Autotrophic Bacteria and the Formation of Acid in Bituminous Coal Mines

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The passage of water through certain bituminous coal mines results in a solution which is extremely acid in reaction because of its high content of ferrous sulfate, some aluminum sulfate, and lesser amounts of manganese sulfate. The ferrous iron is subsequently rapidly oxidized to the ferric state by an autotrophic bacterium. Autotrophic bacteria have also been implicated in the formation of the ferrous sulfate from iron disulfides in the coal mines. Nevertheless, the extent and exact mechanism of bacterial action in the formation of ferrous sulfate remains obscure.

Iron disulfide, which is the original source of the ferrous sulfate, occurs in different forms in the coal and associated strata. These geological and structural aspects of acid formation have been discussed by Temple and Colmer (1951a). Direct bacterial oxidation of iron disulfide has not been demonstrated, although several reports indicate the prevalence of *Thiobacillus thiooxidans* in soils where pyrite is undergoing oxidation (Drewes, 1928; Jensen, 1927; MacIntire et al., 1930; Quispel et al., 1952; Rudolf, 1922). Quispel, Harmsen, and Otzen (1952) postulated the initial surface chemical oxidation of ferrous ions, releasing elemental sulfur which could then be oxidized by *T. thiooxidans*. Early workers at West Virginia University (Carpenter and Herndon, 1933) claimed to have identified *T. thiooxidans* in acid mine waters and this bacterium was definitely established as a normal inhabitant of acid mine waters by Colmer and Hinkle (1947). These authors also found another bacterium in acid mine drainage which was responsible for the oxidation of ferrous iron to the ferric state in acid solutions where ferrous iron was chemically stable; indeed, this unusual reaction led to the discovery of the bacterium. The iron-oxidizing bacterium proved to be an autotroph and has since been described and given the name *Thiobacillus ferrooxidans* n.sp. (Colmer et al., 1950; Temple and Colmer, 1951b).

Leathen, Braley, and McIntyre (1953a) stated that *T. thiooxidans* has no effect upon museum grade pyrite and does not enhance acid formation from "sulfur balls", a type of pyritic concretion commonly found in coal. However, they reported (Leathen et al., 1953b) that the iron-oxidizing bacterium, which we have called *Thiobacillus ferrooxidans*, does increase the acid formed from "sulfur balls" although not from museum grade pyrite. In their experiments both bacteria produced some acid from museum grade marcasite.

The work of Leathen et al. agrees with ours in that *T. thiooxidans* increases the acid formed from marcasite of non-coal origin, and we have confirmed their observation of the effect of *T. ferrooxidans* upon "sulfur balls." However, our results show in addition that *T. thiooxidans* enhances the acid production of certain types of "sulfur balls" and that *T. ferrooxidans* increases the acid formation from museum grade pyrite. An overall mechanism for the acid formation is proposed.

**Materials and Methods**

Museum grade marcasite from a supply house was obtained as crystals upon dolomite and was separated by dissolving the dolomite in warm dilute hydrochloric acid leaving crystals of approximately four mesh size. Museum grade pyrite obtained from a supply house was in the form of moderately disseminated crystals in quartz and was separated by coarse crushing and hand picking. The pyrite was again crushed and sieved to select the material from 14 to 100 mesh. A part of this was ground in a ball mill in the dry state to an undetermined particle size, a fine powder being obtained in 36 hr. Fresh "sulfur balls" were obtained from the picking tables, coal cars, and from the working face of local mines operating on the Pittsburgh seam. These "sulfur balls" differed greatly in their rate of disintegration under moist atmospheric conditions and in their rate of acid formation under experimental conditions. A readily oxidized type of "sulfur ball" was used in the experiments summarized here.

The bacterial cultures were isolated from acid mine water and have been carried in this laboratory for several years. The *T. thiooxidans* appeared identical to a culture obtained from Dr. R. L. Starkey with the possible exception of nitrate utilization. It was maintained upon the customary sulfur medium (Waksman and Joffe, 1922) and was filtered through Whatman No 42 paper to remove sulfur before inoculating experimental units. The *T. ferrooxidans* was maintained upon a solution consisting of FeSO₄·7H₂O, 50 g;
MgSO₄·7H₂O, 1.0 g; (NH₄)₂SO₄, 0.5 g; KH₂PO₄, 0.1 g; distilled water, 1000 ml.

Acid production from marcasite was investigated using approximately five g samples in modified perfusion units (Temple, 1951) containing 400 ml of solution. The solution in this case was unaltered ground water collected as it dripped from the roof of a mine. The inoculated unit received one ml of a *T. thiooxidans* culture. Single units were used.

“Sulfur ball” oxidation was carried out in perfusion units with 5 g of sulfuritic material and 400 ml of solution.

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Days</th>
<th>0</th>
<th>20</th>
<th>58</th>
<th>99</th>
<th>119</th>
<th>145</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. thiooxidans</em></td>
<td></td>
<td>180*</td>
<td>250</td>
<td>700</td>
<td>2450</td>
<td>3000</td>
<td>4700</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>180</td>
<td>180</td>
<td>200</td>
<td>300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Total acidity as ppm Ca CO₃

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>No. of units</th>
<th>Days</th>
<th>Acidity</th>
<th>Standard deviation of mean at 109 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. thiooxidans</em></td>
<td>6</td>
<td>1200</td>
<td>1340</td>
<td>3260</td>
</tr>
<tr>
<td><em>T. thiooxidans</em> and <em>T. ferrooxidans</em></td>
<td>5</td>
<td>660</td>
<td>1390</td>
<td>3720</td>
</tr>
<tr>
<td><em>T. ferrooxidans</em></td>
<td>5</td>
<td>640</td>
<td>1130</td>
<td>3490</td>
</tr>
<tr>
<td>None</td>
<td>2</td>
<td>700</td>
<td>1290</td>
<td>2375</td>
</tr>
</tbody>
</table>

*Mean total acidity as ppm CaCO₃

<table>
<thead>
<tr>
<th>Preparation of Pyrite</th>
<th>Na₂SO₄ Added</th>
<th>Increase in ppm SO₄²⁻</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crushed, 14-100 mesh</td>
<td>+</td>
<td>2700</td>
<td>29</td>
</tr>
<tr>
<td>Crushed, 14-100 mesh</td>
<td>−</td>
<td>6800</td>
<td>29</td>
</tr>
<tr>
<td>Ball mill ground</td>
<td>+</td>
<td>9000</td>
<td>29</td>
</tr>
<tr>
<td>Ball mill ground</td>
<td>−</td>
<td>25000</td>
<td>29</td>
</tr>
</tbody>
</table>

Results

In table 1 the increased acid formation from marcasite in the presence of *T. thiooxidans* is clearly evident. This contrasts strongly with the results using pyrite in that the marcasite was of about four mesh size while pyrite did not react until finely ground. The low initial values were due to the use of neutral roof drips as the solution. At the end of the experiment the bacteria were concentrated about the marcasite particles.

Table 2 presents results of acidity measurements on “sulfur ball” exposed to *T. thiooxidans* and *T. ferrooxidans* singly and together. There was more acid produced in the presence of bacteria with *T. ferrooxidans* showing the greatest effect. A statistical “t” test comparing the four groups indicated a very high probability of the differences between the groups being valid with the exception of the group of flasks inoculated with *T. ferrooxidans* alone and the group of flasks inoculated with both bacteria. Here the probability of a real difference is only about 50 per cent.

Ball mill ground pyrite was much more reactive chemically and was also more susceptible to bacterial action than coarser crushed pyrite. Table 3 indicates some bacterial action in the case of crushed pyrite and much more in the case of ground pyrite. Since the solution contained ferric sulfate at the start, some of the sulfate in the controls may be due to the oxidizing action of ferric ions on pyrite. The values listed are net increases in sulfate. Subsequent determinations showed that the oxidation had tapered off and sulfate formation had practically ceased. Similar experiments with *T. thiooxidans* were negative, indicating no bacterial action.

Discussion

The net increase in acidity from 5 g of “sulfur ball” in 400 ml of solution was 13,750 ppm CaCO₃ in the case of *T. ferrooxidans*, which is equivalent to 0.1375 mole of H₂SO₄ per liter. This may be compared with a net increase obtained by Leathen et al. of 0.1268 mole acid from 50 g of “sulfur ball” in a liter of solution. The greater acidity we noted probably reflects the difference in oxidation rate of the “sulfur balls” used and the faster oxidation rate in perfusion units. Some pyritic “sulfur ball” concretions we have collected were completely stable and did not form acid until after a preliminary treatment with hydrochloric acid. These “sulfur balls” contained a little calcite, which may have been sufficient to prevent acid formation from getting underway.

The acid concentration from 5 g of marcasite in 400 ml of solution exposed to *T. thiooxidans* increased by 4520 ppm CaCO₃ in our experiments, which is equivalent to 0.0452 mole H₂SO₄ per liter in 145 days as compared to an increase of 0.0156 mole in 210 days from 25 g marcasite in 500 ml in Leathen’s experiments. This may be a reflection of the faster oxidation rate in aerated perfusion units than in stationary flasks.

Since particle size is known to be important in the oxidation of sulfur by *T. thiooxidans*, preliminary studies on pyrite involved crushing and screening different particle sizes. No bacterial action was found until fine crushing and ball mill grinding was resorted to, although elemental sulfur of much coarser sizes are readily oxidized. The particle size of the pyrite in “sulfur balls” is unknown but is presumed to be very small since most of the pyrite is not visibly apparent. Some sulfur dioxide was produced in grinding but did not affect the results. Ground pyrite collected and washed on a Buchner funnel showed the same oxidation rate as unwashed pyrite.

The known facts regarding pyrite oxidation and autotrophic bacteria may be summarized as follows: (1) *T. thiooxidans* is without effect on pure pyrite. (2) *T. thiooxidans* increases acid formation from marcasite and from some concretions in coal which have a high pyritic sulfur content. (3) *T. ferrooxidans* increases sulfate formation from pyritic concretions obtained from coal and from pure pyrite of very small particle size. (4) *T. thiooxidans* oxidizes elemental sulfur and thiosulfate very readily but has no apparent direct action on disulfide. (5) *T. ferrooxidans* oxidizes ferrous iron very rapidly in acid solution. (6) Both marcasite and pyrite are slowly oxidized chemically by moist air. (7) Pyrite and marcasite react with oxidizing agents such as ferric sulfate to produce ferrous sulfate and elemental sulfur. (8) Marcasite oxidizes more rapidly than pyrite of the same particle size and marcasite produces more elemental sulfur than pyrite as a result of the oxidation. (9) No ferric ions are to be found in very freshly formed mine waters or in laboratory units where “sulfur balls” or other forms of pyrites are exposed to the action of *T. ferrooxidans*.

The foregoing facts may be coordinated to postulate an overall sequence of acid forming reactions in coal mines. In this sequence the first step is the non-biological chemical oxidation of finely divided pyrite or marcasite to ferrous sulfate.

\[
\text{FeS}_2 + H_2O + 3\frac{1}{2}O_2 = \text{FeSO}_4 + H_2SO_4
\]

This is followed immediately by the bacterial oxidation of ferrous sulfate, a reaction normally carried out by *T. ferrooxidans* in acid solution.

\[
2\text{FeSO}_4 + \frac{1}{2}O_2 + H_2SO_4 = \text{Fe}_2(SO_4)_3 + H_2O
\]

As rapidly as the ferric sulfate is formed, it reacts with the finely divided pyrite present, the ferric sulfate being reduced and the pyrite oxidized in a strictly chemical reaction.

\[
\text{Fe}_2(SO_4)_3 + \text{FeS}_2 = 3\text{FeSO}_4 + 2S
\]

and further,

\[
2S + 6\text{FeSO}_4 + 8H_2O = 12\text{FeSO}_4 + 8H_2SO_4
\]

However the elemental sulfur liberated is more likely to be bacterial oxidized by *T. thiooxidans*.

\[
S + \frac{1}{2}O_2 + H_2O = 2H^+ + SO_4^{2-}
\]

The acidity thus produced favors the continuance of the sequence. Thus the role of *T. ferrooxidans* is that of providing a continuous supply of ferric ions which in turn react with the iron disulfide to give ferrous sulfate. A cycle is thus established which has the net effect of increasing the rate of oxidation of iron disulfide to ferrous sulfate. The action of *T. thiooxidans* is in oxidizing any elemental sulfur formed.

The above hypothesis is strongly supported by the fact that all of the reactions included are actually known to take place under the specified conditions and by the fact that both *T. ferrooxidans* and *T. thiooxidans* are associated with acid formation in the mine at the actual site of iron disulfide oxidation. We have observed freshly exposed neutral strata in coal mines and have found both bacteria absent, while within a few days time large numbers of *T. thiooxidans* and *T. ferrooxidans* developed and the area became highly acid. It is possible, of course, that some reaction or aspect of acid formation has been overlooked. However, this sequence adequately explains the presence of both bacteria without postulating any dubious mechanisms.

Acknowledgments

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**Summary**

*Thiobacillus thiooxidans* increases the acid formation from certain types of the pyritic concretions in coal known as “sulfur balls,” as well as from marcasite. *Thiobacillus ferrooxidans* increases the acid formed from museum grade pyrite of fine particle size as well as from “sulfur balls.” A general mechanism for acid formation in coal mines is proposed.

**References**


