-402	2.7	92.0	89.0	3.1	3.0	8.0	1.9	2.7, 2.5	89.7, 111.6	86.6, 105.5	n.d., n.d.	2.0, 6.6	2.7, 4.1	4.0, 4.0	1.8, 2.1
452-427	2.6	108.8	103.5	6.7	n.d.	-	-	2.7, 2.4	104.4, 137.6	96.3, 117.7	n.d., n.d.	5.9, 10.4	2.9, 4.9	8.0, 32.0	1.6, 2.1
596-452	2.4	n.d.	n.d.	-	n.d.	-	-	2.4, 2.0	n.d., n.d.	n.d., n.d.	n.d., n.d.	- -	n.d., n.d.	- -	- -
620-596	2.5	184.7	168.1	4.5	n.d.	-	-	2.5, 2.0	180.4, 1029.2	168.1, 94.3	n.d., 827.1	4.5, 53.1	n.d., 35.8	- 183.9	- 2.0
644-620[e]	2.8	40.4	39.5	-	n.d.	-	-	2.9, 2.3	32.0, 319.8	31.0, 32.1	n.d., 258.1	- -	1.1, 10.2	- -	2.1, 1.9
738-644	2.7	50.5	46.7	1.1	1.5	-	-	2.8, 2.0	42.8, 768.4	39.5, 32.2	n.d., 687.2	1.1, 47.7	1.5, 27.6	4.3, 185.1	2.0, 2.1
787-738	2.8	57.7	56.7	1.5	n.d.	-	-	2.7, 1.9	46.5, 977.7	44.4, 41.5	n.d., 869.4	0.8, 42.7	2.0, 37.7	10.2, 206.1	2.5, 2.2
811-787	2.8	63.1	59.8	2.3	2.1	8.0	2.0	2.7, 1.9	55.0, 1073.0	49.9, 64.6	n.d., 918.4	3.5, 39.7	n.d., 39.4	- 70.8	- 2.1
906-811	2.6	84.3	80.5	2.3	3.0	9.5	2.1	2.4, 1.8	134.1, 1380.0	96.8, 103.5	37.1, 1086.2	8.3, 32.3	5.3, 53.0	27.7, 143.2	2.3, 2.2

a) In this test series, museum grade pyrite, < 38u, ~ 500 mg in 100 ml of sterile 1/10 m9K was agitated at room temperature. To runs SS-3 and SS-4, 0.316 mg of *T. ferrooxidans* protein was added.
b) Fe^t is the total soluble iron in mg/ml: Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml: $SO_4^=$ is the sulfate ion in mg/ml.
c) These rates are the total soluble iron or sulfate produced per ml of solution per hr during the time interval specified.
d) Not determined.
e) After 620 hr, 50 ml of sterile 1/10 m9K were added.

TABLE XXVI

EFFECT OF MINE *T. FERROOXIDANS* UPON PYRITE SOLUBILIZATION[a]

(Pyrite, 1/10 9K, *T. ferrooxidans*, mine isolated strain VV-10)

Time	Average VV-1 and VV-2 Runs					Average VV-3 and VV-4 Runs					Average VV-5 and VV-6 Runs					
Interval		$Fe^{t,b}$	Fe^{+2}	Rate $Fe^{t,c}$	$SO_4^=$		Fe^t	Fe^{+2}	Rate Fe^t	$SO_4^=$		Fe^t	Fe^{+2}	Fe^{+3}	Rate Fe^t	$SO_4^=$
hr	pH	10^{-3}	10^{-3}	10^{-4}	10^{-1}	pH	10^{-3}	10^{-3}	10^{-4}	10^{-1}	pH	10^{-3}	10^{-3}	10^{-3}	10^{-4}	10^{-1}
0	3.3	13.1	12.6	-	3.2	3.4	11.2	10.6	-	3.5	3.2	5.8	5.3	n.d.[d]	-	3.1
70-0	3.1	19.2	18.3	0.9	3.3	3.1	19.1	18.1	1.1	3.5	3.1	23.8	22.6	n.d.	2.6	3.6
119-70	3.0	24.7	24.3	1.1	3.5	3.0	25.4	25.2	1.3	3.5	3.0	32.9	31.3	n.d.	1.9	3.8
239-119	2.9	44.4	41.7	1.6	4.4	2.9	42.8	40.3	1.5	4.2	2.6	95.2	76.9	n.d.	5.2	11.6
286-239	2.8	52.3	49.8	1.7	5.1	2.8	50.3	27.8	1.6	4.8	2.4	386.2	35.9	309.5	61.9	16.9
310-286	2.8	57.7	52.3	2.3	n.d.	2.8	55.1	49.6	2.0	4.8	2.3	503.9	22.3	436.9	49.0	21.2
335-310	2.7	61.0	57.0	1.3	5.1	2.8	58.6	53.3	1.4	4.9	2.2	633.3	12.9	571.5	51.8	24.2
407-335	2.7	78.3	73.3	2.4	5.4	2.7	72.7	66.6	2.0	5.6	2.0	1050.1	99.9	865.9	57.9	39.7
430-407	2.7	84.4	75.6	2.7	5.7	2.7	77.9	71.1	2.3	5.8	2.0	1118.4	160.6	901.1	29.7	42.8
455-430	2.7	88.7	86.5	1.7	6.3	2.7	82.8	80.5	1.9	6.2	2.0	1169.3	248.1	888.5	20.4	43.7
478-455	2.7	99.3	89.7	4.6	6.5	2.7	89.7	82.6	3.0	5.8	1.9	1225.4	360.7	839.7	24.4	46.9
502-478	2.7	113.9	102.2	6.1	6.8	2.7	98.6	91.7	3.7	6.2	1.9	1253.4	476.9	754.8	11.7	47.3
575-502	2.6	146.8	139.1	4.5	8.4	2.6	131.2	125.3	4.5	7.0	1.8	1294.1	786.7	539.4	5.6	50.8

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a) In this test series, museum grade pyrite, < 38u, ~ 500 mg in 100 ml of sterile 1/10 9K was agitated at room temperature. To runs VV-3 and VV-4, 0.316 mg of *T. ferrooxidans* protein was added and to runs VV-5 and VV-6, 2.25 mg of *T. ferrooxidans* protein was added.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml.

c) These rates are the soluble iron produced per ml of solution per hr during the time interval specified.

d) Not determined.

TABLE XXVII

EFFECT OF MEDIUM UPON THE ACTIVITY OF *T. THIOOXIDANS*[a]

(ATCC strain)

Medium	Inoculum size		Time, hr				
			0	71	99	142	167
1/10 m9K	none	pH	3.3	3.3	3.2	3.3	3.3
		$SO_4^{=,b}$	+	+	+	+	+
1/10 m9K	2.42μg protein/ml	pH	3.3	2.1	1.9	1.7	1.5
	(5-78-106-3)	$SO_4^=$	+	0.69	1.41	2.55	3.73
		$d(SO_4^=)/dt^c$	-	0.23	0.62	0.64	1.13
1/10 9K	none	pH	3.4	3.4	3.4	3.5	3.2
		$SO_4^=$	0.31	0.33	0.33	0.33	0.27
1/10 9K	2.42μg protein/ml	pH	3.5	2.2	2.1	1.9	1.7
	(5-78-106-3)	$SO_4^=$	0.39	0.97	1.23	1.39	1.96
		$d(SO_4^=)/dt$	-	0.20	0.22	0.09	0.55

a) The tests were performed in 1/10 concentration of m9K and 9K medium with ~ 0.5 g of sulfur flowers per 50 ml. The tests were carried out without agitation in an incubator at 28°C.

b) The concentrations are given as mg/ml (values below 0.08 mg/ml are denoted by +).

c) The rate of $SO_4^=$ production is given in mg/ml/24 hr.

TABLE XXVIII

EFFECT OF T. THIOOXIDANS UPON PYRITE SOLUBILIZATION[a]

(Pyrite, water, T. thiooxidans, ATCC strain)

Time Interval hr	Average Q-3 and Q-4 Runs					Average Q-1 and Q-2 Runs[b]				
	pH	$Fe^{t,c}$ 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate $Fe^{t,d}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}
0	4.4	2.9	n.d.[e]	0.7	-	4.1	4.2	n.d.	1.1	-
23-0	3.5	19.7	n.d.	0.7	7.3	3.3	20.9	n.d.	0.6	7.3
71-23	3.1	37.0	34.9	0.9	3.6	3.0	30.5	29.0	1.1	2.0
144-71	2.9	48.8	45.6	n.d.	1.6	3.0	31.5	29.2	2.4	0.1
240-144	2.8	69.3	n.d.	1.4	2.1	2.9	36.9	34.9	1.1	0.6
312-240	2.7	82.5	78.1	1.6	1.8	2.9	43.6	40.9	1.1	0.9
504-312	2.6	111.5	104.2	n.d.	1.5	2.8	63.5	60.2	n.d.	1.0

a) In this test series, museum grade pyrite, < 38μ, ~ 500 mg in 100 ml of sterile deionized water was agitated at room temperature.
b) 2.52 mg of T. thiooxidans protein was added.
c) Fe^{t} is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml.
d) These rates are the total soluble iron produced per ml of water per hr during the time interval specified.
e) Not determined.

TABLE XXIX

EFFECT OF T. THIOOXIDANS UPON PYRITE SOLUBILIZATION[a]

(Pyrite, 1/10 9K, T. thiooxidans, ATCC strain)

Time Interval hr	Average X-3 and X-4 Runs					Average X-1 and X-2 Runs[b]				
	pH	$Fe^{t,c}$ 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate $Fe^{t,d}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}
0	3.1	14.2	11.8	n.d.[e]	-	3.0	15.0	12.7	n.d.	-
96-0	2.8	15.2	14.3	n.d.	0.1	2.6	22.0	21.4	n.d.	0.7
144-96	2.8	19.7	13.8	n.d.	0.9	2.6	25.6	24.2	n.d.	0.8
336-144	2.7	41.4	39.5	n.d.	1.1	2.6	39.1	38.1	n.d.	0.7
504-336	2.7	62.3	58.7	3.1	1.2	2.6	62.0	58.2	3.6	1.4
672-504	2.5	104.4	95.8	4.9	2.5	2.5	89.7	81.5	4.1	1.6

a) In this test series, museum grade pyrite, < 38µ, ~ 500 mg in 100 ml of sterile 1/10 9K medium was agitated at room temperature.

b) 2.46 mg of T. thiooxidans protein was added.

c) Fe^{t} is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml.

d) These rates are the total soluble iron produced per ml of water per hr during the time interval specified.

e) Not determined.

TABLE XXX

EFFECT OF T. THIOOXIDANS UPON PYRITE SOLUBILIZATION[a]

(Pyrite, 1/10 m9K, T. thiooxidans, ATCC strain)

Time Interval hr	Average R-3 and R-4 Runs					Average R-1 and R-2 Runs[b]				
	pH	$Fe^{t,c}$ 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate $Fe^{t,d}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}
0	3.1	9.8	9.1	0.6	-	3.1	10.6	10.5	n.d.[e]	-
24-0	2.9	12.2	11.2	0.6	1.0	2.8	14.9	14.6	0.6	1.8
48-24	2.9	15.1	13.9	0.7	1.2	2.8	18.2	17.1	1.0	1.4
144-48	2.8	25.1	24.1	0.5	1.0	2.8	26.8	25.5	0.7	0.9
336-144	2.8	32.6	31.1	n.d.	0.4	2.7	45.0	43.9	n.d.	1.9

a) In this test series, museum grade pyrite, < 38μ, ~ 500 mg in 100 ml of sterile 1/10 m9K medium was agitated at room temperature.
b) 2.52 mg of T. thiooxidans protein was added.
c) Fe^{t} is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml.
d) These rates are the total soluble iron produced per ml of water per hr during the time interval specified.
e) Not determined.

TABLE XXXI

CHANGES IN pH, TOTAL SOLUBLE IRON AND SULFATE CONCENTRATIONS AND RATES OF SOLUBLE IRON PRODUCTION AS AFFECTED BY MARCASITE CONCENTRATION[a]

(Marcasite, water)

Time Interval hr	500 mg Marcasite, Average BB-1 and BB-2 Runs						1000 mg Marcasite, Average BB-3 and BB-4 Runs					
	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^=$ 10^{-1}	$SO_4^=/Fe^t$ mole ratio	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	$SO_4^=/Fe^t$ mole ratio
0	3.6	90.7	84.6	-	2.6	1.7	3.4	n.d.[d]	n.d.	-	n.d.	-
48-0	3.0	100.0	93.3	1.9	3.8	2.2	2.7	217.6	204.3	-	7.1	1.9
167-48	2.7	180.1	167.1	6.7	6.1	2.0	2.6	428.0	386.2	17.7	13.5	1.8
342-167	2.6	351.5	305.7	9.8	10.9	1.8	2.4	685.3	563.0	14.7	23.4	2.0
505-342	2.4	466.7	410.7	7.1	14.5	1.8	2.3	899.4	708.2	13.1	24.9	1.6

a) In this test series, marcasite from Siglo XX Mine, Llallagua, Bolivia, $< 38\mu$, in 100 ml of sterile deionized water was agitated at room temperature.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml.

c) The rate is the total soluble iron produced per ml of water per hr during the time interval specified.

d) Not determined.

TABLE XXXII

RESPIROMETER TEST SERIES 6R

pH, OXYGEN UPTAKE AND IRON PRODUCTION DATA FOR MARCASITE IN WATER AT 14°C [a]

Sampling Period day	pH[b]	$Fe^{t,b}$ µmoles	O_2[b] µmoles	Mole Ratio of Species Produced during a given time interval Fe	:	O_2
1	3.0	0.26	4.38 (4.05_{13})	1		17
2	3.0	0.32	7.41 (7.85_{11})	1		51
3	2.9	1.25	10.45 (10.78_{10})	1		3.3
6	2.8	2.97	17.58 (17.26_{8})	1		4.1
7	2.8	3.49	17.63 (17.98_{6})	1		0.1
8	2.8	4.68	19.26 (19.31_{4})	1		1.4
9	2.8	5.04	20.98	-		-

a) All the tests were performed at 14°C using marcasite, < 38µ, 25 mg per 5 ml of deionized water. The values given for the specific time periods are corrected for the zero time sampling, i.e., the values obtained at zero time sampling are subtracted out.

b) The value given is the average for two samples, while the value given in parenthesis is the average value for this time period and the subscript value is the number of samples averaged.

TABLE XXXIII

EFFECT OF *T. FERROOXIDANS* UPON MARCASITE SOLUBILIZATION[a]

(Marcasite, water, *T. ferrooxidans*, ATCC strain)

Time	W-7 Run					Average W-1 and W-2 Runs[b]				
Interval hr	pH	$\mathrm{Fe}^{t,c}$ 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate $\mathrm{Fe}^{t,d}$ 10^{-4}	pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}
0	3.9	33.5	31.8	n.d.[e]	-	3.7	26.2	23.6	n.d.	-
23-0	2.8	81.5	76.2	n.d.	20.9	2.8	84.2	79.6	n.d.	25.3
47-23	2.7	130.5	114.5	6.9	20.4	2.7	128.0	113.5	7.8	18.3
119-47	2.6	196.6	166.2	23.8	9.2	2.6	192.6	159.7	22.2	9.0
191-119	2.5	217.1	176.3	27.8	2.8	2.5	231.3	186.0	31.6	5.4
287-191	2.5	258.8	196.7	44.0	4.3	2.4	287.4	93.3	166.0	5.9
359-287	2.4	309.8	248.6	47.0	7.1	2.3	440.2	165.1	306.5	21.2
455-359	2.4	372.0	275.3	75.5	6.5	2.2	654.7	254.8	529.6	22.3

a) In this test series, marcasite from Siglo XX Mine, Liallagua, Bolivia, < 38μ, ~ 500 mg in 100 ml of sterile deionized water was agitated at room temperature.

b) 1.92 mg of *T. ferrooxidans* protein was added.

c) Fe^{t} is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml.

d) These rates are the total soluble iron produced per ml of water per hr during the time interval specified.

e) Not determined.

TABLE XXXIV

EFFECT OF T. FERROOXIDANS UPON MARCASITE SOLUBILIZATION [a]

(Marcasite, 1/10 9K, T. ferrooxidans, ATCC strain)

Time Interval hr	Average Y-3 and Y-4 Runs pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	Average Y-1 and Y-2 Runs pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^{t} 10^{-4}
0	3.0	94.4	90.0	n.d.[d]	-	3.0	86.4	n.d.	n.d.	-
67-0	2.9	168.4	151.6	n.d.	11.0	2.8	169.4	n.d.	n.d.	12.4
235-67	2.7	241.9	210.4	24.6	4.4	2.0	595.1	109.0	461.7	25.3
403-235	2.5	344.9	295.6	49.5	6.1	1.9	905.4	252.7	630.6	18.5

a) In this test series, marcasite from Siglo XX Mine, Llallagua, Bolivia, < 38µ, ~ 500 mg in 100 ml of 1/10 9K solution was agitated at room temperature. To runs Y-1 and Y-2, 2.56 mg of T. ferrooxidans protein was added after 67 hr.

b) Fe^{t} is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml.

c) The rate is the total soluble iron produced per ml of water per hr during the time interval specified.

d) Not determined.

TABLE XXXV

EFFECT OF T. THIOOXIDANS UPON MARCASITE SOLUBILIZATION[a]

(Marcasite, 1/10 m9K, T. thiooxidans, ATCC strain)

Time	Average UU-1 and UU-2 Runs								UU-3 Run							
Interval hr	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	$SO_4^=/Fe^t$ mole ratio	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	$SO_4^=/Fe^t$ mole ratio
0	3.0	80.0	74.1	n.d.[d]	-	2.4	-	1.7	3.1	82.5	75.9	n.d.	-	2.2	-	1.6
71-0	2.8	178.3	162.5	n.d.	13.8	4.7	32.4	1.5	2.2	300.1	233.8	n.d.	30.6	11.0	123.3	2.1
95-71	2.7	208.4	198.2	n.d.	12.5	5.7	41.7	1.6	2.1	417.8	285.3	88.1	49.0	14.6	150.0	2.0
119-95	2.7	242.0	232.4	n.d.	14.0	6.5	33.3	1.6	2.1	440.2	362.8	115.7	9.3	15.4	33.3	2.0
168-119	2.6	319.5	270.6	31.8	15.8	7.5	20.4	1.4	1.9	542.1	356.7	151.0	20.8	18.1	55.1	1.9
287-168	2.5	490.4	400.5	33.5	14.4	11.1	30.3	1.3	1.9	733.7	463.6	257.9	16.1	26.4	69.7	2.1
340-287	2.5	566.6	482.0	96.2	14.4	12.8	32.1	1.3	1.9	825.4	519.7	289.3	17.3	28.8	45.3	2.0
431-340	2.4	703.1	523.8	135.2	15.0	14.3	16.5	1.2	1.8	957.8	540.0	396.3	14.5	35.6	74.7	2.2
479-431	2.4	776.7	560.5	n.d.	15.4	18.0	77.1	1.3	1.7	1024.1	550.3	415.1	13.8	37.6	41.7	2.1
502-479	2.4	805.0	621.6	173.0	12,3	17.9	-	1.3	1.7	1054.6	626.7	418.3	13.3	40.6	130.4	2.2

a) In this test series, ~500 mg of marcasite from Siglo XX Mine, Llallagua, Bolivia, < 38u, in 100 ml of sterile 1/10 m9K was agitated at room temperature. To the run UU-3, 2.42 mg of T. thiooxidans protein was added.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml.

c) These rates are the total soluble iron or sulfate produced per ml of solution per hr during the time interval specified.

d) Not determined.

TABLE XXXVI

ANALYSIS OF SAMPLES FROM AN ABANDONED MINE AND BORE HOLE OVERFLOW

No.	Description		pH	Fe Total ppm	*T. thioparus*[a]	*T. thiooxidans*[b]	*T. ferrooxidans*[c]	*Metallogenium*[d]	$SO_4^=$ reducers[e]	Heterotrophic Population[f]	Yeast Population[g]
1	water, free flow	Bed No. 1	4.6	1.5	+	+	+	-	+	+	+
2	water, muddy	Bed No. 2	5.2	T[h]	+	+	+	-	+	+	+
3	water, stagnant	Bore hole	4.7	107	+	+	+	-	+	+	+
4	water, froth	Bore hole	4.6	67	+	+	+	-	+	+	+
5	water, muddy	Bore hole	4.4	62	+	+	+	-	+	+	+
6	water, free flow	Bore hole	5.5	114	+	-	-	-	+	+	+
7	yellowboy	Bed No. 1	n.d.	n.d.	-	+	+	-	+	n.d.	n.d.
8	"stalactite"	Bed No. 2	n.d.	n.d.	+	+	+	-	+	n.d.	n.d.
9	coal	Bed No. 2	n.d.	n.d.	+	+	+	-	+	n.d.	n.d.
10	rock	Bed No. 2	n.d.	n.d.	-	+	+	-	-	n.d.	n.d.

a) The medium utilized was Vishniac's medium[38].
b) The medium utilized was Silverman and Lundgren's 9K medium[27], using elemental sulfur as the energy source.
c) The medium utilized was Silverman and Lundgren's 9K medium[27], using ferrous iron as the energy source.
d) The *Metallogenium* medium of Walsh and Mitchell[39] was employed.
e) The P3 medium of Postgate[40] was utilized.
f) Tryptone Glucose Extract Agar (Difco) was employed to enumerate aerobic heterotrophs according to the methods of Tuttle, et al.[41]
g) Sabouraud Dextrose Agar (Difco) was utilized to enumerate yeast according to the methods of Tuttle, et al.[41]
h) Values below 0.5 ppm are denoted by T.

TABLE XXXVIII

LISTING OF SAMPLES FROM VV-MINE

Sample Identification	Sampling Site (Fig. 18)	Location Description
VV-1 (coal)	1	coal; bottom of a freshly (< 12 hrs) cut coal face; room 55
VV-2 (coal)	1	coal; upper middle of a freshly (< 12 hrs) cut coal face; room 55
VV-9 (water)	1	water; pool which was 4 to 6 feet from a freshly (< 12 hrs) cut coal face; room 55
VV-12 (water-coal)	1	coal and drip water; freshly (< 12 hrs) cut coal face; room 55
VV-11 (water)	1	water; pond two rooms out by active face; room 53
VV-3 (shale)	2	roof shale; old haulage way
VV-4 (coal)	2	roof coal; old haulage way
VV-5 (coal)	3	wall coal; very old acid region near sump
VV-6 (shale)	3	binder shale; very old acid region near sump
VV-7 (coal)	3	roof coal; very old acid region near sump
VV-8 (shale)	3	roof shale; very old acid region near sump
VV-10 (water)	3	water from sump; very old acid region
W-3 (water)	-	water; inlet into the settling pond
W-4 (water)	-	water; outlet from the settling pond
W-1 (water)	-	well water, chlorinated
W-2 (water)	-	well water, chlorinated

TABLE XXXIX

ANALYSES OF VV-MINE SAMPLES

Sample	Solid Analysis[a]							Water Analysis[b]		
	Pyrite Analysis				Ash Analysis					
	Fe (pyritic)	$SO_4^=$	FeS_2[c]	$SO_4^=/Fe^t$	Ash	Fe^{total}	Ca	pH	$Fe^{t,d}$	$SO_4^{=,d}$
	%[e]	%	%	mole ratio	%	%	%		10^{-2}	
VV-1 (coal)	1.05	3.39	2.24	1.82	7.5	1.15	0.47	8.0	+[h]	0.41
VV-2 (coal)	0.94	3.08	2.03	1.89	5.4	0.94	0.20	8.1	+	0.42
VV-9 (water)	n/a[g]	n/a	n/a	n/a	n/a	n/a	n/a	7.9	+	6.12
VV-12 (water-coal)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7.6	+	0.92
VV-11 (water)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7.9	+	13.76
VV-3 (shale)	1.17	3.02	2.50	1.51	82.5	1.51	0.14	4.3	1.9	9.27
VV-4 (coal)	1.67	5.73	3.58	1.98	16.8	1.98	0.09	2.4	1009.0	24.43
VV-5 (coal)	1.32	4.40	2.83	1.94	7.5	1.52	0.49	2.5	173.0	4.90
VV-6 (shale)	0.46	b.d.l.[f]	0.99	-	91.4	1.25	8.36	3.6	1.1	2.04
VV-7 (coal)	0.83	2.68	1.77	1.82	9.9	1.12	0.33	2.5	286.0	7.71
VV-8 (shale)	0.84	2.20	1.79	1.50	87.3	1.48	23.8	3.7	0.65	3.65
VV-10 (water)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7.0	0.85	4.24
W-1 (water)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7.6	b.d.l.	0.09
W-2 (water)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7.9	b.d.l.	0.09
W-3 (water)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	8.0	b.d.l.	0.43
W-4 (water)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	8.1	b.d.l.	1.17

a) The solid samples (~ 100 g) were washed with deionized water (~ 50 ml) before being ground for analysis; the exception being VV-3 sample which was not washed.

b) Wash solutions from the solid samples were analyzed.

c) FeS_2 was determined indirectly from pyritic iron.

d) Fe^t and $SO_4^=$ are given in mg/ml for the mine water samples; in mg/ml (wash solution)/100 g solid for the solid specimen.

e) All the percentages are given with respect to coal.

f) Below detection limits of the procedure employed.

g) Not applicable.

h) Values below 0.0005 mg/ml are denoted by +.

TABLE XL

VV-MINE DETAILED WATER SAMPLES ANALYSES[a]

	VV-9	VV-10
Sodium	4,800 mg/l	2,025 mg/l
Calcium	881 mg/l	972 mg/l
Aluminum	2,510 mg/l	13 mg/l
Silicon	1,953 mg/l	57 mg/l
Magnesium	216 mg/l	81 mg/l
Iron	(5.6X10^{-2})[b] 104 mg/l	(8.5)[b] 24 mg/l
Barium	<20 mg/l	ND<0.8 mg/l
Boron	6 mg/l	1.8 mg/l
Manganese	53 mg/l	9.7 mg/l
Titanium	318 mg/l	1.1 mg/l
Lead	Trace <1 mg/l	ND<1 mg/l
Chromium	29 mg/l	0.2 mg/l
Nickel	1.3 mg/l	0.1 mg/l
Vanadium	21 mg/l	ND<0.1 mg/l
Copper	22 mg/l	0.3 mg/l
Zirconium	1 mg/l	ND<0.1 mg/l
Cobalt	9 mg/l	0.3 mg/l
Strontium	53 mg/l	14.5 mg/l
Potassium	851 mg/l	Trace>8 mg/l
Other Elements	NIL	NIL
Carbonate	None Detected	None Detected
Bicarbonate	561.2 mg/l	350.75 mg/l
Chlorides	1,418.0 mg/l	1,063.5 mg/l
Nitrate	2.21 mg/l	2.21 mg/l
Total Phosphate	22.6 mg/l	6.10 mg/l
Sulfate	(6,119)[b] 6,036.70 mg/l	(4,242)[b] 4,468.89 mg/l
Fluoride	0.60 mg/l	0.37 mg/l
Total Hardness	600 mg/l	1,825 mg/l
Total Alkalinity	460 mg/l	287.5 mg/l
Total Dissolved Solids	12,220 mg/l	8,268 mg/l
Electrical Conductivity	16,500 mmhos/cm	11,000 mmhos/cm
pH	(7.90)[b] 7.60	(7.03)[b] 6.69

a) All these analyses were performed by Associated Laboratories. The cations were determined by spectrographic analysis; anions by wet chemical analysis using standard EPA water methods.

b) These values were obtained by Ultrasystems, Inc.

TABLE XLI

SIMULATED MINE WATER ANALYSES

	Theoretical	Determined by A. L.[a]	Determined in-house
Sodium	2,000 mg/l	4,000 mg/l	-
Potassium	1,600 mg/l	2,000 mg/l	-
Phosphorus	636 mg/l	500 mg/l	-
Magnesium	200 mg/l	41 mg/l	-
Manganese	80 mg/l	69 mg/l	-
Iron	100 mg/l	14 mg/l	104 mg/l
Aluminum	500 mg/l	13 mg/l	-
Nickel	-	0.33 mg/l	-
Calcium	900 mg/l	44 mg/l	-
Copper	30 mg/l	11 mg/l	-
Chromium	-	trace	-
Other Elements	-	NIL	-
Carbonate	0	NIL	-
Bicarbonate	0	NIL	-
Chloride	1,700 mg/l	1,770 mg/l	-
Nitrate	5,400 mg/l	5,310 mg/l	-
Phosphate	1,950 mg/l	2,250 mg/l	-
Sulfate	4,573 mg/l	4,798 mg/l	4,856 mg/l
Fluoride	-	ND<0.01 mg/l	-
Hardness	-	4,000 mg/l	-
Alkalinity	-	NIL	-
Total dissolved solids	19,033 mg/l	15,800 mg/l	-
Electrical conductivity	-	88,000 mmhos/cm	-
pH	-	1.5	1.4

a) These analyses were performed by Associated Laboratories; cations were determined by spectrographic analysis, anions by wet chemical analysis using standard EPA water methods.

TABLE XLII

RESULTS OF THE BACTERIAL ANALYSIS OF VV-MINE SAMPLES[a]

Mine Sample[b]	pH~7 S-oxid[c]	pH=2.7 S-oxid[d]	pH=2.7 Fe-oxid[e]	Metallogenium[f]	S-red[g]	Heterotrophs[h]	Yeast[i]
VV-1 (coal)	-	-	?<3	-[j]	+	2.6 X 10^4	5.0 X 10
VV-2 (coal)	(+)[k]	-	?<3	-	+	5.4 X 10^4	1.3 X 10^3
VV-9 (water)	$<10^5$-$>10^3$	-	4.0 X 10^2	-	+	7.4 X 10^5	1.0 X 10^4
VV-12 (water-coal)	-	-	?<3	-	+	4.2 X 10^4	8.0 X 10
VV-11 (water)	$<10^7$-$>10^5$	-	4.3 X 10^4	-	+	2.8 X 10^7	5.0 X 10^3
VV-3 (shale)	-	-	?<3	-	+	1.9 X 10^2	1.7 X 10^2
VV-4 (coal)	-	-	?<3,	-	-	1.0 X 10	6.0 X 10
VV-5 (coal)	-	-	?<3	-	-	-	-
VV-6 (shale)	-	-	?<3	-	-	1.1 X 10^3	9.1 X 10^2
VV-7 (coal)	-	-	?<3	-	-	5.0 X 10	-
VV-8 (shale)	-	-	?<3	-	-	2.3 X 10^3	1.7 X 10^3
VV-10 (water)	$<10^7$-$>10^5$	$<10^7$-$>10^5$	9.3 X 10^6	-	+	3.5 X 10^2	2.9 X 10
W-3 (water)	4.3 X 10^3	<3	?<3	-	2.4 X 10^4	3.6 X 10^3	2.4 X 10^2
W-4 (water)	9.3 X 10^4	2.4 X 10^4	4.3 X 10^5	-	4.6 X 10^5	2.9 X 10^4	1.0 X 10^2

a) The bacterial populations were determined using a three-tube MPN method, according to Standard Methods[44].

b) In the case of solid samples, 10 g of solid was washed with 90 ml of 1/10 9K salt solution of Silverman and Lundgren[27], and this solution was then utilized in the MPN evaluations; or 1 g of coal was suspended in 50 ml nutrient solution.

c) The medium utilized was Vishniac's medium[38].

d) The medium utilized was Silverman's and Lundgren's 9K medium[27], using elemental sulfur as the energy source.

e) The medium utilized was Silverman's and Lundgren's 9K medium[27], using ferrous iron as the energy source.

f) The <u>Metallogenium</u> medium of Walsh and Mitchell[39] was employed.

g) The P3 medium of Postgate[40] was utilized.

h) Tryptone Glucose Extract Agar (Difco) was employed to enumerate aerobic heterotrophs according to the methods of Tuttle, et al.[41]

i) Sabouraud Dextrose Agar (Difco) was utilized to enumerate yeast according to the methods of Tuttle, et al.[41]

j) In none of the cultures was <u>Metallogenium</u> observed microscopically, also in no instance was the oxidation of Fe^{++} to Fe^{+++} found to occur.

k) Bacteria were observed microscopically, but the bacterial species was questionable due to unchanged medium pH.

TABLE XLIII

COAL SOLUBILIZATION STUDIES[a]

(Coals VV-1, VV-2, water)

Time Interval hr	Coal VV-1, Average EE-1 and EE-2 Runs				Coal VV-2, Average EE-3 and EE-4 Runs			
	pH	$Fe^{t,b}$ 10^{-3}	$SO_4^{=}$ 10^{-1}	Rate $SO_4^{=,c}$ 10^{-4}	pH	Fe^{t} 10^{-3}	$SO_4^{=}$ 10^{-1}	Rate $SO_4^{=}$ 10^{-4}
0	6.7	+	n.d.[d]	-	6.6	+	n.d.	-
67-0	7.5	+	+	-	7.6	n.d.	+	-
116-67	7.5	+	+	4	7.6	+	0.8	4
238-116	7.5	+	0.8	1	7.6	+	0.9	1
404-238	7.5	+	1.2	2	7.6	+	n.d.	-
476-404	7.2	+	1.3	1	7.4	+	1.0	-
597-476	7.2	+	1.9	5	7.3	+	1.2	2
764-597	7.3	+	2.2	2	7.3	+	2.1	5
937-764	7.1	+	3.1	5	6.8	+	3.1	6
1100-937	7.2	+	n.d.	n.d.	5.9	+	4.5	9
1148-1100[e]	7.1	+	1.2	na[f]	5.7	+	n.d.	na
1268-1148	7.0	+	2.1	8	4.7	+	1.9	-
1340-1268	6.8	+	2.2	1	4.4	0.5	2.0	1
1460-1340	n.d.	n.d.	n.d.	-	3.8	4.0	2.4	3

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a) In this test series, coal VV-1 and VV-2, < 250μ, ~ 5 g in 100 ml of sterile deionized water was agitated at room temperature. Prior to grinding, the coal was washed.

b) Fe^{t} is the total soluble iron in mg/ml (values below 0.0005 mg/ml are denoted by +); $SO_4^{=}$ is the sulfate ion in mg/ml (values below 0.08 mg/ml are denoted by +).

c) This rate is the sulfate ion produced per ml of solution per hr during the time interval specified.

d) Not determined.

e) After 1100 hrs, 50 ml of sterile deionized water was added to each sample.

f) Not applicable.

TABLE XLIV

COAL SOLUBILIZATION STUDIES[a]

(Coal VV-4, water)

Time Interval hr	Average GG-1, GG-2, OO-1, and OO-2 Runs						
	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^{=}$ 10^{-1}	Rate $SO_4^{=}$ 10^{-4}	$SO_4^{=}/Fe^t$ mole ratio
0	4.1	16.5	15.9	-	+	-	-
23-0	3.3	75.3	68.7	25.6	2.5	-	1.9
93-23	3.0	96.5	94.3	3.0	3.1	8.6	1.9
140-93	3.0	111.5	106.3	3.2	3.7	12.8	1.9
280-140	2.9	159.2	137.9	3.4	5.2	10.7	1.9
430-280	2.7	216.3	212.3	3.7	6.6	9.3	1.8
598-430	2.4	312.8	287.7	3.7	7.8	7.1	1.6

a) In this test series, coal VV-4, < 250μ, ~ 5 g in 100 ml of sterile deionized water was agitated at room temperature. Prior to grinding, the coal was washed.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; $SO_4^{=}$ is the sulfate ion concentration in mg/ml (values below 0.08 mg/ml are denoted by +).

c) These rates are the total soluble iron or sulfate ion produced per ml of solution per hr during the time interval specified.

TABLE XL.

COAL SOLUBILIZATION STUDIES[a]

(Coals VV-5 and VV-7, water)

Time Interval hr	Coal VV-5, Average HH-1 and HH-2 Runs					Coal VV-7, Average HH-3 and HH-4 Runs				
	pH	Fe^{t,b} 10^{-3}	Rate Fe^{t,c} 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	pH	Fe^t 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}
0	3.9	5.4	-	1.0	-	3.9	12.6	-	0.8	-
24-0	2.9	93.3	36.6	7.8	283.3	3.0	54.4	17.4	2.0	50.0
119-24	3.0	123.6	3.2	8.1	3.2	3.1	67.5	1.4	n.d.	-
167-119	3.1	136.5	2.7	n.d.	-	3.1	78.0	2.2	2.2	1.4
215-167	3.0	165.0	5.9	8.2	1.0	3.0	86.1	1.7	2.3	2.1
288-215	3.0	188.5	3.2	8.4	2.7	3.0	91.2	0.7	2.5	2.7
359-288	3.0	219.1	4.3	8.9	7.0	3.0	102.7	1.6	2.7	2.8
456-359	3.0	267.0	4.9	9.3	4.1	3.0	121.7	2.0	3.2	5.2
528-456	2.9	305.7	5.4	10.0	9.7	2.8	143.2	3.0	3.9	9.7
624-528	2.9	366.8	6.4	10.5	5.2	2.7	153.3	1.1	4.7	8.3
696-624	2.8	435.6	9.6	11.6	15.2	2.7	167.6	2.0	4.9	2.8
791-696	2.8	517.1	8.6	14.2	27.4	2.6	195.6	3.0	5.4	5.3
961-791	2.7	738.7	13.0	18.1	22.9	2.6	257.8	3.7	6.5	6.5
1055-961[d]	3.1	115.2	-	2.5	-	3.1	34.3	-	1.3	-
1176-1055	2.9	145.7	2.5	3.3	6.6	2.8	49.0	1.2	1.6	2.5
1295-1176	2.9	176.7	2.6	4.4	9.2	2.8	56.1	0.6	2.0	3.4

a) In this test series, coals VV-5 Batch A and VV-7, $< 250\mu$, ~5 g in 100 ml of sterile deionized water was agitated at room temperature. Prior to grinding, the coal was not washed.

b) Fe^t is the total soluble iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml.

c) These rates are the total soluble iron or sulfate produced per ml of solution per hr during the time interval specified.

d) After 961 hr, 50 ml of sterile deionized water was added to each sample.

TABLE XLVI

COAL SOLUBILIZATION STUDIES [a]

(Coals VV-5 and VV-7, water)

Time Interval hr	Coal VV-5, Average GG-3 and GG-4 Runs						Coal VV-7, Average GG-5 and GG-6 Runs					
	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}
0	5.8	n.d.[d]	n.d.	-	+	-	5.7	n.d.	n.d.	-	+	-
18-0	5.1	3.6	3.1	-	1.2	38.8	6.9	b.d.l.[g]	b.d.l.	-	2.6	111.1
91-18	4.0	18.9	18.5	2.1	2.5	17.8	7.0	b.d.l.	b.d.l.	-	2.7	1.4
139-91	3.9	n.d.	n.d.	-	3.1	12.5	7.1	b.d.l.	b.d.l.	-	2.9	4.2
258-139	3.3	80.0	76.3	3.7	4.5	11.8	7.0	b.d.l.	b.d.l.	-	3.1	1.7
330-258	3.1	104.5	100.4	3.4	5.2	9.7	7.2	b.d.l.	b.d.l.	-	3.2	1.4
427-330	3.0	173.7	170.1	7.1	6.2	10.3	7.2	+	b.d.l.	-	3.5	3.1
498-427	2.9	268.5	256.3	13.4	7.4	16.9	7.0	+	b.d.l.	-	4.1	8.5
596-498	2.8	387.2	356.6	12.1	11.3	39.8	6.9	+	+	-	4.6	5.1
692-596	2.8	568.1	537.5	18.8	16.6	55.2	6.5	+	b.d.l.	-	4.9	3.1
786-692[e]	3.1	108.3	105.2	na[f]	3.2	na	5.8	+	b.d.l.	-	1.1	na
858-786	3.0	140.9	118.0	4.5	4.1	12.5	5.0	0.8	0.8	-	1.3	2.8
955-858	2.9	218.6	193.7	8.0	5.3	12.4	4.6	4.2	3.9	0.4	1.6	3.1
1027-955	2.9	269.0	257.8	7.0	6.6	18.1	4.3	8.7	8.2	0.6	1.7	1.3
1147-1027	n.d.	n.d.	n.d.	-	n.d.	-	3.4	27.3	24.6	1.6	2.3	5.0
1267-1147	n.d.	n.d.	n.d.	-	n.d.	-	3.1	72.4	68.1	3.7	4.6	19.2

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a) In this test series, coals VV-5 Batch A and VV-7, < 250u, ~ 5 g in 100 ml of sterile deionized water was agitated at room temperature. Prior to grinding, the coal was washed several times.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml (values below 0.0005 mg/ml are denoted by +); $SO_4^=$ is the sulfate ion in mg/ml (values below 0.08 mg/ml are denoted by +).

c) These rates are the total soluble iron or sulfate produced per ml of solution per hr during the time interval specified.

d) Not determined.

e) After 692 hr, 50 ml of sterile deionized water was added to each flask.

f) Not applicable.

g) Below detection limits.

TABLE XLVII

EFFECT OF PROLONGED COAL EXPOSURE TO MOISTURE UPON ITS DISSOLUTION RATE[a]

(Coal VV-1, water)

Time Interval hr	MM Run					
	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^{=}$ 10^{-1}	Rate $SO_4^{=}$ 10^{-4}
0	6.6	+	+	-	+	-
6-0	6.5	n.d.[d]	n.d.	-	n.d.	-
170-6	7.7	n.d.	n.d.	-	n.d.	-
337-170	7.5	n.d.	n.d.	-	n.d.	-
530-337	7.2	n.d.	n.d.	-	n.d.	-
672-530	6.9	n.d.	n.d.	-	n.d.	-
843-672	4.7	n.d.	n.d.	-	n.d.	-
1009-843	4.0	n.d.	n.d.	-	n.d.	-
1129-1009	3.6	24.2	23.6	-	2.6	-
1177-1129	3.5	28.9	27.5	0.8	3.1	10.4
1297-1177	3.4	39.9	38.2	0.9	3.4	2.5
1370-1297	3.5	47.9	47.8	1.3	n.d.	-
1442-1370	3.4	52.6	51.2	0.5	3.5	0.7
1608-1442	3.3	67.7	66.0	0.9	3.8	1.8
1680-1608	3.3	74.6	72.6	1.0	n.d.	-
1848-1680	3.2	86.4	82.5	0.7	3.9	0.4

a) In this test, coal VV-1, < 250µ, ~ 20 g in 500 ml of sterile deionized water was stirred at room temperature. Prior to grinding and after the grinding, the coal was washed.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml (values below 0.0005 mg/ml are denoted by +); $SO_4^{=}$ is the sulfate ion in mg/ml (values below 0.08 mg/ml are denoted by +).

c) These rates are the total soluble iron or the sulfate ion produced per ml of solution per hr during the time interval specified.

d) Not determined.

TABLE XLVIII

EVALUATION OF THE EFFECT OF MINE WATER AS COMPARED TO DEIONIZED WATER UPON COAL SOLUBILIZATION[a]

(Coal VV-2, water)

Time Interval hr	Aver. KK-1 and KK-2 Runs			Aver. KK-3 and KK-4 Runs			Aver. KK-5 and KK-6 Runs		
	pH	$Fe^{t,b}$ 10^{-3}	$SO_4^=$ 10^{-1}	pH	Fe^t 10^{-3}	$SO_4^=$ 10^{-1}	pH	Fe^t 10^{-3}	$SO_4^=$ 10^{-1}
0	6.1	+	+	8.3	+	51.7	8.2	+	51.4
24-0	7.0	+	0.9	8.3	+	n.d.[c]	8.3	+	n.d.
166-24	7.0	+	0.9	8.3	+	54.5	8.3	+	n.d.
238-166	7.1	+	1.2	8.4	+	n.d.	8.3	+	n.d.
382-238	7.5	+	n.d.	8.4	+	56.4	8.4	+	54.1
502-382	7.3	+	n.d.	8.4	+	n.d.	8.4	n.d.	n.d.
598-502	7.3	+	1.2	8.4	+	56.4	8.4	+	54.2
767-598	7.3	+	1.2	8.3	+	n.d.	8.4	n.d.	n.d.
911-767	7.3	+	1.8	8.3	+	56.4	8.3	+	57.3
1199-911	6.0	+	3.0	8.2	+	61.9	8.2	+	61.4
1559-1199	4.2	n.d.	n.d.	8.2	n.d.	n.d.	8.2	n.d.	n.d.
2376-1559	3.6	n.d.	n.d.	8.1	n.d.	n.d.	8.1	n.d.	n.d.
2904-2376	3.2	71.3	6.0	7.8	+	62.6	7.9	+	108.1

a) In this test series, coal VV-2 (unwashed), < 250µ, ~ 5 g in 100 ml of water were agitated at room temperature. For runs KK-1 and KK-2, sterile, deionized water was used; for runs KK-3 and KK-4, filter sterilized VV-9 water was used; and for runs KK-5 and KK-6, filtered VV-9 water was used.

b) Fe^t is the total soluble iron in mg/ml (values below 0.0005 mg/ml are denoted by +); $SO_4^=$ is the sulfate ion in mg/ml (values below 0.08 mg/ml are denoted by +).

c) Not determined.

TABLE XLIX

SHALE SOLUBILIZATION STUDIES[a]

(Shales VV-3, VV-6, VV-8, water)

Time Interval hr	Shale VV-3 Aver. FF-2, NN-1, NN-2 Runs			Shale VV-6 Aver. II-1 and II-2 Runs			Shale VV-8 Aver. II-3 and II-4 Runs		
	pH	$Fe^{t,b}$ 10^{-3}	$SO_4^{=}$ 10^{-1}	pH	Fe^{t} 10^{-3}	$SO_4^{=}$ 10^{-1}	pH	Fe^{t} 10^{-3}	$SO_4^{=}$ 10^{-1}
0	5.1	+	1.8	6.2	+	n.d.[c]	6.1	+	1.1
24-0	4.6	1.0	2.6	6.9	+	n.d.	6.0	+	1.3
95-24	4.5	2.3	2.8	6.2	+	n.d.	5.9	+	1.4
192-95	4.4	4.7	3.2	6.5	+	n.d.	5.6	+	1.6
312-192	4.0	7.7	3.6	5.8	+	n.d.	5.0	+	1.9
432-312	n.d.	n.d.	n.d.	5.6	+	n.d.	4.9	0.9	2.1
480-432	4.0	11.6	4.5	5.2	+	+	4.5	1.4	2.7
600-480	3.8	17.5	4.9	n.d.	n.d.	n.d.	4.5	1.8	2.8
720-600	3.6	23.6	5.5	5.2	+	+	4.2	2.6	n.d.
841-720	3.5	30.4	6.4	5.5	+	+	4.2	3.5	2.8
1175-841	n.d.	n.d.	n.d.	4.9	+	0.9	3.9	8.0	3.8

a) In this test series, shale VV-3, VV-6, and VV-8, < 250μ, ~ 5 g in 100 ml of sterile deionized water were agitated at room temperature. Prior to grinding, the shales were washed, with the exception of VV-3.

b) Fe^{t} is the total soluble iron in mg/ml (values below 0.0005 mg/ml are denoted by +), $SO_4^{=}$ is the sulfate ion in mg/ml (values below 0.08 mg/ml are denoted by +).

c) Not determined.

TABLE L

EFFECT OF T. FERROOXIDANS UPON COAL SOLUBILIZATION[a]

(Coal VV-1, 1/10 m9K, T. ferrooxidans, ATCC strain)

Time Interval hr	Average JJ-1 and JJ-2 Runs				Average JJ-3 and JJ-4 Runs			
	pH	$Fe^{t,b}$	$SO_4^=$ 10^{-1}	Rate $SO_4^{=,c}$ 10^{-4}	pH	Fe^t	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}
0	4.9	n.d.[d]	+	-	5.1	n.d.	+	-
22-0	6.8	+	+	-	6.9	+	+	-
118-22	6.5	+	+	-	6.6	+	+	-
166-118	6.2	+	+	-	6.2	+	+	-
285-166	5.9	+	+	-	5.9	+	+	-
358-285	5.8	+	1.0	-	5.8	+	1.0	-
479-358	5.8	+	1.1	0.8	5.8	+	1.1	0.8
549-479	5.8	+	1.2	1.4	5.8	+	1.2	1.4
646-549	5.8	+	1.3	1.0	5.8	+	1.3	1.0
814-646	5.6	+	1.7	2.4	5.7	+	1.8	3.0
1125-814	5.6	+	2.1	1.3	5.7	+	2.5	2.3

a) In this test series, coal VV-1, $< 250\mu$, ~ 5 g in 100 ml of sterile 1/10 m9K was agitated at room temperature. To runs JJ-3 and JJ-4, 2.49 mg of T. ferrooxidans protein was added. Prior to grinding, the coal was washed.

b) Fe^t is the total soluble iron in mg/ml (values below 0.0005 mg/ml are denoted by +); $SO_4^=$ is the sulfate ion in mg/ml (values below 0.08 mg/ml are denoted by +).

c) This rate is the sulfate ion produced per ml of solution per hr during the time interval specified.

d) Not determined.

TABLE LI

EFFECT OF T. FERROOXIDANS UPON COAL SOLUBILIZATION[a]

(Coal VV-5, 1/10 m9K, *T. ferrooxidans*, ATCC strain)

Time		Average JJ-5 and JJ-6 Runs					Average JJ-7 and JJ-8 Runs						
Interval hr	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	$SO_4^=$ 10^{-1}	Rate $SO_4^{=,c}$ 10^{-4}	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	$SO_4^=/Fe^t$ mole ratio
0	3.5	n.d.[d]	n.d.	+	-	3.5	+	+	n.d.	-	+	-	-
22-0	4.6	n.d.	n.d.	1.8	82.0	4.7	+	+	n.d.	-	2.0	91.0	-
118-22	4.3	0.9	0.8	2.3	5.2	3.4	+	+	n.d.	-	3.4	14.6	-
166-118	4.3	1.0	0.8	2.4	2.1	3.1	+	+	n.d.	-	3.9	10.4	-
285-166	4.2	1.3	1.1	2.4	-	2.7	5.8	1.5	n.d.	-	6.4	21.0	-
358-285	3.9	1.3	1.1	2.4	-	2.6	46.1	4.2	39.0	5.5	7.5	15.1	-
479-358	3.8	1.5	1.2	2.5	0.8	2.5	167.3	11.5	149.4	10.0	10.2	22.3	-
549-479	3.8	1.6	1.3	2.6	1.4	2.4	274.3	10.2	263.9	15.3	14.8	65.7	-
646-549	3.7	1.7	1.5	2.8	2.1	2.3	497.3	17.6	436.3	23.0	21.6	70.1	2.5
814-646	3.5	2.0	1.8	2.9	0.6	2.2	749.0	3.7	696.6	15.0	30.4	52.4	2.4
1125-814	3.3	2.8	2.5	3.7	2.6	2.2	1080.2	+	965.5	10.6	37.3	22.2	2.0

a) In this test series, coal VV-5 Batch A, < 250u, ~5 g in 100 ml of sterile 1/10 m9K was agitated at room temperature. To runs JJ-7 and JJ-8, 2.49 mg of *T. ferrooxidans* protein was added. Prior to grinding, the coal was washed several times.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml (values below 0.0005 mg/ml are denoted by +); $SO_4^=$ is the sulfate ion in mg/ml (values below 0.08 mg/ml are denoted by +).

c) These rates are the total soluble iron or sulfate produced per ml of solution per hr during the time interval specified.

d) Not determined.

TABLE LII

COAL SOLUBILIZATION AS AFFECTED BY DRY HEAT STERILIZATION[a]

(Coal VV-5, water)

Time Interval hr	Average LL-1 and LL-2 Runs (Sterilized) pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	Average LL-3 and LL-4 Runs (Unsterilized) pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}
0	5.3	1.5	1.4	-	n.d.[d]	-	5.3	0.6	0.5	-	n.d.	-
22-0	4.6	5.3	5.0	1.7	1.9	-	4.6	4.8	4.6	1.9	1.7	-
142-22	4.2	6.1	5.3	<0.1	2.3	3.3	3.9	23.7	23.0	1.6	2.6	7.5
214-142	4.0	7.0	6.6	0.1	2.4	1.4	3.6	44.0	40.3	2.8	3.2	8.3
334-214	3.7	11.0	10.7	0.3	2.9	4.2	3.3	70.3	69.0	2.2	3.8	5.0
454-334	3.6	17.2	16.6	0.5	3.2	2.5	3.2	94.5	92.8	2.0	4.0	1.7
502-454	3.4	21.2	19.8	0.8	n.d.	-	3.1	112.6	107.5	3.8	4.3	6.3
622-502	3.3	33.5	31.4	1.0	3.5	1.8	3.1	159.5	147.2	3.9	5.8	12.5
694-622	3.2	n.d.	n.d.	-	4.4	-	3.0	187.4	157.4	3.9	6.1	4.2
814-694[e]	3.7	31.9	29.7	-	2.6	-	3.3	106.8	101.9	-	n.d.	-
886-814	3.4	37.0	35.6	0.7	2.6	-	3.2	114.5	111.3	1.1	n.d.	-
984-886	3.3	n.d.	n.d.	-	2.9	-	3.1	126.1	114.4	1.2	n.d.	-

a) In this test series, coal VV-5 Batch A, < 250μ, ~ 5 g in 100 ml of sterile deionized water was agitated at room temperature. Prior to grinding, the coal was washed several times. For runs LL-1 and LL-2, the coal was sterilized by dry heat in nitrogen at 160°C for 2 1/2 hr.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml.

c) These rates are the total soluble iron or sulfate produced per ml of solution per hr during the time interval specified.

d) Not determined.

e) After 720 hr, 50 ml of sterile deionized water was added to all flasks.

TABLE LIII

COAL SOLUBILIZATION STUDIES[a]

(Coal VV-5, water)

Time	Average ZZ-1 and ZZ-2 Runs						
Interval hr	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	$SO_4^=/Fe^t$ mole ratio
0	5.2	1.1	0.8	-	+	-	-
22-0	4.5	4.3	4.1	1.5	0.9	-	-
142-22	3.8	28.3	26.5	2.0	1.5	5.0	-
214-142	3.6	42.6	40.8	2.0	1.8	4.2	2.5
310-214	3.4	61.1	59.4	1.9	2.0	2.1	1.9
382-310	3.3	76.3	73.6	2.1	2.5	6.9	1.9
526-382	3.2	100.7	98.6	1.7	n.d.	-	-

a) In this test series, coal VV-5 Batch B, < 250μ, ~ 5 g in 100 ml of sterile deionized water was agitated at room temperature.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml (values below 0.08 mg/ml are denoted by +).

c) These rates are the soluble iron or sulfate produced per ml of solution per hr during the time interval specified.

TABLE LIV

EFFECT OF MINE *T. FERROOXIDANS* UPON COAL SOLUBILIZATION[a]

(Coal VV-5, 1/10 9K, *T. ferrooxidans*, mine isolated strain VV-10)

Time Interval hr	Average XX-1 and XX-2 Runs					XX-4 Run					
	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^=$ 10^{-1}	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}
0	3.4	1.6	1.6	-	3.2	3.4	0.9	0.9	n.d.[d]	-	3.1
75-0	3.9	n.d.	n.d.	-	n.d.	3.7	n.d.	n.d.	n.d.	-	n.d.
167-75	3.8	2.1	2.0	<0.1	4.1	3.3	5.3	4.7	n.d.	0.3	4.1
243-167	3.7	2.4	2.2	<0.1	3.7	3.0	17.5	2.0	n.d.	1.6	5.2
310-243	3.6	2.7	2.6	<0.1	3.7	2.8	42.5	2.9	38.7	3.7	6.7
358-310	3.5	2.9	2.8	<0.1	4.0	2.7	73.7	2.1	68.7	6.5	7.0
405-358	3.6	3.1	2.7	<0.1	4.5	2.6	108.2	2.5	99.7	7.3	9.1
478-405	3.5	3.8	3.7	0.1	5.0	2.6	145.2	4.5	144.0	5.1	10.5
525-478	3.4	4.5	4.1	0.1	5.2	2.5	183.4	10.7	164.8	8.1	11.8
549-525	3.5	4.8	4.3	0.1	5.3	2.5	196.7	3.8	189.3	5.5	12.1
646-549	3.3	7.5	6.8	0.3	n.d.	2.4	262.9	5.1	218.9	6.8	15.9
693-646	3.3	10.6	10.2	0.7	5.6	2.4	302.7	4.4	261.7	8.5	17.0
717-693	3.2	12.9	12.0	1.0	n.d.	2.4	328.1	7.3	286.2	10.6	17.4

a) In this test series, coal VV-5 Batch B, < 250u, ~ 5 g in 100 ml of sterile 1/10 9K was agitated at room temperature. To run XX-4, 0.316 mg of *T. ferrooxidans* protein was added. Prior to grinding, the coal was washed.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml.

c) These rates are the total soluble iron produced per ml of solution per hr during the time interval specified.

d) Not determined.

TABLE LV

EFFECT OF T. FERROOXIDANS UPON COAL SOLUBILIZATION[a]

(Coal VV-5, 1/10 m9K, *T. ferrooxidans*, mine isolated strain VV-10)

Time Interval hr	Average of YY-1 and YY-2 Runs						Average of YY-3 and YY-4 Runs							Average of YY-5 and YY-6 Runs						
	pH	$Fe^{t,b}$ 10^{-3}	Fe^{+2} 10^{-3}	Rate $Fe^{t,c}$ 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}
0	3.7	n.d.[d]	n.d.	-	n.d.	-	3.6	n.d.	n.d.	n.d.	-	n.d.	-	3.7	n.d.	n.d.	n.d.	-	n.d.	-
46-0	4.0	4.0	3.4	-	0.9	-	3.8	1.9	1.1	n.d.	-	0.9	-	3.7	+	+	n.d.	-	1.0	-
143	3.9	n.d.	n.d.	-	n.d.	-	3.5	n.d.	n.d.	n.d.	-	n.d.	-	3.3	n.d.	n.d.	n.d.	-	n.d.	-
166-46	3.8	3.5	3.3	-	0.9	-	3.3	1.3	1.1	n.d.	-	1.7	6.7	3.2	5.6	3.3	n.d.	-	2.1	9.2
216-166	3.7	4.2	4.1	0.1	1.1	4.0	3.2	12.0	8.9	n.d.	2.1	2.5	16.0	3.0	18.6	7.0	n.d.	2.6	2.9	16.0
287-216	3.6	4.2	3.6	-	1.2	1.4	2.9	24.5	7.5	n.d.	1.8	2.9	5.6	2.8	27.8	4.9	n.d.	1.3	3.1	2.8
454-287	3.6	4.4	4.0	<0.1	1.3	0.6	2.7	88.8	6.5	77.9	3.9	5.3	14.4	2.7	82.8	5.4	72.8	3.3	4.7	9.6
478-454	3.6	4.5	4.0	<0.1	1.3	-	2.7	95.3	5.9	92.1	2.7	5.9	25.0	2.7	92.5	5.9	87.8	4.0	n.d.	-
526-478	3.5	4.6	4.3	<0.1	1.3	-	2.7	111.1	6.6	105.7	3.3	6.7	16.7	2.6	123.8	8.2	118.6	6.5	7.2	34.7
550-526	3.4	4.6	4.2	-	1.4	4.2	2.6	120.0	7.8	115.0	3.7	7.4	29.1	2.6	140.9	6.1	130.2	7.1	7.7	20.8
622-550	3.4	5.0	4.3	0.1	1.5	1.4	2.5	161.0	6.0	160.1	5.7	9.1	23.6	2.5	168.9	6.0	157.9	3.9	n.d.	-
648-622	3.4	5.2	4.7	0.1	1.5	-	2.5	187.5	5.7	184.3	10.2	10.3	46.2	2.5	187.0	5.1	187.8	7.0	n.d.	-
671-648	3.4	6.3	5.2	0.5	1.6	4.3	2.5	213.3	6.2	207.0	11.2	10.1	-	2.5	197.9	4.7	186.8	4.7	9.2	12.4

a) In this test series, coal VV-5 Batch B,< 250u, ~ 5 g in 100 ml of sterile 1/10 m9K was agitated at room temperature. To runs YY-3 and YY-4, 0.31 mg of *T. ferrooxidans* was added; to runs YY-5 and YY-6, 2.59 mg of *T. ferrooxidans* was added.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml (values below 0.0005 mg/ml are denoted by +); $SO_4^=$ is the sulfate ion in mg/ml.

c) These rates are the soluble iron or sulfate produced per ml of solution per hr during the time interval specified.

d) Not determined.

TABLE LVI

EFFECT OF T. THIOOXIDANS UPON COAL SOLUBILIZATION[a]

(Coal VV-5, 1/10 m9K, T. thiooxidans, ATCC strain)

Time	Average WW-1 and WW-2 Runs							Average WW-3 and WW-4 Runs							
Interval hr	pH	Fe^{t,b} 10^{-3}	Fe^{+2} 10^{-3}	Rate Fe^{t,c} 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	$SO_4^=$/Fe^t mole ratio	pH	Fe^t 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	Rate Fe^t 10^{-4}	$SO_4^=$ 10^{-1}	Rate $SO_4^=$ 10^{-4}	$SO_4^=$/Fe^t mole ratio
0	3.3	1.4	1.4	-	+	-	-	3.3	1.8	1.7	n.d.[d]	-	+	-	-
75-0	4.1	n.d.	n.d.	-	n.d.	-	-	3.2	n.d.	n.d.	n.d.	-	n.d.	-	-
167-75	3.9	2.2	2.1	-	0.9	-	23.8	2.7	14.5	13.8	n.d.	-	2.9	-	11.6
239-167	3.9	2.1	2.0	-	1.0	1.4	27.7	2.6	20.1	16.9	n.d.	0.8	3.9	13.9	11.3
334-239	3.6	2.4	2.3	-	1.1	1.1	26.7	2.4	28.2	23.8	n.d.	0.9	5.1	12.6	10.5
406-334	3.9	2.7	2.5	-	1.2	1.4	25.9	2.4	34.2	31.2	n.d.	0.8	5.9	11.1	10.0
479-406	3.6	2.9	2.8	-	1.5	4.1	30.1	2.4	43.6	36.7	7.5	1.3	6.2	4.1	8.3
503-479	3.6	3.0	2.9	-	1.5	-	29.1	2.4	46.7	39.1	7.5	1.3	6.4	8.3	8.0
549-503	3.6	3.5	3.2	-	1.4	-	23.3	2.3	59.4	49.4	8.7	2.8	7.0	13.0	6.9
647-549	3.5	5.1	4.1	-	1.6	-	18.2	2.3	84.1	64.0	12.9	2.5	8.1	11.2	5.6
718-647	3.4	5.8	5.4	-	2.0	5.6	20.0	2.3	104.2	86.4	18.7	2.8	8.3	2.8	4.6
815-718	3.4	7.6	7.2	0.2	2.1	1.0	16.1	2.3	152.6	121.3	33.2	5.0	8.9	6.2	3.4
838-815	3.4	8.2	7.7	0.3	n.d.	-	-	2.3	167.1	127.1	37.6	6.3	9.5	26.1	3.3
863-838	3.4	10.1	8.5	0.8	2.2	2.1	12.7	2.3	185.3	154.6	72.0	8.9	13.5	160.0	4.1
982-863	3.3	25.3	23.4	1.3	2.9	5.9	6.7	2.2	332.2	238.1	86.7	12.0	15.1	13.4	2.6

a) In this test series, coal VV-5 Batch B, < 250u, ~ 5 g in 100 ml of sterile 1/10 m9K was agitated at room temperature. To runs WW-3 and WW-4, 2.42 mg of T. thiooxidans protein was added. Prior to grinding, the coal was washed.

b) Fe^t is the total soluble iron in mg/ml; Fe^{+2} is the ferrous iron in mg/ml; Fe^{+3} is the ferric iron in mg/ml; $SO_4^=$ is the sulfate ion in mg/ml (values below 0.08 mg/l are denoted by +).

c) These rates are the total soluble iron or sulfate produced per ml of solution per hr during the time interval specified; rates below 0.1×10^{-4} are not listed.

d) Not determined.

TABLE LVII

RESULTS OF BACTERIAL AND CHEMICAL ANALYSES ON MINE WATER SAMPLE FROM LOWER KITTANNING SEAM[a]

Chemical Data[b]					Bacteria[b]			
pH	Fe^{t} 10^{-3}	Fe^{+2} 10^{-3}	Fe^{+3} 10^{-3}	$SO_4^{=}$ 10^{-1}	pH ~ 7 S-oxid[c]	pH = 2.7 S-oxid[d]	pH = 2.7 Fe-oxid[e]	S-red[f]
6.4	8.6	4.1	6.0	7.2	2.0×10^{2}	< 3	< 3	5.0×10^{2}

a) The bacterial populations were determined using a three-tube MPN method, according to Standard Methods[44]. In none of the cultures was *Metallogenium* observed microscopically. The medium utilized for *Metallogenium* was that described by Walsh and Mitchell[39].

b) All the concentrations are given in mg/ml.

c) The medium utilized was Vishniac's medium[38].

d) The medium utilized was Silverman and Lundgren's 9K medium[27], using elemental sulfur as the energy source.

e) The medium utilized was Silverman and Lundgren's 9K medium[27], using ferrous iron as the energy source.

f) The P-3 medium of Postgate[40] was employed.

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