SUCCESSFUL RECLAMATION USING CONTROLLED RELEASE BACTERICIDES: TWO CASE STUDIES [1]

by

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Abstract. Controlled release bactericides affect reclamation success and provide assurance against post-reclamation water quality problems. They inhibit Thiobacillus ferrooxidans and aid in the establishment of beneficial heterotrophic bacteria necessary to support revegetation of the site. These conditions persist after the bactericide is depleted from the controlled release systems. Two coal refuse disposal areas, one in Ohio and one in West Virginia, were reclaimed using two different generations of controlled release bactericides. Case Study #1, located in Ohio, was reclaimed using first generation products with a release life of two years. Yet, six years after application, the treated area continues to have a dense vegetative cover while the untreated control area has only sparse vegetation. Water quality data from the treated area continue to show a significant improvement versus that from the control area. Case Study #2, located in West Virginia, was reclaimed using third generation products with a controlled release life in excess of seven years. In its third year, the vegetation is lush and healthy except for the control area where vegetation is becoming sparse due to acid toxicity. The water quality data from the treated area corroborates these improvements and justifies the use of bactericides in reclamation.

Additional Key Words: bactericide, reclamation, water quality, Thiobacillus ferrooxidans.

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Introduction

Pyritic materials associated with coal refuse oxidize to form mineral acids when exposed to the atmosphere. This oxidation process is catalyzed by the bacteria Thiobacillus ferrooxidans (Beck and Brown 1968; Baker 19 5) causing acid generation to overwhelm inherent neutralizers in the reject materials. Kleinmann and others (1981) proposed a pH dependent three-stage mechanism of acid generation in pyritic materials. The bacteria, T. ferrooxidans, play a very important role in this process, making bacteria control one method of breaking the acid-generation cycle.

Inhibiting or destroying thiobacilli can significantly slow the rate of acid production. Anionic surfactants, organic acids and food Preservatives (Onysko et al. 1984) act as bactericides and kill these bacteria; however; bactericides degrade over time and are lost because of leaching and runoff. To overcome the inherent 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 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 that competes for both oxygen and moisture with acid-producing bacteria; (2) populations of beneficial heterotrophic soil bacteria and fungi that are reestablished, resulting in the formation of organic acids that are inhibitory to T. ferrooxidans (Tuttle et al. 1977); (3) the action of plant root respiration and heterotrophic 'bacteria activity increase CO_2 levels in the spoil, resulting in an unfavorable microenvironment for growth of T. ferrooxidans.

The objectives of the two case studies are: (1) to demonstrate that bactericides in a combination of liquid spray and controlled release pellets will substantially reduce acid formation; (2) to demonstrate that after controlled release systems are exhausted, the inhibition of acid production continues for prolonged time periods; (3) to demonstrate the feasibility of incorporating these materials into normal reclamation practices.

Case Study #1: Route 43, East Springfield, Ohio

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 and found to be 50.3% total carbon, 1.32% total sulfur, 0.11% sulfate sulfur, 0.43% pyritic sulfur, and 0.78% organic sulfur. No neutralization potential measurement was made.

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 was treated with ProMac Systems. ProMac Systems used for this site had the following formula: (1) ProMac 500SN - 386 kg, (2) ProMac 500PBL - 408 kg, and (3) ProMac 600PBL - 417 kg. This site received 465 ppm of bactericide in controlled release form.

The site, both control and treated areas, was covered with 15.2 to 20.3 cm of topsoil. The topsoil was fertilized with 336 kg/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

Methods. Eight soil moisture samplers (pressure-vacuum lysimeters) were installed at different locations on the site. It was expected that the effects of the ProMac Systems application would be noticeable first in the vadose zone, and then in the drains or the diversion ditch. A rain gauge and an evaporation pan were also installed on the site.

Monitoring included periodic site inspections to ascertain surface vegetation cover and acid "burnout." The site was photographed periodically and sampled for bacterial activity during the past five years.

Soil samples were taken from both treated and untreated areas of the site with a soil corer. Each soil core had all the topsoil layer and part of the refuse material. The areas of the pile, treated and untreated, were sampled according to a grid pattern. Each area was divided into four blocks, avoiding the border zone between treated and untreated. Peripheral areas were also avoided. Five samples were taken from each block, totaling 20 samples per area. Each block was combined to yield a total of four composite samples per area. This reduced the variability between samples that originated from the same area, reduced the number of dilutions needed for testing and enhanced the statistical significance of the test.

Two separate experiments were conducted to measure the most probable numbers (MPN) of heterotrophs and Thiobacillus ferrooxidans. The first was the growth of heterotrophic microorganisms in cover soil of the treated section versus cover soil from the untreated section using a semi-rich medium (TSB). This comparison was conducted for composite samples of the cover soil. The second experiment was the growth of T. ferrooxidans in treated versus untreated sections using a specific medium (9K). This comparison was conducted for composite samples of the refuse layer. Each composite sample consisted of five individual cores. This study was conducted once each year for five years starting in June 1985.

Microbiology. The thickness of the cover soil in the treated and untreated areas of the refuse pile was the same statistically; however, the actual thickness of cover soil was greater in the untreated area (20.3 cm average) than in the treated area (17.3 cm average). The presence and amounts of heterotrophic microorganisms determines the status of the vegetation in a given area. A strong and varied heterotrophic population supports a good vegetative cover. These microorganisms proliferate in areas that contain enough preformed organic matter to sustain their growth. The organic matter in the refuse pile is mostly coal. Coal, especially when present in large particles, is not a carbon source readily utilized by most heterotrophic organisms. Thus, heterotrophs were enumerated only in the cover soil and not in the refuse. Enumeration was done using a general heterotrophic medium amended with sterile soil extract to supplement unknown required growth factors.

The distribution of the heterotrophic organisms in the study site is shown in Figure 1. The untreated area displayed a suppressed number of heterotrophs when compared with treated area. The difference between treated and untreated areas is close to two orders of magnitude except for the drought year of 1988. A very good and healthy heterotrophic microbial population is shown in the treated area except for 1988, when the untreated area was almost equal to the treated.

Thiobacilli are a special group of bacteria that live in a low pH environment, deriving their energy from oxidation of pyritic material and their carbon source from carbon dioxide. In an environment rich in organic matter they are surpassed in growth by heterotrophs. Thus, thiobacilli counts were performed only for the zone starting from the base of the cover soil extending into the upper part of the refuse itself.

The refuse (Figure 2) shows significant differences in numbers of thiobacilli in the treated area compared with the untreated area except for 1986 and during the drought year of 1988. The number of T. ferrooxidans were found to be roughly the same throughout the 12.7 cm thickness of refuse tested in the treated area before the data were composited. This same trend was found in the control area.

Normally, it is expected that as one goes lower in the soil system, lack of oxygen and limiting amounts of nutrients will inhibit or reduce growth. These data from the control area for 1985 and 1986 may indicate a trend of downward movement of thiobacilli to lower depths of the pile. However, the data from 1987 and 1989 would tend to refute that supposition. The data for 1986 may have resulted from the microenvironmental conditions at the time of sampling. This hypothesis will have to be verified in future studies.

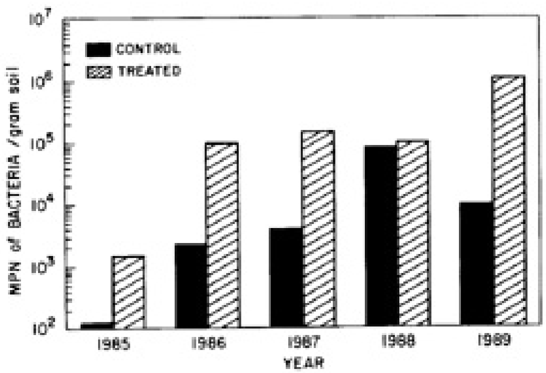

Figure 1. Heterotrophs in the treated and untreated areas at the R. 43 Site

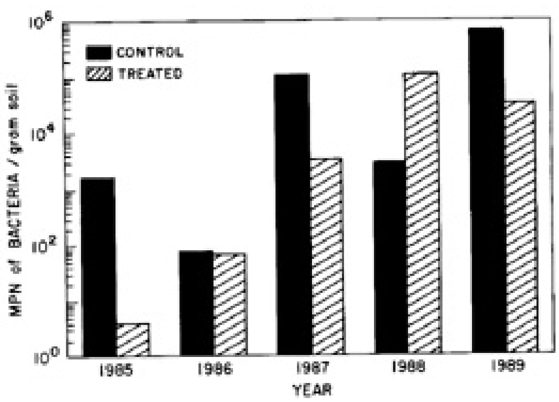

Figure 2. T. ferrooxidans in the treated and untreated areas at the Rt. 43 site

It has been found that specific classes of microorganisms are better presented as a ratio of the total population (Horowitz and Atlas 1976; Walker and Colwell 1976). Specific classes of bacteria will increase in number as a result of a total increase in the bacterial population.

Thus, if the total number of bacteria increases in a specific environment, it is expected that a general increase in every specific group of bacteria will be seen. This situation is well presented in this study. In spite of the fact that higher numbers of thiobacilli were found in 1988 in the treated than in the control areas, the ratio of thiobacilli to heterotrophs was higher in the control area (Figure 3).

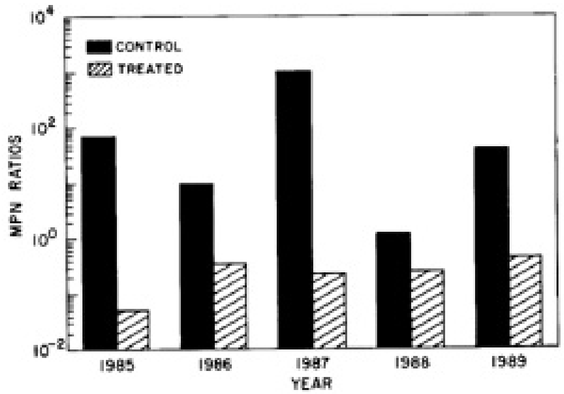

Figure 3. T. ferrooxidans-heterotrophs ratio at the Rt. 43 site

Results of the last five years (Figure 1) show an increase in the number of heterotrophs each year, except for the drought year of 1988, in the treated area. However, in the control area, heterotrophs had a large increase in 1988, but decreased in 1989. The number of thiobacilli in the treated area (Figure 2) increased by more than ten-fold each year through the drought year of 1988, but decreased in 1989. In the control area, the thiobacilli decreased by more than tenfold from 1985 to 1986, then increased by a factor of 1000 from 1986 to 1987, followed by another ten-fold decrease in 1988 and a 100-fold increase from 1988 to 1989. The increases in thiobacilli in the control area are much higher and exhibit an up-and-down effect, while the increases in the treated areas have been consistent until 1988 and 1989.

The ratios of thiobacilli to heterotrophs for a five-year period (Figure 3) make the differences between the treated and control areas very clear. The data indicate a fairly stable and low ratio of thiobacilli to heterotrophs in the treated area. In the control area, the ratios are much higher and follow the same trend exhibited by the thiobacilli populations over the past five years (Figure 2).

Water Quality. Background water samples were taken at points in the ditch that collected drainage and flowed from south to north along the western boundary of the site. The data (Table 1) indicate that a near-neutral to slightly alkaline water enters the site at 0.6 L/s and flows past the gob pile. By the time the water reaches the sampling point on the western edge of the control plot, the flow has slowed and has become very acidic.

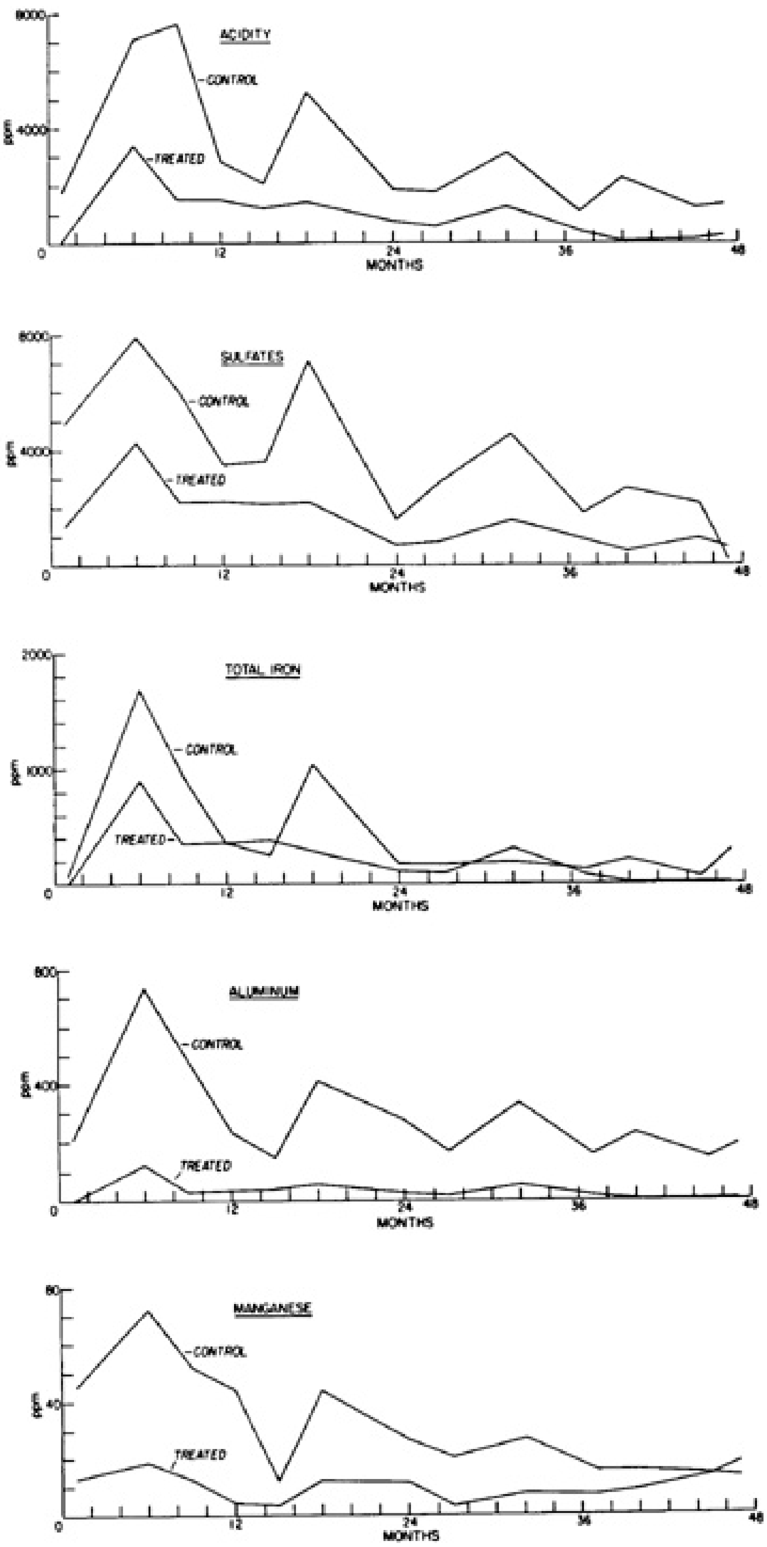

Figure 4. Refuse water quality data from the Rt. 43 site

Table 1. Background Water Quality Data

Parameter	Sample Locations							
	Exit		Treated Plot		Control Plot		Entrance	
	*	**	*	**	*	**	*	**
pH (field)	2.7	-	2.7	-	2.4	-	6.4	-
pH (lab)	2.6	2.7	2.6	2.7	2.4	2.4	7.0	7.3
Acidity (mg/L)	1034	716	1503	1104	1391	941	-	-
Total Iron (mg/L)	-	72	-	60	-	96	-	0
Temperature (^{o}C)	15	22	15	22	13.5	22	11.5	22
Conductivity (umhos/cm)	2650	2150	3750	2950	3500	2850	650	700
Flow (L/s)***	3.1	-	0.06	-	0.06	-	0.63	-

* Water samples taken on 4/1/83
** Water samples taken on 5/18/83
*** Flow was estimated on 4/1/83 only
(-) Indicates no data

Water samples were to be collected throughout the project on a monthly basis; however, the previous water flow pattern was not reestablished after the site was regraded arid recontoured. The only consistent water flow on-site was at the exit from the site. This sample location is northwest of the treated area and is indicative of the untouched portion of the site that was not included in the study.

In June 1984, a soil moisture sampling system (pressure-vacuum lysimeters) was installed for monitoring the water quality of the vadose zone nearest the plant root system, and as a "backup" system for the water quality monitoring of runoff and seepage water, collected by the drainage ditch. The installation and sampling procedures of Parizek and Lane (1970) were followed.

The monthly data for acidity, sulfates, aluminum, manganese and total iron are found in Figure 4. Acidity, sulfates, aluminum and manganese all exhibit a downward trend in both the treated area and the control area; however, the treated area is much lower in all parameters until month 47. Sulfates in the control area decreased below the sulfate concentration in the treated area and manganese in the treated area increased to a higher level than in the control area at month 47. Total iron concentration in the control area is erratic and dips below the total iron concentration in the treated area in months 12, 15, and 32. However, total. iron in the control area always increases sharply after reaching these lows.

The data summarized in Table 2 are the percent reduction in each parameter when the average of all water quality measurements from samples taken out of the four lysimeters in the treated portion of the site are compared with the average of all. water quality measurements from samples taken out of the four lysimeters in the non-ProMac treated portion of the site. The data in Table 2 indicate the ProMac treatment has been very successful in reducing all parameters, except for manganese in 1.987 and 1988. In the second year-, except for total iron, the reductions are not as large as in year one.

Table 2. Reduction in Specific Parameters Comparing Treated to Untreated Areas at Route 43

	Year			
Parameter	1985	1986	1987	1988
Acidity	72%	58%	97.2%	89.0%
Specific Conductivity	35%	32%	67.2%	86.0%
Total Iron	52%	73%	92.1%	99.6%
Manganese	94%	91%	44.0%	15.6%
Aluminum	93%	89%	99.5%	98.9%
Sulfates	68%	58%	81.4%	57.0%

Vegetative Ground Cover Evaluation. Biomass production was measured by running transect lines across the plots. Four quadrats (0.37 m^2 per quadrat) were clipped at ground level where the transect lines intersected. **Total biomass was** measured (dry weight basis) by drying all clippings at 105 degrees Celsius in a forced-air oven until a constant weight was reached. The treated area had a total biomass of 2,915 kg/ha in 1989 while the control area only had a total biomass of 315 kg/ha.

Case Study #2: Dawmont Refuse Area, Dawmont, West Virginia

The Dawmont reclamation project, an abandoned coal refuse disposal area covering approximately 14.2 hectares, was polluting offsite areas with acid mine drainage (AMD) at a flow rate of 0.6 to 1.0 L/s.

Site Treatment

The regraded refuse received a blanket of lime applied uniformly at a rate of 22.4 m.t./ha before the following ProMac treatment was applied: (1) ProMac 2000SB - 3,175 kg, (2) ProMac 2000PN - 2,381 kg, (3) ProMac 2000PB -**3,969 kg, and** (4) ProMac 2000PY - 3,969 kg. The site received 201 ppm of bactericide in controlled release form.

After the lime and ProMac products were applied, the entire site was covered with 30.5 cm of topsoiling materials. Fertilizer was applied to the topsoil at a rate of 1,121 kg/ha of a 10-20-20 commercial fertilizer plus 56 kg/ha of urea (46-0-0). Before the area was seeded and mulched with 4.5 m.t./ha of straw, 13.5 m.t./ha. of lime was disked into the cover material.

Site Monitoring

Twelve pressure-vacuum lysimeters were installed at different locations on the site to measure water quality near the plant root zone. Six samplers were put in the 0.4 hectare control area and six samplers were put in the treated area. Vandals destroyed six lysimeters, causing a small gap in the water quality database.

Cover soil depth is measured once a year when the microbiological studies are done. Although it varies across the site, there is very little difference between the amount of cover on the control when compared to the treated portion of the site. The control area has an average of 24.4 cm of cover material compared to 23.6 cm of cover material in the treated area.

<u>Microbiology.</u> The microbiological studies conducted at this site were the same as the studies conducted at the Route 43 site in Ohio. When the treated and untreated areas were compared, the heterotrophic bacterial counts were significantly greater for the treated area (Figure 5). There was a decrease in heterotrophic counts each year in the untreated area while the heterotrophic counts increased in the treated area in 1988 and sharply decreased in 1989, Next year's data will be needed in order to determine if this is a trend or just a result of climatic conditions in 1988 and 1989.

The data illustrated in Figure 6 showed a trend to have fewer thiobacilli in the treated area than in the untreated area. However, there was a sharp increase in the thiobacilli in the treated area for 1988. It was expected that lower numbers of thiobacilli would be observed as in 1987 and 1989. Possibly the effect of ProMac products on the site was altered by the overall effect of the hot, dry summer of 1988. It seems that thiobacilli flourish better than heterotrophs at lower soil moisture contents or else there was not enough soil moisture to continually activate the control release mechanisms of ProMac pellets.

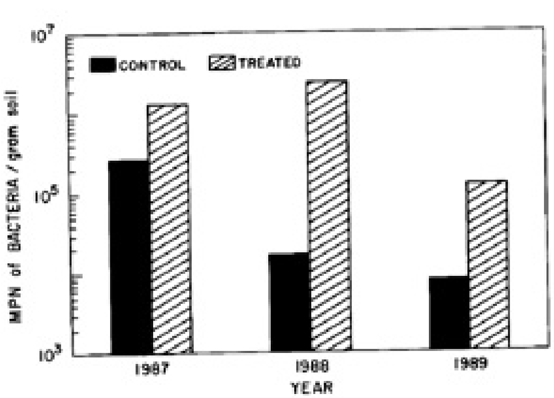

Figure 5. Heterotrophs in the treated and untreated areas at the Dawmont site

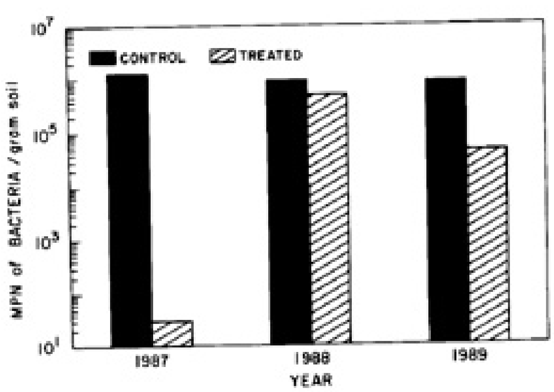

Figure 6. T. ferrooxidans in the treated and untreated areas at the Dawmont site

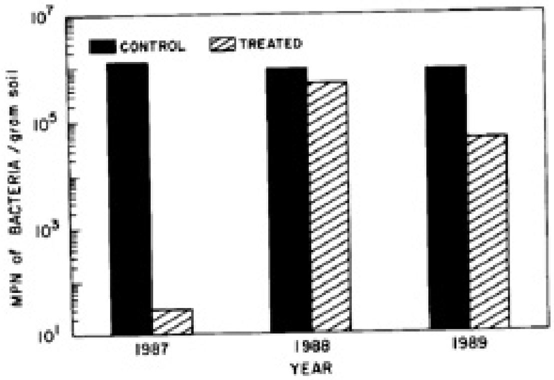

Figure 6. T. ferrooxidans in the treated and untreated areas at the Dawmont site

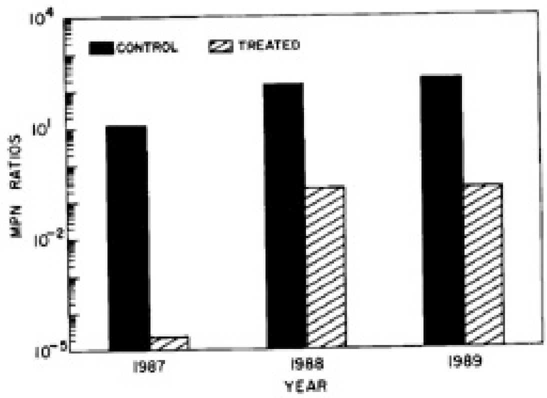

Figure 7. T. ferrooxidans-heterotroph ratio at the Dawmont site

The ratio of thiobacilli to heterotrophs at the interface zone serves as a strong indicator for the overall microbial, picture of the site. It balances situations such as high or low carbon availability or many other non-specific environmental factors that may affect the overall numbers. In most cases, it can be expected that the ratios of a specific bacterial group to the overall population will stay roughly constant.

The ratios of thiobacilli to heterotrophs (Figure 7) in the control area increased 10-fold from 1987 to 1988, and very slightly in 1989. In the treated area, the ratios were very low in 1987, increased 10,000-fold in 1988 and remained the same in 1989. A much lower ratio was expected to be obtained for the treated area in these years. Climatic conditions could be responsible for these unexpected results.

Water Quality. Background water quality data (Table 3) were obtained from a sample of standing water at the base of the unreclaimed refuse site (DM-A) and from a seep that flows from the toe of the regraded refuse area (DM-B). Sample DM-A is indicative of the surface runoff water quality while DM-B is most representative of water infiltrating the refuse, picking up a load of acid salts, and polluting offsite land and water resources. While both water samples are very acidic and contain large amounts of pollutants, sample DM-B would be the most costly to neutralize.

In October 1987, a soil moisture sampling system (pressure-vacuum lysimeters) was installed to monitor water quality in the deep root zone area. The monthly data for acidity, sulfates, aluminum, manganese and total iron are graphically displayed in Figure 8. The trend for all parameters except manganese is that the control area contains higher concentrations than the treated area. Manganese follows the same trend except for month 11 in 1988; the treated area contained more soluble manganese than the control. The drought during the summer months may be responsible for altering this trend.

When viewed from a different perspective (Table 4), the data show a significant reduction in measured parameters. In all parameters, significant reductions are realized when the treated area is compared to the control area.

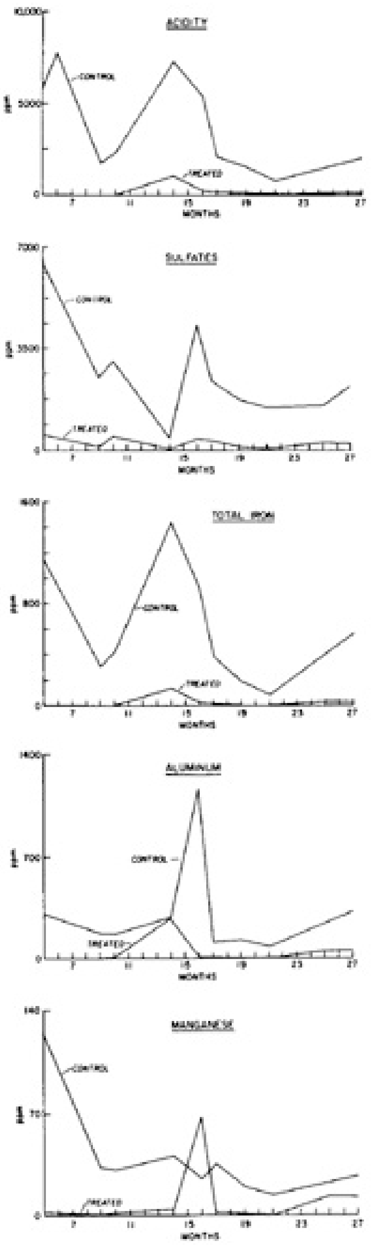

Figure 8. Refuse water quality data from the Dawmont site

Vegetative.. Ground Cover Evaluation. Background data from the refuse (Table 5) indicate why the site was barren. All refuse samples were composites of several smaller samples. Sample DM-1 was from the huge gob pile before it was regraded. Sample DM-2 was taken from the regraded area on the northern part of the site farthest from the railroad tracks while sample DM-3 was taken from the large regraded equipment storage area near the railroad tracks.

Table 3. Background Water Quality Data from Dawmont Site

Sample ID	pH	Conductivity (umhos/cm)	Acidity (mg/L)	SO4 (mg/L)	Fe (mg/L)	Mn (mg/L)	Al (mg/L)
DM-A	2.5	4650	2876	2438	161	8.5	418
DM-B	2.1	18500	20610	3125	3600	290.0	1303

The acid-base account data (Table 5) showed that acid generation had occurred as evidenced by pH values ranging from a low of 2.0 to a high of 3.9. There were no neutralizers present in the refuse to counteract the effects of the acid being generated. The neutralization potential values ranged from a high of +1.79 m.t. $CaCO_3$ equivalent/1000 m.t. of refuse to a low of -10.37 m.t. $CaCO_3$ equivalent/1000 m.t. of refuse. The area (sample DM-3) on the western part of the site seemed to be the least toxic; however, the chemistry of this refuse would kill most vegetation.

Table 4. Reduction in Specific Parameters at the Dawmont Reclamation Site

Parameter	Year 1988	Year 1989	Overall
Acidity	95.8%	95.7%	95.8%
Specific Conductivity	89.9%	86.5%	91.2%
Sulfates	90.5%	90.1%	90.8%
Total Iron	96.1%	93.8%	97.3%
Manganese	74.8%	61.1%	85.6%
Aluminum	85.4%	82.2%	88.5%

The refuse was weathered as indicated by the amount of sulfate sulfur in samples DM-1 and DM-2 (Table 5). Approximately 50% of the total sulfur in these samples was in the sulfate form. Only sample DM-2 had more than 50% of its total sulfur as pyritic sulfur; however, this area was a regraded refuse pile. Moving the refuse around during regrading exposed slightly weathered material that was high in pyritic sulfur. Regardless of the state of weathering, more than enough pyritic sulfur was left to cause acid generation to continue for a long time. Also, there was a large store of acid salts that could be solubilized and leached from the site in the future.

Biomass production was measured in 1989 in the same manner as it was measured at the Route 43 site. Total biomass was measured on a dry weight basis and after a constant weight was reached, the treated area had 1,604 kg/ha while the control area had only 1,033 kg/ha. Considering the materials that make up the deep root zone, one might expect next year's data to show a greater difference between the two areas.

Table 5. Sulfur Forms and Acid-Base Account of Samples Taken at Dawmont Refuse Pile

						Calcium Carbonate Equivalent m.t./1000 m.t. of Material		
Sample Number	Paste pH	Total % S	Sulfate % S	Organic % S	Pyritic % S	Acid Potential from Pyritic % S	Neutralization Potential	Deficiency(-) or Excess(+) Neutralizers
DM-1	2.5	1.79	0.882	0.685	0.223	6.97	- 4.15	- 11.12
DM-2	2.0	18.83	2.200	1.030	15.600	487.50	-10.37	-497.87
DM-3	3.9	3.90	2.160	1.010	0.730	22.81	1.79	- 21.02

Conclusions

The following conclusions can be drawn from these case studies:

1. Bactericides inhibit the proliferation of T. ferrooxidans.
2. A system that combines a liquid spray with controlled release pellets substantially reduces acid formation, allows heterotrophic microorganisms to proliferate and promotes good vegetative growth.
3. The bactericide has residual effect on acid generation long after the controlled release pellets have been exhausted. Generation one pellets used at the Route 43 site were made of natural rubber and had decomposed by the end of the second year of the study. No pellets were found and no trace of the bactericide was found in the surface and refuse water, but effective control of acid generation continues into the sixth year.
4. An integrated system of bactericides can be used as a standard reclamation practice without adding problems to the operation.
5. Without liming the refuse and by adding only minimal cover material, an integrated system of bactericides can help establish and maintain a healthy growth of vegetation.
6. The areas treated with ProMac bactericides show a better water quality in the vadose zone than the control area, providing a non-toxic source of moisture for plant growth.

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