Sulfate reduction and carbon oxidation in model constructed wetlands for metal remediation
by Jason Richard Sturm

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University

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All cells showed decreasing DOC concentrations along the flow path. The control cells and one bulrush cell exhibited increasing DOC concentrations with depth, while the other cells had decreasing DOC concentrations with depth. Sulfate concentrations in the controls and bulrush decreased along the flow path, but increased along the flow path of the cattails. Average first-order DOC degradation constants for all cells ranged from 0.0033 hr^-1 to 0.0100 hr^-1. Average first-order SO42- degradation constants for all cells ranged from -0.0067 to 0.0142.

Wastewater using initial concentrations of 100 mg/L C and 200 mg/L SO42-became carbon limited. Wastewater with initial concentrations of 200 mg/L for both C and SO42- became sulfate limited, with severe limitation experienced below 0.1 mmol SO42-. Sulfate reduction contributed to less than 20% of the carbon oxidation within the cells. Over 95% of the influent zinc was sequestered.

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APPROVAL

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Jason Richard Sturm

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Otto R. Stein, Ph. D. — (Signature) — Date 7/20/00

Approved for the Department of Civil Engineering

Donald A. Rabern, Ph. D. — (Signature) — Date 7/20/00

Approved for the College of Graduate Studies

Bruce R. McLeod, Ph. D. — (Signature) — Date 7-21-00

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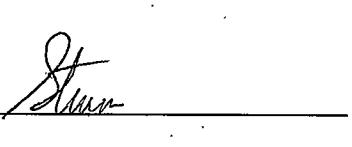
Signature 

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ABSTRACT

Constructed wetlands are an increasingly used tool in the remediation of heavy metal laden wastewater, but the interrelationships between the various chemical and microbial processes occurring in wetlands which influence treatment efficacy are not well understood. Sulfate reduction and carbon oxidation rates were examined to provide additional insight into the internal processes of constructed wetlands for metals removal.

In 1996, six model horizontal subsurface-flow constructed wetlands were constructed and housed in an environmentally-controlled greenhouse. Two were planted with cattail, two with bulrush, and two were left unplanted as treatment controls. From February to June 1998, a synthetic wastewater containing 200 mg/L SO_4^{2-} and 200 mg/L dissolved organic carbon (DOC) was applied to the system. A second wastewater with half the carbon load was run from June through September 1998. Samples were collected from the influent, effluent, and from ports placed within each wetland cell. Samples were analyzed for sulfate, DOC, and zinc concentrations. Change in sulfate and carbon concentration data along the flow path were fitted to a first-order model. Changes in the sulfate to DOC ratio were analyzed as a tool to gauge the impact of alternative electron acceptors. Zinc was tested to determine the effectiveness of the system to remove and retain a divalent metal.

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INTRODUCTION

The use of constructed wetlands is a relatively new tool for the remediation of heavy metal laden water. Although increasingly used as a low cost remedy for many kinds of metal laden waters, constructed wetlands are most commonly used in the treatment of acid mine drainage (AMD). AMD is the result of the oxidation of pyrite upon exposure to oxygen and water and can have disastrous impacts on natural waters. Constructed wetlands have shown an ability to increase pH and remove hazardous heavy metals from AMD, however methods for optimization of the chemical and microbial processes involved in heavy metal removal by constructed wetlands are not yet well understood. One process that appears to be important to heavy metal removal in constructed wetlands is microbially mediated sulfate reduction. Constructed wetlands are capable of providing an environment conducive to high levels of sulfate reduction. Sulfate reduction produces hydrogen sulfide gas that readily couples with many heavy metals to form highly insoluble metal precipitates, simultaneously raising the pH in the process. These precipitates remain in the wetland, effectively removing the metals from the aqueous environment.

Goals and Objectives

Initially, the goal of this research was to look at the effectiveness of the dissimilatory metal-reducing bacteria *Shewanella alga* BrY to reduce, precipitate, and remove metal contaminants from simulated, industrial waste streams within a constructed, vegetated sub-surface bed wetland system. Beginning in April 1997, and

continuing through September 1997, four attempts were made to induce the colonization of three model constructed wetland cells by *Shewanella alga* BrY. Each attempt failed to produce a sustainable population in the wetland cells. A typical graph showing the decreasing populations of the *Shewanella* with time is shown in the appendix. Since further work in this experiment was dependent on the *Shewanella alga* colonization, a change in research focus was required.

The new research objective was designed to contribute to the understanding of sulfate reduction as a means of removing heavy metals from wastewater in a constructed wetland. Six bench-scale constructed wetland cells containing cattail, bulrush, or no plants were supplied with a synthetic zinc-laden wastewater. Samples were taken to measure sulfate, carbon, and zinc concentrations, and the data were analyzed to determine the effect of carbon loading, plants, and other electron acceptor utilization on sulfate reduction and its accompanying zinc precipitation.

Using two wastewater feeds of differing carbon loads, changes in sulfate and carbon concentrations were analyzed with respect to depth and longitudinal distance within the cells. Longitudinally, carbon oxidation and sulfate reduction were independently fitted to a first-order model. The effect of carbon loading, plants, and depth on the reduction rates of both constituents was studied. Alternative electron acceptor utilization was investigated by comparing depth-related changes in the sulfate/DOC ratio along the flow path. The effectiveness of sulfate reduction on heavy metal removal and retention was discerned from measured zinc concentrations.

BACKGROUND

Interest in using constructed wetlands as a wastewater treatment system has continually increased over the past several decades. Most of the focus has been on the usage of wetlands, and more specifically constructed wetlands (CW), for organic matter and nutrient removal. Around the early 1980s, interest was piqued in the possibility of CW being a viable treatment option for acid mine drainage (AMD) (Kittle *et al.*, 1995). Since then, CW have been tested and sometimes used successfully in the remediation of water containing many kinds of metals. Metals of concern include the heavy metals Al, Cd, Cu, Fe, Mn, Ni, Pb, and Zn, which can be toxic to humans or aquatic biota. From AMD to industrial discharge to even municipal wastes, CW have increasingly been used to facilitate removal of these metals.

The more conventional method of water treatment for metals removal includes an active, chemical treatment, such as liming (Webb *et al.*, 1997). By raising the pH of the water, the metals precipitate out as oxides or hydroxides (Stark *et al.*, 1995). However, chemical treatment can be expensive and require constant upkeep. The use of constructed wetlands is an alternative that can provide effective, passive treatment at much less cost. CW can also have the ancillary benefit of providing wildlife habitat or recreational areas (Makos and Hrncir, 1995).

A number of chemical, biological and physical processes occur in a wetland system. Mechanisms occurring in a wetland that aid in the removal of metals are precipitation, adsorption, and plant uptake (Crites *et al.*, 1997; Wildeman *et al.*, 1993; Reed *et al.*, 1995). Each will be examined more closely below.

Plant Uptake and Other Plant Effects

As an integral part of the mosaic that makes up a wetland system, aquatic plants affect the chemical and biological processes of the wetland in many ways. Common emergent plants used in constructed wetlands include cattails (*Typha sp.*), bulrush (*Scirpis sp.*), sedge (*Carex sp.*), and rush (*Juncus sp.*). Although many of the details of their role in treatment are not fully understood, it is clear that plants do impact the biological, chemical, and physical processes of a wetland. Direct plant uptake is a mechanism of metals removal that is probably minor in comparison to precipitation and adsorption, although early studies presumed plant uptake was an important removal process (Wildeman *et al.*, 1993). More recent studies, such as that done by Mitsch and Wise (1998), have found plant uptake to account for less than 5% of total metals retention.

Uptake of heavy metals can have deleterious effects on plants. Elevated concentrations of metals, especially Al, can be toxic to plants and have been found to weaken or kill off aquatic macrophytes (Wieder *et al.*, 1990; Schmidt, 1997; Taylor and Crowder, 1983). Most of the metals taken up by plants are stored in the roots (Taylor and Crowder, 1983; Kadlec and Knight, 1996). This can be significant, especially during senescence, because most roots remain alive while the leaves and stems die. With the eventual death and decomposition of the plant tissues, the metals can be returned to the aqueous solution (WPCF, 1990). For the above reasons, plant uptake and retention is not an effective removal process in the long term, or with waste streams with high metals loading.

Vegetation does affect removal in more indirect ways. Aquatic plants have the ability to transport oxygen to the roots from the stems and leaves (Reddy *et al.*, 1989). Oxygen transport seems to be a way to counteract the negative effects of an anoxic environment associated with living in a flooded environment (Moorhead and Reddy, 1988; Sand-Jensen *et al.*, 1981). Any excess oxygen not needed by the roots then may be transferred into the near-root aqueous solution. This released oxygen creates aerobic microenvironments (Reed and Crites, 1984; Sand-Jensen *et al.*, 1981), but does not appear to affect oxygen concentration of the overall solution, because microbial populations and chemical processes quickly consume any excess oxygen (Steinberg and Coonrod, 1994).

Evidence of oxygen transfer is given by the observation that the roots of wetland plants can retain metals through the formation of an absorptive plaque. Under near-neutral pH conditions, cattails are known to form iron hydroxide coatings (plaques) on their roots (Wieder *et al.*, 1990; Taylor *et al.*, 1984). These plaques are thought to be involved in the immobilization of Ni and Cu (Taylor and Crowder, 1983).

The most important role plants play in wetlands metal removal is as a long-term source of organic matter (OM). OM from decaying plant material serves two purposes. Firstly, the OM can adsorb metals. By adsorption onto OM, the metals are removed from the aqueous solution. This process was the design of many early constructed wetlands. Secondly, the OM is a source of organic carbon for microbial processes. This is particularly important for sulfate reducing bacteria and their ability to remove metals through sulfide production (Schmidt, 1997; Wildeman *et al.*, 1993). Mitsch and Wise

(1998) found sulfate concentrations in their vegetated CW plots to be lower than in the non-vegetated plots.

Adsorption

Adsorption is a mechanism that incorporates many elements of a wetland, and is a metal removal process that can be extremely effective (Mitsch and Wise, 1998). Organic matter, oxyhydroxides, and algae provide typical sorption sites. Many early wetlands constructed for metals removal, often using *Sphagnum* peat moss, were designed so that adsorption would be the dominant removal process (Wieder *et al.*, 1990; Vile and Wieder, 1993). Organic acids (humic and fulvic acids) from decaying organic matter are primarily responsible for the removal. Under the right conditions, the exchange of metals and acid functional groups on the humic and fulvic acids proceeds as:

$$RCOOH \leftrightarrow RCOO^- + H^+ \quad (2.1)$$

$$2\,RCOO^- + Me^{2+} \leftrightarrow Me(RCOO)_2 \quad (2.2)$$

where R is the inert portion of the organic acid RCOOH. Along with the dissociation of the carboxyl ion (COO^-) with the hydrogen ion, there is an exchange with a divalent or trivalent metal ion (Me) (Wildeman *et al.*, 1993; Kadlec and Knight, 1996).

Most heavy metals can be removed, with varying degrees of effectiveness, through adsorption. Makos and Hrncir (1995) found the most toxic form of chromium, Cr(VI) was reduced to Cr(III) and bound with the acid functional groups of humics and

fulvics. However, Wildeman *et al.* (1993) noted that organic adsorption may not be an effective removal process for Mn, Zn, and Cd. With aqueous solutions containing multiple metals, some metals are preferentially sorbed over others. When looking at Fe, Cu, Zn, and Mn from mine drainage water, Machemer and Wildeman (1992) found that initially all of the metals experienced a high level of removal. After a period of time, though, the concentrations of Zn and Mn in the effluent of their cells began to increase. After all the organic exchange sites were filled, the Cu and Fe began to replace the Zn and Mn already bound. There is a limit as to how many exchange sites are available and how much heavy metal can be adsorbed. When the sites are filled, adsorption loses its effectiveness as a metals removal mechanism; therefore, adsorption can not be relied on as a major removal process over the life of a constructed wetland (Machemer and Wildeman, 1992). Perhaps the most effective function of adsorption is to temporarily detain heavy metals thereby giving microbially mediated processes more time to operate (Wildeman *et al.*, 1993).

Adsorption can also occur with algae and oxyhydroxides. Fe(III), Al, and Mn(IV) oxyhydroxides are all capable of adsorbing metal on their surfaces. The oxyhydroxide precipitates are gelatinous and act as a weak acid which attracts hydroxide ions. The hydroxide ions make a negative surface which, in turn, attracts positive ions such as dissociated metals (Wildeman *et al.*, 1993).

Adsorption by algae is more variable. Some metals are toxic to algae, and different types of algae will react with metals in different ways (Kadlec and Knight, 1996). A study by Crist *et al.* (1990) found algae's adsorption of metals to be an ion-

exchange reaction. Two algae studied by Harris and Ramelow (1990), *Chlorella vulgaris* and *Scenedesmus quadricauda*, had similar binding efficiencies for four specific metals. They had the most affinity for Ag, followed by Cu, Cd, and then Zn. Algae also appear to aid in accelerating oxyhydroxide precipitation (Wildeman *et al.*, 1993).

All of the above processes are very much pH dependent. Removal efficiencies are usually directly related to pH. One benefit of these processes is an ability to raise pH. As metals are removed from the aqueous solution in an exchange with ions such as Ca^{2+} or Mg^{2+}, the pH is raised (Stumm and Morgan, 1996) leading to increased removal. Adsorption can act as an effective neutralization process with regards to acidity, but when the exchange sites fill, there will be no more reduction in acidity (Machemer and Wildeman, 1992; Vile and Wieder, 1993). If conditions change too much, such as lowering of pH, the metals will desorb back into solution.

Oxyhydroxide Precipitation

Oxyhydroxides not only remove metals passively through adsorption, but can also actively sequester metals by precipitation. Oxyhydroxide precipitation can be a dominant metals removal process in the aerobic portions of a CW (Webb *et al.*, 1998). The oxidation and precipitation of divalent metals typically occurs along the following reactions (Webb *et al.*, 1998; Stumm and Morgan, 1996; Wildeman *et al.*, 1993):

$$Me^{2+} + 2H_2O \rightarrow Me(OH)_2(s) + 2H^+ \quad (2.3)$$

$$Me^{2+} + H_2O \rightarrow MeO(s) + 2H^+ \quad (2.4)$$

$$4\ Me^{2+} + O_2 + 6H_2O \rightarrow 4MeOOH(s) + 8H^+ \qquad (2.5)$$

The precipitation of oxyhydroxides remove metals from solution, but at the same time produce H^+ ions. The production of H^+ ions thereby decreases the pH of the aqueous solution (Vile and Wieder, 1993; Wildeman *et al.*, 1993). With decreasing pH, the rate of oxyhydroxide precipitation decreases. Therefore, oxyhydroxide precipitation is a self-limiting process (Wildeman *et al.*, 1993). Vile and Wieder (1993) found iron oxyhydroxide deposition occurred more slowly at pH 3.5 than at pH 5.5. Significant iron oxidation occurs at pH values near 7, while manganese oxidation doesn't occur at acceptable rates for adequate removal until pH 9 (Wildeman *et al.*, 1993). Oxyhydroxides are highly soluble in acidic solutions. If changes occur in the wetland environment, metals precipitated as oxyhydroxides can be released back into the aqueous environment.

Sulfate Reduction and Sulfide Precipitation

Within the dynamic wetland system, there are many chemical and biological processes occurring. All of the previously mentioned removal mechanisms play a part in heavy metal sequestration, but for long-term removal of heavy metals by CW, the most effective process appears to be precipitation of metal sulfides. This is brought about by dissimilatory sulfate reduction coupled with the oxidation of an organic substrate occurring in an anaerobic environment. Two by-products of sulfate reduction are hydrogen sulfide and carbonate alkalinity (Schmidt, 1997). Hydrogen sulfide quickly

reacts with most dissolved divalent metals forming metal-sulfide precipitates. The basic equations governing this reaction are (White and Gadd, 1996; Christensen *et al.*, 1996; Wildeman *et al.*, 1993; Webb *et al*, 1998):

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \quad (2.6)$$

$$H_2S \rightarrow H^+ + HS^- \quad (2.7)$$

$$Me^{2+} + HS^- \rightarrow MeS(s) + H^+ \quad (2.8)$$

where CH_2O is an idealized carbon source.

Carbonate alkalinity facilitates the neutralization of solution acidity. Possibly as important as carbonate alkalinity in reducing acidity is the release of H_2S gas to the atmosphere, thereby removing H^+ ions from the aqueous solution. Although Vile and Wieder (1993), found no correlations between changes in H^+ concentration and sulfate reduction in CW cells using *Sphagnum*, straw/manure, sawdust, and mushroom compost, many studies, such as those by Schafer & Associates (1994) and White and Gadd (1996), have found sulfate reduction to significantly increase pH.

Most heavy metals will be effectively removed by sulfide precipitation. This is a great advantage over other removal processes. Sulfide precipitates typically have lower solubilities over a broad pH range than precipitates of oxyhydroxides (Machemer and Wildeman, 1992). One exception to this is manganese. Manganese is a difficult metal to remove by any process, and sulfide precipitation is no exception, as Mn-sulfides are relatively soluble.

Sulfate reduction is an oxidation-reduction (redox) reaction. Redox reactions proceed according to the most favorable electron intensity or energy yield. Thermodynamically, the electron donor (carbon source) supplies electrons to the lowest unoccupied electron level (O_2) first, followed by other levels as electrons are available. This is parallelled biologically with an ecological succession of microorganisms (Stumm and Morgan, 1996). The procession of redox reactions are shown in Table 1.

Table 1. Organic matter decomposition reactions in order of decreasing energy yield.

1. O_2 Reduction: $1/4CH_2O + 1/4O_2 \rightarrow 1/4CO_2 + 1/4H_2O$
2. Denitrification: $1/4CH_2O + 1/5NO_3^- + 1/5H^+ \rightarrow 1/4CO_2 + 1/2H_2O + 1/10N_2$
3. Mn Reduction: $1/4CH_2O + H^+ + 1/2MnO_2 \rightarrow 1/4CO_2 + 1/2Mn^{2+} + 1/8H_2O$
4. Fermentation: $3/4CH_2O + 1/4H_2O \rightarrow 1/4CO_2 + 1/2CH_3OH$
5. Fe Reduction: $1/4CH_2O + FeOOH + 2H^+ \rightarrow Fe^{2+} + 1/4CO_2 + 7/4H_2O$
6. SO_4^{2-} Reduction: $1/4CH_2O + 1/8SO_4^{2-} + 1/8H^+ \rightarrow 1/8HS^- + 1/4CO_2 + 1/4H_2O$
7. Methanogenesis: $1/4CH_2O \rightarrow 1/8CO_2 + 1/8CH_4$

In a plug-flow wetland, the above reactions will occur along the flow path based on the rate organic carbon is utilized. Dissolved oxygen will be the first terminal electron acceptor utilized. Aerobic respiring and faculatively aerobic, fermenting organisms will be dominant. As oxygen is depleted, the microbial population will begin to shift to obligately anaerobic organisms (Odom and Singleton, 1993). The faster the rate of consumption, the smaller each redox zone will be. Sulfate reduction is not a high energy-

yielding reaction. Only after the other higher energy electron acceptors have been consumed will sulfate reduction begin.

Most strains of sulfate reducing bacteria (SRB) utilize only simple organic substrates, and do not actually oxidize them completely to CO_2 (Feng and Hsieh, 1998). SRB utilize low molecular-weight organics, and do not degrade compounds such as proteins, lipids, or starches. They rely on other heterotrophic bacteria to provide the proper fermentation and degradation products (Feng and Hsieh, 1998; Odom and Singleton, 1993). SRBs are a dynamic group of organisms, though. Some will utilize organic acids and alcohols through anaerobic respiration coupled with sulfate to produce acetate, carbon dioxide, and sulfide, while other strains will oxidize acetate to carbon dioxide. Several strains of SRBs are even able to grow fermentatively in the absence of sulfate (Odom and Singleton, 1993).

A number of factors can affect the rate at which sulfate reduction occurs. These include pH, temperature and redox potential. Although SRB activity is greatest at moderate temperatures and near neutral pH, activity occurs at temperatures from 5° C to 50° C and at pH 3.0 to 9.5 (Gyure *et al.*, 1990; Wildeman *et al.*, 1993). Once the redox potential is low enough to facilitate sulfate reduction, the most limiting conditions for SRB activity appear to be the availability of sulfate and carbon. Feng and Hsieh (1998) found SRBs to be active with pore water sulfate concentrations as low as 5 mg/L, but sulfate levels below 30 mg/L seem to affect reduction rates.

In a study using column reactors and very high concentrations of organic carbon and sulfate, > 1200 mg/L and > 3900 mg/L, respectively, Lyew and Sheppard (1999)

measured sulfate reduction rates of between 0.83 mg/L/hr and 2.92 mg/L/hr. Hines *et al.* (1999) found sulfate reduction rates up to 14 mg/L/hr near the surface of marsh sediments. Below 5 cm from the surface, rates were around 2 mg/L/hr. This decrease in rate seems to be due to decreasing concentrations of available carbon with depth. The majority of sulfate reduction occurring during the growing season may be due to organic carbon supplied from the roots of marsh plants (Hines *et al.*, 1999).

Wetlands are a multifaceted system with great potential as a means of removing heavy metals from the aqueous environment. There are many studies looking at the overall effect a wetland has on water quality, but with the possibility of so many processes occurring simultaneously, it is often difficult to quantify what is actually happening within a wetland. With increased knowledge as to their inner-workings, constructed wetlands can be better designed to accentuate the significant chemical and biological processes to achieve the desired effect.

METHODS AND MATERIALS

Design of Wetland Cells

Six polypropylene boxes were constructed to serve as model plug-flow constructed wetlands. The wetland cells (boxes) were constructed of ⅝" (16-mm) opaque polypropylene plastic. Each cell was 91 centimeters long, 62 centimeters wide, and 53 centimeters deep. Attached to the inside front of each cell was a set of racks. The racks held an inlet manifold that could be set at three depths. Each manifold consisted of a piece of one-inch (25-mm) polyvinylchloride (PVC) pipe capped at each end. A half-inch (13-mm) nipple, from which Masterflex size 14 Norprene® tubing was attached, was screwed into each manifold for inflow. Outflow from the manifold was facilitated by a 3-mm slit cut along the length opposite the nipple. Within each cell, 12 centimeters from the front and another 10 cm from the rear, was a baffle to enhance plug flow through the middle of the system. The baffles consisted of a sheet of polypropylene plastic with 24, ¼" (6-mm) holes drilled into it. They also created open water sections at the front (forebay) and rear (afterbay) of the cell to act as complete-stir tank reactors (CSTR).

The middle section of each cell was filled to a depth of 45 centimeters with washed ⅜" to ¾" (10-mm to 19-mm) gravel. The porosity was 0.40. In the middle of the gravel section was a 20-cm diameter, removable basket constructed of ¼" (6-mm) diamond-cut geotechnical screening. The removable basket allowed for internal inspection of the cell with minimal disturbance. Also within the gravel were two rows of

four sampling ports dividing the cells in thirds across the length and width. Each port consisted of a rod of polypropylene plastic with three lengths of ⅛" (3-mm) tubing attached. The tubing reached depths of 8, 23, and 38 centimeters below the gravel surface. At the bottom of the tail open water section was an outlet manifold. It was designed similarly to the inlet manifold, except instead of a nipple, there was a piece of one-inch (15-mm) PVC leading out to an 8" (20-cm) diameter PVC outflow box. Water left the cell through 25-mm plastic tubing maintained at a height of 44.5 centimeters to keep the phreatic water level just below the gravel. Each cell resided on a wooden pallet to facilitate moving if necessary. A schematic of components is shown in Figure 1.

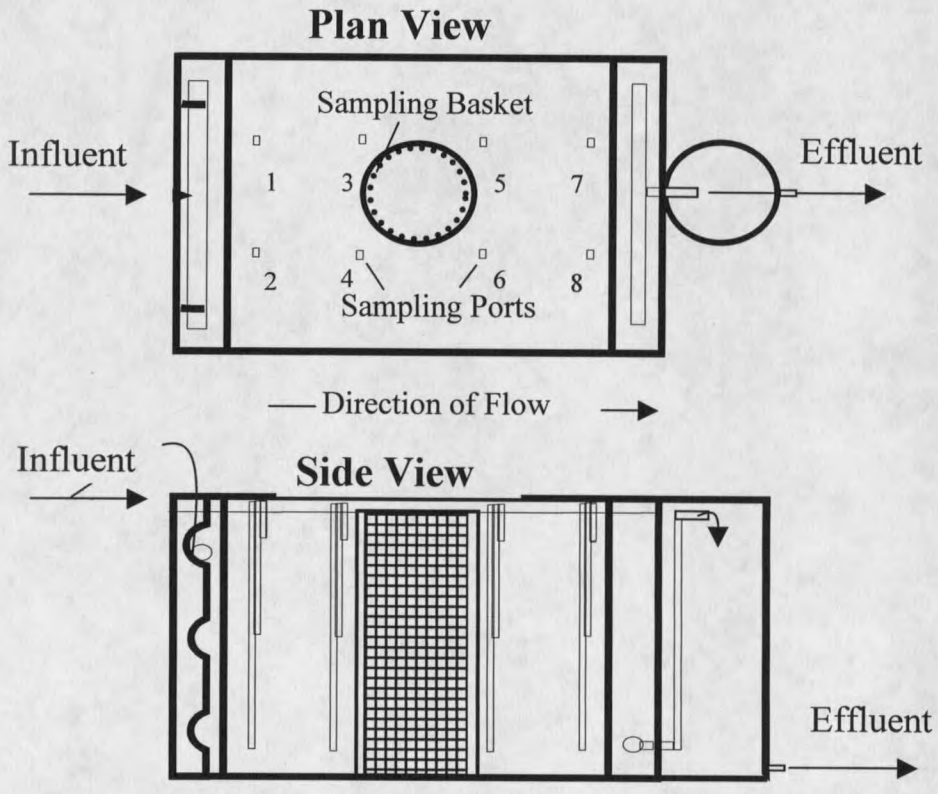

Figure 1. Schematic of constructed wetland cell. (Not to scale)

Experimental Design

Three treatment types were examined. Two cells were planted with cattails (*Typha sp.*), two with bulrush (*Scirpus sp.*), and two were left as unplanted controls. All plants were gathered from a natural wetland near Three Forks, Montana in the fall of 1996. They were transported and planted as root plugs. The plants were then given time to grow and mature as Peter's 50 PPM N 20-10-20 GP (The Scott's Company) fertilizer solution was fed into the cells.

The cells were housed in a greenhouse in the Plant Growth Center at Montana State University. The greenhouse was maintained at a temperature of 70° F (21° C) during the day and 60° (16° C) during the night. From October, 1997 through March, 1998, four hours of artificial light was used to maintain a growing season environment within the greenhouse. Thereafter, only natural light was used to facilitate growth.

Two synthetic wastewater feeds were applied during the course of the experiment. Both feeds were loosely based on water from the Metro Storm Drainage (MSD) in Butte, Montana. The first feed ran from February 9, 1998 to June 5, 1998, while the second feed followed the first and ran from June 5, 1998 to September 24, 1998. Both feeds contained the same constituents with only the carbon concentration changing. The two wastewater feeds are described in Table 2 and Table 3.

Table 2. Wastewater chemicals.

Chemical	Formula
Sucrose	$C_{12}H_{22}O_{11}$
Sodium Sulfate	Na_2SO_4
Zinc Sulfate	$ZnSO_4 \cdot 7H_2O$
Potassium Phosphate	K_2HPO_4
Sodium Nitrate	$NaNO_3$

Table 3. Concentrations of significant wastewater constituents.

Constituent	Feed 1 (mg/l)	Feed 2 (mg/l)
SO_4^{2-}	200	200
C	200	100
Zn^{2+}	24	24
NO_3^-	20	20
P	1:10 (P:N)molar ratio	1:10 (P:N)molar ratio

The wastewater was held in a 230 gallon, polypropylene tank. The tank was connected to a distribution manifold by a length of hose. The wastewater was then supplied to the cells by a Masterflex L/S 6-600 rpm peristaltic, four-roller, cartridge pump (Cole-Parmer Insturment Co., Niles, IL) through Masterflex size 14 Norprene® tubing which ran from the distribution manifold, through the pump, and to the inlet manifold of the cells.

To achieve a desired mean hydraulic residence time of five days within the gravel section of each cell, the flow rate was set at 10 ml/min ±5%. Inflow rates were measured with a graduated cylinder over one minute intervals. Due to variability from many factors, the flow rate had to be recalibrated a minimum of four times a week.

Sampling

Samples were collected every five days for Feed 1, and every seven days for Feed 2. At each sampling date, samples were collected from the inflow line, outlet spout, and a set number of sampling ports. For Feed 1, samples were collected from all depths at ports 2, 3 and 8, positioned as shown in Figure 1, and at one depth for all other ports. This depth was rotated each sampling day. To get more data for a degradation analysis along the length of the cells, the sampling scheme for Feed 2 was altered. Every port was sampled at two depths. The specific depths were rotated on consecutive sampling days. Samples were only collected from the unplanted control cells, until the final three sampling periods of each feed. During the last three sampling times, samples were collected only from the planted cells. Table 4 lists the sampling locations from each sampling date. At the end of each wastewater run, samples were collected from both control cells, Cattail 2, and Bulrush 2 for zinc analysis.

Table 4. Sampling locations on each sampling day

Date	Cells	Longitudinal Depth (cm) All Ports	Vertical Sample Port #	Total Samples
2/24	Controls	38	2, 3, 8	28
3/1/98	Controls	23	2, 3, 8	28
3/6/98	Controls	8	2, 3, 8	28
3/11/98	Controls	38	2, 3, 8	28
3/16/98	Controls	23	2, 3, 8	28
3/21/98	Controls	8	2, 3, 8	28
3/31/98	Controls	38	2, 3, 8	28
4/5/98	Controls	23	2, 3, 8	28
4/10/98	Controls	8	2, 3, 8	28
4/15/98	Controls	38	2, 3, 8	28
4/20/98	Controls	23	2, 3, 8	28
4/30/98	Planted	8	2, 3, 8	56
5/4/98	Planted	23	2, 3, 8	56
5/11/98	Planted	38	2, 3, 8	56
6/10/98	Controls	8	2, 3, 8	28
6/18/98	Controls	23, 38	---	32
6/24/98	Controls	8, 38	---	32
7/1/98	Controls	8, 23	---	32
7/8/98	Controls	23, 38	---	32
7/15/98	Controls	8, 38	---	32
7/30/98	Controls	8, 23	---	32
8/19/98	Controls	8, 38	---	32
8/26/98	Controls	8, 23	---	32
9/2/98	Controls	23, 38	---	32
9/9/98	Planted	8, 23	---	64
9/16/98	Planted	23, 38	---	64
9/23/98	All	8, 38	---	96

Influent and effluent samples were collected by allowing the flow to drip into test tubes. Samples from the ports were collected by use of a 10cc syringe. First, 4 mL were extracted from the tube and wasted. A clean syringe was then used to remove approximately 9.5 mL of sample. All samples were then run through a sterile, Corning® 0.2-micron cellulose acetate membrane syringe filter into a clean, autoclaved, 15 mL test tube and refrigerated at 5° C until analysis. Samples used for zinc analysis were collected in the same manner as described above, but then 0.5 mL of concentrated hydrochloric acid was added to each sample.

Tracer Test

From 2/14/97 to 2/23/97, prior to the wastewater feeds, a tracer test was performed on the wetland cells to gain a better understanding of the internal hydraulics of the system. Each cell was filled with tap water. The tank was filled with a solution of 100 mg/L Br^- from KBr. The flow to the cells was set at 30 mL/min. Samples were taken from the inlet, to ensure uniform mixing in the tank, and from the outlet to gauge the response. After 70 hours, approximately one residence time within the entire cell, the bromide solution was stopped, and the tank was filled with clean water. Samples were taken according to a pre-set schedule. After the first 36 hours, in which samples were taken every 12 hours, sampling was done every 4 to 8 hours to get a good accounting of the breakthrough of bromide concentration. During the run, there were 31 sampling events. Samples were gathered and stored according to the methods previously described for the wastewater feeds.

Sample Analysis

All samples from both wastewater feeds were analyzed for sulfate (SO_4^{2-}), nitrate (NO_3^-), and dissolved organic carbon (DOC). Sulfate concentrations were determined using a Dionex ion chromatograph (IC) equipped with an anion column and suppressor. The IC was connected to a computer equipped with Dionex Peaknet® 4.3 workstation software and an autosampler. A schedule of samples and an analysis method were developed using the software. The schedule and method then controlled the autosampler and IC operations. Standard samples made from sodium sulfate (Na_2SO_4) and sodium nitrate (Na_2NO_3) were run to calibrate the IC. Samples were then run through a filter to remove solids and transferred to vials for analysis. For the bromide tracer test samples, standards were made from potassium bromide (KBr) and run in the same manner.

DOC concentrations were determined using a Dohrman DC-80 carbon analyzer. Samples were acidified with 20% phosphoric acid to a pH below 2, air sparged for six minutes to remove gaseous inorganic carbon and injected into the analyzer. A 1 mL, 10 ppm organic C standard was used to calibrate the machine. The DOC analyzer uses a one point calibration, so readings were kept near 10 for all injected samples. To achieve the target reading (mass), varying volumes of sample had to be injected. The actual DOC concentration was then calculated based on the machine reading and the volume of sample used.

After preparation, zinc samples were taken to the Department of Plant, Soil and Environmental Sciences at Montana State University in Bozeman, Montana for analysis using an inductively coupled plasma spectrometer (ICP).

Statistical Analysis

A paired t-test for means using Minitab® 12 was used to determine if side-to-side differences in concentrations within the cells were statistically significant. Depth and longitudinal differences in the data were analyzed by ANOVA and Tukey's method of pairwise comparisons for means using Minitab® 12 to determine statistical significance. All analyses were done the the 95% confidence level. A p-value less than 0.05 indicates there is a statistically significant difference in the data being tested. Linear regressions performed on longitudinal data sets were done within Excel® to obtain regression constants.

RESULTS AND DISCUSSION

Wetland Hydrodynamics

The bromide tracer test was begun on all six cells, but was finished on only four of the cells. During the run, Control 2 and Bulrush 2 experienced mechanical problems, and the test was discontinued. Because the test was run using only influent and effluent samples of the entire cell system, modeling was based on a complete-mix—plug-flow—complete-mix system described by Equation 4.1:

$$
\begin{aligned}
&C(t) = 0 && \text{for } t < \theta_G \\
&C(t) = C_o(1-e^{-(t-\theta G)(Q/Vf)})(1-e^{-(t-\theta G)(Q/Va)}) && \text{for } \theta_G \le t \le \theta_G + t_p \\
&C(t) = C(\theta_G + t_p)(e^{-(t-\theta G + tp)(Q/Vf)})(1-e^{-(t-\theta G)(Q/Va)}) && \text{for } t > \theta_G + t_p
\end{aligned}
\qquad (4.1)
$$

where t is time; θ_G is the hydraulic residence time within the plug-flow gravel section of the system; C_o is the influent concentration of the tracer; Q is the flow rate; V_f is the volume of the forebay; V_a is the volume of the afterbay; t_p is the total time of tracer input; $C(\theta_G + t_p)$ is the concentration at the time of $\theta_G + t_p$.

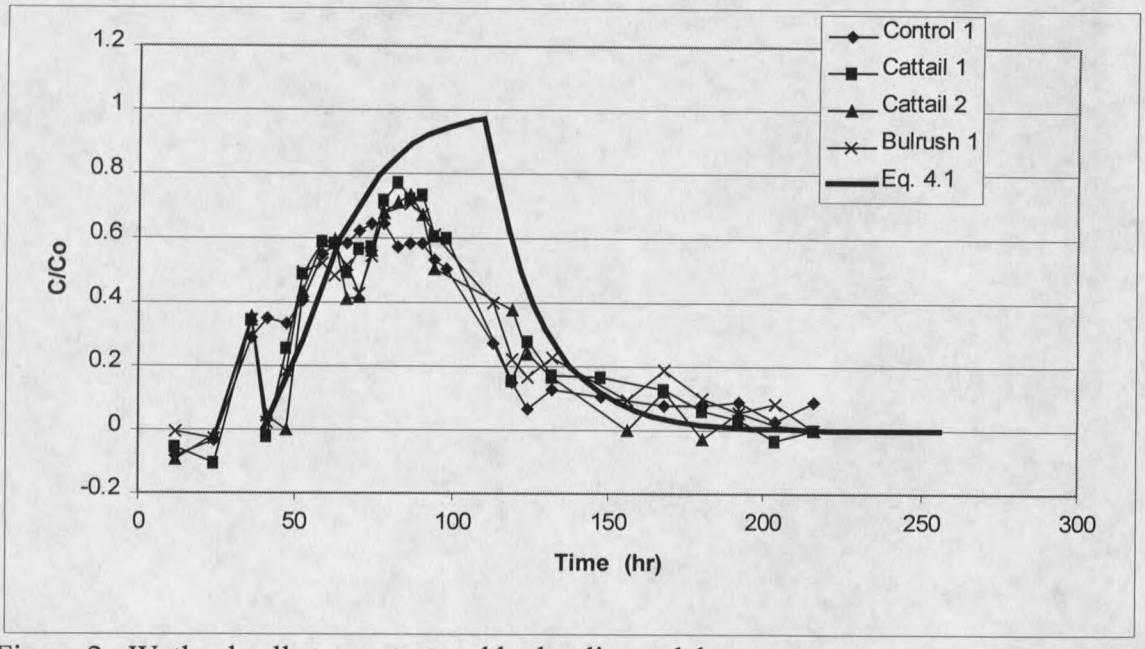

Figure 2. Wetland cells tracer test and hydraulic model.

Measured responses are compared to Equation 4.1 in Figure 2. The actual tracer responses vary from the model probably due to a combination of a lack of true plug-flow within the gravel, and a variation from a true complete-mix in the forebay and afterbay. Mixing, dispersion, detention, and short-circuiting could all have an influence on the system (Kadlec and Knight, 1996) and cause deviation from the model prediction. These factors can cause the tracer to appear sooner and remain longer than would be expected from the theoretical model. Overall, though, the actual response is not greatly different than the predicted model, and should allow for the assumption of plug-flow and complete-mix kinetics with the system. The planted cells were in the early stages of growth, therefore no plant effects were discernable, as all cells exhibited a similar response to the tracer.

Degradation in the Tank

At the design flow rate, the wastewater holding tank took approximately ten days to empty before a new batch was added. During this time, some bacterial activity occurred. Within the tank there were only three electron acceptors, O_2, NO_3^-, and SO_4^{2-}, to facilitate the degradation of the organic carbon. Sulfate concentration never changed within the tank during the study suggesting that nitrate and oxygen, the only other electron acceptors in the tank, were being utilized. Although this system used sucrose ($C_{12}H_{22}O_{11}$) as the carbon source, the idealized version of a carbon source (CH_2O) can be used for ease of calculation without changing the stoichiometric ratios appreciably. Alternate forms of two of the oxidation-reduction (redox) reactions from Table 1 are presented below:

O_2 Reduction:

$$CH_2O + O_2 \rightarrow CO_2 + H_2O \qquad (4.2)$$

NO_3^- Reduction:

$$5CH_2O + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 10H_2O + 2N_2 \qquad (4.3)$$

The stoichiometries of equations 4.2 and 4.3 show one mole of oxygen and 4/5 moles of nitrate are required to oxidize one mole of organic carbon. Assuming the influent water was saturated with oxygen at approximately 8 mg/L and given the influent nitrate concentration of 20 mg/L, then these electron acceptors will oxidize

approximately 3 mg/L and 5 mg/L organic carbon, respectively. As shown in Figure 3, all nitrate was consumed within the tank and supply lines by day 7.

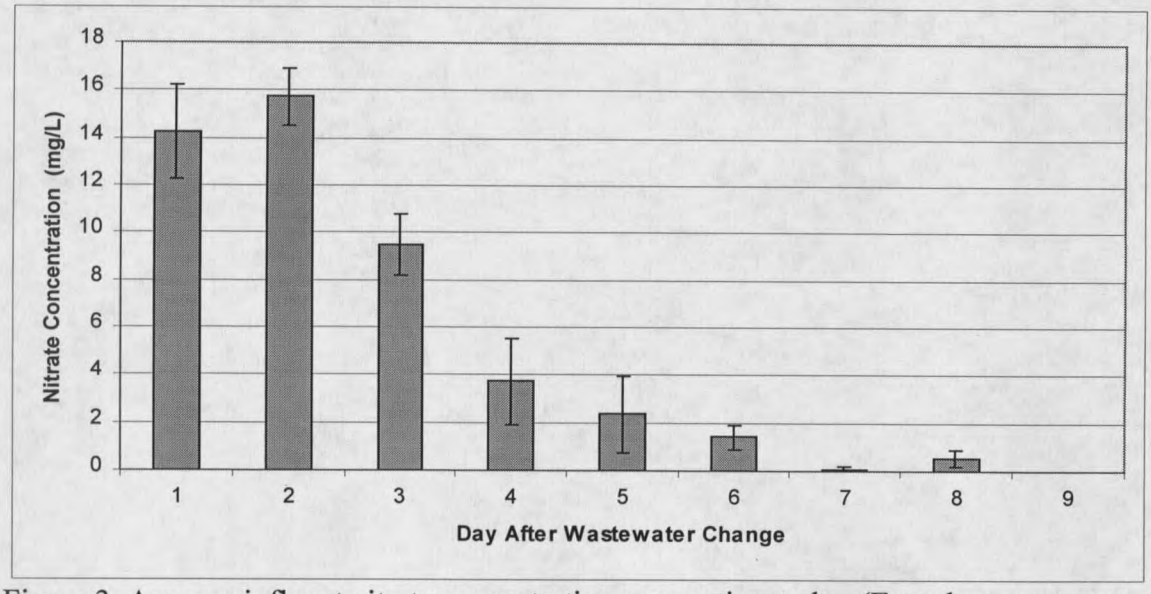

Figure 3. Average influent nitrate concentration over entire study. (Error bars represent +/- one standard error.)

The initial DOC concentration in the tank was 200 mg/L for Feed 1 and 100 mg/L for Feed 2. Measured influent DOC concentrations varied from 184.0 mg/L to 150.8 mg/L for Feed 1, and from 86.3 mg/L to 64.4 mg/L for Feed 2. Averages are shown in Figure 4.

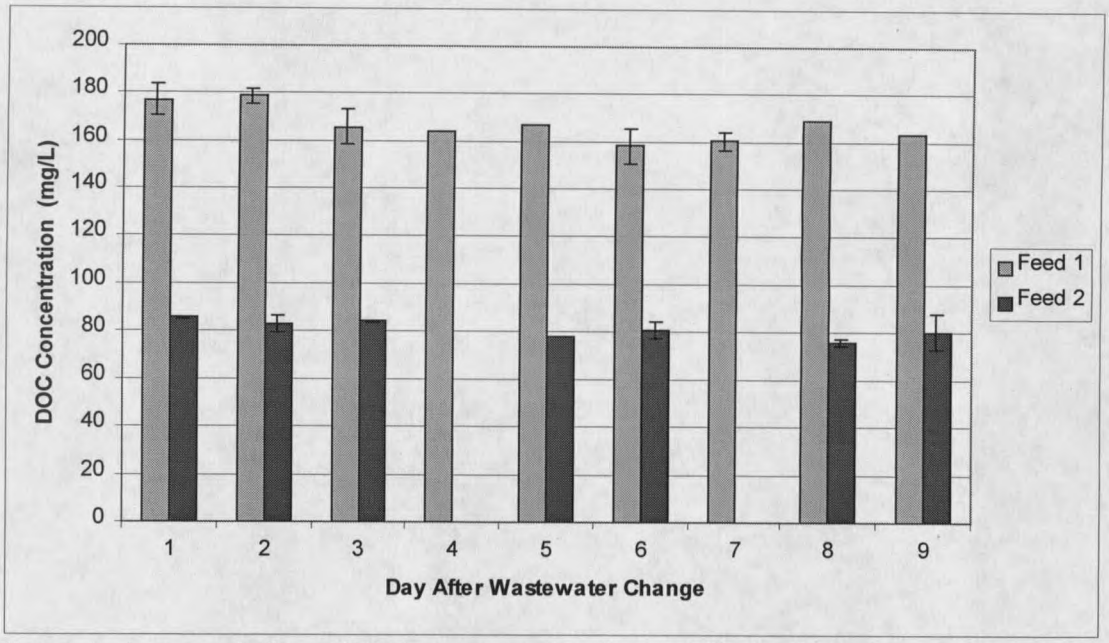

Figure 4. Average influent DOC concentration for each feed. (Error bars represent +/- one standard error.)
*Feed 2 not tested on days 4 and 7.

Clearly more carbon was consumed (average = 32 mg/L over all samples for Feed 1 and 20 mg/L over all samples for Feed 2) than estimated from the available electron donors. The tank was open to the atmosphere through a small hole in the lid, which would allow oxygen to diffuse into the wastewater from the atmosphere. This probably accounts for difference in influent carbon measurements and the stoichiometrically derived values and demonstrates the significance of oxygen transfer in the system.

Degradation within the Cells Prior to the First Set of Ports

The first sampling ports were located within the gravel section of each cell, approximately five centimeters from the inlet baffle. This distance was initially assumed

to have a negligible effect on degradation occurring prior to the sampling ports. This distance corresponds to only 7.5% of the gravel's hydraulic residence time but is 14% of the total residence time of the forebay and five centimeters of gravel. Therefore, although changes in concentrations between the influent and the first set of sampling ports will be stated as occurring in the forebay, it should be noted that the first five centimeters of gravel could have had a substantial effect on the degradation. A considerable amount of degradation took place between the supply system and the first set of ports of each cell. All nitrate not consumed within the supply system was completely removed during this time. Both DOC and sulfate underwent large reductions in concentration. The average influent DOC concentration was approximately 167 mg/L for Feed 1 and 81 mg/L for Feed 2 (Figure 4). Measured DOC values at the first set of sampling ports are shown in Figure 5. 60-85% of the influent DOC in Feed 1 was degraded prior to the first set of ports. Feed 2 experienced a degradation of 73-89%. Sulfate had even higher rates of reduction (Figure 6). 87-97% and 75-85% of the influent sulfate was degraded before reaching the first set of ports for Feed 1 and Feed 2, respectively. The average sulfate concentration at the first set of ports for the planted cells was less than that found in the control cells. This increased microbial activity may have been due to the additional attachment sites provided by the plants, thus allowing for a higher microbial population density. From these results, an overall sulfate reduction rate prior to the first set of ports of all cells, was approximately 3.4 mg/L/hr for Feed 1 and 3.0 mg/L/hr for Feed 2.

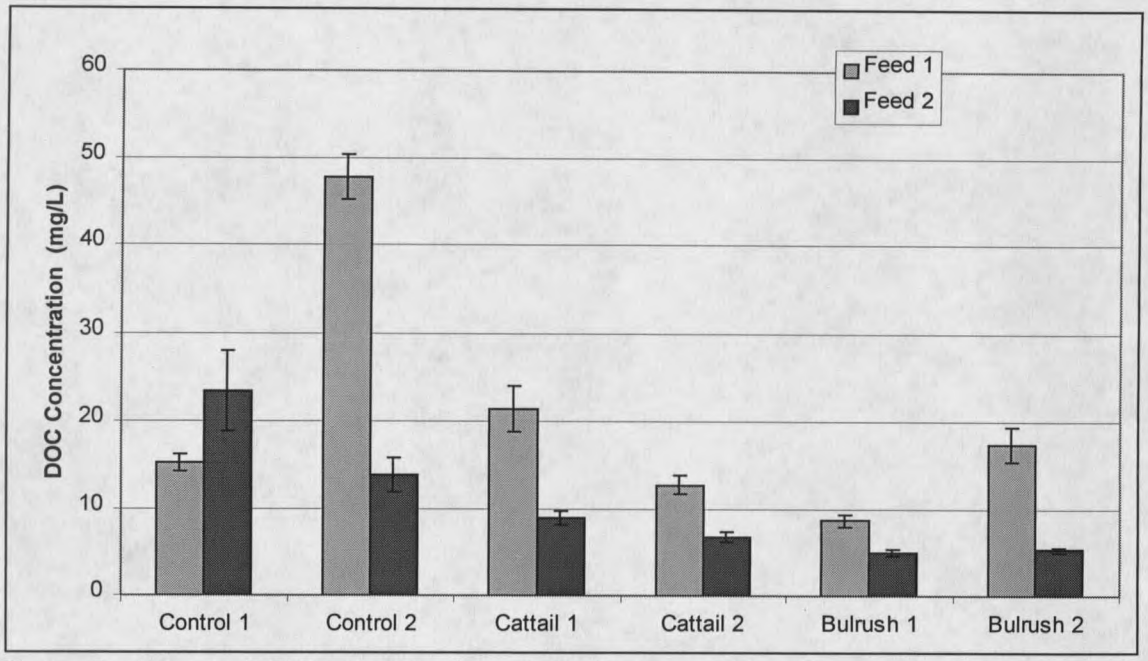

Figure 5. Average DOC concentrations at the first set of sampling ports. (Error bars represent +/- one standard error.)

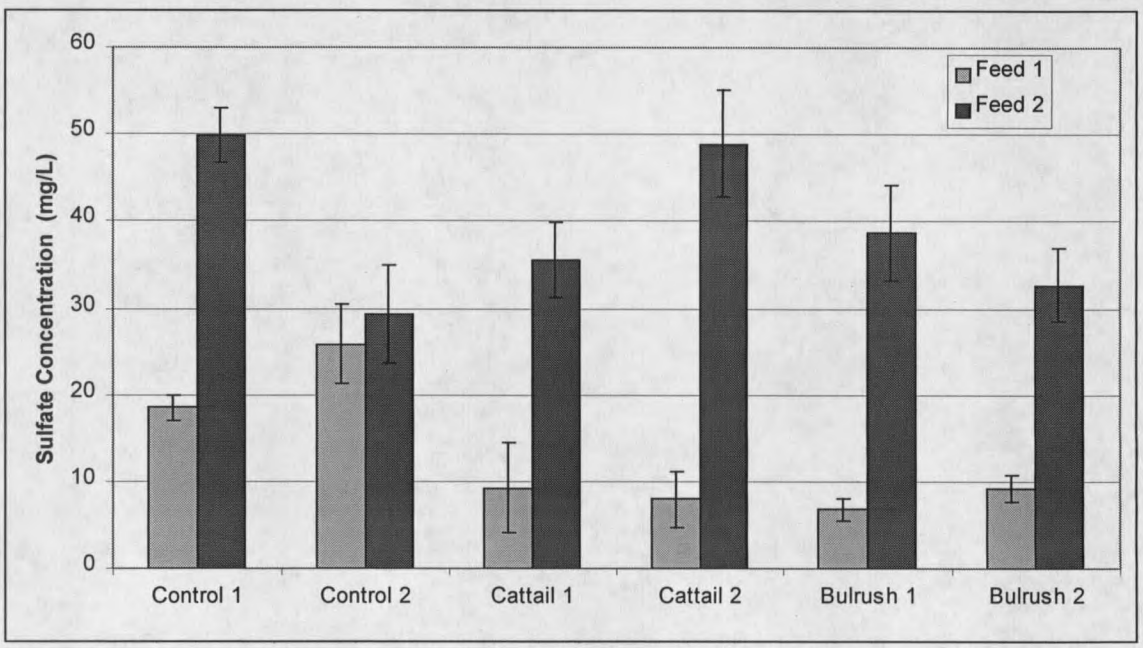

Figure 6. Average sulfate concentrations at the first set of sampling ports. (Error bars represent +/- one standard error.)

Aside from aqueous sample measurements, evidence of sulfate reduction from the entire system was quite evident. The greenhouse contained a strong odor of hydrogen sulfide. Also, within the gravel media were blackened precipitates. Both are common observations during sulfate reduction (Feng and Hsieh, 1998; White and Gadd, 1996).

DOC and Sulfate Concentrations within the Gravel Section

Only samples taken from the control cells on 3/31/98, 4/5/98, 4/15/98, and 4/20/98 (and 3/21/98 for Control 1) were used in the analysis of Feed 1 data. A preliminary analysis indicted a microbial/chemical steady-state had not yet been reached at earlier sampling dates as determined by continually decreasing measured concentrations with time. The samples from 4/10/98 had to be discarded due to contamination. For Feed 2, data were used from 6/18/98, 6/24/98, 7/1/98, and 8/19/98 onward. Ports sampled during these dates are shown in Table 4. The data from 7/8/98, 7/15/98, and 7/30/98 was not used due to a mistake in the influent wastewater preparation.

Side-to-Side Analysis of the Cells

The sulfate and DOC data from the gravel section of each cell were analyzed to determine if there were differences with respect to which side of the cell they were extracted (port 1 vs. port 2, port 3 vs. port 4, etc., Fig. 3.1) which might be taken as evidence of short-circuiting or preferential flow. A paired t-test with a 95% confidence interval was performed on all data which could be directly compared. For each cell over both feeds, DOC and sulfate showed no significant statistical difference ($p \geq 0.06$) in the

left side measurements compared to the right side measurements of the cells. Therefore, all data for each cell was subsequently analyzed without regard to side.

DOC Depth Analysis of the Control Cells

Differences in DOC concentrations for the control cells as a function of depth were analyzed using ANOVA and Tukey's method for pairwise comparisons both at 95% confidence. Both cells during both wastewater feeds, displayed a statistically significant increase in DOC with depth ($p < 0.037$), though the upper two depths showed no significant statistical difference ($p > 0.46$) with respect to each other. This was expected and was probably due to the transfer of oxygen from the atmosphere into the cells. The addition of the high energy electron acceptor would enhance the degradation of organic carbon in the upper areas of the cells. Since oxygen is utilized quickly, its effect on organic carbon concentrations would decrease with depth.

DOC Longitudinal Analysis of the Control Cells

The same techniques performed for the depth analysis were used in the analysis of the data for differences along the length of the control cells. Again, as expected, both cells had significant statistical differences with longitudinal position ($p < 0.004$). With a higher carbon loading, Feed 1 experienced a greater and more consistent decrease in DOC concentration along the length of the cells as illustrated in Figure 7. Although Feed 2 DOC concentrations did decrease longitudinally ($p < 0.003$), the decrease after the second set of sampling ports was minute.

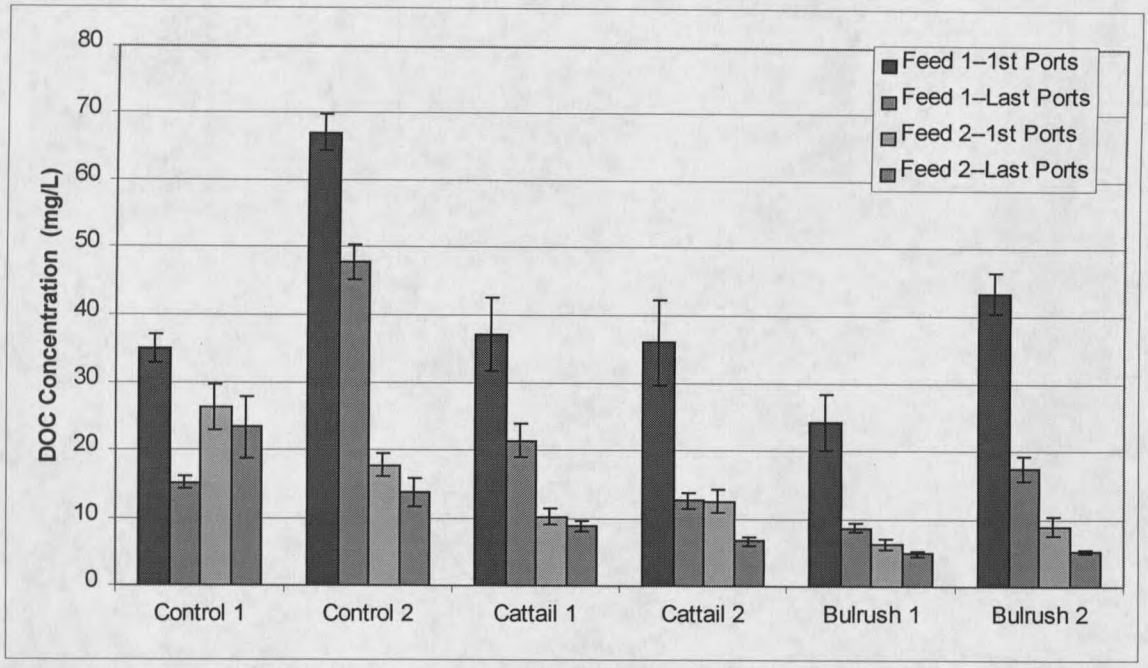

Figure 7. Average DOC concentrations at the first and last set of sampling ports. (Error bars represent +/- one standard error.)

Effect of Plants on DOC Degradation

Overall, the planted cells exhibited more variability than the control cells. The change in average DOC concentrations between the first and last sampling ports is illustrated in Figure 7. The bulrush cells, and more specifically Bulrush 2, behaved more like the controls than the cattail cells. Discernable patterns in the cattail were often difficult to establish. As with the control cells, all of the planted cells had statistically significant ($p \leq 0.047$) increasing DOC concentrations with depth during Feed 2. During Feed 1, three of four planted cells had decreasing DOC concentrations with depth, however, Bulrush 2 had increasing concentrations with depth.

During Feed 1, all cells had an overall decrease in DOC concentration longitudinally within the gravel. Again, Bulrush 2 behaved more like the control cells. Cattail 2 also had an approximately linear decrease in concentration along the flow path, but Cattail 1 and Bulrush 1 had the greatest change in concentration in the front half of the cells and much less decrease in the back half. During Feed 2, Cattail 2 and Bulrush 2 displayed decreasing concentrations, but most of the decrease was in the front half of the cells. The other two cells had no statistically significant ($p > 0.06$) overall decrease in DOC concentrations along the length.

Potential plant effects were undoubtedly influenced by plant status and age of the wetland system. At the time of these experiments, the plants had been growing for approximately a year and a half. Cattail growth was quite vigorous. They reached approximately six feet in height, and thickly colonized the entire gravel section of both cells. The plants were so thick that reaching most sampling ports was difficult. Due to a combination of a mealy bug infestation and the natural scenescense of the plants, almost all of the cattails' leaf material had died off by the end of the summer of 1998.

The bulrush did not seem to thrive quite as well as the cattails during this study. Although they seemed healthy and even developed seed heads, the bulrush only reached a height of three to four feet and never achieved as high a plant density as the cattails. Getting to the sampling ports was never a problem. However, the bulrush never experienced a period of scenescence. In a study using similar wetland cells and plants Biederman (1999) noted that seasonal patterns of COD reduction had not stabilized after three full growing seasons.

Though not measured directly in this experiment, evapotranspiration could also influence the DOC concentration in the cells. In the planted cells, and especially the cattails, evapotranspiration could have removed a significant amount of water. An evapotranspiration rate of up to 16 mm/day was measured from cattails growing under similar conditions (Towler, 1999). This removal would tend to increase concentrations of all solutes in the longitudinal direction by approximately 10%, barring any microbial degradation occurring.

Sulfate Depth Analysis of the Control Cells

The same statistical methods used for the DOC analysis were used for the sulfate analysis. For both control cells during both wastewater feeds, there was no statistically significant difference in sulfate concentration with depth at 95% confidence, contrary to the DOC results. If the observed decrease in DOC concentration near the surface was due to increased oxygen availability, this availability did not seem to inhibit sulfate reduction.

Sulfate Longitudinal Analysis of the Control Cells

For Feed 1, both control cells had a statistically significant decrease ($p = 0.000$) in sulfate concentration along the flow path. During Feed 2 the control cells did not behave similarly. While Control 2 continued to reduce sulfate along the flow path, Control 1 actually experienced a slight increase in sulfate concentrations, although this was not statistically significant ($p = 0.093$).

Effect of Plants on Sulfate Reduction

The only planted cell that had a statistically significant difference ($p = 0.003$ for Feed 1 and $p = 0.001$ for Feed 2) in sulfate concentration with respect to depth was Cattail 2, however the trend reversed depending on the feed. For Feed 1, sulfate concentrations were highest at the upper depth, but for Feed 2 sulfate concentrations were highest at the lower depth.

During Feed 1, bulrush cells reduced sulfate in the longitudinal direction, but cattail cells displayed a tendency to increase sulfate concentration between sampling ports. In batch studies, Allen (1999) reported an ability of planted systems to increase redox potential commensurate with a corresponding increase in sulfate concentration, as organic carbon concentrations decreased. During Feed 2, Cattail 2 and the bulrush cells exhibited increasing sulfate concentrations with distance along the flow path. Cattail 1, on the other hand, displayed an ability to reduce sulfate, counter to what occurred within the same cell at the higher carbon loading. As with observed DOC concentrations, sulfate concentrations would tend to be increased by evapotranspiration in the longitudinal direction. Another possible means of increasing sulfate concentration is from the reoxidation of hydrogen sulfide gas in the head space of the test tubes in which the samples were stored. Mean sulfate concentrations at the first and last sets of ports of all cells are shown in Figure 8.

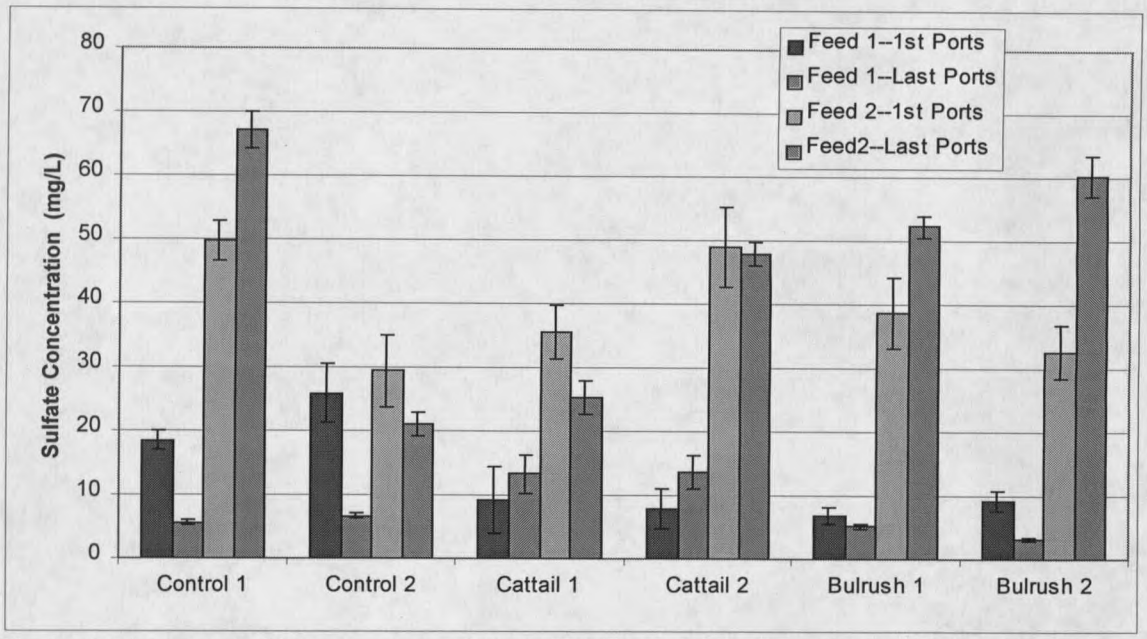

Figure 8. Average sulfate concentrations at the first and last set of sampling ports. (Error bars represent +/- one standard error.)

Based on the average sulfate concentrations during Feed 1 at the first and last set of sampling ports, the controls had an average sulfate reduction rate of approximately 0.15 mg/L/hr, considerably less than the forebay rate (approx. 3 mg/L/hr) and reported literature values of 0.83-2.92 mg/L/hr (Lyew and Sheppard, 1999) and 2-14 mg/L/hr (Hines *et al*, 1999). This was probably due to the limited quantities of both sulfate and carbon. So much degradation occurred in the forebay that reaction rates involving sulfate or DOC became suppressed in the gravel.

Modeling

Although degradation rates can be determined with a simple change in concentration measurement over a specified time period, for predictive modeling, a degradation constant based on an assumed or observed mathematical relationship should be determined. Microbially driven carbon-sulfate redox reactions are typically modeled using a first-order equation that can be found in most water quality related texts (i.e. Tchbanoglous and Schoeder, 1985). A plug-flow system with constant flow rate, as used in this study, permits a distance for time substitution.

The first-order degradation constant can then be found by rearranging the equation to a linear system.

$$\ln(C_t) = -kx + \ln(C_o) \qquad (4.4)$$

where C_t is the measured concentration of the constituent of interest at time, t; C_o is the initial concentration; x is the distance along the flow path; k is the first-order degradation rate constant.

The first-order degradation rate constant can then be found by regression of the natural-log of concentration versus the distance along the flow path.

DOC Degradation

Regressions were run on a set of longitudinal data at a specific sampling date and depth from all cells for Feed 1. Plots of each set of data for each cell are shown in Figure

9. Regression constants and correlation coefficients are shown in Table 5. Planted cells tended to show more deviation from the first-order model, and therefore had lower R^2 values. A number of reasons could account for this. The plants could be a net source of carbon to the system, or conversely they could be supplying additional oxygen to the subsurface, which would stimulate carbon degradation. The root mass of the plants could also affect the flow characteristics of the subsurface. The calculated k values have to be regarded with some caution due to the high amount of variability as displayed by the R^2 values. The regressions yielded k values in units of 1/cm. The more common method of reporting degradation constants is in units of 1/time. The k values in units of 1/hr were calculated from the 1/cm values along with distance and hydraulic residence time.

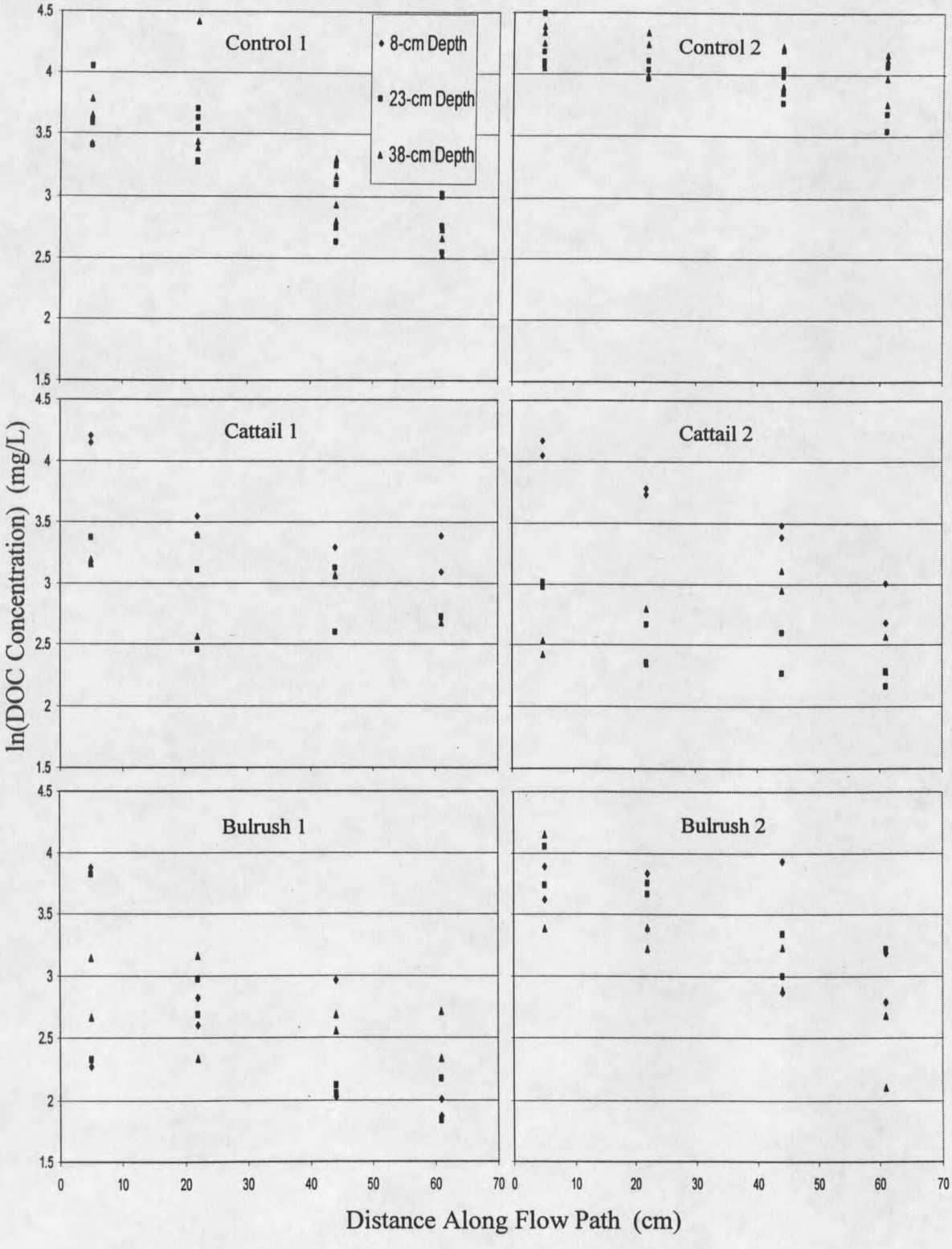

Figure 9. Longitudinal DOC data for Feed 1.

Table 5. DOC degradation constants for Feed 1 (C_o = 200mg/L).

Cell	Date	Depth (cm)	k (1/cm)	k (1/hr)	R^2
Control 1	3/31	38	0.0198	0.0110	0.95
	4/5	23	0.0199	0.0111	0.87
	4/15	38	0.0177	0.0099	0.51
	4/20	23	0.0141	0.0079	0.73
Control 2	3/31	38	0.0041	0.0023	0.91
	4/5	23	0.0021	0.0012	0.46
	4/15	38	0.0049	0.0027	0.58
	4/20	23	0.0124	0.0069	0.84
Cattail 1	4/30	8	0.0166	0.0093	0.75
	5/4	23	0.0085	0.0047	0.31
	5/11	38	0.0072	0.0040	0.24
Cattail 2	4/30	8	0.0220	0.0123	0.94
	5/4	23	0.0124	0.0069	0.73
	5/11	38	-0.0025	-0.0014	0.04
Bulrush 1	4/30	8	0.0204	0.0114	0.43
	5/4	23	0.0221	0.0123	0.54
	5/11	38	0.0072	0.0040	0.24
Bulrush 2	4/30	8	0.0133	0.0074	0.43
	5/4	23	0.0137	0.0076	0.74
	5/11	38	0.0231	0.0129	0.75

The control cells displayed markedly different constants from each other. The degradation constant in Control 2 was less than that determined from any other cell and an order of magnitude less then the other control cell. For some reason, there seemed to be less microbial activity occurring in Control 2 during Feed 1. Actual DOC concentrations were consistently higher in Control 2 than the other cells. Other than Control 2, the cattail cells showed the smallest k values, however this could be a reflection of an addition of carbon by the cattails and not an actual lower degradation rate. Mean values for the regression constants listed in Table 6.

Table 6. Mean DOC k (hr^{-1}) values and standard deviations for Feed 1.

	Control 1	Control 2	Cattail 1	Cattail 2	Bulrush 1	Bulrush 2
Mean	0.0100	0.0033	0.0060	0.0059	0.0092	0.0093
SD	0.0015	0.0025	0.0028	0.0069	0.0045	0.0031

Constants for Feed 2 were not as consistent and generally had lower correlation coefficients than those for Feed 1 as can be seen in Table 7. There were even several negative k values. The trends in degradation rate between cells observed in Feed 1 and shown in Table 6, were not present during Feed 2. Control 2 displayed a higher degradation rate than Control 1, which is counter to what was found for Feed 1. The similarities between the cattail cells exhibited during Feed 1 were not there during Feed 2. The two bulrush cells did display relatively similar k values. These trends are also illustrated by the mean k values listed in Table 8.

Table 7. DOC degradation constants for Feed 2 (C_o = 100 mg/L).

Cell	Date	Depth (cm)	k (1/cm)	k (1/hr)	R^2
Control 1	6/18	23	0.0069	0.0039	0.74
	6/18	38	0.0030	0.0017	0.21
	6/24	8	0.0155	0.0086	0.45
	6/24	38	0.0062	0.0035	0.34
	7/1	8	0.0029	0.0016	0.31
	7/1	23	0.0045	0.0025	0.39
	8/19	8	0.0084	0.0047	0.34
	8/19	38	0.0084	0.0047	0.33
	8/26	8	0.0021	0.0012	0.15
	8/26	23	0.0006	0.0003	0.01
	9/2	23	-0.0024	-0.0013	0.30
	9/2	38	0.0038	0.0021	0.32
	9/23	8	0.0118	0.0066	0.14
	9/23	38	0.0059	0.0033	0.55
Control 2	6/18	23	0.0120	0.0067	0.62
	6/18	38	0.0019	0.0011	0.13
	6/24	8	0.0166	0.0093	0.92
	6/24	38	0.0197	0.0110	0.78
	7/1	8	0.1070	0.0597	0.49
	7/1	23	0.0028	0.0016	0.21
	8/19	8	-0.0132	-0.0074	0.74
	8/19	38	-0.0098	-0.0055	0.61
	8/26	8	-0.0009	-0.0005	0.02
	8/26	23	0.0090	0.0050	0.50
	9/2	23	0.0060	0.0033	0.40
	9/2	38	0.0078	0.0044	0.64
	9/23	8	0.0047	0.0026	0.16
	9/23	38	0.0016	0.0009	0.12
Cattail 1	9/9	8	0.0034	0.0019	0.05
	9/9	23	0.0063	0.0035	0.13
	9/16	23	0.0013	0.0007	0.01
	9/16	38	-0.0006	-0.0003	0.01
	9/23	8	-0.0049	-0.0027	0.19
	9/23	38	-0.0037	-0.0021	0.04
Cattail 2	9/9	8	0.0112	0.0062	0.76
	9/9	23	0.0073	0.0041	0.53
	9/16	23	0.0111	0.0062	0.54
	9/16	38	0.0099	0.0055	0.26
	9/23	8	0.0099	0.0055	0.85
	9/23	38	0.0073	0.0041	0.32
Bulrush 1	9/9	8	0.0077	0.0043	0.43
	9/9	23	0.0052	0.0029	0.70
	9/16	23	0.0016	0.0009	0.29
	9/16	38	0.0135	0.0075	0.63
	9/23	8	0.0015	0.0008	0.05
	9/23	38	-0.0028	-0.0016	0.14
Bulrush 2	9/9	8	0.0069	0.0039	0.14
	9/9	23	0.0074	0.0041	0.30
	9/16	23	0.0085	0.0047	0.44
	9/16	38	0.0058	0.0032	0.44
	9/23	8	0.0021	0.0012	0.04
	9/23	38	0.0144	0.0080	0.43

Table 8. Mean DOC k (hr^{-1}) values and standard deviations for Feed 2.

	Control 1	Control 2	Cattail 1	Cattail 2	Bulrush 1	Bulrush 2
Mean	0.0031	0.0066	0.0002	0.0053	0.0025	0.0042
SD	0.0026	0.0161	0.0024	0.0010	0.0032	0.0022

The skewed nature of the results for Feed 2 compared to Feed 1 for DOC degradation is probably the result of severe carbon limitation. Typical DOC concentrations for Feed 1 were above 12-15 mg/L C at all ports. For Feed 2 though, it was common to see concentrations of 5-7 mg/L at the second set of ports and then little or no reduction through the third and fourth sets of ports. Overall, there was a statistically significant ($p \leq 0.047$) decrease in DOC concentrations along the flow path. The lower concentrations of DOC were probably were insufficient to sustain an appreciable level of microbial activity.

Sulfate Reduction

Regresssion of sulfate data was conducted in a manner identical to the DOC degradation analysis. Plots of each set of sulfate reduction data are shown in Figure 10. Results of the regressions for Feed 1 are shown in Table 9. Control cells displayed less variability with a generally higher rate constant as compared to planted cells. In contradiction to the very different DOC rate constants found between control cells during Feed 1, the sulfate rate constants were quite similar. Although a large portion of the influent sulfate in the system was reduced in the forebays of each cell, conditions within the gravel section of most planted cells were not favorable for continued high rates of

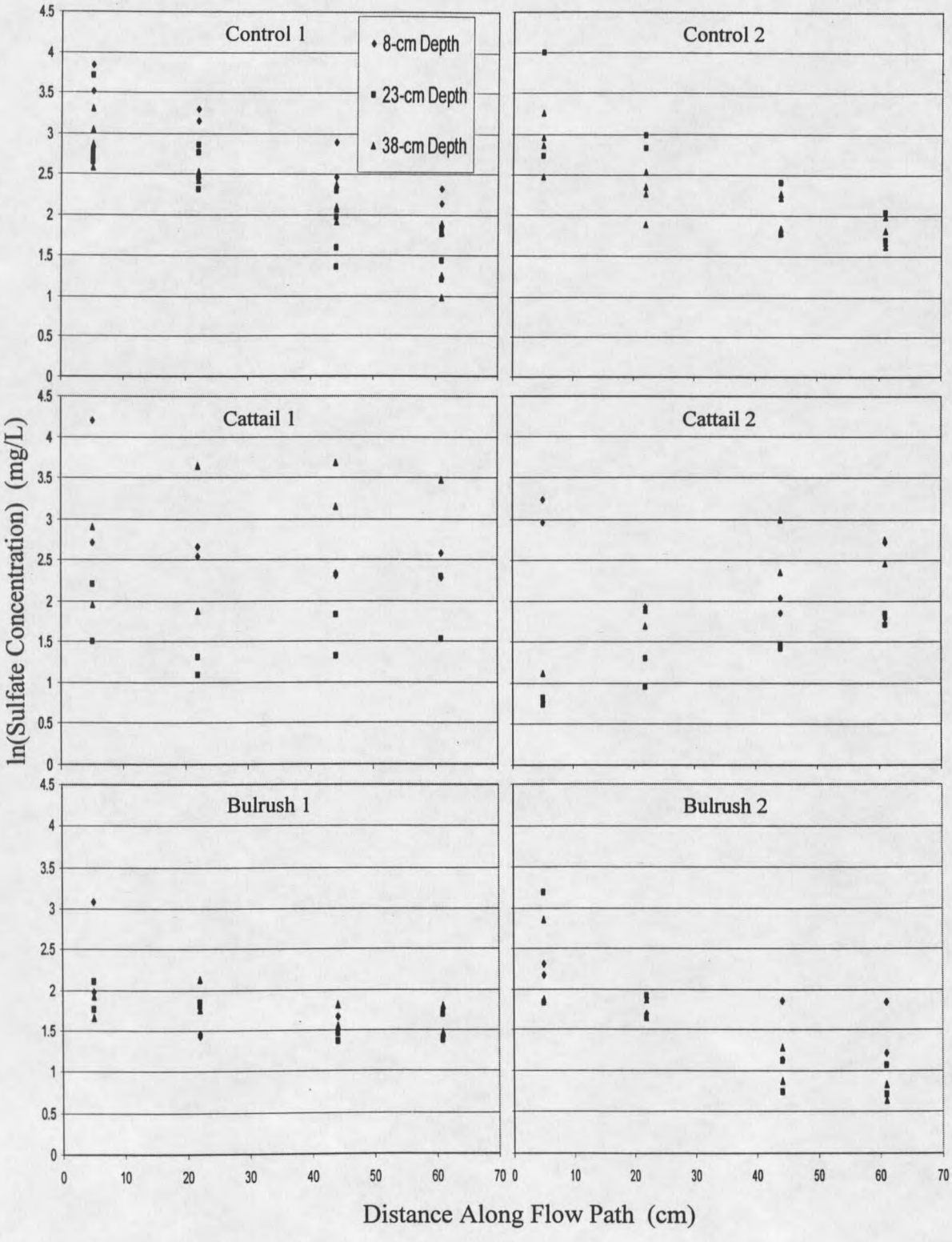

Figure 10. Longitudinal sulfate data for Feed 1.

sulfate reduction. In fact, the gravel section of the cattail cells tended to be a net producer of sulfate (Table 10). The redox potential within the cattail cells might have been too high to sustain sulfate reduction, and some of the reduced sulfide could have reoxidized to sulfate. This apparent increase in sulfate was probably exacerbated by the evapotranspiration loss of water which could account for as much as a 10% increase in concentrations. Both bulrush cells reduced sulfate, but the difference between replicates was large. The mean rate constant for Bulrush 2 was similar to the control cells and was nearly three times greater than that for Bulrush 1 during Feed 1.

Table 9. SO_4^{2-} degradation constants for Feed 1.

Cell	Date	Depth (cm)	k (1/cm)	k (1/hr)	R^2
Control 1	3/21	8	0.0264	0.0147	0.94
	3/31	38	0.0222	0.0124	0.91
	4/5	23	0.0292	0.0163	0.89
	4/10	8	0.0216	0.0121	0.85
	4/15	38	0.0281	0.0157	0.81
	4/20	23	0.0249	0.0139	0.75
Control 2	3/31	38	0.0205	0.0114	0.70
	4/5	23	0.0042	0.0023	0.90
	4/15	38	0.0193	0.0108	0.90
	4/20	23	0.0300	0.0167	0.74
Cattail 1	4/30	8	0.0185	0.0103	0.39
	5/4	23	-0.0028	-0.0016	0.02
	5/11	38	-0.0229	-0.0128	0.36
Cattail 2	4/30	8	0.0132	0.0074	0.26
	5/4	23	-0.0177	-0.0099	0.92
	5/11	38	-0.0313	-0.0175	0.81
Bulrush 1	4/30	8	0.0141	0.0079	0.30
	5/4	23	0.0089	0.0050	0.54
	5/11	38	0.0044	0.0025	0.21
Bulrush 2	4/30	8	0.0128	0.0071	0.48
	5/4	23	0.0300	0.0167	0.68
	5/11	38	0.0295	0.0165	0.81

Table 10. Mean SO_4^{2-} k (hr^{-1}) values and standard deviations for Feed 1.

	Control 1	Control 2	Cattail 1	Cattail 2	Bulrush 1	Bulrush 2
Mean	0.0142	0.0130	-0.0013	-0.0067	0.0051	0.0134
SD	0.0017	0.0033	0.0116	0.0127	0.0027	0.0055

The regressions for Feed 2 data are shown in Table 11. Many of the values were negative, and most of the R^2s were very poor due to the tailing effect of the data. Reaction constants developed from Feed 2 show that there was not enough organic carbon in the system after the forebays to drive sulfate reduction, and there was a greater incidence of increasing sulfate concentrations within the gravel. The carbon limitation makes for interpretation of the data difficult because other processes could now show a greater influence due to the near cessation of sulfate reduction. Mean values for Feed 2 are shown in Table 12.

Table 11. SO_4^{2-} degradation constants for Feed 2

Cell	Date	Depth (cm)	k (1/cm)	k (1/hr)	R^2
Control 1	6/18	23	0.0095	0.0053	0.74
	6/18	38	0.0102	0.0057	0.76
	6/24	8	0.0055	0.0031	0.22
	6/24	38	0.0006	0.0003	0.04
	7/1	8	-0.0051	-0.0028	0.80
	7/1	23	-0.0073	-0.0041	0.69
	8/19	8	-0.0039	-0.0022	0.56
	8/19	38	-0.0061	-0.0034	0.56
	8/26	8	-0.0017	-0.0009	0.31
	8/26	23	-0.0037	-0.0021	0.33
	9/2	23	-0.0065	-0.0036	0.76
	9/2	38	-0.0060	-0.0033	0.57
	9/23	8	-0.0002	-0.0001	0.01
	9/23	38	-0.0028	-0.0016	0.73
Control 2	6/18	23	0.0401	0.0224	0.61
	6/18	38	0.0136	0.0076	0.44
	6/24	8	0.0064	0.0036	0.09
	6/24	38	0.0198	0.0110	0.43
	7/1	8	0.0145	0.0081	0.51
	7/1	23	0.0017	0.0009	0.08
	8/19	8	-0.0202	-0.0113	0.89
	8/19	38	-0.0050	-0.0028	0.36
	8/26	8	0.0023	0.0013	0.37
	8/26	23	0.0131	0.0073	0.50
	9/2	23	-0.0006	-0.0003	0.01
	9/2	38	0.0003	0.0002	0.00
	9/23	8	0.0004	0.0002	0.00
	9/23	38	-0.0035	-0.0020	0.08
Cattail 1	9/9	8	0.0046	0.0026	0.08
	9/9	23	0.0016	0.0009	0.03
	9/16	23	0.0115	0.0064	0.21
	9/16	38	0.0044	0.0025	0.10
	9/23	8	0.0106	0.0059	0.26
	9/23	38	0.0002	0.0001	0.00
Cattail 2	9/9	8	-0.0047	-0.0026	0.08
	9/9	23	-0.0198	-0.0110	0.16
	9/16	23	-0.0113	-0.0063	0.01
	9/16	38	-0.0100	-0.0056	0.09
	9/23	8	0.0024	0.0013	0.14
	9/23	38	-0.0113	-0.0063	0.03
Bulrush 1	9/9	8	-0.0033	-0.0018	0.08
	9/9	23	-0.0014	-0.0008	0.73
	9/16	23	0.0008	0.0004	0.77
	9/16	38	0.0048	0.0027	0.80
	9/23	8	-0.0062	-0.0035	0.07
	9/23	38	-0.0012	-0.0007	0.69
Bulrush 2	9/9	8	-0.0149	-0.0083	0.42
	9/9	23	-0.0161	-0.0090	0.54
	9/16	23	-0.0121	-0.0068	0.45
	9/16	38	-0.0105	-0.0059	0.50
	9/23	8	-0.0140	-0.0078	0.56
	9/23	38	-0.0046	-0.0026	0.49

Table 12. Mean SO_4^{2-} k (hr^{-1}) values and standard deviations for Feed 2.

	Control 1	Control 2	Cattail 1	Cattail 2	Bulrush 1	Bulrush 2
Mean	-0.0007	0.0033	0.0031	-0.0051	-0.0006	-0.0067
SD	0.0032	0.0078	0.0026	0.0042	0.0021	0.0023

Electron Acceptor Usage

A way to look at electron acceptor usage within this system is to compare the ratio of change in sulfate to change in DOC ($\Delta SO_4^{2-}/\Delta DOC$) between sampling locations. Equation (2.6) shows that one mole of sulfate is reduced per two moles of carbon oxidized. The theoretical ratio of $\Delta SO_4^{2-}/\Delta DOC$ is therefore 0.5. This change is assumed to occur with time (or along the flow path). Competition by other electron acceptors (more DOC oxidized than sulfate reduced in stoichiometric balance) or production of sulfate would tend to increase this ratio, while production of DOC by internal sources would tend to decrease this ratio.

Plots of millimolar sulfate concentrations versus millimolar DOC concentrations from Feed 1 are shown for each cell in Figure 11. Due to the severe carbon limitation during Feed 2, further analysis of electron acceptor usage of these data is not warranted. Each point in Figure 11 represents the concentrations taken simultaneously at a given location within the cell. From Figure 11, clearly most cells demonstrate a decrease in DOC concentration as flow moves through the cell, confirming the statistical analysis previously described. Cattail 1, however, appears to have no identifiable trend. Control 1 and the bulrush cells show a consistent, concurrent decrease in sulfate concentration.

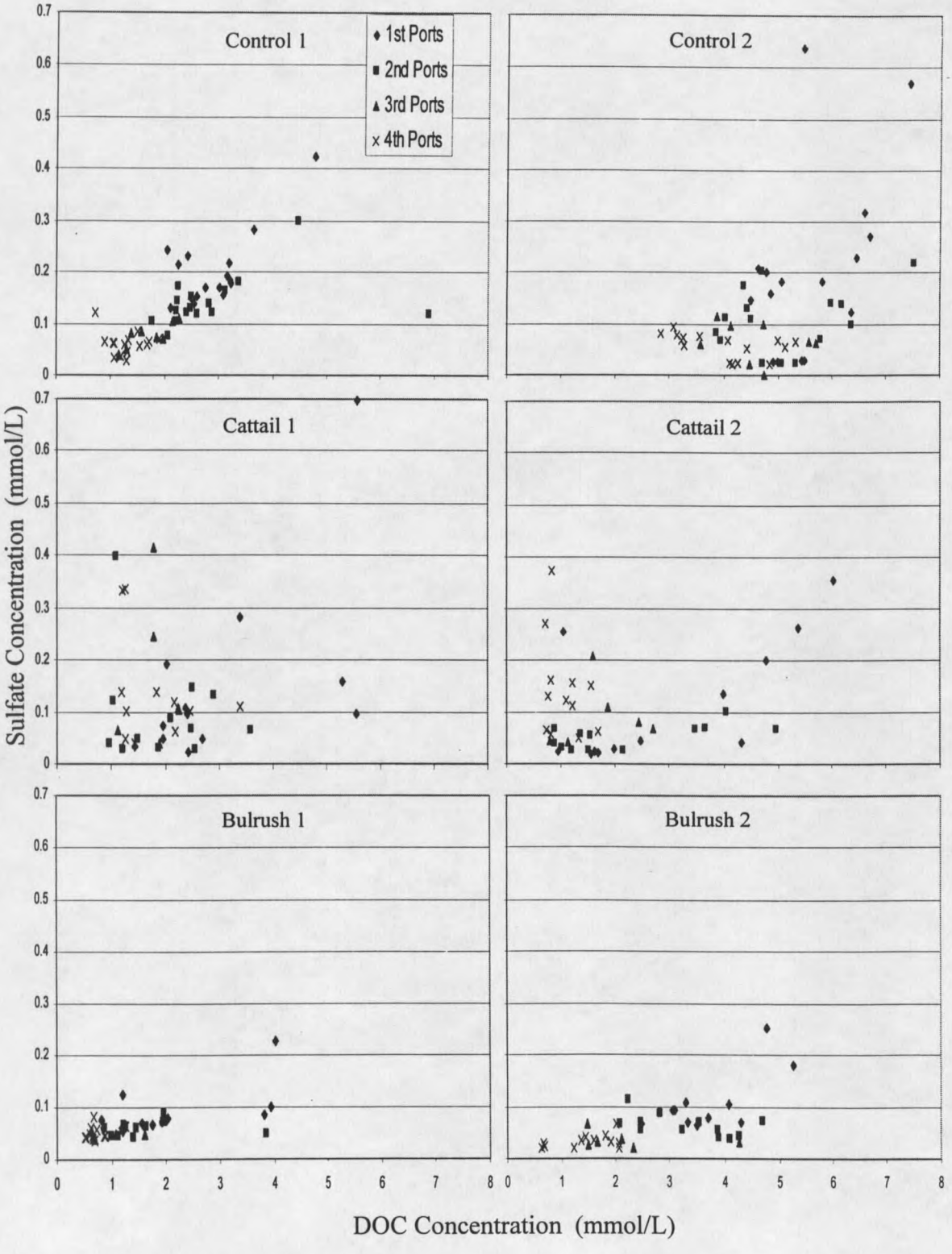

Figure 11. Longitudinally dependent SO_4^{2-} versus DOC data for Feed 1.

Control 2 shows a decrease between the first and second sets of ports but little change after the second set of ports, while Cattail 2 shows a marked increase in sulfate concentration between the third and fourth sets of ports.

The slope of the SO_4^{2-} versus DOC data in Figure 11 provides an average estimate of the $\Delta SO_4^{2-}/\Delta DOC$ in each cell. This, of course, assumes that the change in both DOC and SO_4^{2-} concentrations are related and decrease with time or distance along the flow path. Therefore, the applicability of this analysis on the cattail treatment should be used with caution. Despite this limitation, Table 13 provides statistical results of a linear regresssion of the SO_4^{2-} versus DOC data.

Based on the first-order model in which reaction rates decrease as reactant concentrations decrease, data on the SO_4^{2-} versus DOC graph should get progressively closer together as flow moves through the cell. It should be noted that Figure 11 does indeed demonstrate such a general decrease in reaction rates along the flow path. From the flattening of the data in the y-direction of the SO_4^{2-} versus DOC graph, it would appear that the system during Feed 1 becomes sulfate limited. The limitation is especially pronounced when sulfate concentrations fall below about 0.1 mmol/L (9.6 mg/L) sulfate, and continued sulfate reduction appears to be minimal or completely cease.

Table 13. $\Delta SO_4^{2-}/\Delta DOC$ ratios within the gravel section for Feed 1.

Cell	Date	$\Delta SO_4^{2-}/\Delta DOC$ Ratio	R^2	Average $\Delta SO_4^{2-}/\Delta DOC$ Ratio
Control 1	3/31/98	0.0626	0.71	0.0745
	4/5/98	0.0651	0.96	
	4/15/98	0.0764	0.52	
	4/20/98	0.0937	0.85	
Control 2	3/31/98	0.0840	0.37	0.0921
	4/15/98	0.1189	0.68	
	4/20/98	0.0735	0.52	
Cattail 1	4/30/98	0.0987	0.57	0.0284
	5/4/98	0.0581	0.35	
	5/11/98	-0.0717	0.40	
Cattail 2	4/30/98	0.0272	0.29	0.0159
	5/4/98	0.0470	0.62	
	5/11/98	-0.2640	0.11	
Bulrush 1	4/30/98	0.0463	0.78	0.0215
	5/4/98	0.0112	0.17	
	5/11/98	0.0070	0.17	
Bulrush 2	4/30/98	0.0044	0.05	0.0238
	5/4/98	0.0328	0.41	
	5/11/98	0.0342	0.85	

Table 13 shows that the calculated $\Delta SO_4^{2-}/\Delta DOC$ ratio is consistently less than 0.5. In fact, the data indicate that sulfate reduction contributed only 3-18% of the total DOC oxidized within the gravel. Therefore other processes are responsible for the bulk of the carbon oxidation. Other pathways of carbon degradation in the gravel section of this system include oxygen reduction, fermentation, and methanogenesis. Most strains of SRBs have a difficult time degrading sugars such as the input sucrose. Fermentative bacteria, therefore, are probably breaking down the sucrose to more utilizable forms of carbon, such as acetate. The process, however, only changes the form of the DOC, not

the magnitude. To discern the importance of the other pathways, more information such as COD measurements and testing for methane emission is needed.

Another way to gain insight as to other electron acceptor usage is to look at how the change in the SO_4^{2-}/DOC ratio varies with depth at a given longitudinal position. If oxygen transfer from the atmosphere provides a significant quantity of this alternate electron acceptor near the surface, the SO_4^{2-}/DOC ratio should be more there than at the bottom of the cells. Table 14 presents the mean SO_4^{2-}/DOC ratios at ports 2, 3, and 8 (Figure 1); the only ports that had full depth characterizations.

Table 14. Mean SO_4^{2-}/DOC ratios for each depth at selected ports.

Cell	Port	Depth	mean ratio	Cell	Port	Depth	mean ratio
Control 1	2*	8	0.092591	Control 2	2	8	0.050756
		23	0.05875			23	0.029739
		38	0.061841			38	0.025779
	3	8	0.062108		3	8	0.019536
		23	0.047947			23	0.022039
		38	0.053675			38	0.019666
	8	8	0.088656		8	8	0.018531
		23	0.050987			23	0.01781
		38	0.038812			38	0.015236
Cattail 1	2	8	0.043392	Cattail 2	2*	8	0.047449
		23	0.034004			23	0.013821
		38	0.023565			38	0.016895
	3	8	0.039535		3	8	0.019651
		23	0.024844			23	0.032151
		38	0.172726			38	0.040695
	8	8	0.042768		8	8	0.171834
		23	0.110711			23	0.191808
		38	0.140411			38	0.154559
Bulrush 1	2	8	0.074213	Bulrush 2	2	8	0.029763
		23	0.049355			23	0.023413
		38	0.043861			38	0.021487
	3	8	0.028287		3	8	0.026702
		23	0.047047			23	0.01947
		38	0.050886			38	0.017455
	8*	8	0.114183		8	8	0.032691
		23	0.065429			23	0.023901
		38	0.060792			38	0.024155

*Significant statistical difference with depth at 95% confidence.

From the data in Table 14, only three of the 18 ports analyzed had a significant statistical difference in the SO_4^{2-}/DOC ratio with depth. Within the ports displaying a difference, the 8-cm depth had a significantly higher ($p < 0.05$) ratio than the lower two depths. In all cases the lower two depths had similar ratios. Each of the significant differences occurred in different cells and even different treatments. Overall, there

appears to be at best only limited evidence of preferential carbon oxidation/oxygen reduction near the surface. The rate of DOC oxidation is similar throughout the depth profile of the cells. This would seem to indicate that as oxygen reduction decreases with depth, the rate of methanogenesis increases, or more likely, there is sufficient vertical mixing to uniformly distribute electron acceptors other than sulfate.

Zinc Removal

Zinc was used as a model heavy metal in this system. Zinc forms a highly insoluble precipitate, ZnS, when in the presence of hydrogen sulfide. Combining equations (2.6) and (2.7) and using Zn^{2+} as the divalent metal, equation (4.5) is produced.

$$2CH_2O + SO_4^{2-} + Zn^{2+} \rightarrow 2CO_2 + 2H_2O + ZnS(s) \qquad (4.5)$$

For each mole of sulfate reduced, one mole of aqueous zinc is precipitated as zinc sulfide. To remove all zinc (24 mg/L) from this system, a minimum of 35 mg/L of sulfate needed to be reduced to sulfide. The average removal of sulfate through the entire system was 92 mg/L for Feed 1 and 54 mg/L for Feed 2.

Samples tested for aqueous zinc concentration, when collected, were taken from both control cells, one cattail cell, and one bulrush cell. An ANOVA test performed on each cell showed no statistical difference in the measurements with respect to depth in the cell ($p > 0.30$) or distance along the cell ($p > 0.28$). The lower level of sulfate reduction experienced by the reduced carbon concentration of Feed 2 also had no effect on zinc

removal ($p > 0.11$). The removal efficiencies of zinc were similar during both feeds. Therefore, most zinc removal occurred before the first sampling port, and there was no statistical change in zinc concentration within each cell. Table 15 illustrates the removal effectiveness and retention of zinc by these cells. Although there was evidence of reoxidation of sulfide in the cattail cells, there didn't appear to be any remobilization of zinc. This is further testimony of the effectiveness of wetlands in the removal and retention of heavy metals.

Table 15. Zinc concentrations within the gravel section and removal efficiencies.

Feed 1	Ave. Influent Conc. (mg/L)	High Conc. (mg/L)	Low Conc. (mg/L)	Ave. Conc. (mg/L)	Ave. % Removal
Control 1	26.09	2.19	0.38	1.05	96.0
Control 2	26.09	2.17	0.15	0.65	97.5
Cattail 2	26.09	2.01	0.12	1.04	96.0
Bulrush 2	26.09	1.13	0.22	0.51	98.0

Feed 2	Ave. Influent Conc. (mg/L)	High Conc. (mg/L)	Low Conc. (mg/L)	Ave. Conc. (mg/L)	Ave. % Removal
Control 1	25.12	2.27	0.35	1.05	95.8
Control 2	25.12	0.58	0.21	0.35	98.6
Cattail 2	25.12	2.39	0.46	1.08	95.7
Bulrush 2	25.12	2.37	0.66	1.19	95.3

The removal efficiencies found in this study were similar to many of those reported in other studies. It has commonly been found that 80% or more of zinc can be removed in constructed wetlands relying mainly on sulfide generation (i.e. Crites et al, 1997; Machemer and Wildeman, 1992; Kadlec and Knight, 1996).

CONCLUSIONS AND RECOMMENDATIONS

Initially, it was difficult to find obvious, definitive trends in the data. The extent of the degradation in the forebay was not expected, and therefore the anticipated rates of sulfate reduction and carbon oxidation in the gravel beds were severely diminished. During Feed 1, all cells showed statistically significant decreasing concentrations of DOC along the flow path of the gravel. The control cells and Bulrush 2 exhibited increasing DOC concentrations with depth, while DOC concentrations decreased with depth in the other planted cells. Sulfate concentrations in the control cells and bulrush cells decreased along the flow path, but increased along the flow path in the cattail cells. Depth had no effect on sulfate concentrations with the exception of one cattail cell which had a significant decrease in sulfate concentration with depth.

The presence of plants influenced performance, but the effect was usually small. Plants provided varying degrees of carbon supplementation, oxygen transportation, and evapotranspiration. Overall, cattails seemed to have a more dramatic effect on the system than the bulrush, and therefore the cattails' results tended to differ more in comparison to the control cells.

Sulfate reduction contributed to less than 20% of the carbon oxidation within the gravel, therefore other processes such as oxygen reduction, methanogenesis, and/or fermentation were responsible for the majority of the carbon degradation. Again, quantification of the individual process' effect is difficult, but most of the carbon oxidation is probably due to oxygen reduction. Methanogenesis probably played a minor

role, especially in the planted cells. Fermentation's impact is unknown but possibly significant.

Feed 1, with a carbon load of 200 mg/L, appeared to become sulfate limited, thus drastically reducing sulfate reduction reaction rates. At concentrations below approximately 0.1 mmol SO_4^{2-}, sulfate reduction essentially ceased.

Feed 2, which had half the influent carbon load, reached a point of carbon limitation at approximately 5 mg/L that affected the performance of the plug-flow gravel section of the cells. This limitation resulted in data in which few reliable trends could be extracted.

Even with the depressed rates of sulfate reduction, the system did accomplish its ultimate goal of zinc removal, but virtually all was done in the forebay and not the gravel section. Though there appeared to be reoxidation of sulfate in some cells, no zinc was re-mobilized.

Recommendations for Improvement in this Study

- Increase the initial sulfate and carbon concentrations to diminish effect of the forebay on the degradation within the gravel. The increased concentrations within the gravel would eliminate the possibility of carbon and sulfate limitation.
- Eliminate the addition of nitrate during wastewater preparation to reduce the alternative electron acceptor interaction possibilities for an easier determination of the effect of oxygen reduction and methanogenesis on carbon

oxidation, or alternatively increase the nitrate concentration to limit sulfate reduction until the gravel is reached.

- Add testing of COD and redox potential to gain more information on electron acceptor utilization.
- Add sampling of the forebay and afterbay in addition to influent sampling to provide a more definitive picture of gravel section degradation rates. Sampling the afterbay would allow for a more complete analysis of the degradation within the gravel.
- Run new wastewater feeds for a minimum of two months before beginning sampling to allow for steady-state conditions to be reach and to reduce sampling load.

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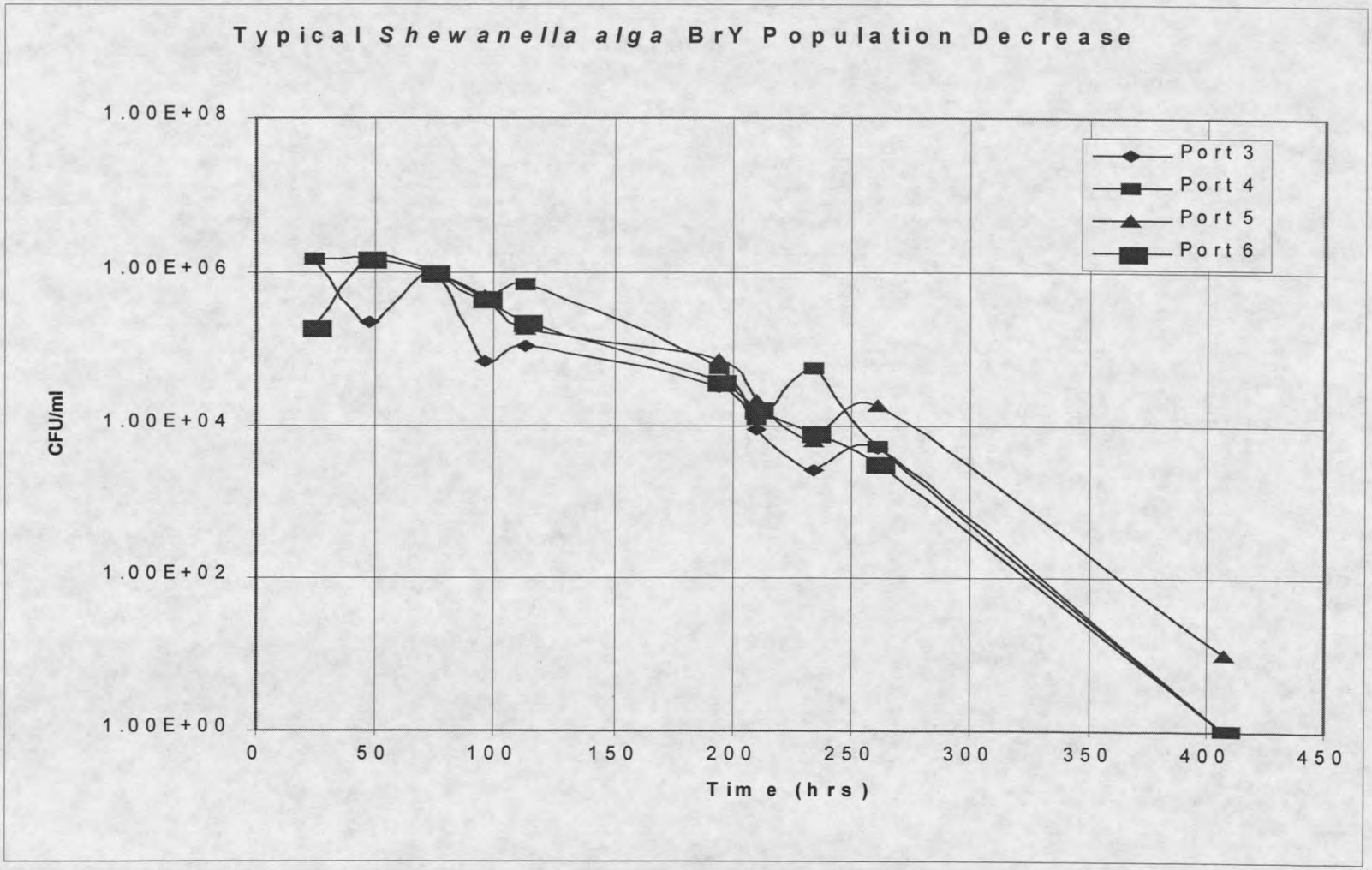
APPENDIX

Typical *Shewanella alga* BrY Population Decrease


Typical Shewanella alga BrY Population Decrease
1.00E+08
1.00E+06
1.00E+04
1.00E+02
1.00E+00
CFU/ml
0
50
100
150
200
250
300
350
400
450
Time (hrs)
Port 3
Port 4
Port 5
Port 6