Effects of supplemental organic carbon addition on sulfate reduction and zinc sulfide precipitation using microcosm and bench-scale constructed wetlands in cold climate regions
by Deborah Jean Borden

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

Abstract:

Two experimental constructed wetland (CW) systems were used to determine the effects of supplemental organic carbon addition on sulfate reduction and zinc precipitation over a simulated growing season.

The first research system consisted of sixteen 20x60-cm, batch-mode, subsurface CW microcosms (columns) that were filled with washed pea gravel and placed in a climate-controlled greenhouse. Eight of the columns were planted with Scirpus acutus (bulrush), four were planted with Typha latifolia (cattail) and four were left unplanted as controls. For one year, beginning in December 2000, all columns were used to treat a sulfate/zinc synthetic wastewater supplemented with two different concentrations of organic carbon. Four of the bulrush columns and all of the cattail and control columns were given 100 mg/L of supplemental organic carbon (sucrose); the remaining four bulrush columns were given 200 mg/L organic carbon. Two 20-day batch incubations were conducted at each of four temperature steps (4°C, 14°C, 24°C, 14°C) to simulate the seasons of the year. Samples were extracted from the sixteen columns on six occasions during each 20-day incubation. A second study, initiated in June 2000, consisted of eight bench-scale horizontal continuous-flow, subsurface wetlands. Three each were planted with cattail and bulrush and two were left unplanted as controls. A synthetic wastewater identical to the 100 mg/L organic carbon, batch-load system water was used. Samples of influent and effluent were analyzed once per hydraulic residence time, every five days. Samples from both systems were analyzed for sulfate (SO_4^{2-}) and chemical oxygen demand (COD). Periodically, samples were also taken for sulfide (S^{2-}), zinc (Zn^{2+}) and pH. In the batch-load system, redox potential (Eh) was automatically recorded in each column at three depths every four hours.

All treatments successfully removed sulfate and zinc in the batch-load system at warm temperatures (24°C). Bulrush plants had negative effects on sulfate and zinc removal at cold temperatures (4°C), due to root-zone oxidation. Cattail plants had similar but less dramatic negative effects on sulfate removal at cold temperatures, also likely due to root-zone oxidation. Bulrush columns with 200 mg/L organic carbon performed significantly better than all treatments with 100 mg/L organic carbon during the summer and fall (14°C). Bulrush columns with 200 mg/L organic carbon performed similarly to control columns with 100 mg/L organic carbon at cold temperatures. The batch-load system performed better than the continuous-flow system for COD and zinc removal. The continuous-flow system performed better than the batch-load system for sulfate removal.

EFFECTS OF SUPPLEMENTAL ORGANIC CARBON ADDITION ON SULFATE REDUCTION AND ZINC SULFIDE PRECIPITATION USING MICROCOSM AND BENCH-SCALE CONSTRUCTED WETLANDS IN COLD CLIMATE REGIONS

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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.

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ABSTRACT

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The first research system consisted of sixteen 20x60-cm, batch-mode, subsurface CW microcosms (columns) that were filled with washed pea gravel and placed in a climate-controlled greenhouse. Eight of the columns were planted with *Scirpus acutus* (bulrush), four were planted with *Typha latifolia* (cattail) and four were left unplanted as controls. For one year, beginning in December 2000, all columns were used to treat a sulfate/zinc synthetic wastewater supplemented with two different concentrations of organic carbon. Four of the bulrush columns and all of the cattail and control columns were given 100 mg/L of supplemental organic carbon (sucrose); the remaining four bulrush columns were given 200 mg/L organic carbon. Two 20-day batch incubations were conducted at each of four temperature steps (4°C, 14°C, 24°C, 14°C) to simulate the seasons of the year. Samples were extracted from the sixteen columns on six occasions during each 20-day incubation. A second study, initiated in June 2000, consisted of eight bench-scale horizontal continuous-flow, subsurface wetlands. Three each were planted with cattail and bulrush and two were left unplanted as controls. A synthetic wastewater identical to the 100 mg/L organic carbon, batch-load system water was used. Samples of influent and effluent were analyzed once per hydraulic residence time, every five days. Samples from both systems were analyzed for sulfate (SO_4^{2-}) and chemical oxygen demand (COD). Periodically, samples were also taken for sulfide (S^{2-}), zinc (Zn^{2+}) and pH. In the batch-load system, redox potential (Eh) was automatically recorded in each column at three depths every four hours.

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CHAPTER 1

INTRODUCTION

Since the early 1980's, constructed wetlands have gained recognition as a cost effective, low maintenance alternative to traditional treatment of waters impacted by mining activities. These waters are often termed acid mine drainage (AMD) and are usually characterized by high concentrations of trace metals and sulfate, and almost always by a low pH. AMD is formed during coal and metal-mining where the disruption of soil and bedrock exposes pyrite-bearing strata to oxygen and water. Pyrite is an iron sulfide mineral (FeS_2) that forms in reducing environments. When exposed to oxygen and water, it will oxidize and release large quantities of iron and sulfate into solution. In addition, H^+ ions are liberated during the oxidation process causing an acidic solution that readily weathers other minerals, releasing other trace minerals into solution (Cohen and Gorman, 1991; Fennessy and Mitsch, 1989; Robb, 1994; Wieder, 1989; Wildeman *et al.*, 1993). The environmental impact of AMD can be severe. It is a major industrial water pollution problem in the coal mining regions of the U.S. and around the world. In the U.S., it impacts many miles of streams and rivers. Often, ferric hydroxide will coat the bottoms of streams and rivers causing serious damage to fish and aquatic plant communities (Robb, 1994). Under acidic conditions, metals may be transported downstream dissolved in water, and when pH changes to neutral or basic, the metals precipitate and end up in aquatic bottom sediments (Cohen and Gorman, 1991).

Remediation of AMD may involve active chemical treatment, such as liming, which increases the pH and subsequently precipitates metals. Chemical treatment requires significant amounts of materials and many hours of maintenance, which results in high operational costs. Alternatively, constructed wetlands can be a low cost, low maintenance treatment system proven to ameliorate AMD chemistry (Webb *et al.*, 1998). Wetlands used for AMD treatment are a fairly new technology and were first proven to improve AMD chemistry in the late 1970's and early 1980's (Huntsman *et al.*, 1978; Wieder and Lang, 1982). These findings led to an increased interest in the potential of constructed wetlands as an alternative to chemical treatment.

In the 1980's, extensive research was conducted to understand the biological and chemical processes in wetlands that may contribute to improvement of AMD chemistry (Stark *et al.*, 1995a; Wieder, 1989). Although research was beginning to explain the processes controlling the effectiveness of wetlands for AMD amelioration, results from wetland studies in the 1980's were highly variable (Hiel and Kerins, 1988; McIntire and Edenborn, 1990). This led many scientists to recommend additional research to improve the understanding of the factors controlling AMD chemistry in wetlands and to improve constructed wetland design for consistent treatment (Henrot and Wieder, 1990; Hiel and Kerins, 1988).

Considerable research continued through the 1990's at which time researchers suggested that sulfate reduction and subsequent metal sulfide precipitation was the predominant metal removal process in constructed wetlands after system start-up. Anaerobic sulfate reduction produces hydrogen sulfide, which chemically reacts with many trace metals to form highly insoluble metal precipitates that remain in the wetland. In addition, sulfate reduction and metal sulfide precipitation consume H^+ ions and raise the pH (Christensen *et al.*, 1996; McIntire *et al.*, 1990; Machemer, 1992; Machemer *et al.*, 1993; Webb *et al.*, 1998). In spite of a better understanding of the factors controlling AMD chemistry in constructed wetlands, treatment is still variable. Consequently, a Directive was developed in 1997 by the Office of Surface Mining, Reclamation and Enforcement (OSMRE) which states that wetlands are viewed as a viable AMD treatment alternative, but that treatment using this technology must be supported by an operable chemical or other conventional treatment system capable of consistently meeting water quality standards in the event that the wetland system does not adequately treat the discharge (OSMRE, 1997). This Directive significantly increases the costs of implementing a constructed wetland system. Future research may produce more consistent results, at which time, a conventional system may not be required.

There are various processes within a constructed wetland that can enhance or interfere with metals removal. Temperature and season, plants and organic matter availability all have significant effects on metals removal in constructed wetlands.

Goals and Objectives

Initially the purpose of this study was to look at the effectiveness of pre-established continuous-flow constructed wetlands for the treatment of a synthetic wastewater high in metals and sulfate without the addition of organic matter. It was hypothesized that internal carbon production would provide some sulfate reduction even without additional influent organic carbon. However, a trial in August 1999 using small concentrations of sulfate found higher sulfate concentrations in the effluent than in the influent, suggesting that added organic carbon is needed for successful sulfate reduction. Since organic carbon is necessary, a change in research focus was required.

The new purpose of the study was to evaluate the effects of plant species, organic carbon concentration, season and temperature on the treatment of a synthetic mine wastewater. The objectives were: (1) compare performance of two plant species and unplanted controls for removal of sulfate and zinc over one year, (2) compare performance of one plant species at two different concentrations of organic carbon, (3) quantify seasonal and temperature variation in removal of sulfate and zinc over one year, (4) compare performance of continuous-flow and batch-load systems.

Two systems were used to accomplish the objectives, a batch-load system and a continuous-flow system. Objectives 1, 2 and 3 were met using a batch-load system. Eight batch incubations were conducted over one year starting in December 1999 and ending in January 2001. Two incubations were conducted at each of four temperature steps simulating the four seasons of the year (4°C, 14°C, 24°C, 14°C). Objectives 1 and 3 were also addressed with the continuous-flow system. Objective 4 was met by comparing the batch-load and the continuous-flow system. The same synthetic wastewater recipe was used in each system to compare performance of sulfate and zinc removal. Due to mechanical problems, data from the continuous-flow system was taken only from June 2000 through the beginning of January 2001. Both systems were located in the same greenhouse and experienced identical environmental conditions.

Background, methods and conclusions for the entire study are presented in Chapters 2, 3 and 7, respectively. Chapter 4 discusses objectives 1 and 2 based on the batch-load system. Chapter 5 discusses objective 3 for the batch-load system. Chapter 6 discusses objective 1 for the continuous-flow system, as well as objective 4 comparing batch-load and continuous-flow systems.

CHAPTER 2

BACKGROUND

Removal Pathways for Metals in Constructed Wetlands

Trace and toxic metals resulting from mining activities may be successfully immobilized in constructed wetlands by a variety of processes. Metals generally enter the wetland environment in water-soluble form and are converted by physical, chemical and biological processes into less mobile forms that, depending on degree, can be classified as readily mobile and available, potentially mobile and available, and essentially immobile and unavailable (Gambrell, 1994). The goal of constructed wetland design is to adequately retain metals in a form that will not be easily released back into the environment.

Readily mobile and available metals consist of water-soluble metals and exchangeable (sorbed) metals. Water-soluble metals may be in the form of soluble free ions, soluble organic or soluble inorganic complexes. These forms are the most mobile and plant available. Exchangeable metals weakly bond to organic and inorganic surfaces and are temporarily immobilized in wetland substrates, but may be released from wetlands if there is a shift in pH or if adsorbed metals come into competition with metals that are more favorable for adsorption (Gambrell, 1994; Wildeman *et al.*, 1993).

The majority of removal pathways in wetlands retain metals in the potentially mobile and available form. These include:

1) Complexation with larger molecular weight organics (humic and fulvic acids)
2) Adsorption to precipitated hydrous oxides (Fe, Mn and Al oxides)
3) Precipitation as inorganic compounds (metal oxides, hydroxides or carbonates)
4) Precipitation as insoluble metal sulfides
5) Uptake by plants

Metals in these forms can be described as effectively immobilized but may potentially become available if there is a shift in pH or oxidation status (Gambrell, 1994; Wildeman *et al.,* 1993; Noller *et al.*, 1994).

Metals in the essentially immobile and unavailable form generally are bound within the crystalline lattice structure of primary minerals. These metals will bind by isomorphous substitution in clay minerals and will only become available due to weathering, generally over long periods of time (Gambrell, 1994).

The processes of exchange, complexation, precipitation and plant uptake that contribute to metals removal in wetlands will be discussed more specifically in the following text.

Exchange of Metals onto Organic Matter and Algae

Organics, generally humic and fulvic acids attached to soil surfaces, can temporarily retain metals in wetlands. During cation exchange processes, a pre-adsorbed cation will dissociate from the organic acid and be replaced with another by chemical binding (Wildeman *et al.*, 1993). Specific cations are preferentially adsorbed to organic matter. Machemer and Wildeman (1992) found that Fe and Cu adsorb more strongly than Zn which, in turn, adsorbs more strongly than Mn. An increase in total metal concentration decreased the percent of Mn and Zn adsorbed as Fe and Cu were preferentially bound. Sorption of metals also varies with pH (Machemer and Wildeman, 1992; Stumm and Morgan, 1996). At low pHs, H^+ ions are more preferentially sorbed than metals (Wildeman *et al.*, 1993).

There is a finite number of sites on organic matter where metals may be temporarily retained, known as the ion exchange capacity. It is defined as the number of moles of ion charge that can be adsorbed to a unit mass of soil under given conditions of temperature, pressure, soil solution composition and soil-solution mass ratio (Stumm and Morgan, 1996). When ion exchange sites are filled, the soil is considered saturated. During initial start-up of a wetland, sorption of metals can be the main removal pathway. Once the organic matter is saturated, this removal pathway is insignificant. Machemer and Wildeman (1992) found that it took approximately four months for their wetland to reach saturation. Once sorption sites were filled, metal sulfide precipitation became the dominant process for metal removal. Christensen *et al.* (1996) found similar results. Adsorption was the dominant metal removal process for the first three weeks after wetland start-up. After this time, metal sulfide precipitation became the dominant removal process. Wildeman *et al.* (1993) suggested that during wetland start-up, metal exchange onto organic matter could serve for temporary retention of metals until sulfate-reducing bacteria have a chance to establish and take over as the dominant metal removal mechanism.

There is growing evidence that algae also remove metals from mine drainage using ion exchange processes (Crist *et al.*, 1990; Wildeman *et al.*, 1993). This metal removal process is not considered significant, but may be considered important for short-term immobilization similar to ion exchange with organic matter (Kadlec and Knight, 1996). Harris and Ramelow (1990) found that two algaes, *Chlorella vulgaris* and *Scenedesmus quadricauda* were efficient at binding silver, copper, cadmium and zinc. Copper, cadmium and zinc binding was pH-dependent while silver was strongly bound over a wide pH range. Crist *et al.*, (1990) found that adsorption of Strontium (Sr) on *Vaucheria* released equivalent amounts of Ca and Mg. Release of protons was observed when Cu was adsorbed, demonstrating additional ion exchange characteristics. Algae have also been known to trap metal oxyhydroxide precipitates, further increasing adsorption of metals in wetlands (Wildeman *et al.*, 1993).

Complexation of Metals with Larger Molecular Weight Organics

Metals complexed with larger molecular weight organics are more tightly bound and effectively immobilized in contrast to exchangeable metals. Complexation occurs by the same processes as adsorption, but complexation reactions are considerably more stable. Evidence suggests that metal complexation is more effective in anoxic and reducing conditions and long-term oxidation may result in a major release of complexed metals (Gambrell, 1994). Henrot and Wieder (1990) found that Fe binding to large molecular weight organics was one of the main removal mechanisms. Similar to adsorption of exchangeable cations, there are limited sites on large molecular weight organics for metal complexation (Henrot and Wieder, 1990).

Adsorption of Metals to Precipitated Hydrous Oxides

Fe(III) and Al hydroxide, as well as Mn(IV) oxyhydroxide precipitates have a strong capability of adsorbing other metal ions onto their surfaces (Stumm and Morgan, 1996). The surface of an oxyhydroxide operates as a weak acid, which attracts hydroxide ions creating a negative surface which, in turn, attracts metal cations. Adsorption to precipitated hydrous oxides can be used in a wetland as a polishing and buffering step to help remove residual metal contaminants (Wildeman *et al.*, 1993). Hydrous oxides form in aerobic environments. When exposed to anaerobic (i.e. low redox potential) environments, hydrous oxides become unstable which, in turn, causes a release of adsorbed metals. Because hydrous oxides are unstable at low redox potential, adsorption to hydrous oxides may only be important in the top few millimeters of wetland sediments, where aerobic conditions exist (Gambrell, 1994).

Precipitation of Metals as Inorganic Oxyhydroxides

Oxyhydroxide precipitation of Fe and Mn can be a major removal mechanism in aerobic environments of wetlands. Iron and manganese enter a wetland generally in the +2 state. Oxidation of Fe(II) and Mn(II) and subsequent hydrolysis create relatively insoluble metal oxyhydroxide precipitates (Wildeman *et al.*, 1993).

$$Fe^{2+} + \frac{1}{4} O_2 + H^{+} \rightarrow Fe^{3+} + \frac{1}{2} H_2O \quad (1)$$

$$Fe^{3+} + 3\ H_2O \rightarrow Fe(OH)_3 + 3\ H^{+} \quad (2)$$

Equations describing Mn oxidation and precipitation may also be found in Wildeman *et al.* (1993). Fe and Mn oxidation is pH dependent and uses oxygen as the electron acceptor (Henrot and Wieder, 1990; McIntire *et al.*, 1990). Fe(II) is autooxidized at pH values greater than 6, while in more acidic conditions, bacteria catalyze the reaction (McIntire *et al.*, 1990). Although Fe(II) may be oxidized to Fe(III) at pH values as low as 3.0, it will not oxidize at acceptable rates until the pH in the system nears 7.0. Mn (II) will not oxidize to Mn(IV) at acceptable rates for pH less than 9.0. Therefore, oxidation of Fe and Mn in low pH solutions, such as AMD, can be difficult and is the limiting step in the oxidation and precipitation sequence (Wildeman *et al.*, 1993). The oxidation and precipitation sequence of Fe and Mn produces H^+ ions, and therefore can be a self-limiting process (McIntire *et al.*, 1990; Stumm and Morgan, 1996; Wildeman *et al.*, 1993). Fe and Mn oxidation and precipitation is also dependent on other wetland parameters such as temperature, flow rate and total metal concentrations of Fe and Mn (Henrot and Wieder, 1990).

Precipitation of Metals as Insoluble Metal Sulfides

In the past ten years, many researchers have acknowledged sulfate reduction and subsequent metal sulfide precipitation as a major contributor to metals removal in wetlands (Christensen *et al.*, 1996; McIntire *et al.*, 1990; Machemer, 1992; Machemer *et al.*, 1993; Webb *et al.*, 1998). Provided conditions remain strongly reducing, metals can be immobilized in wetlands as highly insoluble metal sulfide precipitates (Gambrell, 1994). Mine waters generally contain high concentrations of sulfate and heavy metal cations. In an oxidation-reduction reaction, sulfate-reducing bacteria (SRBs) oxidize decayed organic matter (electron donor) and reduce sulfate (electron acceptor) to sulfide (Odom and Singleton, 1993; Stumm and Morgan, 1996). The produced sulfide will quickly react with trace and toxic divalent metals forming highly insoluble metal sulfide precipitates (Gambrell, 1994; Singh, 1992; Wildeman *et al.*, 1993).

Sulfate reducing bacteria are ubiquitous species. They will tolerate temperatures below -5°C and above 50°C. Their optimal pH range is 5.0 – 9.0, but they have been known to tolerate pH values as low as 2.6 and as high as 9.5. Their optimal oxidation-reduction potential (Eh) is less than –150 mV, but SRBs may be present in soils with Eh as high as –50 mV. The survival of SRBs in adverse conditions is probably due to their ability to form favorable microenvironments on surfaces and particulate material (Bolis *et al.*, 1991; Lyew and Shepard, 1999; Odom and Singleton, 1993; Wildeman *et al.*, 1993). Most SRBs utilize only simple organic substrates as electron donors. The most common of these include primary alcohols (such as ethanol), acetate, lactate, formate, pyruvate, glycerol and some dicarboxylic acids (such as malate and fumarate). They can also utilize

H_2 as an electron donor. Many SRBs cannot completely oxidize organic matter to CO_2 (Odom and Singleton, 1993). An equation for sulfate reduction is given below using lactate as the electron donor:

$$SO_4^{2-} + 2\text{ lactate} \rightarrow 2\text{ acetate} + H_2S + 2\,HCO_3^- \text{ (pH} < 7.0) \qquad (3)$$

The produced sulfide then reacts with a divalent metal (M) to for an insoluble metal sulfide precipitate:

$$H_2S + M^{2+} \rightarrow MS + 2H^+ \qquad (4)$$

Metal sulfides precipitate out of solution in an order inverse to their solubility product (K_{sp}) values:

$$CuS < PbS < CdS < ZnS < NiS < FeS < MnS \qquad (5)$$

Copper has the lowest solubility and will precipitate out of solution first, whereas Mn has a high solubility and does not readily form a metal sulfide precipitate (Christensen *et al.*, 1996; Machemer, 1992; Stumm and Morgan, 1996). Most metal sulfides have a lower solubility than their hydroxide counterpart (exceptions being Al, Fe and Mn) and can precipitate over a broad pH range (Dvorak *et al.*, 1991; Eger, 1992). They also have a higher sludge density than hydroxide precipitates, resulting in lower sludge handling costs (Christensen *et al.*, 1996).

Generally, the sulfate reduction step is rate limiting. Although SRBs are ubiquitous and tolerate a wide range of environmental conditions, rates of sulfate reduction in wetlands have been found to be extremely variable and are dependent on many wetland factors (Webb *et al.*, 1998). These include pH, redox potential, temperature, concentration of dissolved sulfate, quantity and type of organic matter and the surface area available for SRB and fermentative bacteria attachment (Lyew and Sheppard, 1999; Westrich and Berner, 1988).

Uptake of Metals by Plants and Other Plant Effects

Initially thought to be a significant pathway, a number of results suggest that uptake of metals by plants accounts for removal of less than 1% of the total metal load applied to a wetland (Gersberg *et al.*, 1985; Hiel and Kerins, 1988; Mitsch and Wise, 1998; Sencindiver and Bhumbla, 1988; Wildeman *et al.*, 1993). Metal uptake in sufficient quantities can be toxic to plants. Plants will form iron oxide coatings on their roots to prevent excess metal uptake (Sencindiver and Bhumbla, 1988; Wildeman *et al.*, 1993).

There is contradictory evidence as to where plants store metals in their tissues. Taylor and Crowder (1983) and Hiel and Kerins (1988) suggested that plants retain most metals in the roots and not in the rhizomes or aboveground biomass. Sencindiver and Bhumbla (1988) suggested that Fe and Mn accumulate in the rhizomes and aboveground biomass and not in the roots. To explain these contradictions, Wildeman *et al.* 1993 suggested that as a survival mechanism, plants shift contaminants from the roots to the senescing leaves at the end of the growing season.

Although plants may not play a significant role in metals removal by uptake directly, they serve other important roles in the wetland environment. Plants provide the biomass necessary for other processes that can remove metals. Decayed plant materials break down by enzymatically catalyzed microbial processes to a soluble form of organic matter useable for sulfate reduction. The decaying organic matter also provides additional sorption sites for metals (Eger, 1992; Mitsch and Wise, 1998; Noller *et al.*, 1994; Wildeman *et al.*, 1993).

Plants can also provide oxygen to the substrate. Plant roots need oxygen for aerobic respiration and cannot obtain the oxygen they need from diffusion through the wetland surface (Reddy *et al.*, 1989). Instead, they must transport oxygen from the aboveground leaves to the roots through air-filled intercellular spaces (aerenchyma) that extend from foliar parts down to the roots (Armstrong, 1971). Many researchers have speculated that oxygen transported from aerial tissue into the root zone may exceed plant demands. If so, oxygen may diffuse out of submerged roots into wetland sediments (Callaway and King, 1995; Dunabin *et al.*, 1988; Flessa and Fisher, 1992; Moorhead and Reddy, 1988; Reddy *et al.*, 1989; Sorrell and Armstrong, 1994; Steinberg and Coonrod, 1994). It has been suggested by a number of researchers that the higher the sediment oxygen demand, the greater the sink for oxygen released from plant roots (Dunabin *et al.*, 1988; Reddy *et al.*, 1989; Sorrell and Armstrong, 1994).

For the purposes of metals removal, rhizosphere oxidation has advantages and disadvantages. Positively, rhizosphere oxidation detoxifies H_2S along with Fe and Mn, which may reach phytotoxic concentrations in waterlogged soils (Armstrong, 1971; Gambrell and Patrick, 1978; Reddy and D'Angelo, 1994). Aerobic microsites may allow for the oxidation of iron and manganese and subsequent precipitation as hydrous oxides, which have a high surface area and adsorptive capacity for phosphorus and metals (Patrick and Gambrell, 1976). Negatively, rhizosphere oxidation increases the redox potential of the wetland environment, which adversely affects anaerobic sulfate reduction/metal sulfide precipitation, the major pathway for metals removal in anaerobic wetlands (Cohen and Gorman, 1991; Wildeman *et al.*, 1993). Sulfate reducing bacteria are generally obligate anaerobes and cannot survive aerobic conditions (Odom and Singleton, 1993). In addition, oxygen is a more thermodynamically favorable electron acceptor than sulfate and will consume soluble organic compounds that would be used for sulfate reduction had oxygen not been present. The organic compounds oxidized by oxygen may limit the amount of organic compounds available for sulfate reduction, in effect, reducing the amount of metals removed in an anaerobic wetland. Cohen and Gorman (1991) and Wildeman *et al.* (1993) suggested that plants are not necessary or may be counter-productive for the purposes of sulfate reduction and metals removal in wetlands. Wildeman *et al.* (1993) suggested that for sulfate-reducing treatment systems, the most important role plants play is for aesthetic considerations.

Plants have several other advantages. They provide additional surface area for the attachment of microbial communities (Fennessy and Mitsch, 1989). They also act as a living filtration system for suspended solids and increase water retention time in wetlands, allowing for sedimentation processes to occur (Fennessy and Mitsch, 1989; Noller *et al.*, 1994; Reddy *et al.*, 1989). The many advantages and disadvantages of adding plants to a constructed wetland for mine water treatment can make it difficult to decide whether plants would be beneficial for metals removal.

Alternate Electron Acceptors

There are numerous oxidation-reduction (redox) reactions in a wetland that can utilize organic carbon in quantities that may limit the availability of organic carbon for sulfate reduction. Microbially mediated oxidation-reduction reactions oxidize organic matter using various inorganic electron acceptors such as O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-} and CO_2. The electron acceptors are used in a specific order based on the potential energy release from the reaction. Reactions that have greater energy release will be used first. Each redox reaction has a specific standard free energy change, ΔG°, that describes how much energy is released. The more negative ΔG°, the greater the energy release. The potential energy release from the oxidation of organic matter coupled with different electron acceptors can vary significantly as shown below in Table 1 (Reddy and D'Angelo, 1994; Snoeyink and Jenkins, 1980). Redox potential (Eh) (Table 1) is widely used as an indicator of the type of redox reactions occurring in a solution. It is measured as the potential of an electrode, usually in volts or millivolts. Eh is a function of ΔG° in which highly positive Eh values correspond to highly negative ΔG° values.

Aerobic decomposition using O_2 will proceed first because the reaction has the most negative ΔG° and therefore produces the highest energy release. Once O_2 is depleted, the microbial population will begin to shift to facultative and anaerobic organisms such as nitrate and manganese reducers (Odom and Singleton, 1993). The lower the energy release, the more slowly decomposition will occur (Reddy and D'Angelo, 1994). Sulfate reduction is not a high energy releasing reaction; all electron

Table 1. Selected types of microbial oxidation-reduction reactions in wetlands.

Process	Electron Acceptor (EA)	End Products	Moles of e^-/mole of EA	ΔG° (kJ/mole of electron)	Redox Potential - Eh (mV)
Aerobic respiration	O_2	H_2O, CO_2	4	-125.1	200 to 800
Nitrate reduction	NO_3^-	N_2, CO_2	5	-118.8	200 to 700
Manganese reduction	MnO_2	Mn^{2+}, CO_2	2	-94.5	200 to 500
Iron reduction	$Fe(OH)_3$	Fe^{2+}, CO_2	1	-24.3	0 to 300
Sulfate reduction	SO_4^{2-}	H_2S, CO_2	8	-25.4	-200 to -50
Methanogenesis	CO_2	CH_4, CO_2	8	-23.2	-200 to –100

acceptors with higher energy release will be used before sulfate. Diffusion of oxygen from the wetland surface and from plant roots, the presence of other electron acceptors as well as methanogenesis may deplete the amount of organic matter available for sulfate reduction. Burgoon *et al.* (1995) found that oxidation of organic matter by aerobic processes and methogenesis can be significant. They found that electron acceptors in the influent to a wetland (i.e. manganese, iron and sulfate) oxidized less than 2% of the organic matter. The remaining 98% was oxidized by electron acceptors found or produced in the wetland (i.e. oxygen and CO_2 addition and consumption by aerobes and methanogens).

Sulfate Reduction vs. Methanogenesis

Although the theoretical parameter, ΔG°, suggests that sulfate reduction is a slightly more favorable reaction than methanogenesis, SRBs and methane-producing bacteria (MPB) compete for simple organic carbon. The outcome of the competition is crucial for systems designed to remove metals using sulfate reduction. If MPBs outcompete SRBs, little sulfate reduction will occur and few metals will be removed from the influent waters. The outcome of the competition can be unpredictable and is dependent on many parameters. These include the carbon source available, the ratio of COD:S, pH, sulfide concentration and solids retention time (SRT) (Omil *et al.*, 1998). COD, chemical oxygen demand, is a measurement of the organic matter available for redox reactions. Fox and Ketha (1996) determined that the competition between SRBs and MPBs depends on the type of organic matter available. SRBs competed better for glucose and intermediates of isopropyl acetate (IPA). MPBs competed better for a fermentative pharmaceutical wastewater up to a concentration of 1:9 (wastewater:water) when the MPB population rapidly decreased from inhibition and SRBs became dominant. Research suggests that the COD:S ratio is very important in determining competition outcome. Generally, as the COD:S ratio decreases, the percentage of COD used for sulfate reduction increases (Fox and Ketha, 1996; Omil *et al*, 1998; Mizuno *et al.*, 1994; Vroblesky *et al.*, 1996) . Vroblesky *et al.* (1996) and Mizuno *et al.* (1994) found that at a COD:S ratio of 1.5, SRBs are dominant and at a COD:S ratio greater than 6.0, MPBs are dominant. Small concentrations of sulfide become toxic to MPBs before SRBs, therefore, when sulfide is present in quantities toxic to MPBs, SRBs will dominate (Capone *et al.*, 1983; Fox and Ketha, 1996; Mizuno *et al.*, 1998). As pH increases, SRBs outcompete MPBs. In a favorable environment for SRBs, as SRT decreases, the time for SRBs to outcompete MPBs decreases (Omil *et al.*, 1998).

Organic Matter Dynamics and Supplementation

Decomposition of organic matter can determine the rates of sulfate reduction in wetlands (Reddy and D'Angelo, 1994). The accumulation of organic matter in wetlands designed for sulfate reduction can be due, in part, to the decomposition of dead plant matter, which accumulates on the wetland surface and within the substrate. During decomposition, the plant matter is broken down into more simple, soluble forms by a number of processes, including leaching, fragmentation and consumption by fungi, bacteria and soil fauna. Fermentative bacteria produce extracelluar enzymes, which further decompose the plant matter to simple organic molecules which are readily used by aerobic and anaerobic microorganisms (Feng and Hsieh, 1998).

Rates of organic matter decomposition are dependent on the chemical makeup of the organic substrate, electron acceptor availability, temperature, pH, Eh and nutrient availability. Westrich and Berner (1988) suggested that the chemical makeup of the organic substrate is the main factor controlling the rate of decomposition. Plant matter is composed mainly of cellulose and lignin, two complex organic molecules. The cellulose fraction degrades more readily, therefore, substrate cellulose content decreases more rapidly during decomposition. The higher the cellulose fraction of the plant material, the faster decomposition will occur. Plant decomposition is also affected by the availability and type of electron acceptors. Greater concentrations of electron acceptors that produce lower energy-releasing oxidation-reduction reactions will decrease the Eh and, in turn, eventually decrease the rate of microbial activity responsible for decomposition (Table 1). A decrease in temperature or nutrient availability will also inhibit microbial activity and subsequently, decrease decomposition rates (Reddy and Patrick, 1975).

Commonly, the rate-limiting step for sulfate reduction is the rate at which fermentative bacteria decompose complex organic molecules to simple organic molecules (Westrich and Berner, 1988). Therefore, if organic matter from plants is the only carbon source, the chemical makeup of complex organics undergoing degradation is the main factor controlling sulfate reduction (Feng and Hsieh, 1998; Westrich and Berner, 1988).

In a metal treatment wetland, the organic matter supplied from decomposing plant material may not be sufficient to meet the carbon demand necessary for sufficient sulfate reduction to precipitate the metals of concern (Taritus and Unz, 1994). Adding a readily decomposable substrate (i.e. peat, mushroom compost, horse manure) to a constructed wetland will contribute to the organic carbon pool, increasing sulfate reduction rates. Taritus and Unz (1994) used decomposition kinetics to model the removal of mine water pollutants in constructed wetlands. Model simulations indicated that wetlands constructed with an organic substrate are initially capable of removing far greater amounts of metals than wetlands built with less biodegradable substrates (i.e. gravel). However, after three to five years of operation this difference becomes negligible. When the easily degradable fractions of the organic matter became exhausted, sulfate reduction became limited by the availability of simple organic substrate. They suggested that without regular carbon input, periodic additions of decomposable organic matter would be required for long-term metals removal using constructed wetlands. Several researchers have suggested that a regular input of soluble organic carbon with influent wastewater can significantly stimulate sulfate reduction and increase the longevity of a constructed wetland designed for metals removal (Christensen *et al.*, 1996; Dvorak *et al.*, 1991; McIntire *et al.*, 1990; Stark *et al.*, 1991; Stark *et al.*, 1995a; Stark *et al.*, 1995b). Stark *et al.* (1995a) recommend that soluble carbon supplementation should be seriously considered when designing a wetland for metals removal. Soluble forms of dairy whey and sodium lactate were both shown to stimulate sulfate reduction and subsequently remove metals when added to a wastewater influent.

Temperature and Seasonal Effects

Generally, metals removal efficiency decreases during the winter months of wetland operation. It is well known that, other factors being equal, biological processes, such as fermentative breakdown of organic matter and sulfate reduction, may slow down or stop as temperatures decrease. As a consequence, metals removal may be limited by the slowing of microbial activity. An experimental wetland in Fayette County, Pennsylvania demonstrated one to two orders of magnitude difference in sulfate reduction rates from summer to winter (McIntire and Edenborn, 1991). Wieder (1993) found that sulfate reduction occurred at substrate temperatures as low as 4°C, albeit at relatively slow rates. Henrot and Wieder (1990) demonstrated that iron removal efficiency in peat increased linearly with temperature over the 4 to 25°C range.

Plant processes that vary with temperature may also affect metals removal efficiency. Callaway and King (1996) demonstrated that oxygen leakage from plant roots of *Typha Latifolia* is greater in the winter than in the summer. This increased oxygen will promote aerobic processes, precipitating metals as oxides instead of sulfides. Plants may also supply more soluble organic carbon to the wetland during certain seasons of the year. Kadlec and Knight (1996) suggested that the organic carbon contribution from plants was significantly higher in the winter and spring compared to the summer and fall. Increased plant organic carbon in the spring and winter may increase microbial activity and subsequently increase metals removal.

In winter, wetlands may freeze if cold temperatures precede snow accumulation. If snow accumulates before cold temperatures, an ice layer will not tend to form because of the insulating properties of snow (Kadlec and Knight, 1996). Delivery systems, inlet and outlet structures are also subject to freezing. Wildeman *et al.* (1993) give wetland design guidelines for successful winter operation.

Hydraulic Problems

Rapid, catastrophic hydraulic failures have occurred in wetland systems due to clogging of pore spaces. Hydraulic conductivity, K, decreases over time due to substrate compaction and organic matter and precipitate accumulation (Drury and Mainhausen, 2000), resulting in short-circuiting, lowered retention times and subsequent decreases in metals removal (Hiel and Kerins, 1998). Experimental wetlands in Butte, Montana had a 90% decrease in hydraulic conductivity over a 2.5 year period (Drury and Mainhausen, 2000). Tanner and Sukias (1995) found that in four gravel-bed constructed wetlands, organic matter accumulation resulted in an average effective porosity reduction of 50% in the first two years of operation. Commonly used organic substrates have low hydraulic conductivities. For example, the K value for an experimental gravel wetland in Butte, Montana was 600 ft/d while the K value for a wetland with 50% compost was 12 ft/d (Drury and Mainhausen, 2000). The low hydraulic conductivity of organic substrates, intended to enhance treatment, may instead cause serious hydraulic problems. Wildeman *et al.* (1993) suggest that the permeability of a substrate is a critical design variable for successful operation. Higher hydraulic conductivity substrates such as gravel would be preferable to organic substrates because they are less susceptible to clogging. If lower hydraulic conductivity substrates must be used, Burgoon *et al.* (1995) recommend that periodic drain cycles may flush accumulated solids and extend the life of a wetland. Although this may decrease clogging problems, the drain/fill cycle can reoxidize sulfide and should be used as a last resort for anaerobic constructed wetlands for metals removal.

Accumulation of precipitates can be a major source of clogging problems. Fe oxides tend to form readily at the influent end of a wetland, lowering the hydraulic conductivity (McIntire and Edenborn, 1990; Sanders *et al.*, 1998; Wieder *et al.*, 1990; Wildeman *et al.*, 1993). Metal sulfides tend to cause much less clogging than metal oxide precipitates (Wildeman *et al.*, 1993). Sanders *et al.* (1998) suggested that to prevent clogging of wetlands by iron oxides, aerobic pretreatment of Fe might be necessary followed by a settling pond for precipitation of iron oxides. From the settling pond, AMD may flow to a subsurface anaerobic wetland for removal of other trace metals.

Wetland Design for Metals Removal

When constructed wetlands were first implemented for metals removal, they were designed to precipitate metals as oxyhydroxides. Shallow ponds were designed to aerobically precipitate Fe and Mn as oxyhydroxides. Problems with decreasing pH were solved by adding a base such as lime, limestone, sodium hydroxide or sodium carbonate. Although very effective at removing these and other metals, the labor and chemical expenses are high (Henrot and Wieder, 1990; Wieder, 1993). During the late 1980's, anaerobic wetlands gained recognition as an effective way to remove metals at significantly less cost. Anaerobic wetlands not only remove metals, but they also raise the pH (McIntire *et al.*, 1990). Design of anaerobic wetlands minimizes metal oxide precipitation, which is generally restricted to the upper sediments where oxygen can diffuse (Henrot and Wieder, 1990). Metal oxide precipitation may also occur in the plant rhizosphere where oxygen may diffuse from plant roots (Mitsch and Wise, 1998). In the presence of reducing conditions, metal oxides generally dissociate and can significantly increase soluble Fe and Mn in the effluent of anaerobic wetlands if Fe and Mn oxides are present in the influent (Taritus and Unz, 1995).

Today, most wetlands for metals removal are designed to promote sulfate reduction. Anaerobic wetlands can successfully remove metals to levels that meet water quality standards, and contrary to aerobic processes, anaerobic sulfate reduction generates alkalinity and can raise the pH of wetland effluent (Eger, 1992; Machemer, 1992; McIntire *et al.*, 1990; Webb *et al.*, 1998).

To promote anaerobic conditions, many wetland designs have incorporated some form of organic rich substrate such as peat, mushroom/municipal compost, horse manure or wood shavings. The organic substrate (1) rapidly depletes oxygen creating a reduced environment favorable for sulfate reduction and (2) provides the electron donor for SRB activity. It also may contain $CaCO_3$ (limestone) which can add to the neutralization potential of a wetland (Dvorak *et al.*, 1991; McIntire *et al.*, 1990). Additional alkalinity can be achieved by including crushed limestone along with an organic substrate. Alkalinity generation and pH increase in a wetland also depend on the influent AMD pH, alkalinity, flow rate and residence time (Wieder, 1993). An experimental anaerobic wetland studied by McIntire and Edenborn (1990) increased pH from 2.9 to 6.5 and alkalinity from 0 to 1077 ppm. Increases in pH, in turn, increased metal retention. Dunabin *et* al. (1988) found that metal retention in an anaerobic wetland increased from 49% to 99% when pH increased from 4.3 to 7.5.

Although organic substrates and limestone can add alkalinity, many researchers have determined that their neutralization capabilities become exhausted very quickly. McIntire *et al.* (1990) found that the neutralization potential of mushroom compost in their wetland was exhausted within three months. They suggested that sulfate reduction processes would be a more long-term solution for alkalinity generation than limestone and other alkalinity producing substrates. Wildeman *et al.* (1993) suggested that during wetland system start-up, organic substrate could add alkalinity in sufficient quantities until SRBs have a chance to colonize and start adding alkalinity themselves. Hedin *et al.*

(1994) suggested using an anoxic limestone drain (ALD) to raise the pH before entering an anaerobic wetland since SRBs tend to perform optimally at near neutral pH.

Problems can arise from attempting to raise the pH using sulfate reduction. In order for alkalinity to be generated, sulfate reduction must be in excess of metal precipitation. Metal precipitation produces 2 moles of H^+ counteracting the 2 moles of HCO_3^- produced from sulfate reduction (Dvorak *et al.*, 1991; Eger, 1992; Singh, 1992). Unfortunately, excess sulfate reduction produces excess sulfide that is not precipitated as a metal sulfide. The excess may accumulate to concentrations toxic to fish, macrophytes and bacteria. Free sulfide can also produce an undesirable rotten egg odor (Singh, 1992). To counteract sulfide toxicity, Singh (1992) suggests producing metal hydroxides instead of metal sulfides by raising the pH using lime.

There are advantages and disadvantages to designing wetlands for either metal hydroxide or metal sulfide precipitation. Metal sulfide precipitation seems to be gaining the most interest, but decisions as to the best removal process for design should be determined on a case-by-case basis.

Even though the understanding of mechanisms within wetlands for metals removal has improved significantly in recent years, performance continues to be inconsistent and, as a consequence, researchers have not come to any firm agreement on sizing criteria for a successful wetland, even at the same pH (Stark *et al.*, 1991). For this reason, sizing coefficients (which take the form of metal loading factors) should be used with caution. Physical, chemical and biological factors all play a role in the success of a wetland for metals removal. There are many recommendations in the literature to optimize metals removal in constructed wetlands but much more research must be conducted before design criteria can be used with confidence.

CHAPTER 3

MATERIALS AND METHODS

Experimental Overview

Two wetland experiments were conducted simultaneously in a single greenhouse unit of the Plant Growth Center at Montana State University in Bozeman, MT. Both experiments were conducted using a simulated mine-impacted water modeled after runoff/groundwater discharged to the Metro Storm Drain (MSD) in Butte, Montana. Contaminants in the MSD most likely originate from infiltration through areas of mine waste into the local alluvial aquifer. Typical of acid mine drainage, the water has high concentrations of metals and sulfate but atypically has a pH that is almost neutral (~ 6.6). The experiments were conducted to determine the effects of plant type, temperature and carbon concentration on sulfate reduction for the removal of zinc.

The first experiment consisted of sixteen microcosm subsurface constructed wetlands (columns) operated in batch-load mode. Eight of the columns were planted with *Scirpus acutus* (hardstem bulrush), four with *Typha latifolia* (broadleaf cattail) and four were left as unplanted controls. A series of eight 20-day incubations was conducted during a one year period. The second experiment consisted of eight horizontal continuous-flow constructed wetlands operated with a hydraulic residence time of five days. Three cells were planted with *Scirpus acutus,* three with *Typha latifolia* and two were left as unplanted controls. This experiment was conducted to compare continuous-flow and batch-load operation to determine the effect of hydraulic flow regime on various wastewater parameters.

Various wastewater parameters were analyzed in both systems to characterize each system's removal efficiency for sulfate and zinc. The greenhouse temperature was set at 24°C for the three summer months (June, July and August), 14°C for the three fall months (September, October and November) and three spring months (March, April and May) and 4°C for the three winter months (December, January and February). The batch-load study was initiated in December 1999 and concluded in January 2001. The continuous-flow study was initiated in June 2000 and concluded in January 2001. Natural variation in light and seasonal differences in temperatures were sufficient to produce typical patterns of plant dormancy in the winter and regrowth in the spring.

Batch-Load System

Column Design and Construction

Thirty-two constructed wetland microcosms "columns" were built in April 1997 (Allen 1999); sixteen of which were used in the current experiment. The columns, 60 cm tall, were constructed from 20 cm polyvinyl chloride (PVC) pipe capped on the bottom and filled to 50 cm with 3-13 mm diameter washed pea gravel. Porosity was 0.27 with a resulting pore volume of 4.3 L, and did not differ significantly among replicates (Allen, 1999). Three solution sampling ports (3-mm inner diameter vinyl tubing) and three access tubes (11-mm inner diameter PVC pipe) for platinum redox electrodes were installed vertically in the center of each column at three depths: 5, 15 and 30 cm. A water delivery system was used to replace evaporative losses and continuously maintain water levels just below the gravel surface through the addition of tap water to the bottom of the columns (Figure 1).

The columns had been used in a previous study looking at removal of organic carbon from a secondary wastewater source. The study was conducted for three years prior to the initiation of the current study. For details about plant collection and establishment within the columns, see Allen (1999).

Wastewater Application, Sampling, and Analysis Procedures

Two synthetic wastewater feeds, based on water from the MSD in Butte, Montana, were created by adding constituents to dechlorinated tap water and differed only in organic carbon concentration (Table 2). Organic carbon was supplied as sucrose. Zinc, supplied as $ZnSO_4 \circ 7H_2O$, was the only divalent metal introduced as representative of this class of contaminants. Remaining sulfate was supplied as Na_2SO_4. Ammonium chloride and potassium phosphate supplied appropriate nutrients for microbial and plant growth. The low-carbon wastewater was administered to four of the eight bulrush columns and all of the cattail and control columns. The high-carbon wastewater was administered to the remaining four bulrush columns to evaluate effects of carbon concentration.

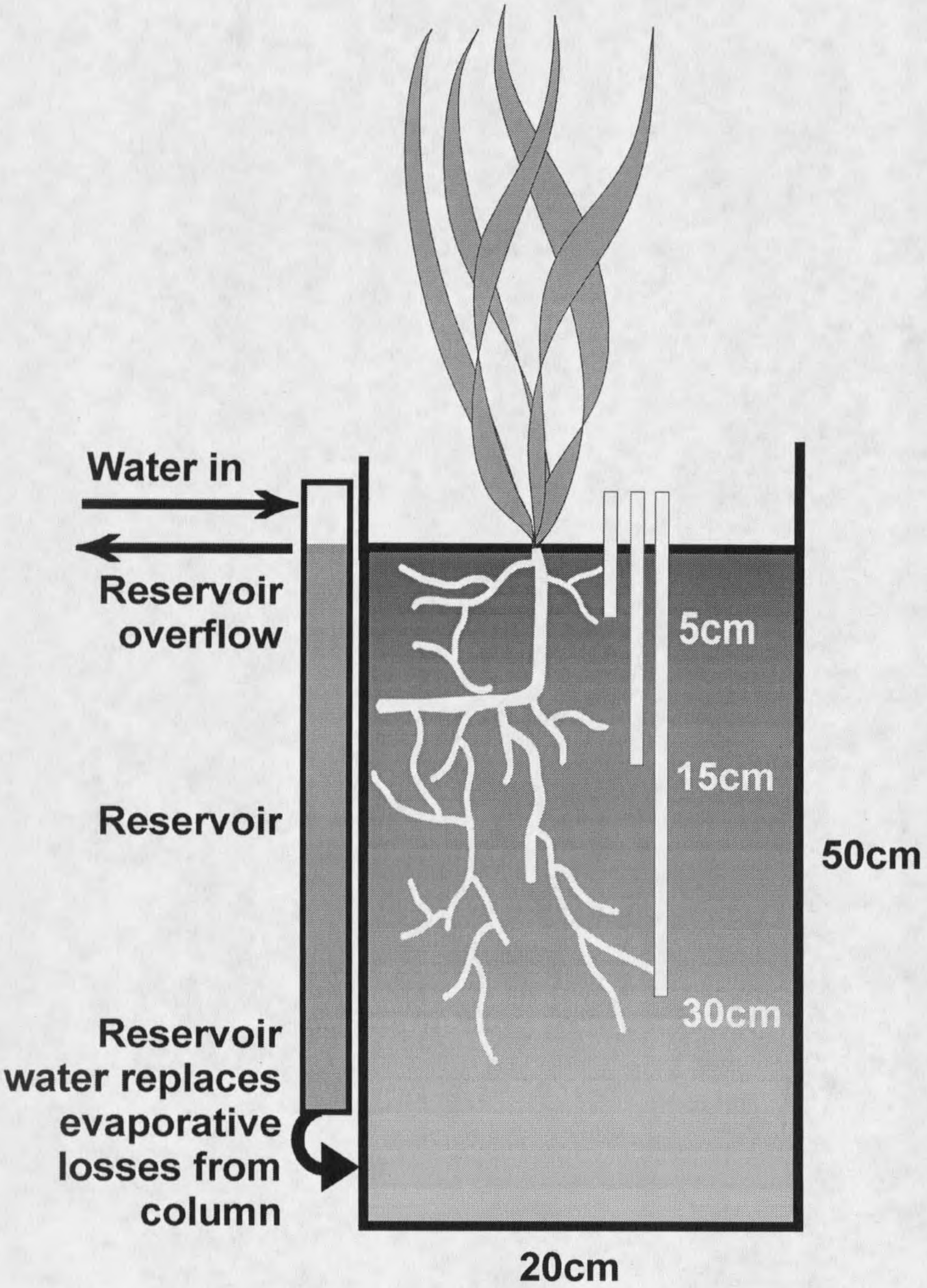

Figure 1. Schematic of column design and water delivery system (Allen, 1999).

To establish a consistent starting point, the columns were drained and filled with fresh wastewater three days prior to the start of a 20-day incubation, which was initiated upon a second drain and fill sequence. Two 20-day incubations were conducted at each of the four temperature steps (4, 14, 24 and 14°C) for a total of eight incubations over the year (Figure 2). A preliminary incubation (Incubation 0), at 4°C, was conducted prior to Incubation 1 for the purposes of practicing protocols.

Table 2. Concentrations of significant wastewater constituents.

Constituent	Low C wastewater (mg/L)	High C wastewater (mg/L)
SO_4^{2-} (SO_4-S)	200 (67)	200 (67)
Organic C (COD)	100 (267)	200 (534)
Zn^{2+}	24	24
N	10	10
P	1	1

The previous study using the columns (Allen, 1999) indicated that there were no measurable vertical gradients of chemical constituents; therefore, samples were drawn from 15 cm only in this study. Samples were collected with a 60-ml syringe, after three sampling tube volumes (approximately 15 ml) had been withdrawn and discarded, on days 0 (immediately after filling), 1, 3, 5, 10 and 20 of each incubation and were analyzed for sulfate and COD. The influent wastewater feeds were sampled for sulfate, COD and zinc prior to filling the columns. Samples for zinc, sulfide (S^{2-}) and pH were analyzed on day 5 only. Results from the sulfide samples were inconclusive, therefore, are not discussed in the results and discussion.

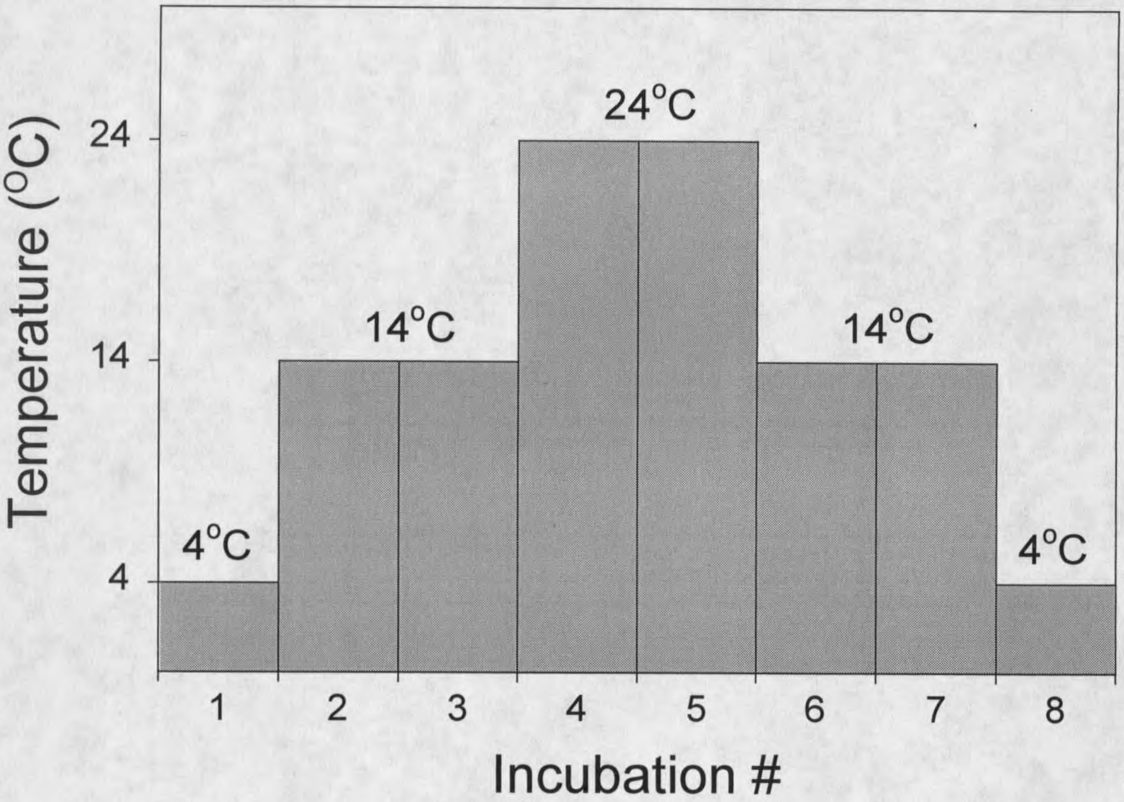

Figure 2. Timeline and temperature steps for the eight incubations.

Solution samples were placed in 15-ml glass test tubes. Two milliliters of sample were immediately apportioned to COD reagent vials (0-1500 mg/L; Hach Company, Loveland, CO) and digested at 150°C for two hours. COD concentrations were then found by colorimetric determination. The remaining 13-ml was filtered into sterile test tubes through an 0.2-micron cellulose acetate membrane filter and refrigerated at 5°C.

The filtered samples were analyzed for concentrations of sulfate using ion chromatography (Dionex Co.).

On Day 5, samples for zinc and sulfide were also taken. For zinc, 19 ml of sample was collected in 20-ml glass scintillation vials using a 20-ml non-gasketed syringe. For preservation, 1 ml of concentrated hydrochloric acid (HCl) was added to the 19 ml sample. The scintillation vials were sealed with a cone-shaped plastic liner lid. The non-gasketed syringe and plastic lids were used to eliminate exposure of zinc samples to any type of rubber that could absorb zinc. Samples were analyzed at the Soil, Plant & Water Analytical Lab at Montana State University using inductively coupled plasma spectrometry (ICP). Sulfide concentrations were measured using the Methylene Blue Method (0-0.600 mg/L; Hach Company, Loveland, Co.). 25 ml of sample was collected in 118-ml polypropylene containers using a 60-ml syringe. Immediately after collection, 1 ml of Hach sulfide reagent 1 and 1 ml of Hach sulfide reagent 2 were added to each sample. Sulfide concentrations were then found by colorimetric determination. Samples that exceeded 0.600 mg/L sulfide were appropriately diluted.

Redox Measurement

Platinum redox electrodes were installed in the three access tubes of each column at 5, 15 and 30 cm depths. Electrodes were made by welding 1.5 cm lengths of 20-gauge platinum (Pt) wire to 12-gauge copper wire and sealing the junction with epoxy, leaving approximately 1 cm of Pt wire exposed (Faulkner et al. 1989). Each electrode was soldered to the center conductor of a coaxial cable, the junction was sealed with epoxy, and the electrodes were connected to a personal computer via an analog multiplexer and a Labworks II computer interface system (SCI Technologies, Bozeman, MT). The outer conductor of the coaxial cable acted as an electromagnetic radiation shield (Allen, 1999). The columns were connected with a salt bridge and two saturated calomel reference electrodes (SCE) were located along the centerline of the salt bridge (Veneman and Pickering 1983). Electrode potential (E_m) was measured and recorded automatically every 4 hours. To estimate redox potential (Eh), measured E_m values were corrected by adding 244 mV (Stumm and Morgan 1996). Because pH was consistently circumneutral in this study (average = 6.5 with plants and 6.7 without plants) and the effect of temperature on E_m is relatively small, measurements were not corrected for pH or temperature. Before each incubation, redox probes were checked against a ferrous-ferric standard solution to ensure proper function (Light, 1972). Redox potential (Eh) ranges from –400 mV in strongly reducing environments to +700 mV in well oxidized environments, and measurements are more reliable in reducing conditions (Gambrell and Patrick 1978).

Continuous-Flow Constructed Wetlands

Design and Construction

Eight subsurface horizontal continuous flow constructed wetlands (cells) were created in 1995 (Biederman, 1999). Each cell was constructed of 16-mm polypropylene to the dimensions of 152 cm long, 76 cm wide and 53 cm deep and filled to 47 cm with 13-19 mm diameter gravel (Figure 3). Initial porosity was 0.40. 19-mm (0.75") PVC distribution and collection manifolds were constructed with 3-mm slots on the crown and placed horizontally across each cell to ensure an even flow distribution. The distribution and collection manifolds were placed at 41 cm and 5 cm above the bottom of the cell, respectively. The collection manifold led to a separate tank (outflow box) which allowed for adjustment of the water surface. The water surface levels in each cell were set at 46 cm for the duration of the experiment. To measure air and water temperature, differential thermocouples were placed in the greenhouse and in each cell at a depth of 36 cm measured downward from the gravel surface. Air and water temperatures were recorded every 30 minutes by a Campbell Scientific 21X datalogger with Multiplexer and SM192 Storage Module (CSI, Logan, UT). The continuous-flow system was used in a previous study that looked at organic carbon removal efficiency using a secondary wastewater source. For details on plant collection and establishment in the cells, see Biederman (1999).

Wastewater Mixing and Delivery

Three 1000-L polypropylene tanks, connected in parallel, served as the reservoir for the wastewater, which was identical to the synthetic low carbon (100 mg/L O.C.) wastewater used in the column study. Although intermittent due to mechanical and electrical problems, input of the low carbon wastewater feed was initiated in August 1999 and concluded in January 2001. Wastewater was mixed in batches of 3000 L at a time and delivered to the cells at a rate of 600 ml per 20 minutes (30 ml/min) via a dosing water delivery system. A flowrate of 30 ml/min corresponded to a hydraulic residence time of five days in each cell. Tanks were refilled every eight days with fresh wastewater, which was apparently subject to degradation by microbes residing in the holding tanks. Figure 4 shows how the sulfate concentration degraded over one eight-day emptying period in the summer when degradation was most significant.

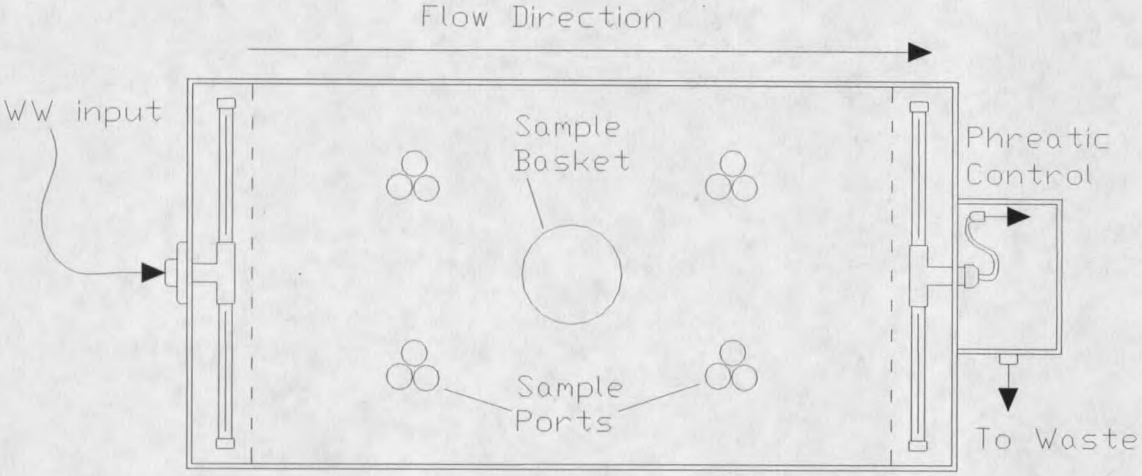

Figure 3. Plan view schematic of a continuous-flow constructed wetland cell (Biederman, 1999).

From the holding tanks, water flowed by gravity to a centrifugal pump and a pressurized 45-litre holding tank. Pressurized flow was delivered to eight 46-cm tall reservoirs, one for each cell, constructed from 8-cm PVC pipe capped on the bottom. The reservoirs were designed to fill to exactly 600 ml within 15 minutes. They drained

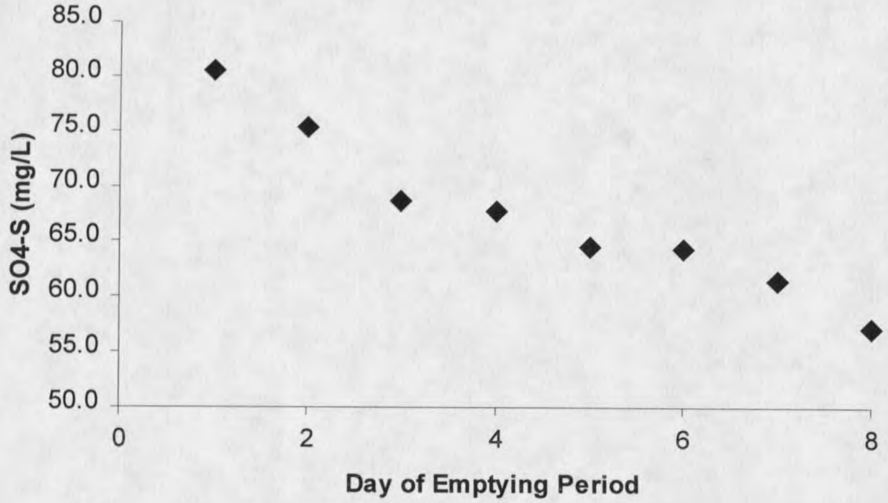

Figure 4. Degradation of influent sulfate in the holding tanks over an eight-day emptying period.

completely to each cell in a five minute period, thus delivering an average 30 ml/min (approximately continuous) flow rate.

A detailed description of the water delivery system, a circuit diagram, instructions on operation of the timer and specifications on the centrifugal pump are given in Appendix A.

Sampling and Analysis Procedures

Influent from three randomly chosen cells and effluent from all eight cells were sampled once per hydraulic residence time, every five days, and analyzed for sulfate and COD. Every twenty-five days, or every fifth hydraulic residence time, three randomly chosen influent samples and effluent samples from all eight cells were sampled for sulfate, COD, zinc and pH. Samples were collected and analyzed using the same methods as described for the batch-load system samples.

Statistical Analysis

Batch-Load System

Multivariate repeated measures analysis of variance (MANOVAR) was performed on sulfate data using SAS version 8.0 (SAS Institute, Inc., Cary, NC). Repeated measures analysis accounts for the fact that observations were taken on the same columns on different dates, and were not independent. Two analyses were conducted. The first analysis compared treatments within each incubation to determine effects of plant treatment and carbon concentration. The second analysis compared treatments across incubations to determine overall treatment differences over the year. In the first analyses, the sample size was $n=4$ and the number of sampling times over the 20-day incubation (number of dependent variables) was $p=6$. In the second case, the sample size was $n=4$ and the number of incubations was $p=8$. Potvin *et al.* (1989) demonstrate that MANOVAR analysis of time trends are valid only when the sample size, n, is equal to or greater than the number of dependent variables, p. To meet these requirements, only three of the six sampling days over the 20-day incubation were used in the first statistical analysis (Days 1, 5 and 20). To meet the requirements of MANOVAR for the second analysis, comparisons were made among four incubations, one from each season (Incubations 3, 5, 7 and 8). In all analyses, treatments were compared using planned contrasts. Differences were considered statistically significant at $p \leq 0.05$.

Continuous-Flow System

Analysis of variance (ANOVA) was performed using Minitab version 13.20 (Minitab, Inc., State College, PA) using a general linear model with day considered as a block effect. Within ANOVA, Bonferroni's method of pairwise comparison was used. Differences were considered statistically significant at $p \leq 0.05$. Statistical analysis was conducted on sulfate and zinc data only.

Comparison of Batch-Load and Continuous-Flow Systems

The two systems were compared graphically. Concentrations of sulfate, COD and zinc on Day 5 in the batch-load system were compared to effluent concentrations in the continuous-flow system for the same parameters. The days used for comparison from the continuous-flow system were an average of two days bracketing the sampling date in the batch-load system.

CHAPTER 4

PLANT SPECIES AND CARBON LOAD EFFECTS IN THE BATCH-LOAD SYSTEM

Results

The presence and species of plants, as well as carbon load, affected sulfate removal in the batch-loaded columns (Figure 5, Table 3), but both effects varied with temperature and time of year (Table 4). Table 4 shows treatment effects on overall performance and on time trends. "Overall effects" refers to statistical differences in treatment means over each 20-day incubation. "Time-trend effects" refer to statistical differences in the rate at which sulfate is reduced. Differences among planted columns and unplanted controls were not observed at 24°C, but differences were observed at 14°C (in the fall) and 4°C. Differences between high and low carbon loads were observed at 24°C, 14°C (in the fall) and 4°C (second winter). Patterns of plant and carbon loading effects were generally established in the first three days of each 20-day incubation and remained consistent over the entire period. To satisfy the requirements of repeated-measures multivariate analysis of variance (MANOVAR), only three of the six sampling days, Days 1, 5 and 20, were used for statistical analysis. Because trends were generally consistent over the 20-day incubation, this requirement did not bias statistical results.

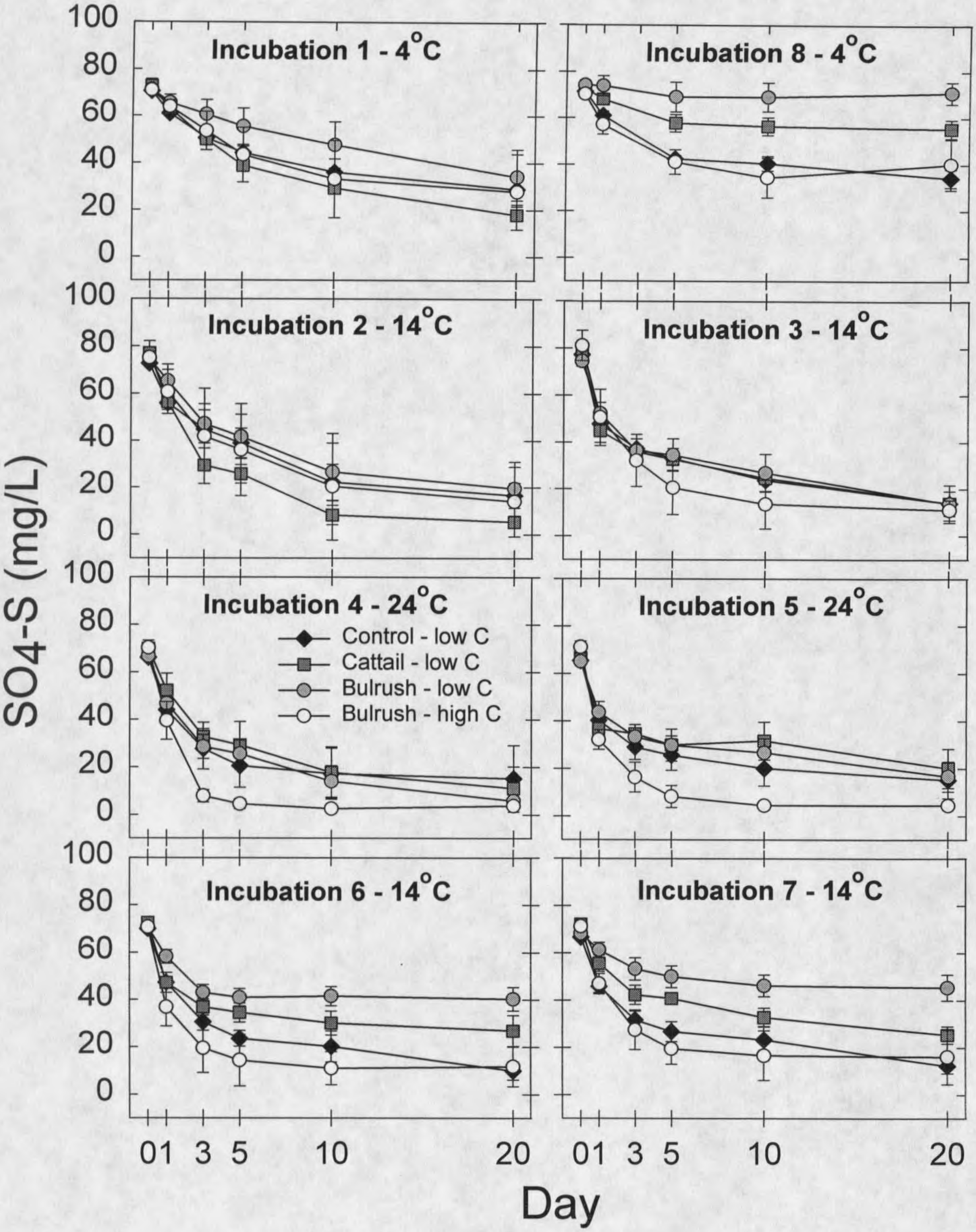

Figure 5. Sulfate concentrations over eight 20-day incubations. Symbols represent the mean of four replicates. Error bars represent one standard deviation of the mean. Symbols for day zero represent sulfate concentrations for samples taken immediately after filling the columns with fresh wastewater. Theoretical influent concentration = 67 mg/L SO_4-S.

Table 3. Percent sulfate removal for Incubations 1-8. Percentage values represent means of four replicates. Values in parenthesis represent one standard deviation of the mean. Values for Day 0 represent removals immediately after filling the columns with fresh wastewater.

Plant Treatment	**Incubation 1 - 4°C**	**Incubation 2 - 14°C**	**Incubation 3 - 14°C**	**Incubation 4 - 24°C**
	SULFATE REMOVAL (%)	SULFATE REMOVAL (%)	SULFATE REMOVAL (%)	SULFATE REMOVAL (%)
Day 0				
Control - low C	0 (0)	-4 (1)	-4 (3)	0 (1)
Cattail - low C	-4 (4)	-7 (7)	-6 (5)	-5 (5)
Bulrush - low C	-2 (1)	-3 (3)	-1 (3)	-1 (2)
Bulrush - high C	2 (2)	1 (1)	8 (8)	2 (2)
Day 1				
Control - low C	13 (2)	23 (3)	35 (1)	34 (3)
Cattail - low C	10 (4)	22 (5)	39 (7)	21 (11)
Bulrush - low C	7 (5)	10 (10)	29 (6)	30 (5)
Bulrush - high C	9 (4)	17 (13)	36 (15)	43 (11)
Day 5				
Control - low C	37 (4)	46 (9)	55 (4)	69 (14)
Cattail - low C	45 (2)	65 (6)	56 (6)	56 (15)
Bulrush - low C	21 (11)	42 (13)	53 (10)	62 (9)
Bulrush - high C	39 (16)	51 (27)	74 (14)	94 (3)
Day 20				
Control - low C	59 (10)	77 (7)	81 (7)	77 (21)
Cattail - low C	74 (9)	93 (2)	81 (7)	83 (13)
Bulrush - low C	51 (17)	73 (16)	82 (11)	91 (2)
Bulrush - high C	61 (23)	81 (20)	86 (5)	95 (1)

Table 3. Continued.

Plant Treatment	Incubation 5 - 24°C	Incubation 6 - 14°C	Incubation 7 - 14°C	Incubation 8 - 4°C
	SULFATE REMOVAL (%)	SULFATE REMOVAL (%)	SULFATE REMOVAL (%)	SULFATE REMOVAL (%)
Day 0				
Control - low C	1 (1)	-6 (1)	-1 (0)	-8 (1)
Cattail - low C	-1 (1)	-9 (2)	-9 (4)	-10 (1)
Bulrush - low C	2 (1)	-7 (1)	-3 (0)	-12 (2)
Bulrush - high C	4 (3)	1 (1)	1 (1)	1 (1)
Day 1				
Control - low C	39 (3)	29 (2)	31 (4)	8 (3)
Cattail - low C	45 (6)	29 (6)	16 (5)	-3 (3)
Bulrush - low C	34 (7)	12 (4)	8 (5)	-11 (6)
Bulrush - high C	54 (6)	47 (11)	33 (6)	18 (6)
Day 5				
Control - low C	61 (10)	64 (5)	60 (6)	36 (2)
Cattail - low C	55 (10)	48 (6)	38 (3)	13 (5)
Bulrush - low C	55 (6)	38 (5)	25 (7)	-4 (9)
Bulrush - high C	88 (7)	79 (15)	72 (9)	41 (8)
Day 20				
Control - low C	77 (12)	85 (5)	81 (5)	48 (6)
Cattail - low C	70 (13)	59 (9)	62 (5)	16 (6)
Bulrush - low C	75 (10)	39 (7)	31 (8)	-7 (7)
Bulrush - high C	94 (0)	83 (12)	77 (16)	43 (16)

Table 4. Within-incubation MANOVAR analysis for sulfate removal. Treatments with the same letter are not significantly different ($p < 0.05$). Letters a-d represent overall effects from best to worst. Letters w-z represent rate of removal from best to worst.

Plant Treatment	Incubation 1 - 4°C		Incubation 2 - 14°C	
	Overall Effects	Time Trend Effects	Overall Effects	Time Trend Effects
Control - low C	ab	wx	a	w
Cattail - low C	a	w	a	w
Bulrush - low C	b	x	a	w
Bulrush - high C	ab	w	a	w

Plant Treatment	Incubation 3 - 14°C		Incubation 4 - 24°C	
	Overall Effects	Time Trend Effects	Overall Effects	Time Trend Effects
Control - low C	a	x	b	w
Cattail - low C	a	x	b	x
Bulrush - low C	a	x	b	x
Bulrush - high C	a	w	a	w

Plant Treatment	Incubation 5 - 24°C		Incubation 6 - 14°C	
	Overall Effects	Time Trend Effects	Overall Effects	Time Trend Effects
Control - low C	b	x	b	x
Cattail - low C	b	x	c	y
Bulrush - low C	b	x	d	z
Bulrush - high C	a	w	a	w

Plant Treatment	Incubation 7 - 14°C		Incubation 8 - 4°C	
	Overall Effects	Time Trend Effects	Overall Effects	Time Trend Effects
Control - low C	a	x	a	w
Cattail - low C	b	x	b	wx
Bulrush - low C	c	y	c	x
Bulrush - high C	a	w	a	w

Plant Species Effects on Sulfate Removal

During the first five incubations of the study, significant overall and time trend differences between low-carbon treatments were generally not observed. Incubations 1 and 2 had similar overall and time trend treatment effects, with cattail performing best followed by control and bulrush-low (Figure 5), but with little significant difference (Table 4). All treatments typically improved sulfate removal in Incubations 3, 4 and 5 (Table 3), but improvement was most dramatic in the controls and least in the cattails.

In contrast to the first five incubations, overall and time trend differences among planted columns and unplanted controls at the low carbon load were highly evident in Incubations 6, 7 and 8 (Table 4 and Figure 5). All three low-carbon treatments differed significantly. Control columns performed best in all three incubations followed by the cattail columns. Bulrush-low columns consistently removed less sulfate than the other two treatments over the last three incubations. Time trends of sulfate removal were significantly different for all three treatments in Incubation 6 with bulrush-low columns having the least rapid sulfate removal followed by cattail and then by control. Bulrush-low columns had significantly less rapid sulfate removal compared to the cattail and control columns in Incubation 7. Control columns had significantly more rapid sulfate removal compared to bulrush-low columns in Incubation 8 but had similar time trends as the cattail columns.

MANOVAR was used to determine whether the observed sulfate removal differences among treatments were significant over the entire year. In order to satisfy the requirements of MANOVAR, Day 5 data was used from four representative incubations, one from each season (Incubations 3, 5, 7 and 8). Results indicated that control columns performed significantly better than bulrush-low columns (p=0.0029) and cattail columns (p=0.0455). Cattail columns tended to perform better than bulrush-low columns, but were not significantly different (Table 5). Treatment effects of time trends resulted in significantly less rapid sulfate removal for cattail (p=0.0269) and bulrush-low (p=0.0006) columns compared to control columns, but not to each other.

Table 5. Between-incubation MANOVAR analysis for sulfate removal. Treatments with the same letter are not significantly different ($p < 0.05$). Letters a-d represent overall sulfate removal effects from best to worst. Letters w-z represent rate of removal from best to worst.

Plant Treatment	All Incubations	
	Overall Effects	Time Trend Effects
Control – low C	b	X
Cattail – low C	c	Y
Bulrush – low C	c	y
Bulrush – high C	a	W

Carbon Load Effects on Sulfate Removal

Sulfate removal in the high organic carbon bulrush columns (influent COD = 534 mg/L) was compared to low-carbon bulrush, cattail and control columns (influent COD = 267 mg/L). Carbon load had little effect on overall sulfate removal in Incubations 1-3 but removal was more rapid with high carbon loading in Incubations 1 and 3 (Figure 5, Table 4). Results for Incubations 1 and 3 reflect that sulfate was removed more rapidly with high carbon from Days 1 to 5, but mean sulfate on days 1 and 20 were similar between high and low carbon loading. Bulrush-high columns began to perform better than all low-carbon treatments in Incubation 3. This trend continued in Incubations 4, 5 and 6 where bulrush-high columns performed significantly better than all low-carbon treatments. On Day 5, percent sulfate removal in bulrush-high columns averaged 94%, 88% and 79% for Incubations 4, 5 and 6, respectively, while percent sulfate removal averaged over all low carbon treatments was 62%, 57% and 50%, respectively (Table 3). Control columns began behaving similar to bulrush-high columns in Incubations 7 and 8. On Day 5, the average percent sulfate removal for bulrush-high and control columns was 57% and 48% in Incubations 7 and 8, respectively, while cattail and bulrush-low columns averaged 26% and 11%, respectively.

Differences in time trends for sulfate removal between bulrush-high columns and low-carbon treatments were observed in all incubations, except Incubation 2 (Table 4). Sulfate removal in the bulrush-high columns was significantly more rapid than in all the low-carbon treatments for Incubations 3, 5, 6 and 7. For both incubations at 4°C (1 and 8), sulfate removal was significantly more rapid in the bulrush-high columns compared to the bulrush-low columns but was not significantly different for bulrush-high columns compared to cattail and control columns.

Organic carbon (COD) for all low-carbon treatments in all incubations decreased to a residual value $\approx$ 35 mg/L COD by Day 3 and by Day 5 in the high-carbon treatment. COD never reached zero because of highly recalcitrant organic matter that does not oxidize readily (Biederman, 1999). COD removal was rapid and similar for all treatments in all incubations (Figure 16, Appendix B), therefore, further analysis of COD was not conducted.

Results for zinc will be discussed in Chapter 5. Comparisons of sulfate removal to redox potential will be given in the following discussion.

Discussion

Sulfate reduction, the process mainly responsible for sulfate and metals removal, is an oxidation-reduction (redox) reaction that cannot take place until more thermodynamically favorable electron acceptors have been depleted (i.e. oxygen, nitrate, manganese). Organic carbon, or another electron donor, must also be present for any of the redox reactions to take place. Redox values will vary based on a balance between the quantity and type of electron acceptors available and the quantity and type of organic carbon. In wastewaters with high sulfate concentrations and minimal electron acceptor concentrations of higher thermodynamic favorability, high Eh values, which indicate more aerobic conditions, are generally explained by diffusion of oxygen from the wetland surface and from plant roots (Reddy *et al.*, 1989). Low Eh values are explained by an absence of oxygen which, in turn, provides a thermodynamically favorable environment for microbially mediated sulfate reduction. For the synthetic wastewater feeds in this study, large quantities of sulfate were present without the presence of other more favorable electron acceptors except oxygen, suggesting that redox potential should have been in the zone for sulfate reduction unless oxygen interference was abundant. According to Table 2, if sulfate reduction was taking place, redox potential should have been between -50 mV and –200 mV. Comparisons of sulfate removal and redox potential will be discussed in the following sections.

Plant Species Effects on Sulfate Removal

In fall and the second winter (Incubations 6, 7 and 8), planted treatments performed significantly worse than the unplanted controls, but differences were not observed during the first winter, spring or summer (Incubations 1-5). These results generally agree with those from a high-COD, low-sulfate experiment conducted previously in the same batch-load system (Allen, 1999) in which, bulrush columns removed less sulfate than cattail or control columns at 4°C, but at 24°C no significant treatment differences were found.

Sulfate concentration and redox (Eh) for four incubations (3, 5, 7 and 8) representing 14°C-spring, 24°C-summer, 14°C-fall and 4°C-winter seasons for this study are shown in Figure 6. Measured redox potential generally correlates with measured decreases in sulfate. In spring and summer, Eh values were low and similar for all three low-carbon treatments and corresponded to high sulfate removal. Warm summer temperatures, 24°C, presumably increased microbial activity which, in turn, increased sulfate removal for all treatments. Cattail columns performed better in the spring than the control columns, possibly due to an increase in easily degradable plant organic matter. Living plant biomass typically peaks in the summer followed by a peak of standing dead biomass in the fall. This standing dead biomass turns to litter in the winter and spring, adding soluble organic carbon to the system in spring (Kadlec and Knight, 1996).

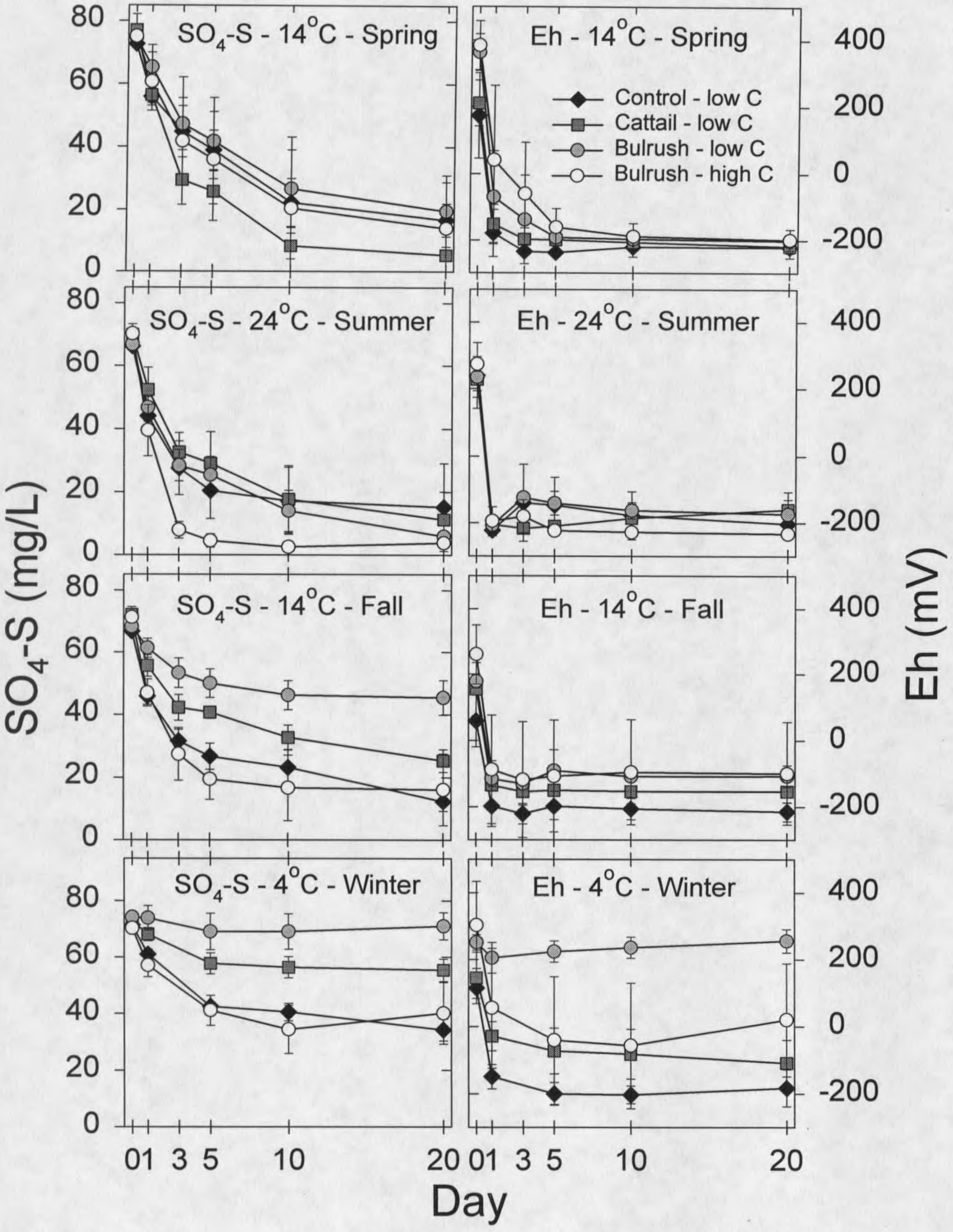

Figure 6. Sulfate concentration and root-zone oxidation over 20-day incubations for spring, summer, fall and winter. Symbols for sulfate and redox potential (Eh) represent the mean of four replicates. Error bars represent one standard deviation of the mean. Symbols for sulfate on day zero represent data taken immediately after filling the columns with fresh wastewater.

In the spring, when Eh values were low, the increased soluble organic carbon in the cattail columns could have allowed for increased sulfate reduction compared to control columns. Bulrush columns were likely providing soluble organic carbon to the wetland substrate during spring as well, although, not in quantities that affected sulfate removal. It is also possible that carry-over effects from a previous experiment allowed for increased sulfate reduction in the spring for cattail columns. This possibility will be discussed in detail in the following chapter.

In the fall (14°C), sulfate removal was significantly less for the two low-carbon planted treatments (bulrush and cattail) than for the unplanted controls (Table 4). Eh values were slightly higher for bulrush-low and cattail compared to control, although, not dramatically. Likely, root-zone oxidation was occurring but available oxygen was being consumed rapidly by aerobic respiration and therefore did not accumulate in the sediments, thus Eh remained low. Aerobic respiration likely consumed organic carbon that could have been used for sulfate reduction, reducing sulfate removal in the planted columns to less than that in the control columns. Callaway and King (1996) found indirect evidence that oxygen diffusion from the plant roots of cattail may be greater at cold temperatures than at warm temperatures. Allen (1999) found redox potential to be higher for bulrush than for cattail at colder temperatures. Plant roots tend to provide more oxygen to the substrate in the winter because root respiration is less, possibly allowing more oxygen to diffuse into the wetland substrate. In addition, Gersberg *et al.* (1985), as quoted by Kadlec and Knight (1996), demonstrated that bulrush species have the capability to provide more oxygen to the root zone (a higher plant aeration flux) than cattail species. The effect on sulfate reduction is consistent with these observations, although Eh did not provide as accurate a gage for evidence of root-zone oxidation in this case.

The possibility that plants were providing oxygen to the wetland system through their roots became apparent for sulfate removal and Eh at 4°C (Incubation 8). Sulfate removal in the bulrush-low columns was significantly less than in the cattail columns (Table 4), which was significantly less than control columns. Eh values reflected these observations. Eh values were >200 mV for bulrush-low columns, a value much greater than the maximum Eh value of -50 mV at which sulfate reduction will occur. A very small amount of sulfate reduction was observed in the bulrush-low columns, which probably occurred in lower redox microzones. Eh values for cattail were between -25 mV and -100 mV over the 20-day incubation. Controls varied from –150 mV to –200 mV. Taken together, the sulfate removal and redox results suggest that cattail and, especially, bulrush species are providing oxygen to the wetland substrate through their root zones during the winter. Only direct measurement of oxygen diffusion from plant roots could verify this.

Carbon Load Effects on Sulfate Removal

Significant differences in sulfate removal between the high and low carbon treatments in bulrush were not observed in the first three incubations but significantly more sulfate removal was observed in the bulrush-high treatment during the last five incubations. An increased carbon load should theoretically provide more carbon for both aerobic and anaerobic respiration processes. If oxygen diffusion from the wetland surface and plant roots is similar for both bulrush treatments with high and low carbon loads, and oxygen is depleted with the low carbon treatment, then the higher carbon load should increase sulfate reduction. However, several factors may confound this statement. First, researchers have suggested that oxygen diffusion from plant roots and the wetland surface is dependent on the sediment oxygen demand (i.e. carbon load). When sediment oxygen demand is increased, the sink for oxygen release from plant roots and the wetland surface is increased (Dunabin *et al*., 1988; Reddy *et al*., 1989; Sorrell and Armstrong, 1994), thereby decreasing the amount of organic carbon available for sulfate reduction. Second, although methanogenesis has a slightly lower thermodynamic favorability than sulfate reduction, the two processes occur simultaneously. Sulfate reducing bacteria (SRBs) compete with methanogenic bacteria (MPBs) for available organic carbon which can diminish sulfate reduction and subsequent metals removal. The competition outcome is dependent on (among other things) the ratio of COD:S, where high COD:S ratios (i.e. high carbon loads) create a favorable environment for methanogens to outcompete sulfate reducers (Fox and Ketha, 1996; Omil *et al*., 1998; Mizuno *et al*., 1994; Vroblesky *et al*., 1996). Researchers have found that for COD:S ratios < 1.5, sulfate reducing bacteria are dominant and for COD:S ratios > 6.0, methanogenic bacteria are dominant (Mizuno *et al*., 1994; Vroblesky *et al*., 1996). Bulrush-low columns had an initial COD:S ratio of 4.0 and the bulrush-high columns had an initial COD:S ratio of 8.0; however, sulfate reduction still occurred in both treatments. Possibly this is due to differences in experimental methods. COD:S ratios are based on uniform well-mixed slurries, whereas, the batch-load system may have microenvironments where SRBs are present and MPBs are not, allowing sulfate reduction to occur under unfavorable conditions.

Temperature also affects the competition outcome. SRBs can function at temperatures near 4°C, albeit, at slow rates while MPBs will not function at temperatures this low. MPBs perform best at temperatures near the optimum for mesophilic (30°C to 40°C) or thermophilic (50°C to 60°C) microorganisms, with deviations from these temperatures resulting in significant reductions in microbial activity (Leslie Grady *et al.*, 1999). Updegraff *et al.* (1998) found that methanogenesis increased sharply with temperature from 6°C to 30°C in two peat soils; fen and cedar swamp. Likely, methanogenesis plays a more important role in the warm summer incubations and has little influence in the cold winter incubations. Organic carbon may also be uptaken by plants and volatilized to the atmosphere, adsorbed to substrate surfaces and biofilms or simply volatized from the substrate to the atmosphere, depleting organic carbon for sulfate reduction.

The synthetic wastewater for this experiment should have been stoichiometrically limited by sulfate for both the high and low carbon loads. Only 134 mg/L COD would be required to reduce 200 mg/L sulfate compared to the 267 mg/L COD and 534 mg/L COD supplied, assuming sulfate reduction was the only oxidation-reduction process utilizing organic carbon. In actuality, organic carbon appeared to limit sulfate reduction, indicating that organic carbon was being consumed by other oxidation-reduction reactions such as aerobic respiration and methanogenesis.

The quantity of COD oxidized for bulrush-low and bulrush-high on Day 5 by sulfate reduction and by other oxidation-reduction processes for the eight incubations is shown in Figure 7. COD used for sulfate reduction averaged 70 mg/L for bulrush-high and 32 mg/L for bulrush-low. COD used for other processes averaged 470 mg/L for bulrush-high and 250 mg/L for bulrush-low. Clearly, most of the COD oxidized for both high and low carbon loads was used for processes other than sulfate reduction. Significantly more sulfate was reduced using the high carbon load in Incubations 4-8 but there was also a considerably higher amount of COD used for processes other than sulfate reduction as compared to low carbon load.

Though redox potential gives some indication as to whether oxygen diffusion is taking place, it cannot estimate oxygen diffusion as a function of carbon load. A redox potential value reflects the balance between the carbon added and the number and type of electron acceptors available for reduction. Conceptually, an increase in carbon load would be expected to decrease the redox potential; however, by increasing oxygen sink strength, an increase in carbon load also increases root zone oxygen leakage, potentially buffering the redox potential. Measured redox potential is influenced by electron acceptor delivery and consumption, which cannot be separated. However, inferences can be made by comparing redox potential values for the bulrush high and low carbon treatments to the low-carbon control treatment, since controls have no plant-mediated oxygen diffusion and presumably little total oxygen diffusion.

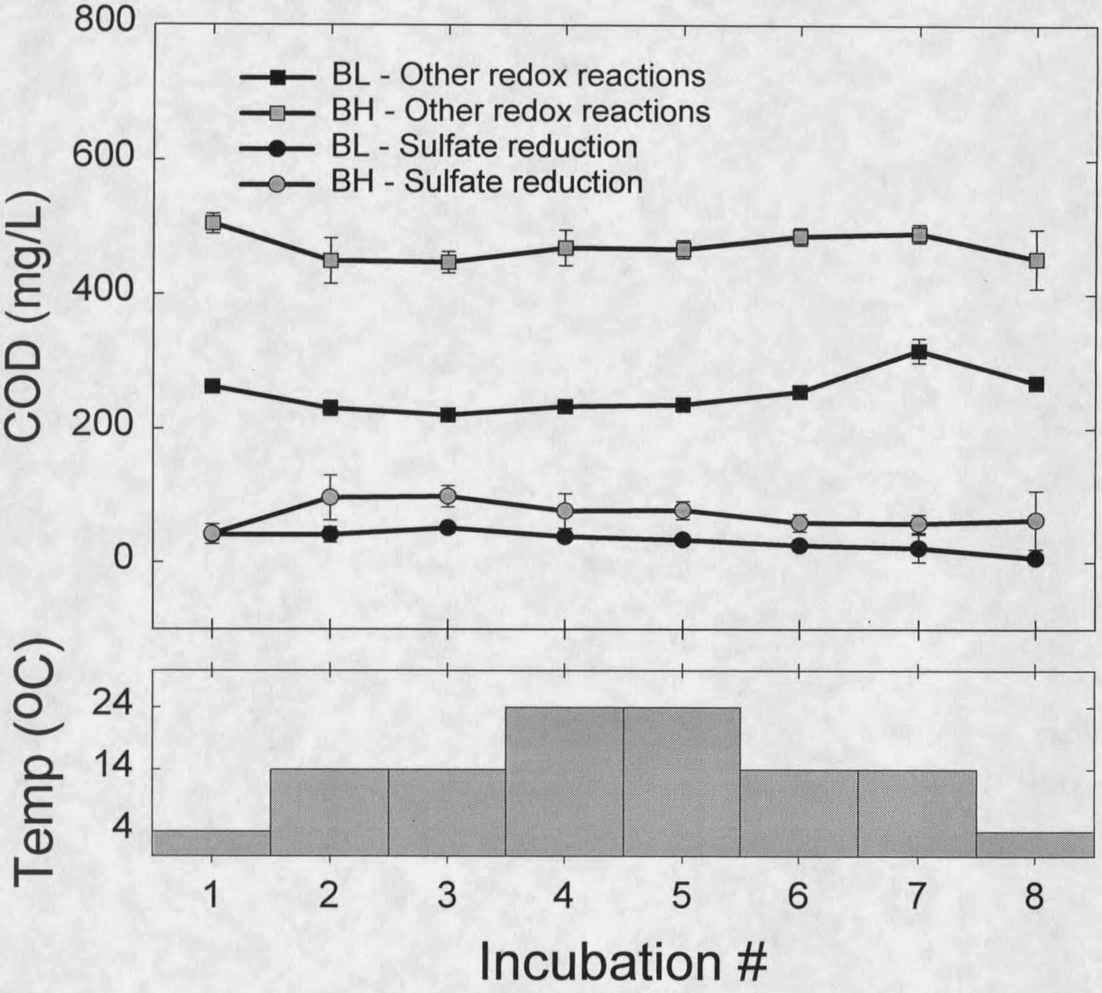

Figure 7. COD concentrations used for sulfate reduction and other redox reactions up to Day 5 for Incubations 1-8 in the bulrush-low and bulrush-high columns. Symbols represent the mean of four replicates. Error bars represent one standard deviation of the mean. Theortical influent concentrations for BL and BH were 267 mg/L and 534 mg/L COD, respectively.

In the spring and summer (14°C and 24°C) redox potentials in the bulrush (high and low) and cattail columns were not dramatically different than the control columns and sulfate removal was relatively high for all treatments (Figure 6) suggesting that oxygen diffusion did not interfere drastically with sulfate reduction. Methanogenesis and other organic carbon removal processes, not including aerobic respiration, were likely responsible for the additional COD consumed that was not used for sulfate reduction.

In the fall, redox potential was slightly higher for the bulrush-low and bulrush-high compared to cattail and control and sulfate removal was generally significantly different for all treatments. Likely, root-zone oxidation was occurring but the oxygen was being used rapidly for aerobic respiration and did not have a chance to accumulate in the substrate and raise the Eh significantly.

In the winter, the control treatments had lower redox potential than either bulrush or cattail treatment (Figure 6) suggesting that significant oxygen diffusion by bulrush and cattail was occurring. The increased oxygen diffusion was likely significant enough for aerobic respiration and oxygenation of the substrate. Redox potential was highly aerobic during the entire 20-day incubation for the bulrush-low columns and increased after day 10 for the bulrush-high columns, suggesting an even greater increase in oxygen diffusion in winter over fall in the bulrush treatments. In winter, bulrush oxygen diffusion was great enough to raise Eh and oxidize some sulfide back to sulfate after day 10. Bulrush-high columns performed statistically similarly to the control columns in Incubations 7 and 8 suggesting that the increased oxygen from bulrush species at cold temperatures consumed the extra carbon in the bulrush-high columns resulting in similar sulfate removal as the low-carbon controls (Table 4). Root-zone oxidation was considerably greater in the bulrush-high columns compared to the cattail columns raising redox potential above that in the cattails even though organic carbon concentration was twice as much for the bulrush-high. Although redox potential was lower for cattail, sulfate reduction was better for bulrush-high likely because higher organic carbon concentration effects outweighed effects of root-zone oxidation. In the fall and winter, aerobic respiration and other organic carbon removal processes, not including methanogenesis, were likely responsible for consumption of organic carbon not attributed to sulfate reduction. Despite an increase in COD used for other processes, sulfate reduction was more successful at the high carbon load versus the low carbon load for most incubations.

CHAPTER 5

TEMPERATURE AND SEASONAL EFFECTS IN THE BATCH-LOAD SYSTEM

Results

Temperature and season had significant effects on the overall performance of the batch-load system for sulfate reduction (Figure 8) and subsequent metal sulfide precipitation (Figure 9). Incubation 0, a preliminary incubation ran at 4°C for the purposes of practicing protocols and methods, was included in the seasonal sulfate and zinc removal graphs to extend the period of observation and better evaluate seasonal patterns. It was not included in the statistical analysis because methods and protocols differed slightly from other incubations. Zinc data was taken on Day 20 for Incubations 0 and 1 and on Day 5 for the rest of the incubations.

Sulfate Removal

The best removal for all treatments was generally observed at the warmest temperatures (Incubations 4 and 5) on all three days graphed, with the exception of cattail (Figure 8) which generally performed best in early spring (Incubation 2). Statements concerning comparisons between seasons are subjective and not based on a statistical

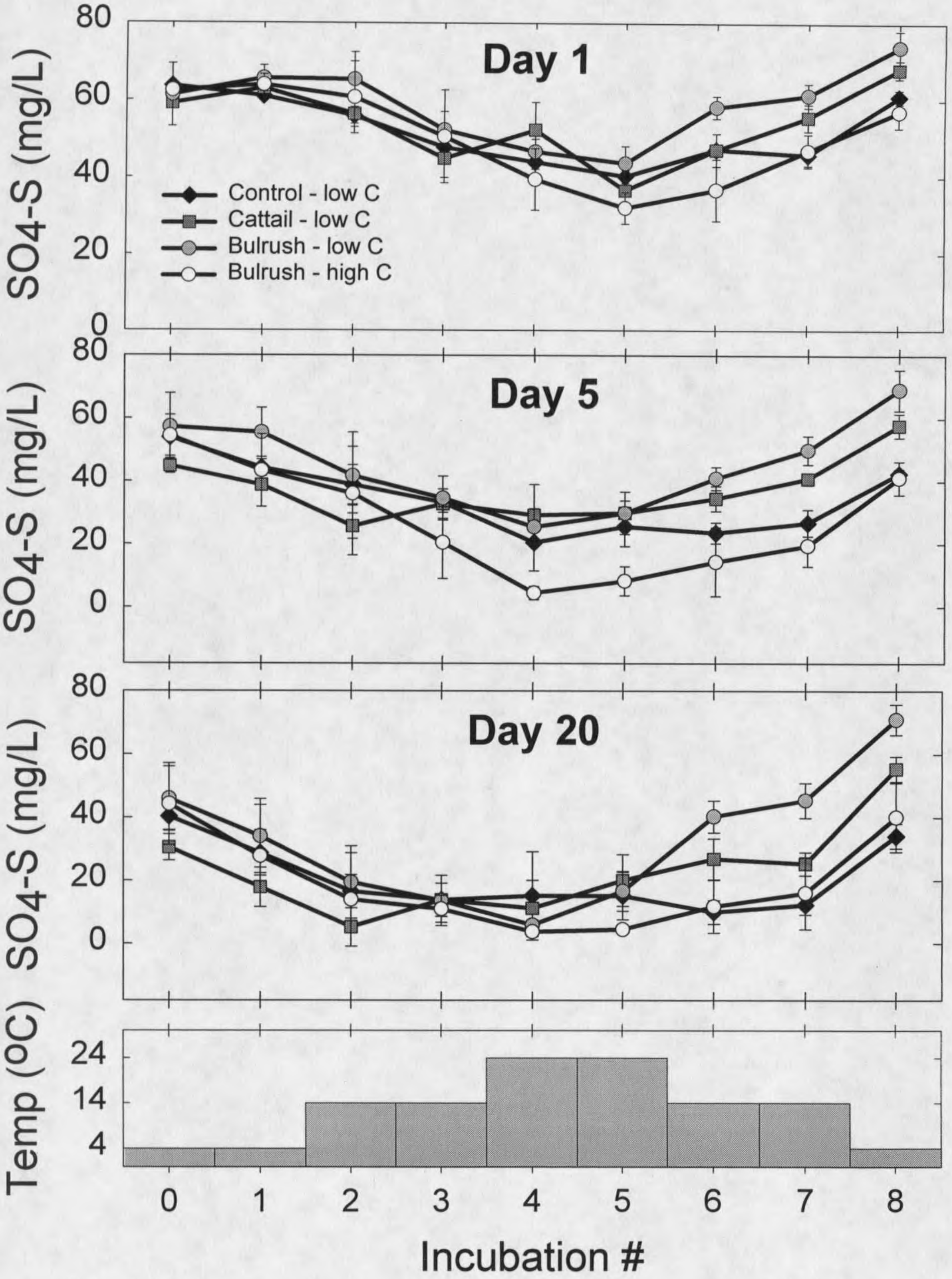

Figure 8. Sulfate concentrations for all incubations on Days 1, 5 and 20. Symbols represent the mean of four replicates. Error bars represent one standard deviation of the mean. Mean influent concentration was 67.7 mg/L.

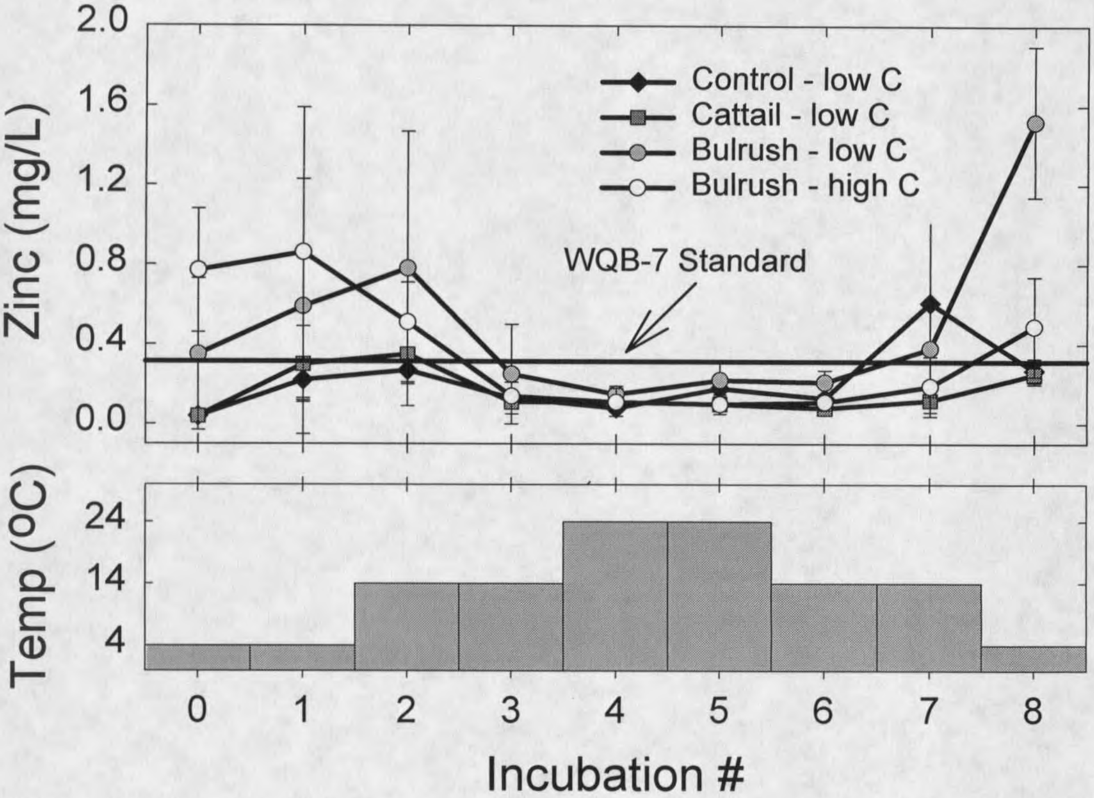

Figure 9. Zinc concentrations on Day 20 for Incubations 0 and 1 and on Day 5 for Incubations 2-8. Symbols represent the mean of four replicates. Error bars represent one standard deviation of the mean. Mean influent concentration was 24 mg/L. The horizontal line represents the Montana WQB-7 Water Quality Standard for zinc = 0.31 mg/L based on an average measured hardness of 302 mg/L as $CaCO_3$.

analysis. The worst removal for all treatments was generally observed at low temperatures, particularly during the second winter (Incubation 8).

Differences due to season were generally most dramatic in the bulrush-low columns and least dramatic in the control columns throughout the yearlong study. Control and bulrush-high columns had the most significant within-treatment variation on Day 5 while cattail and bulrush-low columns had the most significant within-treatment variation on Day 20.

Within each treatment, sulfate removal was different between first and second winters and between spring and fall (Figure 8). Cattail and bulrush-low treatments had better sulfate removal during the first winter (Incubations 0 and 1) and spring (Incubations 2 and 3) but control and bulrush-high treatments had better or similar sulfate removal in the fall (Incubations 6 and 7) and second winter (Incubation 8). Control columns tended to have similar removal for the same temperature in one season, whereas, planted columns frequently varied from incubation to incubation even during the same season (Figure 8). In general, variation from one incubation to the next was more dramatic at colder temperatures. The most dramatic change in sulfate removal occurred from Incubation 7 to Incubation 8 (14°C and 4°C) for all treatments on all three days.

Consistent between-treatments differences established during Incubations 0-2 were generally not repeated in the in the rest of the study. Between treatment differences were similar for Incubations 3-5, when low-carbon treatments performed similarly, and not as well, as the bulrush-high treatment. The last three incubations followed a different trend than the first five incubations, with all treatments generally performing differently. Control and bulrush-high columns had the highest sulfate removal.

Zinc Removal

Zinc concentrations, measured on Day 20 for Incubations 0 and 1 and on Day 5 for Incubations 2-8, were always less than 10% of influent values. Removal was best during the warmest incubations (Incubations 3-6) and averaged > 99%. During the colder months (Incubations 0-2, 7 and 8), removal tended to be less. The worst observed removal (93%) occurred in the bulrush-low treatment in Incubation 8. Average zinc removal for each treatment and incubation was compared to the Montana WQB-7 standard (Figure 9). The Circular WQB-7 Montana Water Quality Standards (MT DEQ, 1999) were adopted from the EPA National Recommended Water Quality Criteria for zinc (USEPA, 1999) and are based on hardness. The WQB-7 standard for zinc is 0.31 mg/L for water used in this study, based on an average measured hardness of 302 mg/L as $CaCO_3$. Average zinc removal for all treatments was in compliance with WQB-7 during warm Incubations 4-6. Bulrush treatments tended to be out of compliance during colder months. Control and cattail treatments were each out of compliance in one incubation over the year.

Discussion

Microbial sulfate reduction is enhanced by warmer temperatures and larger concentrations of carbon originating from influent sources or decayed plant material and is decreased by aerobic and methanogenic processes. Unplanted columns are affected by only temperature-dependent microbial activity, whereas planted columns also are affected by plant processes, which vary on a seasonal basis. In the spring and winter, organic carbon supply from decayed plant material may increase sulfate removal, while in the winter and fall, root-zone oxidation may decrease sulfate removal (Allen 1999; Callaway and King, 1996; Kadlec and Knight, 1996). Increases in microbial sulfate reduction tend to increase the amount of zinc removed as a metal sulfide. The following discussion will explore how microbial and plant processes were affected by temperature and season and how this affected sulfate and, subsequently, zinc removal in the microcosm constructed wetlands.

All treatments performed best at warm temperatures (Incubations 4 and 5) except for cattail, which performed best in the spring (Table 3, Figure 8). Better Zn removal in the summer was likely due to increased microbial sulfate reduction rates, as well as decreased root-zone oxidation (Summer Incubation, Figure 6). Sulfate removal was likely limited by carbon availability and not by microbial reaction rates. Increased sulfate reduction rates rapidly depleted available carbon, which presumably limited further sulfate removal. Since bulrush-high columns had more available organic carbon than the low-carbon treatments, these columns were not limited by carbon as quickly or may not have been limited by carbon at all, allowing sulfate removal to reach almost 100%.

In the fall (Incubations 6 and 7), sulfate removal was worse than in the summer but better than in the winter (Incubation 8) for all treatments (Table 3, Figure 8). Sulfate removal was the least during the winter. Decreasing temperatures from summer to fall and from fall to winter, decreased microbial sulfate reduction rates. Root-zone oxidation was beginning to take place in the fall and was significant in the winter, depleting available organic carbon for sulfate reduction in the planted columns, further contributing to lower sulfate removals (Figure 6). Evidence for carbon limitation and/or oxygen inhibition can be seen in the constant or increasing sulfate concentrations during the later stages of Incubations 6, 7 and 8. Sulfate removal in the control columns did not seem to be carbon limited during the fall and winter since root-zone oxidation was not a factor and therefore was not depleting available organic carbon, allowing sulfate removal to continue throughout the two fall incubations (Figure 5). Decreases in removal from summer to fall to winter in the controls were likely due solely to temperature-dependent decreases in sulfate reduction rates.

During the first winter and early spring (Incubations 0-2), sulfate removal was potentially affected by start-up and carry-over factors; therefore results from the first winter and early spring may not be representative of normal sulfate removal dynamics occurring during these seasons. Sulfate removal was generally greater in the first winter (Incubations 0-1) than the second (Incubation 8) for the low-carbon planted treatments and was generally similar or worse for the control and bulrush-high columns. Possibly, additional organic carbon was sequestered in the columns from a previous study by Allen (1999) which supplied 470 mg/L COD every 20-days for close to three years. The sequestered organic carbon could have provided additional electron donor, increasing removal in the low-carbon planted columns compared to the second winter. Sulfate removal over the 20-day period for Incubation 1 suggests that carbon was not limiting since removal was continuing for all treatments on Day 20 (Figure 5), compared to Incubation 8 where all treatments, except controls, were not removing sulfate after Day 10. All treatments, including bulrush-high columns, performed similarly to the controls in Incubation 1, suggesting that sequestered carbon may have overwhelmed the affects of winter root-zone oxidation and that the added carbon in the bulrush-high columns was insignificant since all treatments had sufficient carbon. An observation that confounds this explanation is that the control and bulrush-high columns tended to perform better in the second winter compared to the first but perhaps SRB populations were not completely established in the first incubations.

Incubation 2 in spring was probably affected by the sequestered organic carbon as between-treatment trends were similar in Incubations 0-2 (Figure 8). Trends changed in Incubation 3 to resemble trends in the summer, suggesting that Incubation 3 may not have been affected by carry-over affects. During the summer, evidence suggests that carbon was limiting and that all sequestered carbon had been consumed. If Incubation 3 was not affected by sequestered carbon, this incubation may more accurately describe spring sulfate removal dynamics.

From another viewpoint, differences between the first and second winters may have been solely due to the natural variation in the columns. Seasonal and temperature effects were still evident during the first winter, as seen by lower sulfate removal. The explanation for carry-over and start-up effects may explain differences between the first and second winters although this suggestion is just speculation and therefore, should not be relied upon too heavily.

Seasonal effects from plant processes tended to have more influence on the lower organic carbon planted columns. Within-treatment variation of sulfate removal between summer and winter was most dramatic in the bulrush-low columns, likely due to significant root-zone oxidation in the winter. All low-carbon treatments performed similarly in the summer, but as temperatures decreased in the fall, root-zone oxidation affected bulrush-low columns most dramatically, resulting in the least sulfate removal of all low-carbon treatments. Seasonal variation was observed in the cattail columns, but it was not as dramatic as the bulrush-low columns. Bulrush-high columns had a less dramatic difference between summer and winter compared to bulrush-low because the higher carbon load dampened the effect of root-zone oxidation in the winter. All planted treatments had a more dramatic change in sulfate removal from high to low temperatures compared to control columns. Control columns were not affected by root-zone oxidation; therefore, changes in sulfate removal from summer to winter were only a function of changes in temperature-dependent microbial reaction rates.

Many researchers have suggested that after system start-up, when adsorption is the dominant metal removal process, sulfate reduction and metal sulfide precipitation become the dominant process in wetlands for metals removal (Christensen *et al.*, 1996; Machemer and Wildeman, 1992; Wildeman *et al.*, 1993). Once sulfate reduction is dominant, sulfate removal should correlate with zinc removal. In this study, zinc removal did not always correlate with sulfate removal (Figure 8 and Figure 9).

At warm temperatures (Incubations 3-6), sulfate removal correlated well with zinc removal. Zinc concentrations were in compliance with Circular MT WQB-7 Water

Quality Standards for all treatments. Conversely, zinc removal did not always correlate with sulfate removal at cold temperatures. During the first winter, early spring and second winter (Incubations 0-2 and 8), the average zinc concentrations for bulrush-low and bulrush-high columns were higher than the WQB-7 standard of 0.31 mg/L (Figure 9). Figure 10 shows sulfate and zinc removed on Day 5 of Incubations 1-8. Equivalent or greater molar concentrations of sulfate must be removed for all zinc removal to be attributed to zinc sulfide precipitation. Sulfate removal was always greater than zinc removal except for bulrush-low in Incubation 8 when sulfate removal was not enough to attribute all zinc removal to zinc sulfide precipitation. In Incubation 8, on average, 17.25 mg/L of the total influent zinc concentration of 24 mg/L was removed by processes other than zinc sulfide precipitation. Based on zinc removal for the bulrush-low columns in Incubation 8, it seems possible that adsorption was playing the major role in removing most of the metals during the entire experiment but that sulfate reduction was removing the additional zinc necessary to meet WQB-7 standards. Decreased zinc removal for bulrush-low did correlate with decreased sulfate removal in Incubation 8 but did not correlate for bulrush-high, since sulfate reduction was satisfactory but bulrush-high columns were out of compliance in Incubation 8. Possibly, root-zone oxidation affected the chemical reaction between zinc and sulfide or the more aerobic environment reoxidized some sulfide, decreasing zinc sulfide precipitation in winter. Cattail columns performed better than all other planted treatments for zinc removal all year, possibly because of high organic matter concentrations from plant debris during colder months,

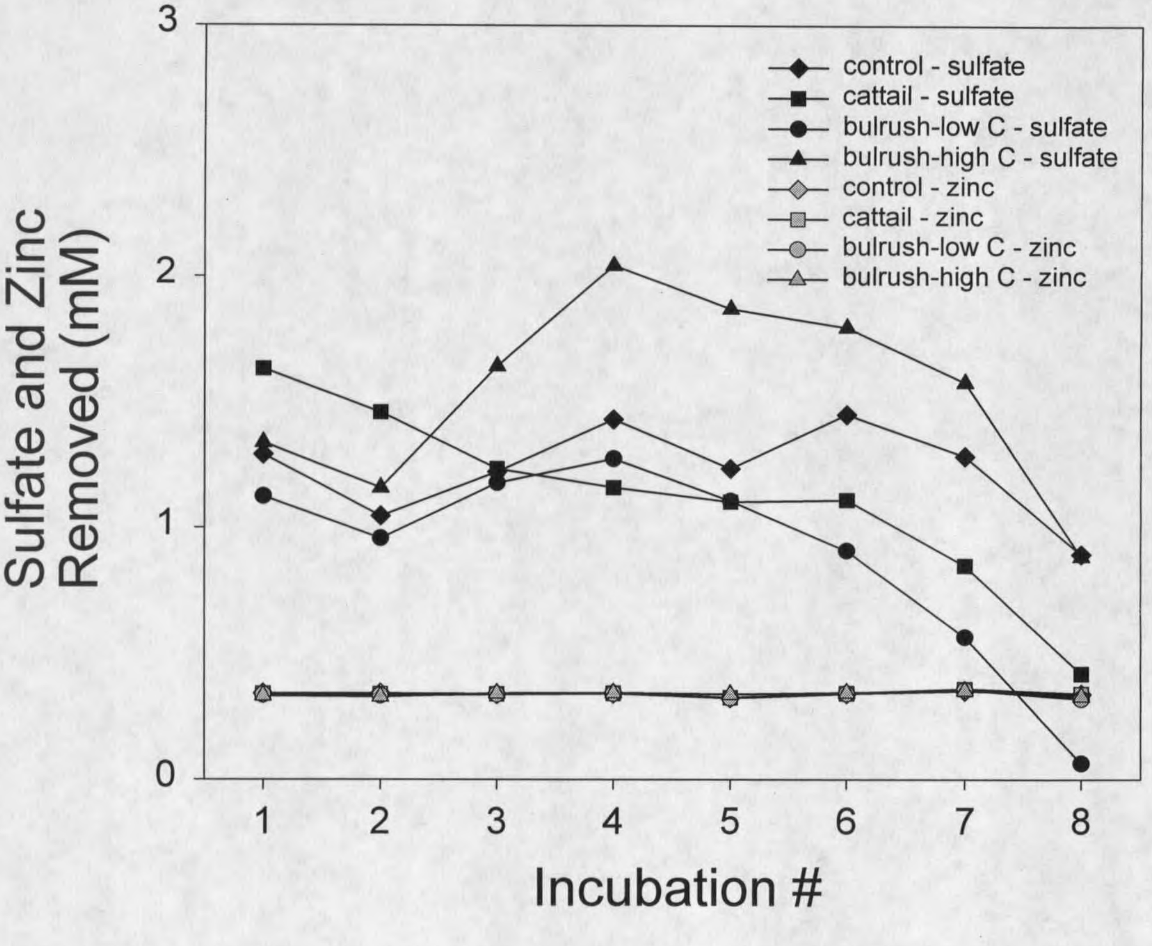

Figure 10. Molar concentration of sulfate and zinc removed by Day 5 for Incubations 1-8. Molar concentrations of sulfate equal to or greater than molar concentrations of zinc are necessary to precipitate all zinc as zinc sulfide. Symbols represent the average of four replicates.

which may have increased the adsorptive capacity for zinc when sulfate removal was fairly poor. Control columns generally performed well all year, since sulfate removal was sufficient even at cold temperatures.

Based on superior sulfate removal results, bulrush-high columns should have had superior zinc removal all year. In actuality, this was not the case suggesting that sulfate removal cannot always be used as an indicator of zinc removal. There was generally good zinc removal all year for the control columns at the low carbon concentration suggesting that there is a more positive impact on metals removal by removing plants than there is from raising the organic carbon concentration when plants are present. This statement may not be true for cattail plants since zinc removal was generally good all year.

CHAPTER 6

CONTINOUS-FLOW SYSTEM RESULTS AND COMPARISONS TO THE BATCH-LOAD SYSTEM

Results

The continuous-flow system (cells) was used to more accurately simulate full-scale wetland systems as compared to the smaller batch-load (columns) system. Treatment and seasonal comparisons can be made between cells and systems with influent COD = 267 mg/L. Differences between the systems aside from basic hydraulics are size, age and number of replicates. Data collection in the continuous-flow system was initiated in June 2000 and ended in January 2001.

Sulfate removal for all continuous-flow treatments demonstrated highly significant differences from each other in the order of cattail > control > bulrush (Table 6, Figure 11). Interestingly, zinc removal was in the order of cattail > bulrush > control with only controls demonstrating significant differences from the other two treatments (Table 6, Figure 12). At no time during the study did zinc concentrations meet the MT WQB-7 Standard for zinc.

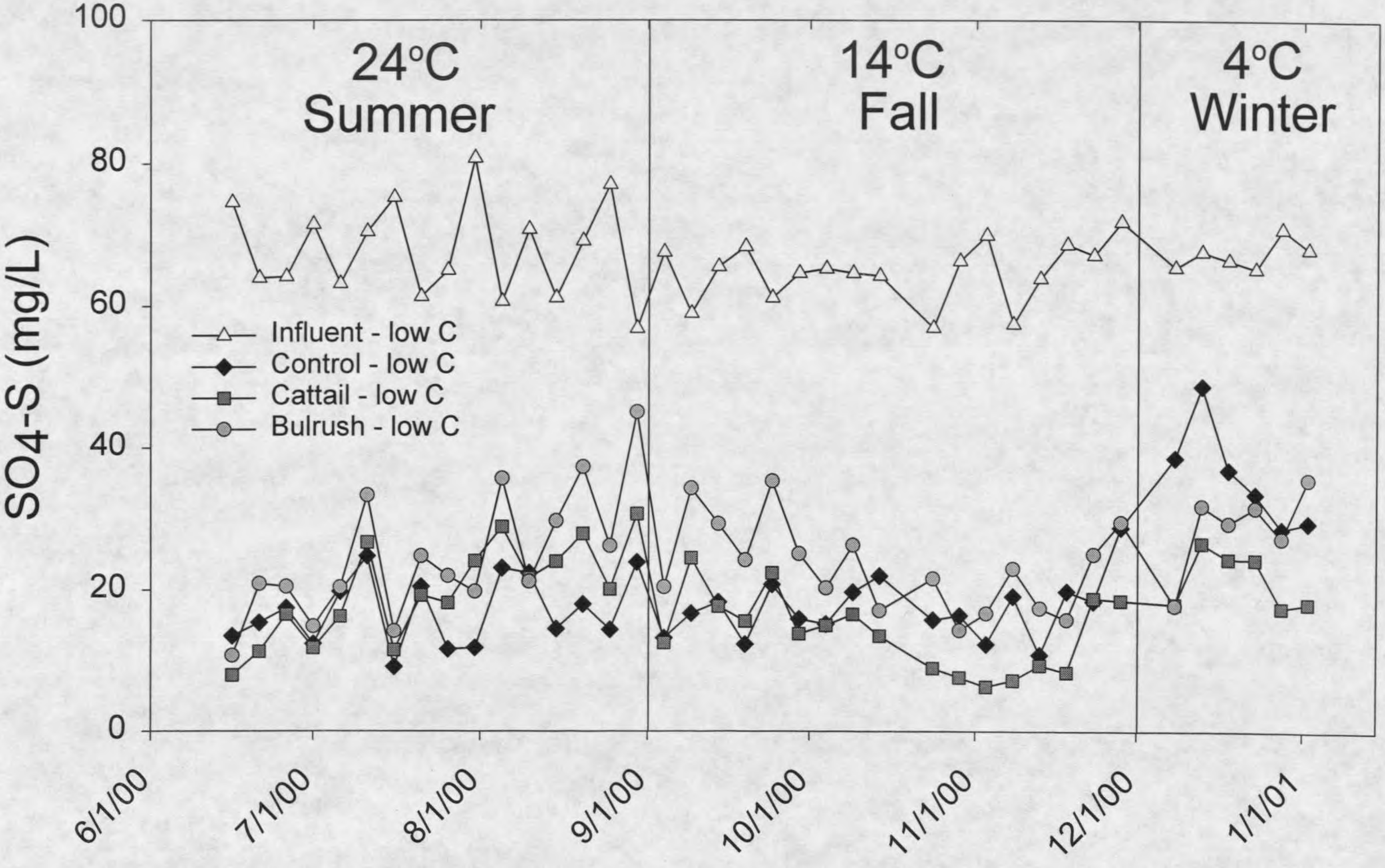

Figure 11. Mean influent and effluent sulfate concentrations for continuous flow cells. Influent concentrations are means of three random cells. Effluent concentrations for cattail and bulrush are means of three cells each. Effluent concentrations for control cells are means of two cells. Theoretical influent concentration = 67 mg/L SO_4-S.

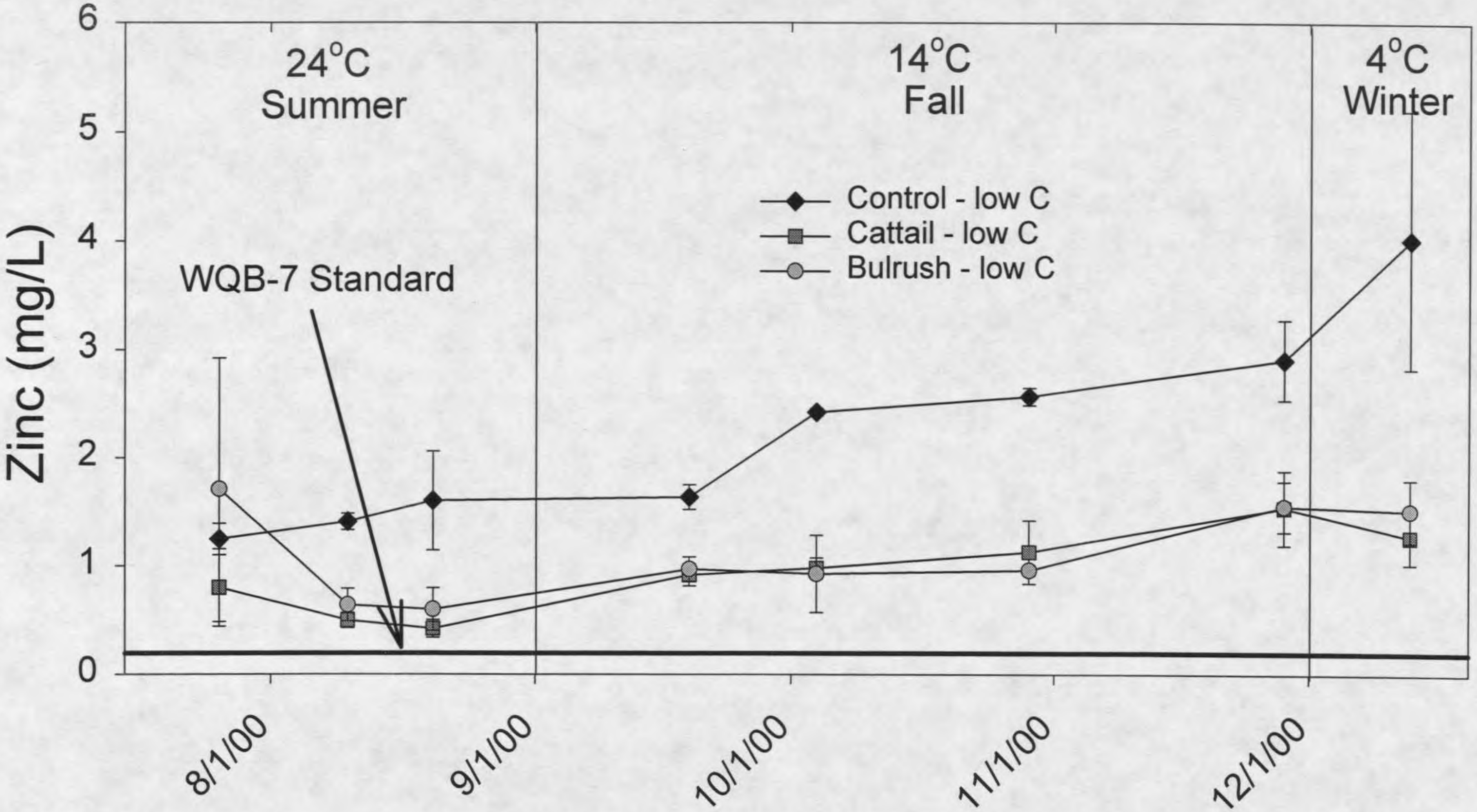

Figure 12. Mean effluent zinc concentrations for the continuous-flow cells. Effluent concentrations for cattail and bulrush are means of three cells. Effluent concentrations for control cells are means of two cells. Error bars represent one standard deviation of the mean. Theoretical influent concentration = 24 mg/L zinc. Horizontal line represents the Montana WQB-7 Standard for zinc = 0.19 mg/L based on an average hardness of 171 mg/L as $CaCO_3$.

Table 6. Percent sulfate and zinc removal and ANOVA analysis for the continuous-flow cells, overall, and in summer, fall and winter. Percentage values represent means of three replicates for cattail and bulrush and two replicates for control. Values in parenthesis represent one standard deviation of the mean. Treatments with the same letter are not significantly different ($p < 0.05$). Letters a-c represent removals from best to worst.

Plant Treatment	**Overall - Sulfate Removal**		**Summer - Sulfate Removal**		**Fall - Sulfate Removal**		**Winter - Sulfate Removal**	
Control - low C	70 (5)	b	75 (4)	a	73 (5)	b	47 (9)	b
Cattail - low C	73 (7)	a	70 (5)	a	78 (7)	a	68 (8)	a
Bulrush - low C	62 (7)	c	63 (7)	b	64 (6)	c	57 (9)	b

Plant Treatment	**Overall - Zinc Removal**		**Summer - Zinc Removal**		**Fall - Zinc Removal**		**Winter - Zinc Removal**	
Control - low C	88 (2)	b	93 (1)	b	87 (1)	b	82 (4)	b
Cattail - low C	95 (1)	a	97 (1)	a	94 (1)	a	94 (3)	a
Bulrush - low C	94 (2)	a	95 (3)	ab	94 (1)	a	92 (1)	a

Treatment differences for sulfate removal in the continuous-flow system varied with temperature and season. In the summer, treatment was more variable because influent sulfate concentration varied due to degradation in the holding tanks (Figure 4), as seen by the typical one residence time lag between changes in influent and changes in effluent (Figure 11). Fluctuations in effluent concentrations were less in the fall compared to the summer because fluctuations in influent concentrations decreased. Treatment effects on sulfate removal were not consistent over the study period. In the summer, controls performed best, whereas, in the fall and winter, cattails performed best. Sulfate removal was similar or better in the fall than in the summer for cattail and bulrush but was worse for control. Sulfate removal decreased for all treatments from fall to winter, although control cells had the most dramatic decrease.

Treatment effects on zinc removal were consistent across seasons (Figure 12). At all temperatures, cattail and bulrush treatments were statistically similar and significantly better than the controls (Table 6). For all treatments, performance decreased with decreasing temperature but this effect was most apparent in the controls.

Comparisons between batch-load (BL) and continuous-flow (CF) system performance are shown as scatter plots of the ratio of Day 5 concentration divided by influent concentration for sulfate, COD and zinc in Figures 13, 14 and 15, respectively. Comparisons are made for Incubations 4-8, representing summer through winter operation. The Day 5 column data was used for the comparison because the CF system had a hydraulic residence time of five days. To minimize influences of season, temperature and CF influent variation, averages of CF sampling dates bracketing the Day

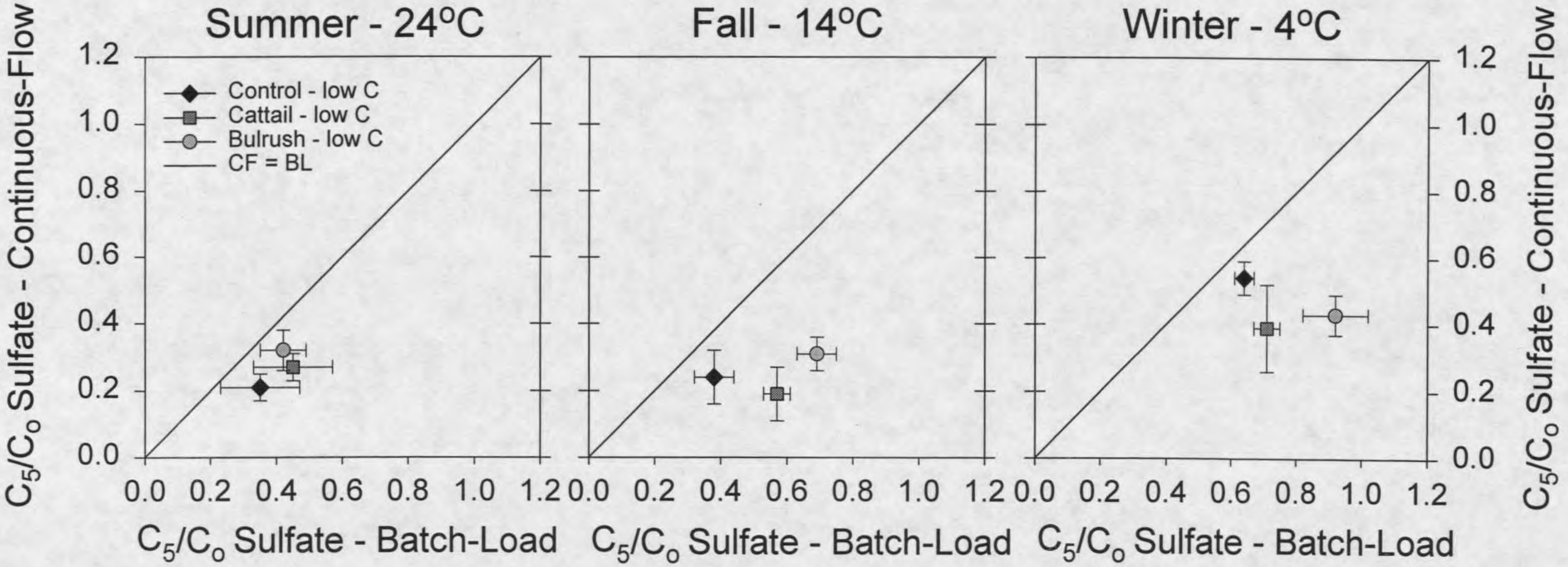

Figure 13. Fraction of sulfate remaining on Day 5 for the CF system compared to the BL system for summer, fall and winter. Error bars represent one standard deviation of the mean based on four (winter) or eight (summer and fall) replicates for the BL system and four (control, winter), six (planted, winter), eight (control, summer and fall) or twelve (planted, summer and fall) replicates for the CF system. Points below the diagonal 1:1 line represent greater sulfate concentrations for BL compared to CF.

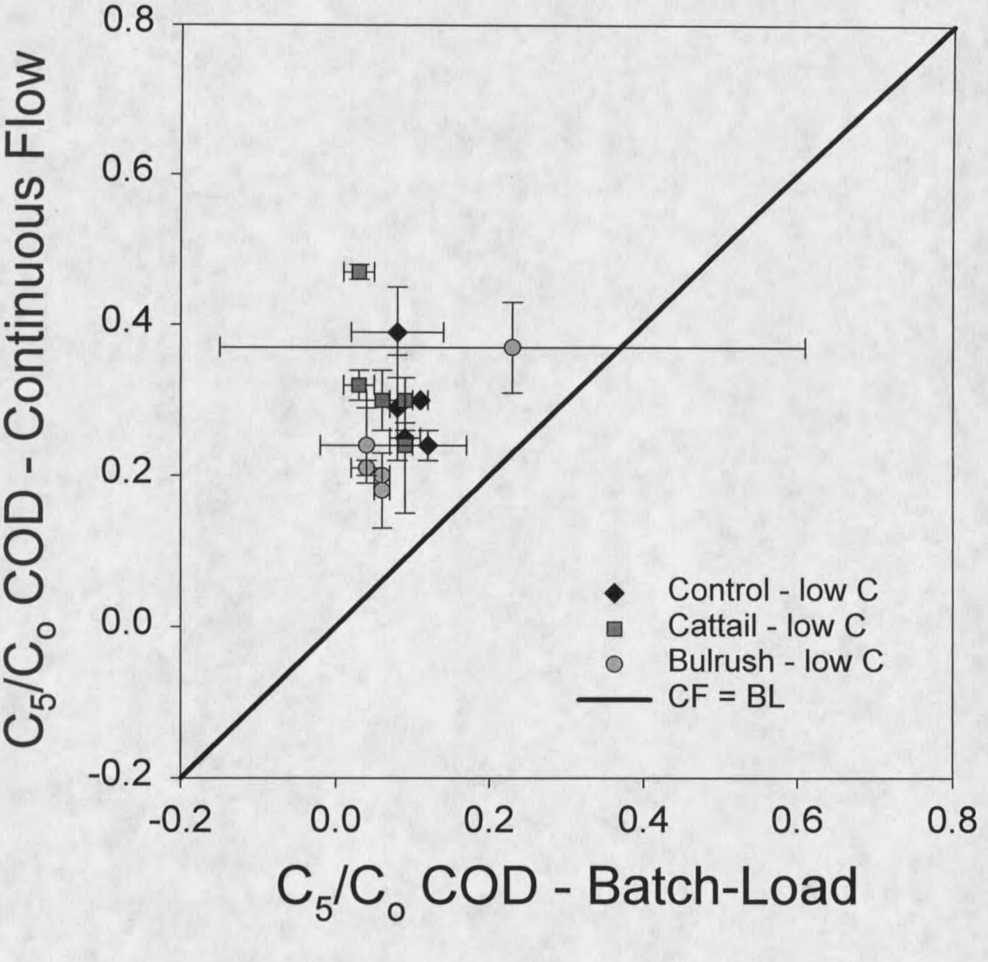

Figure 14. Fraction of COD remaining on Day 5 for the CF system compared to the BL system for five incubations. Error bars represent one standard deviation of the mean based on four replicates for the BL system and four (control) or six (planted) replicates for the CF system. Points above the diagonal 1:1 line represent greater COD concentrations for CF compared to BL.

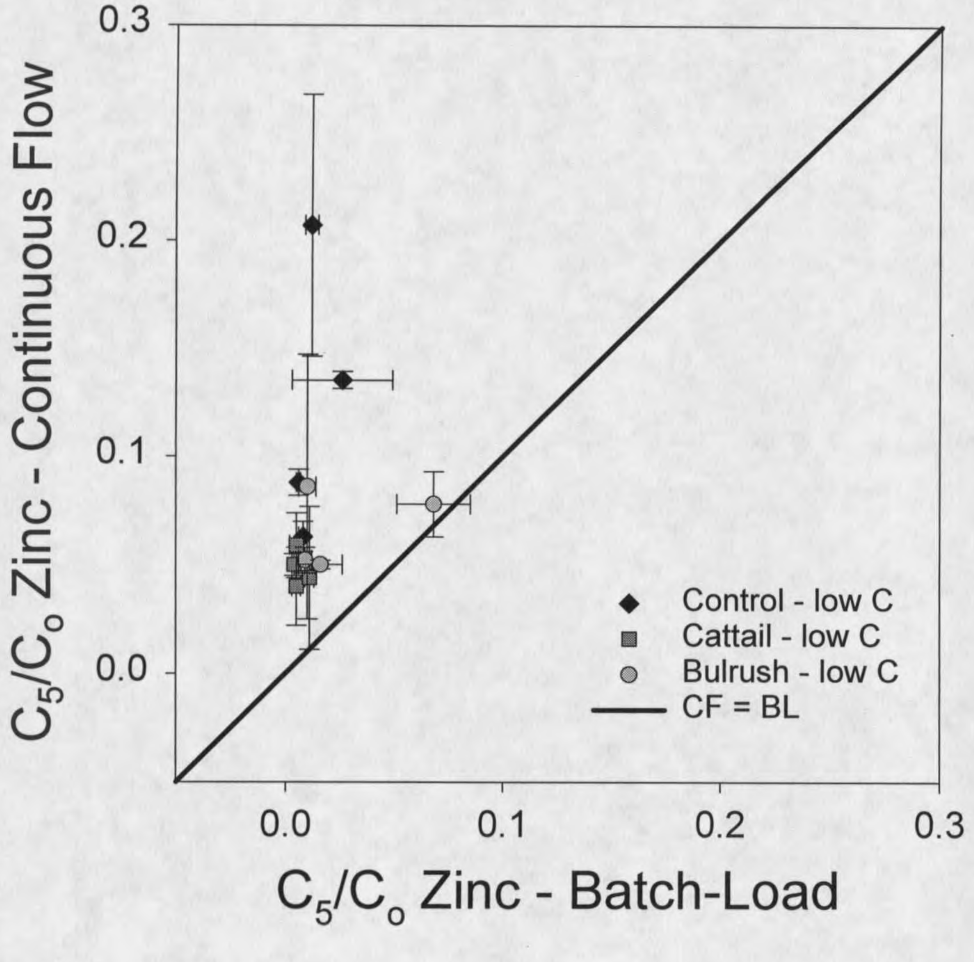

Figure 15. Fraction of zinc remaining on Day 5 for the CF system compared to the BL system. Error bars represent one standard deviation of the mean based on four replicates for the BL system and two (control) or three (planted) replicates for the CF system. Points above the diagonal 1:1 line represent greater zinc concentrations for CF compared to BL.

5 BL sampling date were used for sulfate and COD comparisons. Zinc was sampled less frequently; therefore, the CF sampling date closest to the Day 5 BL sampling date was used for comparison. Relative performance of sulfate removal between systems varied with season and treatment; therefore, comparisons were separated by season and values from Incubations 4 and 5 (summer) and 6 and 7 (fall) were combined to better illustrate the seasonal effect.

The CF system removed more sulfate than the BL system on Day 5 for all treatments and seasons (Figure 13). Both systems displayed a general decrease in sulfate removal with temperature and season, especially between fall and winter, but BL planted treatments were most responsive. No seasonal variations in relative performance of the BL and CF systems were apparent for COD and zinc removal; therefore, data were compared for each Incubation 4-8. Converse to sulfate removal, COD removal was always superior in the BL system (Figure 14). Zinc removal was also superior in the BL system with no discernable treatment trends in BL (Figure 15). Interestingly, zinc removal was the best for controls in the BL system, but was the worst for the CF system. More variability was observed in the CF system compared to the BL system with planted treatments typically removing more zinc than controls.

Discussion

Clearly the type of hydraulic loading has a dramatic and complex effect on wetland performance for metals removal and recommendations that would be based on results. BL columns always removed more COD and zinc but always less sulfate. The influence of plants in the CF system were dampened, in turn dampening temperature and seasonal variation compared to the BL system. BL control columns outperformed planted columns for sulfate removal in winter while CF planted cells outperformed control cells. In addition, the planted cells showed the most variability between systems, whereas control BL columns were more likely to behave like control CF cells at any season and temperature. Differing results from the two systems suggest that extrapolation of conclusions from the BL system may not describe what is happening in a different flow regime and/or on a larger scale.

There are several possible explanations for why hydraulic loading regime influences wetland performance. The interaction between loading rate, treatment and seasonal effects is of particular interest. Influent COD concentration was the same for all columns and cells compared, but total COD loaded to the CF system was six times that in the BL system over the study period. The CF system was continually loaded with 267 mg/L COD every five days for over a year for a total mass of COD per volume of wetland = 9.12 kg/m^3. The BL system was only loaded with low and high (534 mg/L) COD for nineteen batches over the year-long study for a total mass per volume of COD = 1.39 kg/m^3 and 2.78 kg/m^3 for low and high carbon concentrations, respectively. Likely, the larger mass load of COD per volume for the CF system contributed to increases in sulfate reduction and decreases in COD reduction compared to that found in the BL system.

Another possibility for increased COD removal and decreased sulfate removal in the BL system is that there was more oxidation of the gravel substrate in the BL system from the drain and fill cycling. Additional oxidation of the gravel substrate from the drain and fill cycle (BL) was suggested by Burgoon (1989). The increased availablity of thermodynamically favorable oxygen would outcompete sulfate for available organic carbon, increasing COD removal and decreasing sulfate removal in the BL system (Burgoon, 1991). If less oxygen was available in the CF system than in the BL system, then higher sulfate removal would be the expected result. This possibility was addressed during the previous study on the columns (Allen, 1999) and it was determined that this contribution of oxygen is fairly minor.

Another possibility, consistent with all results, may be that there is a positive interaction between the BL system and a plant's ability to oxygenate the rhizosphere. If true, controls would behave similarly in BL and CF while planted treatments in BL would demonstrate lower sulfate removal, due largely to a higher redox potential during winter operation compared to CF. An explanation of why BL would increase the plant's ability to oxygenate that wetland remains elusive. In reality, all the possibilities discussed may contribute to differences between BL and CF systems. Likely, the greater mass load of COD per volume is the major reason the CF system removes sulfate more efficiently than the BL system, but drain/fill oxygenation and plant mediated oxygenation also likely contribute to making the BL system poorer for sulfate removal.

Explanations as to why the BL system is better at zinc removal, while not as efficient at sulfate removal, are even more elusive. Either there is a zinc removal mechanism in the BL columns that does not exist in the CF system or is greatly reduced, or perhaps something in the CF system is inhibiting the precipitation of zinc when sulfide and zinc concentrations are in excess of their solubility product. Alternatively, zinc removal may have been better in the BL system because the CF system had reached adsorption capacity. The CF system was continually loaded with 24 mg/L of zinc every five days for over a year for a total mass of zinc per volume of wetland = 820 g/m^3. The BL system was only loaded with 24 mg/L zinc in nineteen batches over the year-long study for a total mass of zinc per volume = 125 g/m^3. Similar to COD, the total mass load of zinc in the CF system was more than six times that in the BL system suggesting that it is much more likely that adsorption sites were filled in the CF system. Based on conclusions in Chapter 4, zinc removal tends to be enhanced when adsorption and sequential sulfate reduction and metal sulfide precipitation are taking place concurrently. Adsorption may explain why zinc removal was better in the BL system even though sulfate removal was less. Yet another possibility is that the BL system, unlike the CF system, was shock loaded with wastewater on the first day of an incubation which may somehow influence zinc removal processes. Mechanisms responsible for observed differences between CF and BL systems require further research.

CHAPTER 7

CONCLUSIONS AND IMPLICATIONS

Conclusions

Plant Treatment Effects – Batch-Load System

Overall, control columns performed significantly better for sulfate removal than cattail and bulrush-low columns. Cattail columns performed better than bulrush-low columns, although not significantly. Root-zone oxidation from planted columns was most significant at cold temperatures. Bulrush-low columns provided the most plant-root oxidation, followed by cattail. Root-zone oxidation significantly decreased sulfate removal in the fall and winter in the planted columns.

Carbon Load Effects – Batch-Load System

Overall, bulrush-high columns performed significantly better for sulfate removal than all low-carbon treatments. In late fall and winter (Incubations 7 and 8) control columns performed similarly to bulrush-high columns suggesting that oxygen diffusion from plant roots was consuming the extra carbon available in the bulrush-high columns resulting in similar sulfate removal.

Temperature and Seasonal Effects – Batch-Load System

Sulfate and zinc removal were best in the summer for all treatments except cattail, which performed best in spring, and removal was the worst during the winter for all treatments. Differences in sulfate removal and subsequent zinc removal during the different seasons of the year may be attributed to (1) temperature-dependent microbial reaction rates, (2) seasonally-dependent quantity and quality of organic carbon from plant debris and (3) seasonally-dependent plant-root oxidation. Temperature and seasonal variation for sulfate removal was greatest in the planted treatments, especially in the bulrush-low columns, likely due to variation in root-zone oxidation. Temperature and seasonal effects were slightly dampened in the bulrush-high columns due to the additional carbon. Zinc removal was within the MT WQB-7 Water Quality Standards at warm temperatures (Incubations 3-6) for all treatments. Bulrush treatments tended to be out of compliance at cold temperatures. Cattail and control columns removed zinc successfully all year except in one incubation each when concentrations were out of compliance.

Batch-Load vs. Continuous-Flow

The continuous-flow (CF) system consistently removed more sulfate than the batch-load (BL) system, whereas the BL system consistently removed more COD and zinc than the CF system. Less sulfate removal in the BL system may be attributed to a lower organic carbon loading rate, oxidation from plant roots, oxidation from the drain/fill cycle and less organic carbon from plant debris. Better zinc removal in the BL system may be attributed to adsorption. Zinc removal in the CF system never met MT WQB-7 Standards.

Implications

- Based on data from the batch-load system, caution is warranted for using plants in constructed wetlands for metals removal where removal depends on sulfate reduction.
- Based on data from the continuous-flow system, plants improve zinc removal in constructed wetlands for metals removal where removal depends on sulfate reduction.
- Pros and cons of plants are not clear based on contrary behavior between the two systems.
- Based on batch-load data, a lower influent carbon concentration can be used when plants are not present.
- Preliminary tests should be conducted to determine appropriate influent carbon concentrations for successful sulfate reduction and zinc precipitation since insufficient organic carbon will decrease sulfate/zinc removal and grossly excess organic carbon will not significantly improve removal, rather, it will be used by other undesirable processes (i.e. aerobic and methanogenic processes).
- Design criteria should be based on sulfate and zinc removal efficiencies in the winter.
- Sulfate and zinc removal do not always correlate. Caution is warranted in extrapolating metals removal capabilities based on sulfate removal data.

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APPENDIX A

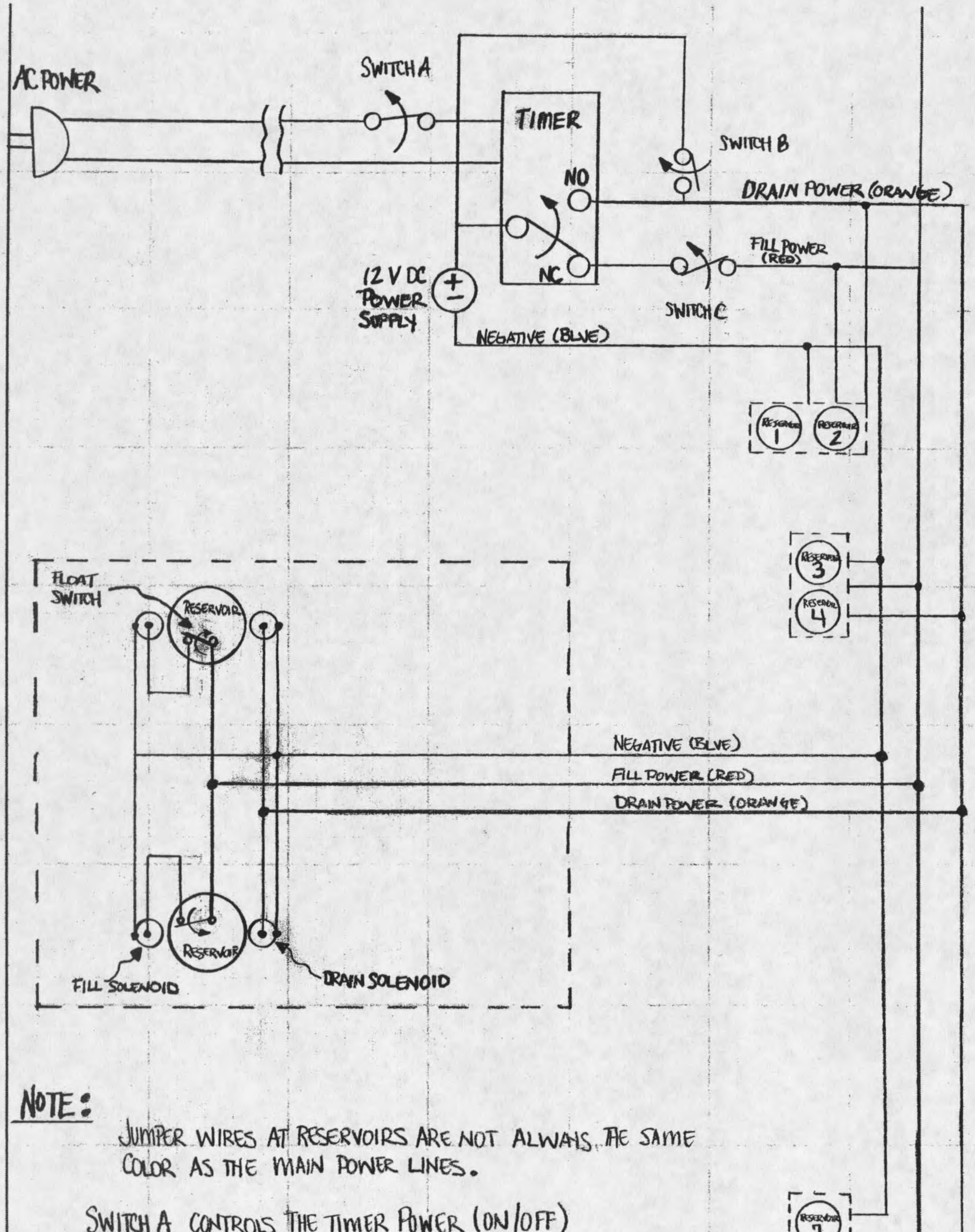
WATER DELIVERY SYSTEM

Detailed Description of Water Deliver System

From the three 1000 L holding tanks, wastewater was transported to a 12 gallon horizontal water tank (W.W. Grainger, Inc.) and Teel centrifugal pump, model number 3P577A, (W.W. Grainger, Inc., Lake Forest, IL) unit equipped with a 5-21 p.s.i ranged pressure switch (Square D Company, Palatine, IL). The storage tank was used to dampen pressure differentials so to minimize pumping intervals. A check valve was placed between the tank and the pump to ensure that the wastewater could not flow back towards the tanks. From the pump, water was distributed via ¼" PVC pipe to eight 18" tall reservoirs constructed from 3" PVC pipe capped on the bottom. Each reservoir was placed in a corner on the influent side of each cell and elevated 12" above the cell using cast-iron brackets. Each reservoir had an entry for incoming water and an exit at the bottom where water drained into the manifold of each cell via 3/8" vinyl tubing. There were 42-103 selector solenoid valves (Joseph Pollak Corp., Boston, MA) installed at the entrance and exit of each reservoir to control inflow and outflow from the cells. Each reservoir was equipped with a Miniature Vertical Liquid Level (float) Switch (Madison Company, Branford, CT). An adjustable timer, set on 20-minute intervals, was used to control when each solenoid valve would open and close. At time 0 of a 20-minute cycle, the inflow solenoid valve would open and the outflow solenoid valve would close. This allowed the reservoirs to fill. The correctly adjusted float switch would shut off flow to the reservoir when the correct volume had entered (600 ml) by closing the inflow solenoid valve. For this experiment, the pressure switch was adjusted to turn the pump on at 5 p.s.i. and turn it off at 7 p.s.i., therefore, if pressure in the water tank dropped below 5 p.s.i. during the filling process, the pressure switch would initiate the pump to start pumping until all reservoirs had been filled and pressure in the line had reached 7 p.s.i.. At this time, the pressure switch would shut the pump off. A maximum of 7 p.s.i. in the line was chosen because manufacturer specifications suggest that the solenoid valves can only handle a maximum pressure of 8 p.s.i. To ensure that the solenoid valves would function properly, a maximum of 7 p.s.i. was chosen. Once all reservoirs were filled, the timer would continue in the same position until 15 minutes had passed. At this time, the timer would open the drain solenoids (fill solenoids closed) and allow the 600 ml of wastewater to enter into the manifolds of the eight cells. The timer allowed the reservoirs to drain for 5 minutes, completing the 20-minute cycle. The timer would then close the drain solenoids and open the fill solenoids starting the next 20-minute cycle. By allowing 600 ml of wastewater to enter the cells every 20 minutes, this is equivalent to continuous flow of 30 ml/min, the desired flowrate.

The timer was equipped with manual switches which would override the automatic 20-minute cycle and allow for manual filling and draining of the reservoirs during wastewater changing or in case of malfunctioning. There are three switches on the timer box, switches A, B and C. Switch A controls the timer power (on/off). Switch B can manually turn (on/off) the drain cycle if switch A is off. Switch C can manually turn (on/off) the fill cycle if switch A is off. A 12 V DC power supply is used to power the timer, fill/drain solenoids and the float switches.

During wastewater changing, the system was taken off-line and bleach water was pumped through the ¼" PVC and into the reservoirs to keep the system clean and free of buildup on walls from biofilm. The lines were then flushed with plenty of fresh water before being put back on-line. Solenoid valves were cleaned using a syringe to prevent buildup of biofilm and to ensure proper function. The float switches were calibrated to 600 ml/min. Periodically, float switches were removed and cleaned.



NOTE:

JUMPER WIRES AT RESERVOIRS ARE NOT ALWAIS THE SAME COLOR AS THE MAIN POWER LINES.

SWITCH A CONTROLS THE TIMER POWER (ON/OFF)

SWITCH B CAN MANUALLY TURN (ON/OFF) THE DRAIN CYCLE

SWITCH C CAN MANUALLY TURN (ON/OFF) THE FILL CYCLE

Instructions for Timer Operation

Two times must be set in the timer, time to fill and time to drain. The timer is always running so it is important to set the time to fill while the drain time is running or vice versa. To set the fill time, hold the set button and the OFF button. To set the drain time, hold the set button and the ON button. Hold the appropriate buttons down until the desired time is displayed. If you overshoot the desired time, there is no reset button to start over. In order to get back to zero you must let the time go all the way to 99:99 then it will start back at 00:00.

It is very important to make sure that the timer is set for drain or fill during the time that the opposite is running. If, for example, you are trying to set the fill time and you are setting it while the drain time is running but then the running time switches over to fill, the time that you are on is the time that will be set for the fill time. There is no way to reset the time at this point and the timer will have to run to zero. This may be a long time. Shutting off the timer will only reset to the set time.

Specifications Information and Replacement Parts Manual **1P852, 1P853, 1P854, 1P952, 1P953 and 3P577A**

Please read and save this Replacement Parts Manual. Read this manual and the General Operating Instructions carefully before attempting to assemble, install, operate or maintain the product described. Protect yourself and others by observing all safety information. The Safety Instructions are contained in the General Operating Instructions. Failure to comply with the safety instructions accompanying this product could result in personal injury and/or property damage! Retain instructions for future reference.

Teel® Centrifugal Pumps

Refer to form 554042 for General Operating and Safety Instructions and applicable Warranty.

Description

Teel centrifugal pumps are designed to handle sediment-laden liquids and semi-solids up to 3/8" diameter. For use with nonflammable liquids compatible with pump component materials.

The pumps incorporate a dual volute design which allows pumps to self-prime (up to 20 ft. lift). Pump head is cast iron with semi-open clog resistant cast iron impeller. A Buna-N mechanical seal (comprised of ceramic seat, carbon head and stainless steel spring) isolates the motor from the pump housing. All units are manual models, no controls are supplied.

⚠WARNING ***Make certain that the unit is disconnected from the power source before attempting to service or remove any component!***

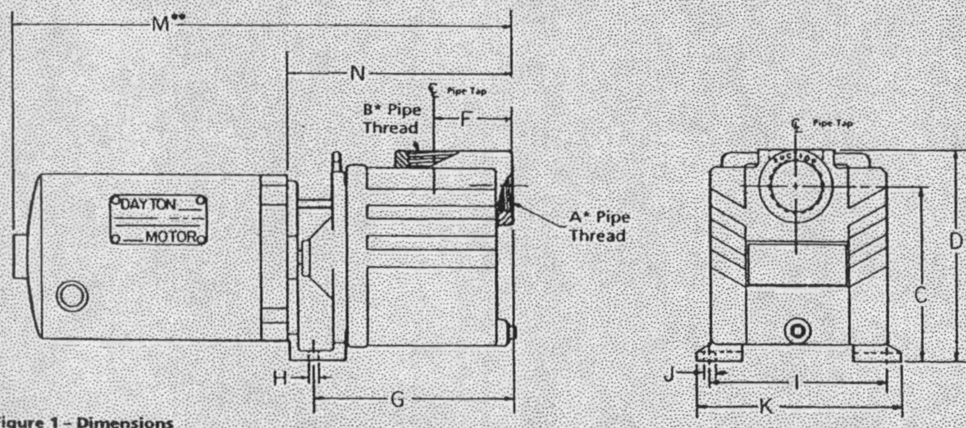


Figure 1 – Dimensions

Dimensions

Model	A*	B*	C	D	F	G	H	I	J	K	M**	N	Motor #	HP	Type
1P852	1 1/4"	1 1/4"	6 1/8"	7 5/16"	2 13/16"	6 7/8"	7/16"	6"	7/16"	7"	17 7/16"	7 1/8"	6K704	1/2	Capacitator Start
1P853	1 1/4	1 1/4	6 1/8	7 5/16	2 13/16	6 7/8	7/16	6	7/16	7	18 1/8	7 1/8	6K706	3/4	Capacitator Start
1P854	1 1/4	1 1/4	6 1/8	7 5/16	2 13/16	6 7/8	7/16	6	7/16	7	18 7/8	7 1/8	6K709	1	Capacitator Start
1P952	1 1/4	1 1/4	6 1/8	7 5/16	2 13/16	6 7/8	7/16	6	7/16	7	16 9/16	7 1/8	9N096	3/4	Three Phase
1P953	1 1/4	1 1/4	6 1/8	7 5/16	2 13/16	6 7/8	7/16	6	7/16	7	16 7/8	7 1/8	9N097	1	Three Phase
⇒ 3P577A	1	1	6 1/8	7 5/16	2 13/16	6 7/8	7/16	6	7/16	7	17 1/8	7 1/8	6K695	1/3	Capacitator Start

(*) Standard NPT pipe threads.

(**) This dimension may vary due to motor manufacturing specifications.

NOTE: Dimensions are ± 1/8".

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Teel® Centrifugal Pumps

Specifications & Performance

		Motor Data					GPM at Total Head in Feet						
Model	Port Size NPT	HP	Voltage	Phase	Thermal Protection	RPM	10'	20'	30'	40'	50'	60'	Max* Head
1P852	1¼"	1/2	115/230V, 60 Hz	1	Automatic	3450	4000	3600	3000	2200	1200	–	59 Ft.
1P853	1¼	3/4	115/230V, 60 Hz	1	Automatic	3450	4650	4300	3700	3000	2000	830	65
1P854	1¼	1	115/230V, 60 Hz	1	Automatic	3450	4900	4400	3800	3200	2450	1500	70
1P952	1¼	3/4	230/460V, 60 Hz#	3	None	3450	4650	4300	3700	3000	2000	830	65
1P953	1¼	1	230/460V, 60 Hz#	3	None	3450	4900	4400	3800	3200	2450	1500	70
3P577A	1	1/3	115V, 60 Hz	1	Automatic	3450	3000	2500	2000	1400	–	–	52

(#) Three phase motors are capable of operating on 50 Hz power, resulting in approx. 20% reduction in flow performance.

(*) Shutoff; to convert to PSI, divide by 2.31.

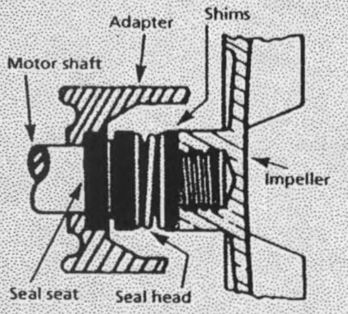

Figure 2 – Models 3P577A, 1P852, 1P853 & 1P854

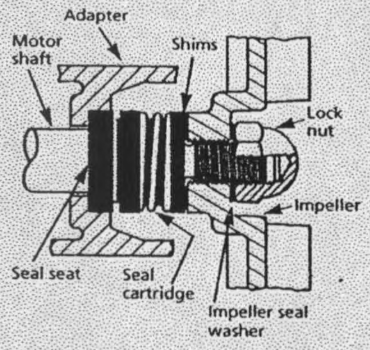

Figure 3 – Models 1P952 & 1P953

MECHANICAL SEAL REPLACEMENT

Should the mechanical seal, which consists of seal seat and seal head (Ref. No. 6), require replacement, proceed as follows and refer to Figures 2, 3, and 4.

When replacing mechanical seals in Models 1P952 & 1P953, a new impeller seal washer should also be installed.

IMPORTANT: Always replace both the seal seat and the seal head to ensure proper mating of mechanical seal components!

1. Unthread cap screws (Ref. No. 13) and remove pump casing (Ref. No. 11) from adapter (Ref. No. 3).
2. Unscrew impeller (Ref. No. 8) from the motor shaft.

NOTE: Some motors use an open end 7/16" wrench across the flats on the rear of the motor shaft (remove bearing cap for access) to prevent shaft from turning. Other motor shafts have a screwdriver slot instead of the flats.

   a. For Model Nos. 3P577A, 1P852, 1P853 and 1P854 - Unscrew impeller (Ref. No. 8) from the motor shaft.
   b. For Model Nos. 1P952 and 1P953 - Unscrew impeller nut (Ref. No. 10) and remove impeller seal washer (Ref. No. 9). Then unscrew impeller (Ref. No. 8) from the motor shaft.

3. Unthread cap screws (Ref. No. 4) and remove the adapter from the motor mounting face. The seal head and impeller shims (Ref. No. 7) will come loose at this time.
4. Push seal seat from the adapter recess with a screwdriver.
5. Clean the adapter recess before inserting a new seal seat.
6. Carefully wipe the ceramic surface of the new seal seat with a clean cloth.
7. Wet the rubber portion of the seal seat with a light coating of soapy water.
8. Press the new seal seat squarely into the cavity in the adapter. If the seal seat does not press squarely into the cavity, it can be adjusted in place by pushing on it with a piece of pipe. Always use a piece of cardboard between the pipe and the seal seat to avoid scratching the seal seat (this is a lapped surface and must be handled very carefully).
9. After the seal seat is in place, ensure that it is clean and has not been marred.
10. Using a clean cloth, wipe the shaft and make certain that it is perfectly clean.

Models 1P852, 1P853, 1P854, 1P952, 1P953 and 3P577A

MECHANICAL SEAL REPLACEMENT (Continued)

NOTE: If removed, slide flinger washer (Ref. No. 2) onto the shaft until it is located approximately 1/8" from the face of the motor bearing hub.

11. Secure the adapter (Ref. No. 3) on the motor mounting face. Carefully guide motor shaft through the seal seat.
12. Apply a light coating of soapy water to the inside rubber portion of seal head and slide onto the shaft (with the sealing face first) so that the rubber portion is just up over the shaft shoulder.

⚠ CAUTION ***Do not touch or wipe the face of the carbon (black) part of the seal head.***

13. Replace any impeller shims (Ref. No. 7) which may have been removed in disassembly. (See "Shim Adjustments".)
14. Screw the impeller (Ref. No. 8) back in place, tightening until it is against the shaft shoulder.
15. For Model Nos. 1P952 and 1P953 only: Replace the impeller seal washer (Ref. No. 9) and impeller nut (Ref. No. 10). Tighten until snug.

NOTE: A new impeller seal washer (Ref. No. 9) should be used every time this assembly is taken apart. Do not put this new washer in until the impeller has been shimmed properly. Use old washer for shimming purposes. Once shimming is finished, remove oid washer and put on new one and tighten.

16. Remount the pump casing (Ref. No. 11) on the adapter (Ref. No. 3). (See "Shim Adjustment," if motor or impeller was replaced.)

IMPORTANT: Always inspect the O-ring gasket (Ref. No. 5) for cracks or cuts when unit is disassembled. Replace if damaged.

SHIM ADJUSTMENT

When installing a replacement motor (Ref. No. 1), adapter (Ref. No. 3), impeller (Ref. No. 8), or casing (Ref. No. 11), it may be necessary to adjust the number of shims (Ref. No. 7) to ensure proper running clearance between the impeller and the casing volute. Proceed as follows:

NOTE: A proper running clearance is less than 0.010".

1. For component replacement other than the motor, add one 0.010" shim in addition to the shims removed originally.
2. For motor replacement add two shims 0.010" in addition to the shims removed during disassembly.
3. Reassemble the pump as described in steps 14, 15 and 16.

IMPORTANT: Insure that the pump housing is snugly in place and check the shaft to make sure it is turning freely (use the screwdriver slot in the motor to turn the shaft). If it turns freely, check to ensure that the adapter and pump casing are fitted "metal to metal" where they meet on the outside. If they are not "metal to metal," tighten the cap screws (Ref. No. 14) and recheck the shaft for free turning. Tighten carefully, turning the shaft while tightening so that the motor bearings are not damaged in the event that too many shims were installed. If shaft seizes before fasteners are completely tight, disassemble the pump and remove one (1) shim and repeat reassembly.

Limited Warranty

Dayton One-Year Limited Warranty. *Teel® Centrifugal Pumps, Models covered in this manual, are warranted by Dayton Electric Mfg. Co. (Dayton) to the original user against defects in workmanship or materials under normal use for one year after date of purchase. Any part which is determined to be defective in material or workmanship and returned to an authorized service location, as Dayton designates, shipping costs prepaid, will be, as the exclusive remedy, repaired or replaced at Dayton's option. For limited warranty claim procedures, see PROMPT DISPOSITION below. This limited warranty gives purchasers specific legal rights which vary from jurisdiction to jurisdiction.*

Limitation of Liability. *To the extent allowable under applicable law, Dayton's liability for consequential and incidental damages is expressly disclaimed. Dayton's liability in all events is limited to and shall not exceed the purchase price paid.*

Warranty Disclaimer. *Dayton has made a diligent effort to provide product information and illustrate the products in this literature accurately; however, such information and illustrations are for the sole purpose of identification, and do not express or imply a warranty that the products are merchantable, or fit for a particular purpose, or that the products will necessarily conform to the illustrations or descriptions.*

Except as provided below, no warranty or affirmation of fact, expressed or implied, other than as stated in the "LIMITED WARRANTY" above is made or authorized by Dayton.

Product Suitability. *Many jurisdictions have codes and regulations governing sales, construction, installation, and/or use of products for certain purposes, which may vary from those in neighboring areas. While Dayton attempts to assure that its products comply with such codes, it cannot guarantee compliance, and cannot be responsible for how the product is installed or used. Before purchase and use of a product, review the product applications, and all applicable national and local codes and regulations, and be sure that the product, installation, and use will comply with them.*

Certain aspects of disclaimers are not applicable to consumer products; e.g., (a) some jurisdictions do not allow the exclusion or limitation of incidental or consequential damages, so the above limitation or exclusion may not apply to you; (b) also, some jurisdictions do not allow a limitation on how long an implied warranty lasts, consequentially the above limitation may not apply to you; and (c) by law, during the period of this limited warranty, any implied warranties of implied merchantability or fitness for a particular purpose applicable to consumer products purchased by consumers, may not be excluded or otherwise disclaimed.

Prompt Disposition. *Dayton will make a good faith effort for prompt correction or other adjustment with respect to any product which proves to be defective within limited warranty. For any product believed to be defective within limited warranty, first write or call dealer from whom the product was purchased. Dealer will give additional directions. If unable to resolve satisfactorily, write to Dayton at address below, giving dealer's name, address, date, and number of dealer's invoice, and describing the nature of the defect. Title and risk of loss pass to buyer on delivery to common carrier. If product was damaged in transit to you, file claim with carrier.*

Manufactured for Dayton Electric Mfg. Co., 5959 W. Howard St., Niles, Illinois 60714 U.S.A.

Teel Specifications Information and Replacement Parts Manual

1P852, 1P853, 1P854,
1P952, 1P953 and 3P577A

For Replacement Parts, call 1-800-323-0620

24 hours a day – 365 days a year

Please provide following information:
-Model number
-Serial number (if any)
-Part description and number as shown in parts list

Address parts correspondence to:
Grainger Parts Operations
P.O. Box 3074
1657 Shermer Road
Northbrook, IL 60065-3074 U.S.A.

ENGLISH

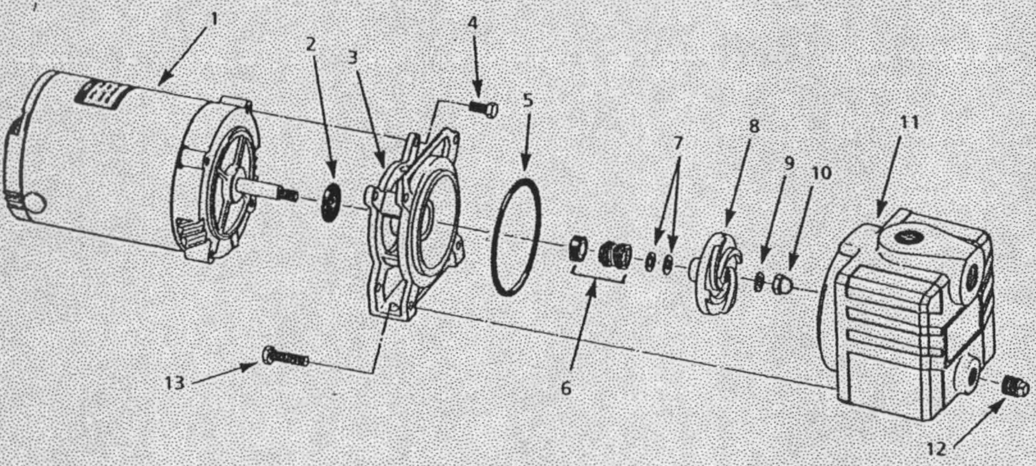

Figure 4 – Replacement Parts Illustration

Replacement Parts List

Ref. No.	Description	Part Number For Models: 1P852	1P853	1P854	1P952	1P953	3P577A
1	Motor	6K704	6K706	6K709	9N096	9N097	6K695
2	Slinger washer	1534-000-00	1534-000-00	1534-000-00	1534-000-00	1534-000-00	1534-000-00
3	Adapter	1560-000-01	1560-000-01	1560-000-01	1560-000-01	1560-000-01	1560-000-01
4	3/8-16 x 3/4" Hex hd cap screw (4 req.)	*	*	*	*	*	*
5	O-ring gasket	1531-000-00	1531-000-00	1531-000-00	1531-000-00	1531-000-00	1531-000-00
6	Shaft seal assembly, Buna-N	1R303	1R303	1R303	1R303	1R303	1R303
	† Shaft seal assembly, Viton	1R300	1R300	1R300	1R300	1R300	1R300
7	Shim package, 0.010, 0.020, 0.030 (1 of each)	1657-000-90	1657-000-90	1657-000-90	1657-000-90	1657-000-90	1657-000-90
8	Impeller	1537-000-01	1537-000-02	1538-000-01	1541-000-03	1542-000-01	1535-000-01
9	Impeller seal washer	–	–	–	1471-020-00	1471-020-00	–
10	Impeller nut	–	–	–	1784-000-00	1784-000-00	–
11	Casing	1558-000-02	1558-000-02	1558-000-03	1558-000-02	1558-000-03	1558-000-01
12	1/2" NPT Drain plug	*	*	*	*	*	*
13	3/8-16 x 2" Hex hd cap screw (4 req.)	*	*	*	*	*	*

(*) Standard hardware item, available locally.

(†) Optional.

Manufactured for Dayton Electric Mfg. Co.
Niles, Illinois 60714 U.S.A.

TEEL®

APPENDIX B

COD DATA

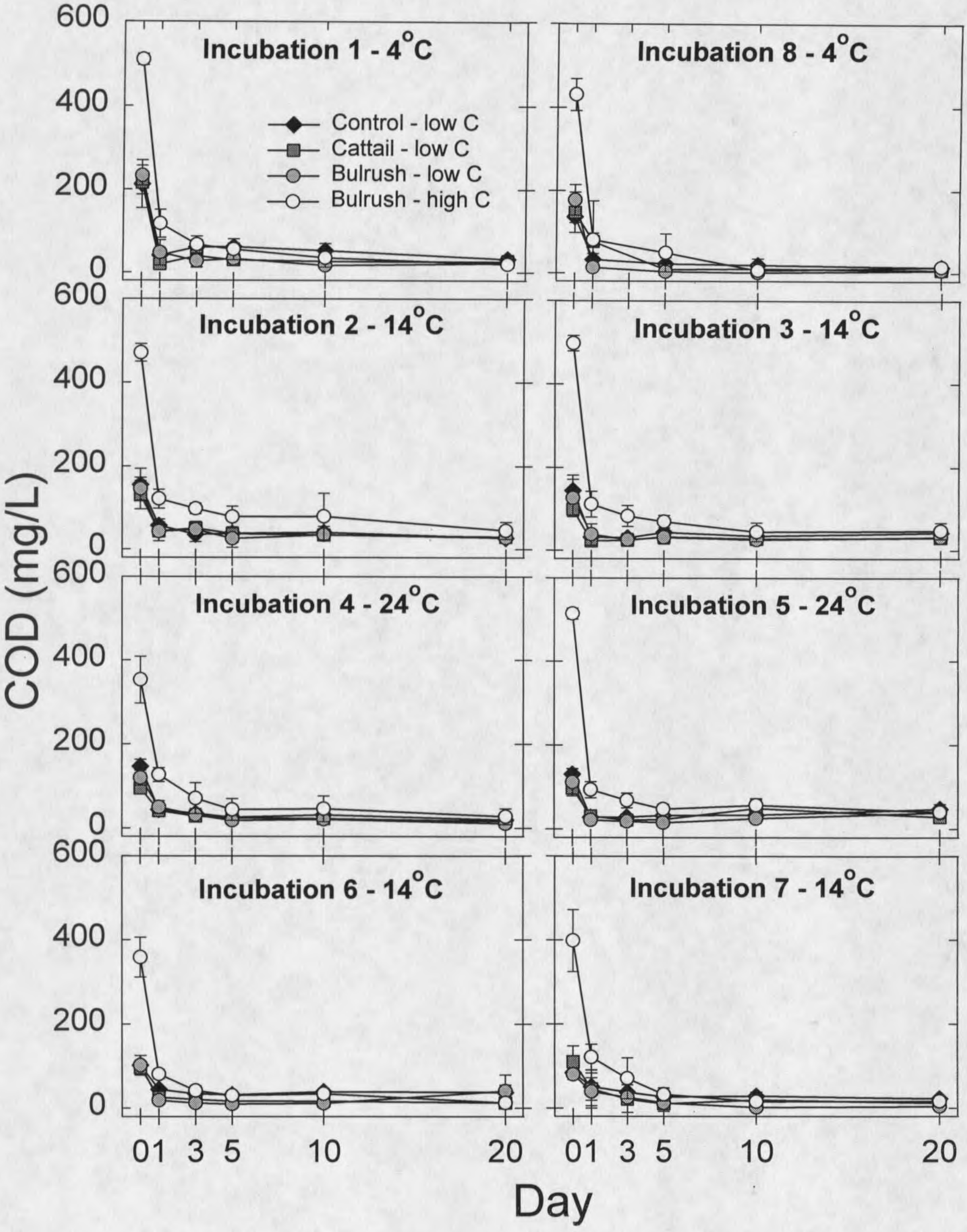

Figure 16. COD concentrations over eight 20-day incubations. Symbols represent the mean of four replicates. Error bars represent one standard deviation of the mean. Symbols on day zero represent COD concentrations for samples taken immediately after filling the columns with fresh wastewater. Theoretical influent concentration = 267 mg/L COD and 534 mg/L COD for low and high carbon, respectively.