Introduction

Unusually heavy rainfall in the last week of May, 2004, caused extensive flooding, resulting in numerous physical changes in the stream systems. As debris dams were broken by the rapidly moving waters, stream bed erosion accelerated, resulting in new exposure of the coal seam in the three streams mentioned above. Alex Cherry (University of Kentucky graduate student of Forestry), discovered via limited measurements from another experiment, that changes in stream-water pH differed substantially after the new exposure of coal. From related studies, a correlation has been suggested between pH and the oxidation of pyrite contained in coal. Therefore, this experiment was designed to either confirm or refute preliminary observations, as well as to investigate possible mechanisms of interaction between stream water and newly exposed coal.

Pyrite oxidation is a naturally occurring sequence of reactions present in mining areas, where coal is exposed to surface and/or groundwater. These reactions can be biologically catalyzed by the presence of *Thiobacillus ferrooxidans*, which is an iron-oxidizing acidophilic bacterium. As mines are abandoned a substantial amount of pyrite remains on the surface. When oxygen and water come into contact with the coal, acidic drainage (AD) is created. The general accepted reactions for pyrite oxidation are as follows:\(^1\)

\[
2\text{FeS}_2(s) + 7\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{Fe}^{2+} + 4\text{SO}_4^{2-} + 4\text{H}^+ \quad [1]
\]

\[
\text{Fe}^{2+} + .25\text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + .5\text{H}_2\text{O} \quad [2]
\]

\[
\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3(s) + 3\text{H}^+ \quad [3]
\]

\[
\text{FeS}_2(s) + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+ \quad [4]
\]

---

\(^1\) Barton p.106
Pyrite combines with oxygen and water to produce ferrous iron, sulfate and acidity [1]. Ferrous iron is then converted to ferric iron [2]. This reaction is the rate-determining step, and is dependent on oxygen availability. As pH decreases, this reaction is catalyzed in the presence of *Thiobacillus ferrooxidans*.² Ferric iron then becomes a possible electron acceptor which contributes to further acid production via hydrolysis [3] and/or further pyrite oxidation [4]. As demonstrated by reaction [4] the oxidation of pyrite can continue as long as ferric iron is present.³

Acid drainage releases iron, sulfate, and other hazardous substances into surface and ground water.⁴ The hazardous water inevitably has a negative effect on the entire ecosystem in areas where mining has occurred. However, remedial strategies have been developed which slow the process of pyrite oxidation. Prior research indicates that a dry cover composed of organic material (soil, compost, etc.) can be placed above mining waste, which decreases oxygen availability. Oxygen is crucial for the initial step of pyrite oxidation [1]. The addition of compost also decreases phytotoxicity by complexing aluminum and iron, thereby reducing the activity of these elements in their free state.⁵ Compost, when applied in extremely high amounts, acts as a buffer, therefore reducing the pH. This process is known as nitrification.⁶ As a result of nitrification, the rate of pyrite oxidation may be reduced.

The parameters studied were chosen on the basis that they were likely to detect the degree of pyrite oxidation occurring in these streams. According to the reactions involved in the oxidation of pyrite, sulfate and iron are produced. Electrical conductivity was chosen in order to evaluate the concentration of metals in the water. The concentration of iron in the

---

² Kohfahl p.B11
³ Barton p.107
⁴ Kohfahl B11
⁵ Biocycle
⁶ Biocycle
water will also aid in detecting pyrite oxidation, as iron is a product. Dissolved oxygen is important in the first two reactions of pyrite oxidation. A decrease in pH is indicative of pyrite oxidation because acidity is a product. Sulfate is also a product of pyrite oxidation; therefore its concentration may also prove useful in observing the effects of exposed coal in the stream beds.

Temperature, along with pH, affects the activity of *Thiobacillus ferrooxidans* and was therefore evaluated. When pH is less than four, *Thiobacillus ferrooxidans* activity increases, due to its acidophilic nature. Although such acidic conditions did not occur during the study period, it may be possible that small, quiescent volumes of groundwater may be trapped in the coal seam and therefore be more acidic than the surface water being analyzed. If these bacteria are present in the three chosen streams, pyrite oxidation may increase significantly under optimal conditions, such as an acidic environment due to a sudden exposure of coal, which contains FeS$_2$(S). There are numerous other parameters, such as manganese aluminum, and zinc, which are important in the oxidation of pyrite. However, after preliminary tests were conducted, these parameters were not chosen for analyses due to their very low concentration in the streams and time constraints in analysis procedures.

**Methods**

Water samples were collected on July 28, August 9, August 17, August 20, September 30, and October 28, 2004. Five samples from each of three streams, Field Branch, Booker, and Goff, were collected at each of the above dates. Two sample locations were upstream and two downstream of the coal seam exposure, with one location atop the fresh exposure. Sampling locations were in five meter increments. Bottles were acid washed, rinsed thoroughly with deionized water, and allowed to air dry between collection dates. At the time of collection, an
YSI instrument, model YSI 85, was used to determine water temperature, dissolved oxygen, and electrical conductivity at each site, at the time sampling. Samples were then frozen. Iron concentration (FerroVer, Method 8008), sulfate concentration (Sulfa Ver 4, Method 8051), and pH, were evaluated using the HACH CEL/890 Advanced Wastewater Laboratory.\textsuperscript{7}

Method 8008, found in the HACH handbook, noted that two steps be taken prior to analysis of samples for iron concentration; a digestion process and an adjustment of pH to 3 – 5.\textsuperscript{8} The digestion process and pH adjustment was performed on five samples for Clarks Run, a Danville, Kentucky stream of similar composition to those evaluated in this project. Five samples were also randomly selected to be tested from the September 30 and October 28 collection dates. These tests were constructed to determine the need for such adjustments, as called for by the HACH handbook. The handbook also cited that in order to store the samples one should acidify them.\textsuperscript{9} However, the samples were frozen upon collection, therefore achieving the same effect as acidification.

Samples collected on September 30 and October 28, 2004 were evaluated for the presence of \textit{Thiobacillus ferrooxidans}. This analysis was chosen because the pH in these streams may fluctuate over the course of the year. Selective culture medium was prepared in accordance to the protocol for \textit{Thiobacillus ferrooxidans} Medium.\textsuperscript{10}

**Data Tables**

Tables providing all raw data are analyzed and arranged by each stream separately (Appendix I) and for a composite of all these streams (Appendix II). Results for a method

\textsuperscript{7} HACH Handbook p.231, 543  
\textsuperscript{8} HACH Handbook p.231  
\textsuperscript{9} HACH Handbook p.231  
\textsuperscript{10} Atlas p.889-890
validation study are also provided (Appendix III). The raw data was analyzed to detect trends by several approaches. Tables 1-16 present the results of these analyses.

<table>
<thead>
<tr>
<th>Table #</th>
<th>Table Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Overall Chang DO Btwn Above and Below</td>
</tr>
<tr>
<td>2</td>
<td>Overall Chang Ec Btwn Above and Below</td>
</tr>
<tr>
<td>3</td>
<td>Overall Chang pH Btwn Above and Below</td>
</tr>
<tr>
<td>4</td>
<td>Overall Chang [Iron] Btwn Above and Below</td>
</tr>
<tr>
<td>5</td>
<td>Overall Chang [Sulfate] Btwn Above and Below</td>
</tr>
<tr>
<td>6</td>
<td>All Charts Combined Avg Change Btwn Above and Below</td>
</tr>
<tr>
<td>7</td>
<td>Average [Sulfate] Per Stream at Given Point</td>
</tr>
<tr>
<td>8</td>
<td>Average pH Per Stream at Given Point</td>
</tr>
<tr>
<td>9</td>
<td>Average [DO] Per Stream at Given Point</td>
</tr>
<tr>
<td>10</td>
<td>Average [Iron] Per Stream at Given Point</td>
</tr>
<tr>
<td>11</td>
<td>Average Ec Per Stream at Given Point</td>
</tr>
<tr>
<td>12</td>
<td>Overall DO</td>
</tr>
<tr>
<td>13</td>
<td>Overall Ec</td>
</tr>
<tr>
<td>14</td>
<td>Overall [Sulfate]</td>
</tr>
<tr>
<td>15</td>
<td>Overall pH</td>
</tr>
<tr>
<td>16</td>
<td>Overall [Iron]</td>
</tr>
</tbody>
</table>

**Interpretation**

Based on the data compiled to test for the necessity of performing a digestion prior to analysis of iron, it was concluded that the difference is not sufficient to affect the data when the
digestion is not performed. The result of adjusting the pH was also insufficient to include as a step in the protocol. Differences were within the standard deviation of +/- .02 mg/L, determined by the HACH.

No growth of *Thiobacillus ferrooxidans* was observed. This negative result may have been due to substitutions in the manufacturing of the media which isolates this bacterium. The recipe for the media called for hydrated forms of ingredients that were not available, so others hydrated forms were substituted. This resulted in the chemicals not going into solution and a very turbid broth after autoclaving. However, these results may be due to a false negative. After consulting with Dr. Peggy Richey, Microbiology professor at Centre College, it was concluded that the broth neither proved nor disproved the presence of *Thiobacillus ferrooxidans* because no change in turbidity of the broth was observed.

Tables 1–6 were compiled to evaluate the average difference between conditions above and below the coal seam exposure. Calculations were made based on the following formula:

\[
\text{Difference} = \frac{(\text{Avg}(A+B) - \text{Avg}(D+E))}{(\text{Avg}(A+B+D+E))}
\]

This analysis was done to evaluate the difference in each parameter above and below the coal exposure. The data in Tables 1–6 does not confirm an overall increase or decrease for any of the five parameters studied.

Tables 7–11 illustrate the average of each parameter, per stream, over the entire sampling period. This was done for each location, A being ten meters upstream, C being atop the exposure, and E being ten meters downstream of the exposure. This analysis was done to evaluate any recurring patterns in how each parameter in each stream changes over the twenty meter gradient. Although some general patterns of change were observed, more analysis was

---

11 Appendix I
12 HACH Handbook p.234
needed to either prove or disprove the hypothesis. Assumptions could not be made about the effect of the coal exposure because all streams did not provide consistent results at each collection point.

Therefore, Tables 12–16 combine the averages from all three streams, per each point along the twenty meter gradient. This composite approach strongly suggests a change in each parameter at point C, directly above the coal seam exposure. Tables 12–16 illustrate either a minimum or maximum in the data at the particular point C. This transient change indicates a microenvironment change immediately over the coal seam. This transient change then dissipates shortly downstream, resulting in downstream values for each parameter similar to those above the exposure, as represented in Appendix II.

Conclusion

Based on the data collected and analysis performed, there is overwhelming evidence that the hypothesis, which is that there will be a change in water quality parameters due to an exposure of the coal seam in the streams, is confirmed. It is clear that in Tables 12 – 16 there is a change where the coal seam is exposed. Although, some differences do occur from stream to stream, the three stream average shows an extreme value at position C, directly over the fresh coal exposure. This behavior is consistent with a pyrite oxidation process influencing the water chemistry in these microenvironments. Overall patterns prove the presence of pyrite oxidation. Levels of the parameters downstream of the exposure are expected to reflect those above the exposure due to other chemical and biological activities in the streams. After consulting with Dr. Christopher Barton at the University of Kentucky, a possible explanation about water chemistry is offered. A high concentration of iron and sulfate over the coal seam exposure (Point C), with accompanying low electrical conductivity and high pH, suggests that an iron
oxyhydroxysulfate precipitate is likely being formed. This “releases some protons acidity, which, downstream, will lower pH, redissolve the precipitates, and increase the electrical conductivity.”

Acknowledgements

First of all I would like to thank Dr. Christopher Barton (University of Kentucky Department of Forestry) and Dr. Preston Miles (Centre College Professor of Chemistry). Ideas and guidance provided by each of you has proven crucial to the success of this experiment. Thank you also to Alex Cherry and David Collett for assistance and contributions to the experimental design and collection of water samples. Lastly, assistance in evaluating microorganisms by Dr. Peggy Richey (Centre College Professor of Microbiology) is also appreciated.

13 Barton, Email
Abstract

This project evaluated the possible effects of recently exposed coal seams in perennial streams of the Eastern Kentucky Coal Field Region. Three streams, Goff, Booker and Field Branch, in Robinson Forest (University of Kentucky Research Facility) were sampled upstream and downstream of the exposed coal. Parameters monitored include pH, electrical conductivity, dissolved oxygen, iron and sulfate concentration, and temperature. Data was compiled for five months following a heavy runoff event that produced fresh exposure of coal seams in the beds of these three streams. Our data indicates an extreme in dissolved oxygen, electrical conductivity, pH, iron and sulfate concentrations at the sampling site directly over the coal exposure. We suggest a mechanism based upon oxidation of pyrite contained in the coal. The formation of an oxyhydroxide sulfate precipitate above the coal exposure will produce the changes observed.
Bibliography


HACH. *DR/820 – DR/850 – DR/890 Datalogging Colorimeter Handbook*