COMPARISON OF BACTERIA POPULATIONS WITH METAL AND SULFATE CONCENTRATIONS IN A SCALE MODEL VERTICAL FLOW POND[1]

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Abstract: The pattern of alkaline addition, metal removal and sulfate reduction was analyzed in a model of a vertical flow pond (VFP) aerobic wetland system that was a scale model of the system constructed at the Jennings Environmental Education Center. Samples were obtained from four locations in the VFP and at three locations from the aerobic wetland. Acidity removal occurred in the upper 60 cm of the substrate resulting in a net alkaline discharge. The system was effective in iron and aluminum removal. Iron and manganese concentrations were inversely correlated with iron and manganese bacteria populations. Sulfate concentrations did not vary significantly throughout the VFP, and with sulfate concentrations was correlated with anaerobic sulfate bacteria. A core was obtained from the VFP and the metal and sulfate concentrations compared with concentrations in the water samples. Alkaline addition appeared to be a result of limestone dissolution with little if any contribution from sulfate reduction. However, bacteriological activity is an important but little understood mechanism involved in the function of aerobic and anaerobic passive treatment systems.

Key Words: passive treatment, bacteria, iron, manganese, aluminum and sulfate reduction

Introduction

Constructed aerobic wetlands have been used for over two decades for the treatment of acid mine drainage (Kleinmann *et al.,* 1983; Burris *et al.*, 1984; Gerber *et al.*, 1985). Many of these early systems were based on observations of natural or volunteer *Sphagnum* wetlands receiving mine drainage, but most of these constructed *Sphagnum*-based wetlands ceased to be effective in improving water quality after several months. As a result of this early work, almost all acid mine drainage passive treatment systems constructed during the last two decades were planted with cattails (*Typha latifolia*), which are tolerant of a wide range of water conditions (Sencindiver and

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Bhumbla, 1988; Samuel *et al.*, 1988; Hedin, 1989; Brenner *et al.*, 1993, 1995). Although numerous wetlands were constructed during this period, little research has been undertaken to understand the mechanism operating within these systems.

Most constructed wetlands are designed to remove metal by oxidation and hydrolysis (Hedin, 1989). Wieder and Lang (1986) reported that 93 and 27% of iron and manganese accumulation, respectively, was in oxidized forms. In drainages with a pH less than 6, the abiotic oxidation rates are slower and oxidizing bacteria become an important component of these treatment wetlands (Kleinmann and Crerar, 1979; Brenner *et al.*, 1995). These chemautotrophic and chemheterotrphic bacteria have been reported to increase the oxidization of ferrous iron, thereby enhancing the formation of iron precipitates (Brenner *et al.*, 1993, 1995). Brenner *et al.* (1995) reported that a greater amount of iron oxidizing bacteria occurred in association with cattail rhizomes than elsewhere in the substrate, suggesting increased iron oxidation in the rhizosphere. But this increase in oxidation associated with the rhizosphere may be due to a combination of plant induced oxygenation and iron bacteria (Sencindiver and Bhumbla, 1988; Brenner *et al.*, 1995). In addition to iron oxidizing bacteria, manganese oxidizing bacteria and fungi have been shown to be the primary source of manganese removal from AMD in constructed wetlands (Brenner *et al.*, 1995; Robbins *et al.*, 1999).

During the last decade, vertical flow ponds (VFPs) also referred to as vertical flow wetlands (VFWs) and sequential alkaline producing systems (SAPS) have been used to treat AMD, but the mechanisms operating within these systems are not completely understood. Based on the initial design of these systems proposed by Kepler and McCleary (1994), VFPs provide alkaline addition through the dissolution of limestone and sulfate reduction, resulting in the precipitation of iron and aluminum in the substrate. Brenner (2001a,b) reported that VFPs are effective in removing over 90% of the iron and aluminum, but they are not as effective in removing manganese. Demchak (1998) indicated that the efficiency of some of these systems began to decline after 18-24 months due to a reduction in limestone availability and iron accumulation in the substrate and similar results were reported by Brenner (2001a,b) and Brenner *et al.* (2002) for a scale model of these systems. When a scale model was dissected, iron precipitates had accumulated in the upper third of the substrate and the efficiency of the system in both alkaline addition and metal removal had declined in the second year of operation (Brenner 2001a,b). Demchak (1998) also reported the overall effectiveness of the VFPs varied seasonally especially as the systems aged. In theory, these systems function anaerobically. But in a recent study, Brenner *et al.* (2002) reported that populations of iron and manganese bacteria under both aerobic and anaerobic conditions were correlated with iron and manganese concentrations within the system, suggesting that occurrence of both oxidative and reductive processes are occurring in VFPs.

The objective of the current study was to evaluate the efficiency and the role of bacteria in the treatment of AMD using a scale model of an operating VFP and aerobic wetland system located at the Jennings Environmental Education Center in Butler County, PA.

Methods

In the spring of 1998, a scale model VFP and aerobic wetland was constructed at the Jennings Environmental Education Center, Butler County, Pennsylvania (Fig. 1). The system was designed proportionally to a passive AMD treatment system on the property. The scale model was designed for an average flow of 2.1 L/min with an average potential acidity of 300 mg/L and average iron, manganese and aluminum concentrations of 32, 15 and 19 mg/L, respectively. The VFW was constructed using a l.5 m tall by 1 m diameter fiberglass septic tank using substrate consisting of a mixture mushroom compost and AASHTO#9 (~ 1cm) Vanport marine limestone (90+ % Ca) at a ratio of 1 compost: 1.4 limestone by weight. Ninety centimeters of the compost-limestone mixture was placed over 30 cm of AASHTO #57 washed river gravel and a 30 cm water cap was maintained on the surface to the system. The water distribution system consisted of over and underdrains constructed of 2.5 cm perforated PVC pipes. Sampling ports were installed at depths of 30, 45, and 90 cm. A 2.42 x 1.21 x 0.3 m deep aerobic wetland was constructed using treated 1.5 cm thick plywood. The water distribution system was constructed using 2.5 cm PVC pipe installed in river gravel and sampling ports were installed at the influent, 1.2 m and the effluent. Samples were collected once a week from each sampling port in both the VFP and aerobic wetland. Samples were analyzed for acidity, alkalinity, sulfate, iron, manganese and aluminum according to the established procedures (Greensburg *et al.*, 1992). Samples were analyzed for total iron, manganese and aluminum by atomic absorption and by spectrophotometric procedures. Samples for bacteriological analysis were collected in 150 ml polypropylene bottles and cultured aerobically and anaerobically on iron, manganese, and sulfate medium. One ml of each sample was cultured aerobically and anaerobically on iron isolation, # 2 manganese and sulfate-reducing media at 20 ^{o}C for 5 to 7 days as described by Greensburg *et al.* (1992).

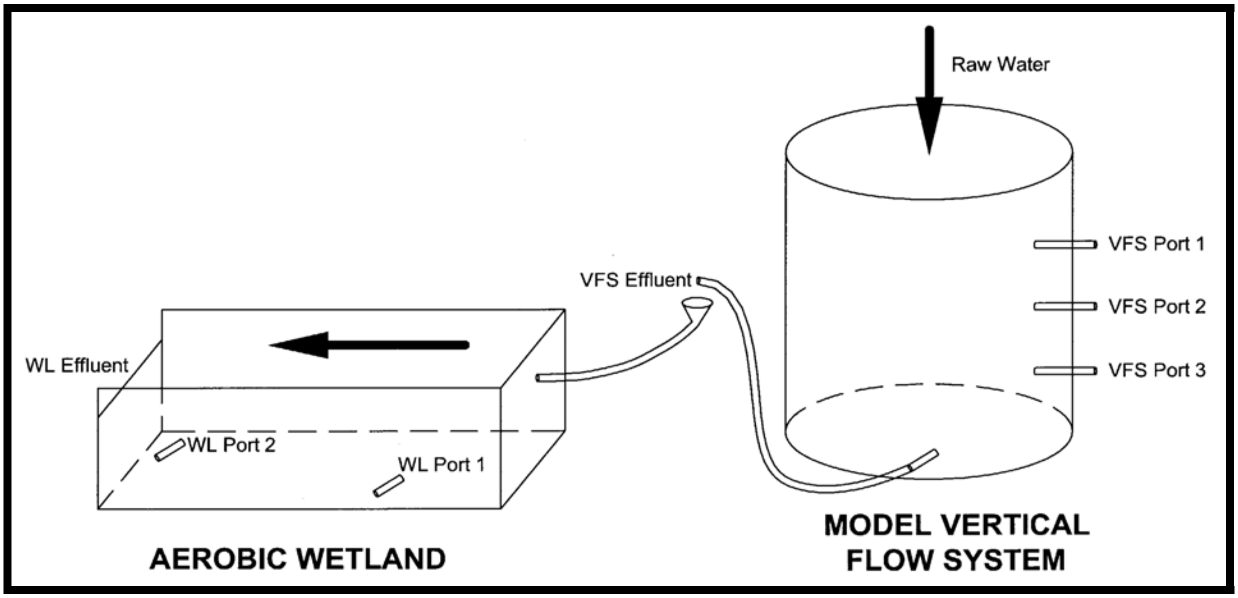

Figure 1: Schematic of the scale model vertical flow pond and test bed wetland.

In August 2002, a core was removed from the VFP using 2.5 cm PVC pipes. Once the core was removed, it was logged into three distinct zones (A,B,C) based on its visual appearance and photographed. A sample was removed from each zone and transported to an analytical chemistry laboratory for analysis. The samples were dried and passed through a series of sieves to separate limestone and substrate fragments from the metal precipitates. The final pan samples were analyzed for iron, manganese, aluminum, silica, calcium and other trace metal concentrations using a-ray fluorescence. The concentration of each chemical compound was expressed as g/100g. Once these analyses were completed, chemical speciation was determined by x-ray diffraction. To determined loss on ignition (L.O.I.) as representative of the volatile organics in each zone, a known weight of sample was placed in a Muffle furnace at 950 C for 2 hrs and expressed as a percent of sample weight. Sulfates were determined using the gravimetric methodology with the ignition of the residue (Standard Methods 4500 SO_4 C). The remainder of the core was sealed and frozen for additional analysis at a later date.

Results and Discussion

As reported by Brenner *et al.* (2002), the combination of the VFP and aerobic wetland was effective in removing acidity and reducing metal concentrations in the final discharges, but there was a reduction in the efficiency of the system during the second year of operation. Overall, the mean pH increased from 3.72 to 6.65, with over 74% of the this increase occurring in the VFP with a corresponding decrease in acidity from 300 mg/l in the influent to less than 50 mg/l in the final discharge resulting in a net alkaline discharge of 40 to 90 mg/L. The majority of acidity reduction and increase in pH occurred between sampling ports 2 (45 cm) and 3 (90 cm) of the substrate with alkaline addition occurring in the lower 45 cm of substrate and in the aerobic wetland (Brenner *et al.*, 2002). Conductivity and total dissolved solids did not vary among the different sampling ports with the average conductivity varying between 1372 and 1019 μohms/cm (overall mean = 1191 μohms/cm) and the average total dissolved solids ranging between 900 and 680 mg/L (overall mean = 746 mg/L) (Table 1).

Table 1: Mean concentrations of the chemical parameters obtained from the scale model vertical flow pond and aerobic wetland (N=64).

	Vertical Flow Pond					**Aerobic Wetland**		
Parameter	**Influent**	**Port 1**	**Port 2**	**Port 3**	**Effluent**	**Port 1**	**Port 2**	**Effluent**
pH (s.u.)	3.73	3.48	3.86	5.98	6.05	6.54	6.66	6.58
Alkalinity (mg/L)	0.0	0.0	0.0	37.7	47.1	77.9	90.9	46.7
Fe (mg/L)	38.0	40.2	25.7	17.8	16.6	12.6	2.2	8.9
Manganese (mg/L)	19.0	19.2	18.8	18.3	18.6	15.6	12.3	16.3
Sulfates (mg/L)	938.5	890.4	850.0	861.5	859.6	1212.5	1145.5	959.5
Conductivity (μohms/cm)	1194.7	1199.7	1161.9	1196.4	1188.6	1019.1	1372.3	1194.8
Total Dissolved Solids (mg/L)	680	710	680	700	700	900	860	740

Overall, the combination of the VFP and aerobic wetland reduced iron and aluminum concentrations from 32 to 4 mg/L (88.8%) and from 17 mg/L to less than 1mg/L in the final discharge, respectively but, the system only removed an average of 2 mg/L of manganese The efficiency of the system in iron removal varied seasonally with 19 mg/L being removed during months of March, April and May compared to 33 and 24 mg/L removed during the summer-fall and winter months, respectively. The efficiency in iron removal also varied between the two years of operation with 34 mg/L (87%) being removed during the first year compared to 17 mg/L (46%) the second year.

The amount of volatile organics, as represented by the loss on ignition (L.O.I.), was highest in the B Zone (62.36%) than it was in either the A (54.19%) or the C zone (54.00%) which may be a result of the mixing of the limestone and compost or decomposition occurring in the C zone of the VFP. The metal concentrations in the different zones of the core corresponded to the metal concentrations obtained from each sampling port of the VFP. The A zone was a reddish brown color and it was located in the upper 7.5 cm of the core; the middle zone B was dark gray in color and was located between the 7.5 and 30.5 cm; the lower C zone comprised the lower 14.1 cm of the core. In the A zone, the iron concentration was 87.06 g/100 g compared to 6.13 g/100 g and 12.50 g/100 g in the B and C zones, respectively. Based on x-ray diffraction, a portion of the iron precipitate was iron oxide (Fe_2O_3), but an iron hydroxide ($FeOH_3$) fraction may also be present in an amorphous state that cannot be detected by x-ray diffraction. The aluminum precipitate also existed in an amorphous state, but it was probably $AlOH_3$. Aluminum and silica (SiO_2) concentrations in the A zone were 1.44 g/100 gm and 11.88 g/100 gm, respectively, but in the B zone, both aluminum and silica concentrations increased to 43.19 g/100 g and 46.77 g/100 g, respectively. In the C zone, the aluminum concentration decreased to 19.41 g/100 g, but the silica concentrations were similar to the B zone, with a concentration of 48.04 g/100 g. Other chemical compounds occurring in the core were Cr_2O_3 ($\sum$ all zones 0.003 g/100 g), P_2O_5 ($\sum$ all zones 1.42 g/100 g), TiO ($\sum$ all zones 2.9 g/100 g), NiO ($\sum$ all zones 0.182 g/100 g).

Calcium concentrations ($CaCO_3$) increased throughout the core from 0.30 g/100 g in the A zone to 4.28 g/ 100 g and 19.38 g/ 100 g in the B and C zones, respectively which corresponds to acid neutralization in the upper zones (port 1 and 2) via limestone dissolution with alkaline addition occurring in the lower C zone (Port 3) of the substrate (Figure 2).

The removal of aluminum was a function of increases in pH and alkalinity. The pH and alkalinity along with bacteriological activity interact in the removal of iron and manganese in VFPs and aerobic wetlands (Figure 3). Previously, Brenner *et al.* (2002) reported that bacteria populations was inversely correlated with iron (aerobic, $r = -0.795$, $P < 0.01$; anaerobic, $r =$

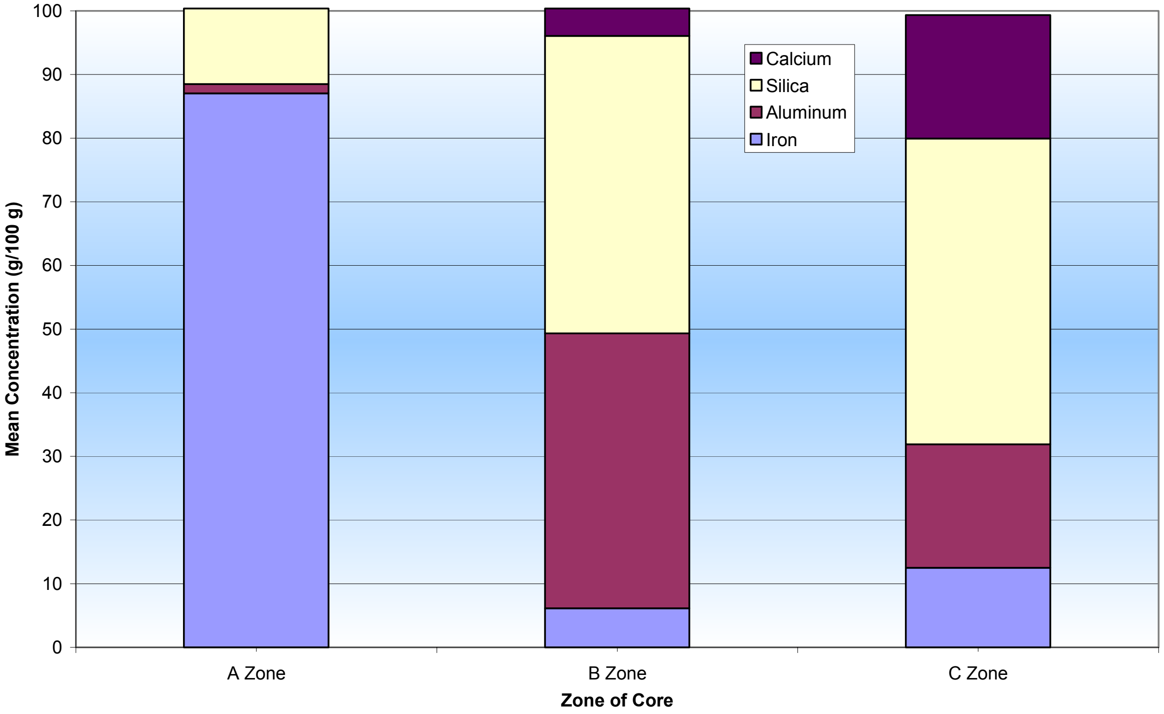

Figure 2: Mean concentrations of chemical compounds within the three zones of the core from the scale model vertical flow pond.

0.796, P <0.001 and manganese (aerobic, r = -O.657; anaerobic, r = -0.730, P < 0.01) concentrations in the discharges, indicating that a reduction in the number of bacteria resulted in elevated metal concentrations in the discharges. Conversely, increased iron bacteria populations were positively correlated with iron (aerobic, r = 0.726, P < 0.001; anaerobic, r = 0.642, P < 0.05) removal, but since the system only removed 1 or 2 m g/L of manganese, there was not a significant correlation between manganese removal and manganese bacteria populations. In the system (aerobic r = 0.0471, P > 0.50; anaerobic r = 0.375, P >0.1) (Figure 4). Although there is an initial reduction in iron and manganese in the aerobic wetland, concentrations of both metals increased between the second sampling port and the final discharge, with the largest increase occurring during the winter months. These increases in iron and manganese may be due, at least in part, to the redissolution of iron, and/or the accumulation and flushing of iron in the discharge, and/ or the desorbing and resorbing of manganese in the substrate (Brenner *et al.*, 1993; 1995).

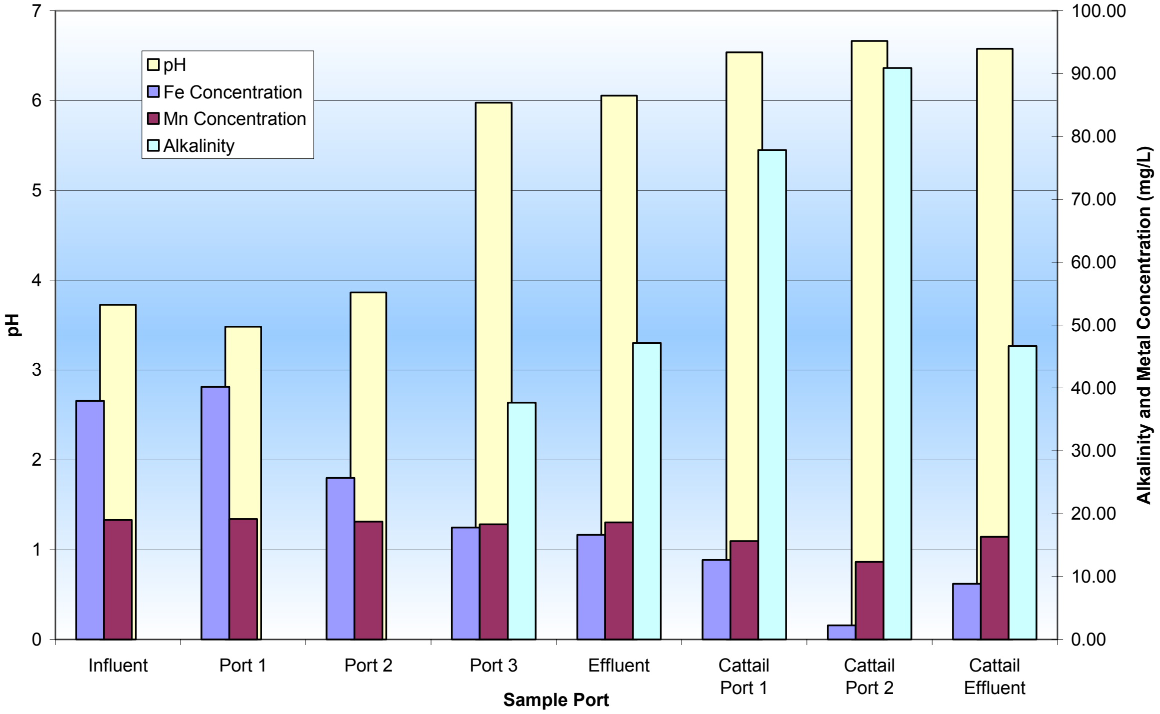

Figure 3: pH, alkalinity, and average metal concentrations through scale model VFP and test bed wetland from 1998-2000.

Sulfate concentrations did not vary among the different sampling ports in the VFP, but sulfate concentrations in the cores increased from 1.08 g/100 g in zone A to 7.77 g/100 g in zone B and decreased to 5.28 g/100 g in the zone C. The original concept of VFPs was that the organic compost would provide a source for bacterial sulfate reduction. In theory, the reduction of sulfates to sulfides results in the formation iron precipitates as either FeS or FeS_2 and elemental sulfur along with neutralizing mineral acidity and generating bicarbonate alkalinity. Duplicate samples obtained from a full scale operational VFP from 1997-1999 and analyzed by two different laboratories produced varying results. The results reported by the Pennsylvania Department of Environmental Protection (PA DEP) indicated that sulfate concentrations in the effluent increased by an average of 113 mg/L, whereas the U.S. Department of Energy Laboratory (DOE) laboratory reported an average sulfate reduction of 35 mg/L over the same three-year period (Jennings Water Quality Improvement Coalition, 1999). Since sulfides were not detected in any of the cores obtained from the model or the full scale VFP (Brenner *et al.*,

2003) and the low sulfate concentrations in both sets of cores, it is unlikely that sulfate reduction was occurring within either VFP. Sulfate concentrations decreased initially in the aerobic wetland and then increased at sampling port 2 and in the effluent. Both aerobic and anaerobic sulfate bacteria increased in the VFP and aerobic wetland, with the greatest increase occurring in the anaerobic component of the bacterial flora in the lower 45 cm of the substrate in the VFP, but these increases in bacteria populations did not result in lower sulfate concentrations. Although both aerobic and anaerobic bacterial increased in the aerobic wetlands, sulfate concentrations also increased at these sampling locations. Sulfate bacteria may be contributing to the changes in sulfate concentrations in portions of the VFP and aerobic wetland, but the precise role of these bacteria in sulfate metabolism has not been defined for these systems.

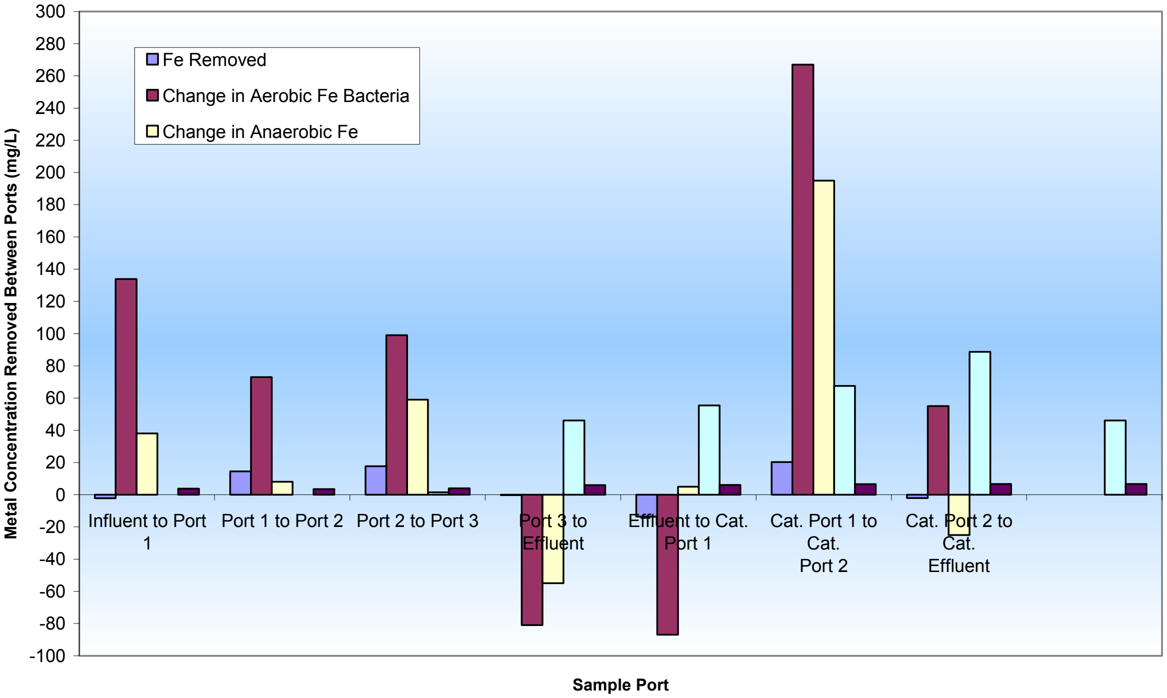

Figure 4: Comparison of the change in the number of iron bacteria and the amount of iron removed between ports in scale model VFP and aerobic wetland system.

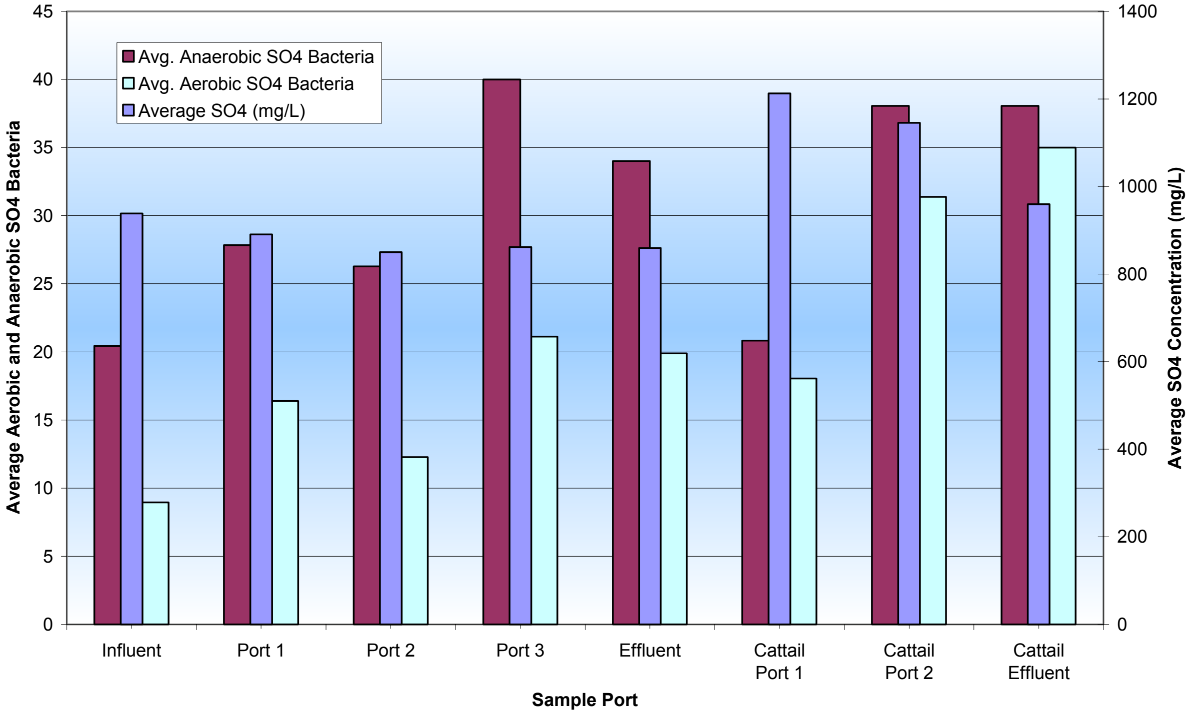

Figure 5: Comparison of mean sulfate concentration, aerobic sulfate bacteria, and anaerobic sulfate bacteria throughout the vertical flow pond and aerobic wetland

Conclusions and Recommendations

There was a three-unit increase in pH and removal of 250 mg/L of acidity in the upper 45 cm of the VFP, but the majority of alkaline addition occurred between 45 and 90 cm of the substrate. Although the efficiency of the VFP varied seasonally, as well as between years, overall the system was effective in the removal of iron and aluminum, but not manganese. The removal of both iron and manganese was correlated with aerobic and anaerobic bacteria populations within the system. The results of this study, as well as those of Brenner *et al.* (1993, 1995) suggest that chemoauthotropic and chemoheterotropic bacteria are important components of both VFPs and aerobic wetlands. Although in theory VFPs function anaerobically, the isolation of both aerobic iron, manganese and sulfate bacteria from these systems suggest that the systems are not completely anaerobic or that these bacteria are functioning facultative. But, it has suggested that the aerobic bacteria are able to survive but not function in anaerobic environments.

Previous authors (Brenner *et al.* 1993, 1995; Hedin *et al.* 1994) have suggested that the manganese removal is a sequential process and occurs only after the removal of iron. Brenner and Pruent (1999) reported that manganese concentrations were reduced from 11.3 mg/L to 4.8 mg/L in a surface limestone drain, settling pond and aerobic wetland system with the majority of the iron being removed by the limestone drain/settling pond with manganese removal occurring in the aerobic wetland. The results of the current and previous studies (Brenner *et al.*, 1993, 1995, 2002; Hedin *et al.*, indicate that additional alkaline addition after the removal of iron and aluminum is necessary to facilitate additional manganese removal. Since higher pH and alkalinity appears to be correlated with bacteriological activity and corresponding metal removal these components should be considered in the design of any passive systems for the treatment of acid mine drainage.

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