TEN YEAR RESULTS FROM BACTERICIDE-TREATED AND RECLAIMED MINE LAND¹

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Abstract: In 1984 a reclamation project was undertaken by the Ohio Department of Natural Resources on abandoned coal refuse near East Springfield, Ohio. The site was divided into two areas and each was reclaimed using standard, accepted reclamation practices. However, one area also received a controlled release bactericide treatment of ProMac Products to prevent acidification. Perforated PVC pipe drains were installed in each area at a depth of about 8m from the surface to create artificial seeps which could be monitored. Over a period of five years water quality, soil microbiology and vegetative biomass were measured and compared between the bactericide treated and untreated sides. Results from the first five years were previously reported when monitoring was terminated. Five years later, in 1994, ten years after reclamation, the site was revisited. Water quality, soil microbiology, and vegetation were analyzed to determine the long term effects of controlled release bactericide treated and the untreated parcels. Especially evident are the differences in vegetation. Volunteer vegetation has become established on the bactericide treated side, whereas loss of vegetation has led to severe erosion on the untreated side.

Additional Key Words: Acid mine drainage, Bactericides, Biomass, Reclamation, Revegetation, Soil Microbiology.

Introduction

Inhibiting or destroying *Thiobacillus ferrooxidans* bacteria can significantly slow the rate of acid production from pyritic materials. Anionic surfactants, organic acids and food preservatives (Onysko et al. 1984) act as bactericides and destroy these bacteria; however, bactericides biodegrade over time and are lost because of leaching and runoff. To overcome this short duration effectiveness of spray applications, controlled release systems to provide the bactericide slowly over a long time period were developed (Sobek et al. 1985). Control of acid generation for prolonged periods greatly enhances reclamation efforts and can reduce reclamation costs by reducing the amount of topsoil and lime needed to establish vegetation. Three natural processes resulting from strong vegetative cover for three years or more can break the acid production cycle. These processes are: (1) a healthy root system competing with acid producing bacteria for both oxygen and moisture; (2) reestablished populations of beneficial heterotrophic soil bacteria and fungi forming organic acids that are inhibitory to *Thiobacillus ferrooxidans* (Tuttle et al. 1977); (3) plant root respiration and heterotrophic bacteria activity that increase CO_2 levels in the spoil, resulting in an unfavorable microenvironment for growth of *Thiobacillus ferrooxidans*.

For successful reclamation at acid producing sites it is essential that the acid and metals salts which cannot be tolerated by new growth vegetation be inhibited from damaging the plants during the initial stages of development. Relying on the depth of cover or the effectiveness of neutralizers may be inadequate to assure successful vegetation. In 1984 in conjunction with the Ohio Department of Natural Resources AML Division, a site was selected off Route 43 near East Springfield, Ohio, as a test site for a first generation time release bactericide product with sodium lauryl sulfate as the active ingredient and an effective release rate of approximately two years.

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Site Conditions

The site contains coal refuse to a depth of 15 m. The site had been abandoned since about 1960 and was polluting streams and the nearby Wolf Creek with highly acid and metal contaminated run-off. The paste pH of the coal refuse was less than 3.0 across this site while 10:1, water:refuse extracts had pH values ranging from 2.5 to 3.5. A composite sample of refuse was analyzed for sulfur forms with the following results: pyritic sulfur=0.43%; sulfate sulfur=0.11%; organic sulfur=0.78%; total sulfur=1.32%.

Site Treatment

No amendments were added to the regraded refuse pile before ProMac Systems was applied. The 0.9 hectare at the southern end of the site was left as a control plot while the 1.0 hectare adjacent to the northwest side of the control area was treated with ProMac Systems (Figure 1). The treated side received a bactericide dosage of 225 kg/ha of 88% active bactericide powder made into a water based solution and 575 kg/ha of controlled release bactericide formulations containing from 16% to 28% active ingredient. The bactericide application was made in a single step using a hydroseeder after the refuse had been graded, but before the soil cover was applied.



Figure 1. A sketch of the Rt. 43 site in southeast Ohio which was reclaimed as a demonstration site in 1984. The sketch shows the layout of the bactericide treated side and the untreated control side and the locations of the drains installed to create artificial seeps for monitoring.

The site, both control and treated areas, was covered with 15.2 to 20.3 cm of topsoil obtained from the same borrow area adjacent to the site. Using pan loaders, material was taken from the borrow area and dumped and spread in a left to right direction starting at the bottom of the slope across and up both areas at the same time. The topsoil was fertilized with 336kg/ha of a 16-16-16 fertilizer and 6.9 m.t./ha of lime. The area was seeded with 61.6 kg/ha of seed mixture followed by an application of 4.5 m.t./ha of hay mulch.

Site Monitoring

The site was monitored from 1985-1989 to ascertain variations in water quality, soil microbiology and vegetative biomass. Results of those analyses were previously reported (Sobek et al. 1990). Following the report of that data, monitoring was terminated at the end of 1989. In 1994 it was decided to revisit the site for the purpose of conducting a ten year evaluation. Visits were made in June, July, and September of 1994.

In June, 1994 photographic comparisons of the control and treated site were taken, and soil samples were collected to ascertain the strength and presence of heterotrophic and *Thiobacillus ferrooxidans* bacteria. Vegetation was collected to compare biomass production, and an attempt was made to obtain water samples from the two artificial seeps which had been installed at the time of reclamation. A slight trickle allowed for a water sample to be taken on the control side, however, no water was flowing on the treated side.

In July, 1994 another site visit was made to attempt to obtain comparative water samples, but neither side was producing drainage. Additional photographs were also taken. In September, 1994 the site was revisited to obtain samples from deeper within the refuse because the June samples reflected very little *Thiobacillus ferrooxidans* activity on either side. It had been predicted in 1987 that with the passage of time these bacteria might migrate deeper into the refuse, and the September samples were collected to test that hypothesis. Once again both seeps on the site were dry.

Observations

Within the first year after the reclamation had been completed it was evident that areas existed on both the control and treated sides which would have difficulty supporting vegetation. During the 1985-1989 monitoring period it was evident that vegetation was burning out in an expanding area on the control site. After 10 years 35-40% of the area on the control site is barren of any vegetation and is characterized by erosion gullies. On the treated side, one spot consisting of less than 10% of the treated site area has sparse to no vegetation, and no significant erosion has occurred.

The PVC pipes which were used to create seeps for water collection exhibit marked contrasts in appearance. The pipe on the control side has no vegetation growing in the outfall area and the bottom of the interior of the pipe contains considerable red staining. The lack of vegetation and the stain are both indicative of iron rich ferruginous acid mine drainage that continued to occur after reclamation. The pipe on the control side is overgrown by vegetation and exhibits no staining. This indicates improved water quality resulting from bactericide treatment.

In addition to the grasses which were used to seed both sites, volunteer vegetation is also very evident, although the variety and density is significantly greater on the treated than on the control site.

Results

<u>Microbiology</u>: It has been found that specific classes of microorganisms are better presented as a ratio of the total population (Horowitz and Atlas, 1976). Specific classes of bacteria will increase (or decrease) in number as a result of a total increase (or decrease) in the bacterial population. It is, therefore, important to examine the ratio of *Thiobacillus ferrooxidans* to heterotrophs for the bactericide treated and the untreated- control sides. These ratios, for the first five years and for 1994, are presented in Table 1. These data are the arithmetic means from five samples from each side chosen from random locations.

Two important observations can be made. First, the impact of bactericide treatment on change in the microbiology of the site continued to persist beyond the two year life of the controlled release pellets. Second, that after ten years the *Thiobacillus ferrooxidans* bacteria count is very small on both sides. However, higher heterotrophic bacteria population on the treated side is a reflection of the better vegetation

resulting from the bactericide treatment. The effect of bactericides was not short term. It is possible that the low populations of *Thiobacillus ferrooxidans* on both treated and control sides is due to the depletion of their food source (pyrite) over time.

Table 1. Post-reclamation ratio of	Thiobacillus ferrooxidans to	heterotrophic bacteria over	ten years (in
MPN/g of dry soil).		-	

Side	1985	1986	1987	1988	1989	1994
Bactericide Treated	5 x E-2	3.5 x E-1	2.2 x E-1	2.5 x E-1	4.3 x E-1	1.4 x E-4
Untreated- Control	7 x E+1	1 x E+1	1 x E+3	1.2 x E+0	4.1 x E+1	2 x E-4

<u>Water Quality:</u> Table 2 shows water quality from the drains in 1989, five years after reclamation as well as the sample that was obtained from the control site in June, 1994. At that time the bactericide treated side was not running and so no water sample was available.

Table 2. Water quality from drains (in ppm except pH, and conductivity, which is in umhos).

Sample	pН	Conduct.	Acidity	Sulfate	Iron	Mn	Al
1989-Treated	5.9	590	19	100	0.2	0.3	0.5
1989-Control	2.6	2,910	844	2,040	104	6.1	38.7
1994-Control	3.4	851	112	9	16	1.6	9.3

<u>Vegetative Ground Cover Evaluation</u>: Table 3 shows the biomass data obtained in 1989, five years after reclamation and then again in 1994. Biomass results were obtained by harvesting three $1m \times 1m$ plots on each side. The biomass collected was oven dried at 105° C until a constant mass was reached. Because of the large areas where no vegetation exists on the control site, biomass production is expressed in a range from 0 to the maximum measurable amount. The vegetation on the treated side has much tertiary volunteer vegetation which has produced rhododendrons and locust trees.

Table 3. Biomass yield in kg/ha from the bactericide treated side and its comparison with yield from the untreated control side.

1989		1994		
Bactericide Treated Side	Untreated-control Side	Bactericide Treated Side	Untreated-control Side	
2,915	0 to 315 max	4,118	0 to 1,895 max	

Vegetation quality can best be shown with photographs. Figures 2 shows pictures of the drains installed to create artificial seeps for monitoring taken in 1987 (Figure 2(a) and 2(b)) and again in 1994 (Figure 2(c) and 2(d)). These pictures show two important results of this site treatment. First, the quality of vegetation on the bactericide treated side has been much better in terms of health and density over the life of the site. Second, the drain on the untreated-control side and the ground around it show staining from iron rich flow over the years, whereas the drain on the treated side shows no evidence of polluted effluent.



igure 2(a). 1987 photo of drain on the treated side.



Figure 2(b). 1987 Photo of drain on the untreated-control



igure 2(c). 1994 photo of drain on the treated side.



Figure 2(d). 1994 photo of drain on the untreated-control

Rt.43 site photographs from 1987 and 1994 showing the difference between treated and untreated sides in revegetation quality nce of effiuent quality from drains installed to create artificial seeps. Note iron staining on the untreated-control side.



Figure 3(a) shows an aerial photo of the site taken in 1987. The vegetation quality difference again is very apparent. Figure 3(b) shows vegetative cover on the treated side in 1994 and compares it with the mostly barren and eroded untreated-control side in Figure 3(c).

As the above information collected over ten years indicates, the life of bactericide treatment in reclamation is long lasting. The change in microbiology is the result of the cycle shown in Figure 4 which is started by bactericide application but outlasts the controlled release systems.



Figure 4. A model of the site recovery cycle initiated by the use of bactericides in mine land reclamation. The cycle illustrates why a single application of bactericide can produce successful long lasting results.

Discussion

What is evident in studying this site over a ten year period is the importance of the reclamation plan in dealing with acid abatement. If the initial revegetation is not successful, serious site degradation will occur over time. The first few years are a critical period in determining the ultimate success or failure of a reclamation site. In order for tertiary volunteer vegetation to succeed, the microbiology and ecology of the site must be changed to provide an acceptable environment for growth.

The only difference between the two sides was the additional use of controlled release bactericide on the treated side. Both the active ingredient then used and the rubberized pellets first developed have subsequently evolved to a third generation product which utilizes sodium dodecylbenzene sulfonate as the active ingredient encapsulated in polyethylene. This formulation utilizes a longer lasting bactericide with a slower biodegradation rate, and one which does not hydrolyze in an acid environment as the earlier surfactant would. This makes it a more effective bactericide and the polyethylene matrix gives it improved control release characteristics which allow the bactericide to be released for a period of more than six years. The following conclusions have been formulated based upon field observations, and laboratory analysis:

1. Bactericides were an effective means of preventing acid drainage, at this site.

2. The effect of bactericides thus far had lasted 10 years which is beyond the life span of the controlled release systems, which on this site was 2 years.

3. The use of bactericides encourages the development of heterotrophic bacteria populations and promote strong, healthy vegetation.

4. The use of bactericides significantly improve water quality by preventing acid formation and the resultant leaching of metal contaminants harmful to revegetation efforts.

5. Over time, *Thiobacillus ferrooxidans*, can reduce in population as their food source (pyrites) is depleted. However, this time period can be decades long and deterioration of water and vegetation occur while they remain active.

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