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Seasonal influence on sulfate reduction and zinc sequestration in subsurface treatment wetlands

Otto R. Stein[a,b,*], Deborah J. Borden-Stewart[c], Paul B. Hook[b,d], Warren L. Jones[a,b]

[a]Department of Civil Engineering and Center for Biofilm Engineering, Montana State University Bozeman, MT 59717-3900, USA
[b]Center for Biofilm Engineering, Montana State University Bozeman, MT 59717-3900, USA
[c]Geosyntec Consultants, 55 SW Yamhill St, Portland OR 97204, USA
[d]Intermountain Aquatics, Inc, 85 S. Main St, Driggs, ID 83422, USA

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ABSTRACT

To characterize the effects of season, temperature, plant species, and chemical oxygen demand (COD) loading on sulfate reduction and metals removal in treatment wetlands we measured pore water redox potentials and concentrations of sulfate, sulfide, zinc and COD in subsurface wetland microcosms. Two batch incubations of 20 day duration were conducted in each of four seasons defined by temperature and daylight duration. Four treatments were compared: unplanted controls, *Typha latifolia* (broadleaf cattail), and *Schoenoplectus acutus* (hardstem bulrush), all at low COD loading (267 mg/L), plus bulrush at high COD loading (534 mg/L). Initial SO_4-S and zinc concentrations were 67 and 24 mg/L, respectively. For all treatments, sulfate removal was least in winter (4 °C, plant dormancy) greatest in summer (24 °C, active plant growth) and intermediate in spring and fall (14 °C), but seasonal variation was greater in cattail, and especially, bulrush treatments. Redox measurements indicated that, in winter, plant-mediated oxygen transfer inhibited activity of sulfate reducing bacteria, exacerbating the reduction in sulfate removal due to temperature. Doubling the COD load in bulrush treatments increased sulfate removal by only 20–30% when averaged over all seasons and did not alter the basic pattern of seasonal variation, despite tempering the wintertime increase in redox potential. Seasonal and treatment effects on zinc removal were broadly consistent with sulfate removal and presumably reflected zinc-sulfide precipitation. Results strongly suggest that interactive effects of COD loading rate, temperature, season, and plant species control not only sulfate reduction and zinc sequestration, but also the balance of competition between various microbial consortia responsible for water treatment in constructed wetlands.

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1. Introduction

Water contaminated by mining activities is usually characterized by a low pH and high concentrations of soluble metals and sulfate. Early research demonstrated that constructed wetlands can be a cost-effective, low maintenance alternative to traditional chemical addition treatment that raises pH to produce metal oxide and hydroxide precipitates (Wieder, 1989; Stark et al., 1995; Webb et al., 1998); however, results from field applications in the 1980's were highly variable (Hiel

*Corresponding author. Tel.: +1 406 994 6121; fax: +1 406 994 6105.
E-mail address: ottos@ce.montana.edu (O.R. Stein).

Abbreviations: COD, chemical oxygen demand; MSD, metro storm drain; MPB, methane producing bacteria; PVC, polyvinyl chloride; SRB, sulfate reducing bacteria

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and Kerins, 1988; McIntire and Edenborn, 1990). More recent research has shown that of the many documented metal removal processes in constructed wetlands (Wildeman et al., 1993; Gambrell, 1994), microbial sulfate reduction and subsequent metal sulfide precipitation is often dominant (McIntire et al., 1990; Machemer and Wildeman, 1992; Machemer et al., 1993; Christensen et al., 1996; Webb et al., 1998). Though the role of sulfate reduction for metals removal in constructed wetlands is reasonably well characterized, the combined influence of seasonal variation, plant species selection and chemical oxygen demand (COD) loading on sulfate reduction and competing processes has not been described.

The sulfate reducing bacteria (SRB) responsible for the production of sulfide are ubiquitous, tolerating temperatures below −5 °C and above 50 °C and pH values as low as 2.6 and as high as 9.5, though optimal ranges are narrower. Most SRB utilize only simple organic substrates as electron donors including lactate, acetate, and primary alcohols such as ethanol (Odom and Singleton, 1993). A typical sulfate reduction reaction using lactate as the electron donor is:

$$SO_4^{2-} + 2\ \text{lactate} \rightarrow 2\ \text{acetate} + H_2S + 2\ HCO_3^- \quad (pH < 7.0). \tag{1}$$

Sulfide then reacts with a divalent metal (M^{2+}) to form an insoluble metal sulfide precipitate:

$$H_2S + M^{2+} \rightarrow MS + 2H^+. \tag{2}$$

Metal sulfides precipitate out of solution in an order inverse to their solubility product values:

$$CuS < PbS < CdS < ZnS < NiS < FeS < MnS. \tag{3}$$

Copper has the lowest solubility and will precipitate out of solution first, whereas Fe and Mn have a high solubility and do not readily form a metal sulfide precipitate (Christensen et al., 1996; Machemer and Wildeman, 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). The advantages of the sulfide precipitation products keep constructed wetlands a viable alternative to chemical precipitation for metals removal despite a somewhat greater uncertainty of performance.

Although SRBs are ubiquitous and tolerate a wide range of environmental conditions, evidence suggests that rates of sulfate reduction in wetlands are extremely variable and depend on many factors including pH, redox potential, type of organic matter, and the ratio of organic carbon (COD) to sulfur (Westrich and Berner, 1988; Webb et al., 1998; Lyew and Sheppard, 1999). Redox potential is especially critical. Microbes utilizing different metabolic pathways compete for available organic carbon, and success is largely dictated by the amount of energy released when different inorganic electron acceptors are utilized (Reddy and D'Angelo, 1994; Snoeyink and Jenkins, 1980). Energy release is directly related to optimal redox and typically sulfate reduction does not occur at higher redox levels when other more favorable electron acceptors are present. Thus, the presence of nitrate and/or oxidized forms of Fe and Mn in the influent and especially oxygen transfer from the wetland surface and from plant roots can severely limit sulfate reduction.

Methane-producing bacteria (MPB) and SRB have a similar energy yield and operate at a similar redox level, thus they typically occur together and compete for available organic carbon. Competition between SRB and MPB depends on many factors including pH, sulfide concentration, the type of available organic carbon, and the COD:S ratio, (Omil et al., 1998). Relative SRB activity is typically enhanced by higher pH and sulfide concentrations and when simpler organic substrates are available (Capone et al., 1983; Fox and Ketha, 1996; Mizuno et al., 1998). 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, SRB are dominant, and at a COD:S ratio greater than 6.0 MPB are dominant. In the wetland environment, organic carbon is derived from decaying plant litter and plant root exudates, supplies of which vary by plant type and season and are likely insufficient for significant sulfate reduction when metal sequestration is a goal. Therefore, carbon is typically added as a compost material during wetland construction or in soluble form to the influent during operation. Hydraulic plugging due to compost and precipitate formation in early studies (Drury and Mainzhausen, 2000) has favored the addition of soluble organic carbon to influent in more recent applications.

Sulfate reduction may also play an important role in the removal of organic carbon from domestic wastewater in wetland systems. Large reductions in sulfate concentrations during treatment of domestic wastewater have been documented (Allen et al., 2002; Hook et al., 2003; Garcia et al., 2003, 2005). When input sulfate concentrations are high and redox conditions are favorable, a considerable fraction of the organic carbon reduction may be attributed to sulfate reduction.

The purpose of this study was to evaluate the effects of plant species, organic carbon concentration, season and temperature on the treatment of water impacted by mining activity. The objectives were: (1) compare performance of two plant species and unplanted controls for removal of sulfate and zinc, (2) compare performance of one plant species at two different concentrations of organic carbon and (3) quantify seasonal and temperature variation in removal of sulfate and zinc over a one-year cycle of plant growth and temperature variation.

2. Methods and materials

This study was conducted from December 1999 to January 2001 utilizing 16 sub-surface flow constructed wetland microcosms ("columns") that had been operating in a controlled temperature greenhouse at Montana State University since April 1997. Columns were constructed from 60 cm tall × 20 cm diameter polyvinyl chloride (PVC) pipe and filled to a 50 cm depth with washed, noncalcareous alluvial gravel (0.3–1.3 cm) resulting in a pore volume of 4.3 L. Three solution sampling tubes (0.3 cm diameter vinyl tubing) and three platinum redox electrodes (contained within 0.11 cm

inner diameter PVC access tubes) were installed from above near the center of each column with openings at 5, 15, and 30 cm below the gravel surface. No vertical gradients in water chemistry were evident in previous experiments with the same columns (Allen et al., 2002) so solution samples were taken from the 15 cm depth only for this study. Details of column construction and planting, greenhouse environmental variability, and prior operation are described in Allen et al. (2002) and Hook et al. (2003). Except as noted below, operation was identical during this study.

Eight columns were planted with *Schoenoplectus acutus* (hardstem bulrush), four with *Typha latifolia* L. (broadleaf cattail), and four were unplanted controls. Replicate columns of all treatments were interspersed and randomized. Seasonal cycles of plant dormancy and growth were induced by varying the greenhouse set temperature in parallel with natural variation in day length. During winter months (December, January, and February), the ambient air temperature was set at 4 °C. During the spring (March, April, and May) and fall (September, October, and November), the set temperature was 14 °C. During summer months (June, July, and August), the set temperature was 24 °C. There were approximately week-long 5 °C steps between all simulated seasons. Though actual temperatures showed some diurnal and spatial variability, set temperatures represent average daily greenhouse temperatures well.

Synthetic mine-impacted water modeled after runoff discharged from the metro storm drain (MSD) in Butte, Montana, USA (Gammons et al., 2005) was created by adding constituents to tap water. All columns received water containing 24 mg/L zinc, supplied from $ZnSO_4 \cdot 7H_2O$ and 200 mg/L SO_4 (67 mg/L SO_4-S) with 35 mg/L SO_4 supplied from $ZnSO_4 \cdot 7H_2O$ and the remainder supplied from Na_2SO_4. As with MSD water, pH was circumneutral (6.5–6.7). Nitrogen (10 mg/L) and phosphate (1.0 mg/L) were added for microbial and plant growth as NH_4Cl and K_3PO_4, respectively. Sucrose ($C_{12}H_{22}O_{11}$) was added at two rates to create distinct organic carbon concentrations. Four bulrush columns and all cattail and unplanted control columns were given a low carbon concentration of 100 mg/L C (COD = 267 mg/L, "low-COD" treatments); the remaining four bulrush columns were given a high carbon concentration of 200 mg/L C (COD = 534 mg/L, "high COD" treatment).

Chemical constituents from all four replicates of the four treatments were tracked during nine 20-day incubations conducted throughout the year-long study. A preliminary incubation (Incubation 0) was conducted at 4 °C to test protocols and methods before performing the incubations reported here (Incubations 1–8). Two incubations were conducted during each of the four seasons. The two winter incubations (Incubations 1 and 8) were split between the first and second winters, while pairs of spring, summer and fall incubations were each done within one 3-month period. Columns were gravity drained three days prior to each incubation and then again at the start of each incubation on day 0. Within 1 h after draining, the columns were re-filled with synthetic wastewater. Solution samples were taken from the influent (prior to filling) and from each column on days 0 (immediately after filling), 1, 3, 5, 10, and 20. A syringe was used to draw samples from the vinyl sampling tubes after three sampling tube volumes (approximately 15 mL) were withdrawn and discarded.

All samples were analyzed for sulfate (SO_4) and COD. Influent water and day 5 samples (day 20 samples in Incubations 0 and 1) were also analyzed for zinc, sulfide (S^{2-}), and pH. Samples were immediately analyzed for COD (0–1500 mg/L) and sulfide using colorimetric procedures (Hach Corp., Loveland, CO). Zinc samples were acidified with 5% hydrochloric acid (HCl) and later analyzed using inductively coupled plasma spectrometry (ICP). Sulfate samples were filtered through a 0.22 μm cellulose acetate filter, stored in sterile test tubes at 2 °C, and later analyzed using high performance liquid chromatography (Dionex model DX-500, Dionex Corp., Sunnyvale, CA).

To distinguish oxidizing and reducing environments, electrode potential (E_m) was automatically measured and recorded every 4 h during Incubations 2–8. To estimate redox potential (E_h), measured E_m values were corrected by adding 244 mV (Stumm and Morgan, 1996). Because pH was consistently circumneutral (6.5–6.7) 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). Details of redox electrode construction and operation are described in Allen et al. (2002) and Borden (2001).

Multivariate repeated measures analysis of variance (MANOVAR), which accounts for the interdependence of sequential observations taken on the same column, was used to test effects of plants and COD loading on sulfate removal during individual incubations. The statistical power of MANOVAR is compromised when the number of repeated observations on the same individual is large compared to the number of replicates (Potvin et al., 1990); therefore, only three sampling days (Days 1, 5, and 20) were used in the analysis of each incubation. Two sets of tests were performed to evaluate effects of plants and COD loading: (1) differences in sulfate concentrations averaged over an incubation and (2) differences in slopes of time trends (i.e. differences in the removal rates based on change in concentration). Treatments were compared using planned contrasts to test (1) the effects of plants on removal rates at low COD loading (i.e. pairwise comparisons of control, cattail, and low-COD bulrush treatments) and (2) the effects of high versus low COD loading on removal rates in bulrush columns. Differences were considered significant at $p \leqslant 0.05$. Analyses were performed with SAS version 8.0 (SAS Institute, Inc., Cary, NC).

3. Results

Time series for COD, SO_4-S and redox potential within each season (Incubations 1, 2, 4, 7, 8) are shown in Fig. 1. The full set of eight incubations indicated some random, non-seasonal variations in redox patterns not shown in Fig. 1; otherwise the results shown are quite representative. Virtually no plant or seasonal effects on COD removal were discernable. In fact, little difference in COD concentration between low-COD and high-COD bulrush treatments was apparent after 3 to 5 days even though the high-COD

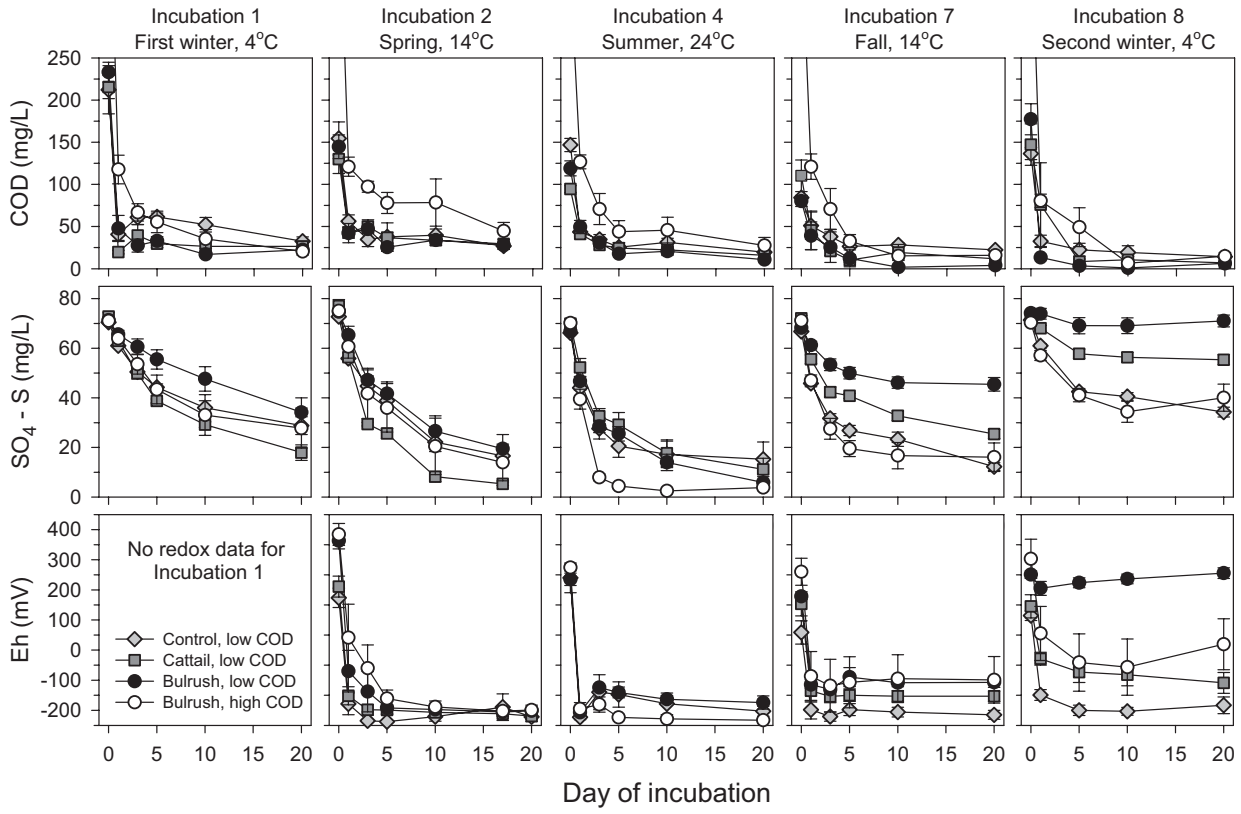


Fig. 1 – Representative time series of COD, sulfate, and redox potential for each season (means ± 1 standard error for days 0, 1, 3, 5, 10, and 20). COD scale is truncated at 250 mg/L; for high-COD bulrush treatments, day 0 samples collected after refilling columns averaged 442 mg COD/L. No redox data were taken during Incubation 1.

treatment had twice the initial concentration ($C_0 = 534$ versus 267 mg/L). Final COD concentrations were typically less than 40 mg/L.

Effects of plants and carbon loading on sulfate removal were generally established within the first three days of each 20-day incubation and remained consistent thereafter (Fig. 1). For columns with low COD loading, the presence and species of plants affected sulfate removal significantly in the fall and winter (14 °C and 4 °C, respectively) but usually not in the spring or summer (14 °C and 24 °C, respectively; Table 1). When differences were significant, sulfate removal was usually greatest in unplanted control columns, intermediate in cattail columns and least in low-COD bulrush columns. Differences in organic carbon loading affected sulfate removal in bulrush columns during most incubations. Sulfate removal rates were significantly faster in high-COD than low-COD bulrush columns during all but one incubation, and average sulfate concentrations were significantly lower in high-COD columns in Incubations 4–8. Averaged across incubations and days, sulfate removal rates were 26% higher with high COD. Sulfate removal in high-COD bulrush columns also averaged 16% more than those with cattails and 9% more than unplanted controls.

Redox potentials were typically less than −100 mV after 24 h and around −200 mV after 5 days (Fig. 1). Redox values usually displayed no clear variation between treatments in the warmer spring, summer, and fall incubations, but large differences between treatments were observed in the second winter when planted treatments, especially low-COD bulrush columns, had redox levels higher than considered optimal for sulfate reduction at all times during the incubation.

Sulfate concentrations measured on day 5 of each incubation varied seasonally (Fig. 2) and showed a relatively strong relationship with temperature (Fig. 3). Less sulfate remained during the warmer growing season incubations than during colder dormant season incubations across all treatments. Cattail columns removed less sulfate than controls at all temperatures but their seasonal temperature response was virtually identical, while bulrush columns at both low- and high-COD loadings showed much stronger cold-temperature inhibition. Differences generally were greater between 4 and 14 °C than between 14 and 24 °C suggesting that the coldest temperatures and plant dormancy had the largest inhibitory effect on sulfate removal. Only at 24 °C was average sulfate removal in the low-COD bulrush columns similar to low-COD cattail and control columns.

Effects of plants and season on sulfate removal were largely associated with variations in redox potential. When all low-COD incubations were analyzed together, redox potential accounted for over half the variation among treatments and seasons in days 1–10 sulfate removal ($R^2 = 0.67$, 0.52, and 0.52 for days 1, 5, and 10, respectively); this relationship weakened by day 20 ($R^2 = 0.30$). High-COD loading counteracted the tendency of low-COD bulrush columns to raise redox potential and decrease sulfate removal in winter (Incubation 8, Fig. 1) although redox potential did not explain the overall

Table 1 – Summary of seasonal effects of plants and COD loading on sulfate removal

Treatment comparisons	Incubation, temperature, and season															
	1 4 °C winter		2 14 °C spring		3 14 °C spring		4 24 °C summer		5 24 °C summer		6 14 °C fall		7 14 °C fall		8 4 °C winter	
	Mean	Rate	Mean	Rate	Mean	Rate	Mean	Rate	Mean	Rate	Mean	Rate	Mean	Rate	Mean	Rate
(a) *Effect of plant treatments at low-COD loading*																
Control, low COD	AB	ab	A	a	A	a	A	a	A	a	A	a	A	a	A	a
Cattail, low COD	A	a	A	a	A	a	A	b	A	a	B	b	B	a	B	ab
Bulrush, low COD	B	b	A	a	A	a	A	b	A	a	C	c	C	b	C	b
(b) *Effect of COD loading rate*																
Bulrush, high COD versus low COD	n.s.	x	n.s.	n.s.	n.s.	x	X	x	X	x	X	x	X	x	X	x

Each column includes the results of a repeated measures analysis of variance of one incubation using data for days 1, 5, and 20. Treatment effects on sulfate concentrations (averaged across days) and removal rates (trends of sulfate concentrations over time) were tested with planned contrasts. Upper case letters represent results of contrasts for average concentrations; lower case letters represent results for removal rates. (a) Pairwise comparisons among low-COD treatments: treatments with the same letter are not significantly different. (b) Comparisons of low-COD versus high-COD bulrush treatments: significant effects of loading rate are indicated by the letters X and x (n.s. indicates not significant).

Fig. 2 – Seasonal patterns of sulfate, sulfide, and zinc concentrations (means ± 1 standard error). Values are for day 5 of each incubation except for Incubation 1 sulfide and zinc concentrations, which are for day 20.

Fig. 3 – Relationships between sulfate removal and temperature. Within each temperature, symbols are offset to reduce overlap; regression lines are not offset.

effect of COD loading on sulfate removal across all incubations. Sulfate removal in high-COD treatments was usually greater than would be expected based on the relationship with redox potential found for the low-COD treatments; approximately 80% of sulfate removal values for high-COD

columns were above the 95% confidence band for regression models describing sulfate removal as a function of Eh in low-COD columns, and this was true for days 1–20.

Seasonal variations in sulfide and zinc were apparent but less consistent than those for sulfate (Fig. 2). For low-COD treatments, average sulfide concentrations were generally higher in spring and summer than fall or winter. Sulfide levels were consistently higher in unplanted controls than low-COD bulrush or cattail columns. For high-COD bulrush columns, sulfide concentrations were relatively steady from spring through fall and lower in winter. High- and low-COD bulrush columns were similar during the first three incubations and diverged thereafter. Averaged over all treatments, seasonal patterns of zinc concentrations were similar to sulfate, with better removal during the growing season than winter. However, closer inspection suggests that only the bulrush columns, especially those with low-COD, displayed a clear seasonal pattern. Overall zinc removal was high across treatments and seasons. Average zinc concentrations were $\leqslant$0.5 mg/L ($\geqslant$98% removal) in all treatments during the warmest incubations and in cattail and control treatments throughout the year. In the low-COD bulrush treatment, zinc increased to 1.6 mg/L (93% removal) during the second winter.

4. Discussion

The decrease in sulfate concentration observed in all incubations was almost certainly due to the activity of sulfate reducing bacteria (SRB). SRB are ubiquitous, especially in reduced wetland environments. Elevated concentrations of sulfide and high zinc removal by day 5 are further evidence of SRB activity. However, SRB activity is influenced by temperature, the availability of more efficient electron acceptors, especially at higher redox levels, and competition with methane producing bacteria (MPB) at low redox levels. Organic carbon load can influence redox conditions via microbial oxygen consumption and the competition between SRB and MPB via changes in C:S ratios. In this discussion we elucidate how environmental and plant factors likely influenced SRB activity and the resultant observed changes in water chemistry.

Typically, the conditions that inhibited sulfate reduction—cold temperatures and presence of plants, especially bulrush—were those with higher redox levels. We found similar patterns in a previous study using high COD loading, but much lower sulfate concentrations (Stein and Hook, 2005). Hook et al. (2003) hypothesized that measured redox potentials in constructed wetlands would not increase until combined oxygen demand of the plant root and the aerobic biofilms surrounding the root were less than the rate of plant-mediated oxygen supply, and that seasonal dormancy of some wetland plant species at cold temperatures might reduce the internal plant demand enough to enhance oxygen availability in the surrounding solution. Data of others (Howes and Teal, 1994; Callaway and King, 1996; Moog and Brüggemann, 1998) support at least the second contention. In our planted columns, especially those with bulrush, higher redox potentials during periods of cold temperature and plant dormancy made possible increased aerobic microbial activity, in direct competition with SRB for available organic carbon. By inhibiting SRB activity at lower temperatures, bulrush exacerbated the inherent effect of cold temperature that was seen in the control columns. Redox potential for cattail indicated a similar, but less dramatic, increase in aerobic microbial activity as compared to bulrush.

Higher COD loading in the bulrush columns allowed anaerobic processes, including SRB activity, to proceed at rates similar to or greater than unplanted control columns even though redox was sometimes elevated compared to controls (Incubations 7 and 8). However, both high-COD and low-COD bulrush columns displayed greater seasonal variation in sulfate reduction and redox potential than cattail and control columns (Figs. 2 and 3), presumably due to the interacting effects of temperature, which affected all treatments, and enhanced root zone oxidation during the dormant season, which is most significant for bulrush (Allen et al., 2002; Hook et al., 2003; this study).

An assumption that all of the observed sulfate removal is due to SRB activity allows for a comparison between competing microbial consortia for available COD. Stoichiometrically, SRB require only 133 mg/L COD to consume all of the 200 mg/L sulfate supplied in the influent (2 mol oxygen is equivalent to 1 mol of sulfate); therefore, added COD was well in excess of the amount required by SRB to utilize all available sulfate (267 mg/L for low-COD and 564 mg/L for high-COD influent). However, COD removal was nearly complete in most treatments and incubations (after 5 days, concentrations were $<$45 mg/L for low-COD and $<$80 mg/L for high-COD treatments), whereas sulfate removal was never complete and varied with season, temperature, plant species and carbon load (Fig. 1). It follows that the contribution of sulfate reduction relative to other metabolic pathways for COD removal was also affected by these factors.

Using the data from day 5 (when COD removal was nearly complete), the COD:sulfate stoichiometry relation was used to estimate the fraction of COD removed by sulfate reduction (Fig. 4). Based on this estimate, the majority of COD was consumed by processes other than sulfate reduction in all treatments and incubations. Across all treatments, the contribution of sulfate reduction to COD removal was greatest in summer, least in winter, and intermediate in spring and fall. The fraction of COD removal attributable to sulfate reduction was greatest in unplanted controls year-round, varying from about 40% of the observed COD removal at 24 °C to only 25% at 4 °C. In winter, sulfate reducers used a larger fraction of COD in low-COD cattail than low-COD bulrush columns. As the only available electron acceptors in our columns were oxygen, sulfate and protons, only aerobic bacteria, SRB and MPB likely competed for organic carbon (fermentative microbes likely changed the species of organic carbon but not the overall oxygen demand). Micro-environments within the gravel-root matrix probably allow all three groups to survive and grow simultaneously.

Only unplanted control columns lacked potential confounding factors involving plants, such as increased organic carbon supply due to plant exudates and detritus or interference from plant-mediated oxygen transfer. Redox measurements suggest competition for COD in the unplanted controls was limited primarily to SRB and MPB (redox was

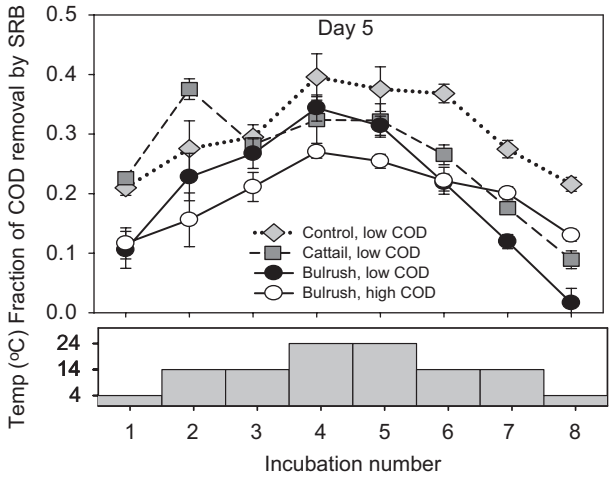


Fig. 4 – Seasonal variation in the fraction of day 5 COD removal potentially attributed to sulfate reduction (means ± 1 standard error). Values were calculated as the ratio of (a) mass of COD oxidized via sulfate reduction over (b) mass of COD removed. Mass of COD oxidized via sulfate reduction was estimated from change in mass of sulfate assuming 2 mol of COD per 1 mol of sulfate.

approximately −200 mV year-round). However, some oxygen diffusion across the air–gravel interface undoubtedly occurred and we previously estimated that a few milligrams of oxygen were supplied daily with water replacing evaporative losses in the same columns (Allen et al., 2002). Based on this, we estimated that 5–10% of the observed COD removal in unplanted control columns can be attributed to the activity of aerobic bacteria.

Redox and sulfate measurements in the planted treatments, especially in bulrush columns in winter, strongly suggest plant-mediated oxygen transfer stimulated aerobic bacteria above the levels estimated in the unplanted control columns. Assuming that SRB and MPB are affected about equally by oxygen availability, an increase in aerobic activity due to plants can be estimated by comparing low-COD planted and unplanted treatments in Fig. 4. Based on this approximation, aerobic COD removal was roughly 20% during the height of the growing season when redox potential was uniformly low and increased to 60–90% of the total COD removal during the dormant season, depending on species. While we have confidence in our estimate of the fraction of COD attributable to SRB activity (Fig. 4), fractioning the rest of the COD removed between MPB and aerobic microbes is based on a set of assumptions that cannot be verified from the available data, therefore the magnitudes of relative aerobic activity are only approximations. Regardless, the seasonal variation of the estimated aerobic activity in planted columns is remarkable and is consistent with our previous studies (Allen et al., 2002; Hook et al., 2003).

Doubling the COD load in bulrush columns approximately doubled the total mass of both sulfate and COD removed in most incubations (Fig. 1). However, the fraction of COD removal by SRB relative to other microbial consortia was greater for low-COD bulrush than high-COD bulrush in winter but less for low-COD than high-COD bulrush in summer (Fig. 4). Our influent COD:S ratios were 4 and 8 for the low-COD bulrush, and high-COD bulrush respectively, placing initial conditions at intermediate or MPB-favoring ratios, respectively (Fox and Ketha, 1996; Omil et al., 1998; Mizuno et al., 1994; Vroblesky et al., 1996). During the summer growing season when redox data suggested aerobic respiration was at a minimum, the higher COD loading may have shifted the balance for COD removal from SRB to MPB. During the winter season, lower temperatures and increased oxygen transfer inhibited SRBs (and presumably MPBs) regardless of COD load, but the higher COD load overwhelmed the oxygen transfer capacity of the bulrush, resulting in continued significant SRB activity.

Wetland plants have the potential to stimulate SRB activity by generating organic carbon as exudates and detritus and to inhibit SRB activity by transferring oxygen to the root zone. Our results strongly suggest that the net effect of plants is inhibitory. In almost all cases where plants had detectable effects, their presence decreased sulfate and zinc removal, sulfide concentrations, and the fraction of COD removal attributable to SRB activity. This was most apparent for bulrush and is likely to be true with other species that are capable of significant oxygen transfer during the dormant season (Allen et al., 2002; Hook et al., 2003). Seasonal variation in production of detritus and exudates may also affect these processes but was not evaluated.

The general similarity between seasonal sulfate and zinc removal patterns suggests that sulfate reduction played an important role in the sequestration of zinc. However, other known zinc removal processes such as sorption to gravel, roots and organic detritus (Christensen et al., 1996; Machemer and Wildeman, 1992; Wildeman et al., 1993) probably also played a role, especially during the dormant season. The quantity of sulfide produced in the low-COD bulrush columns during the last winter incubation was stoichiometrically insufficient to react with the observed quantity of zinc removed, implying that other removal mechanisms were involved. In all other cases sulfate reduction exceeded the amount needed for zinc removal, typically by a factor of 3–6.

5. Conclusions

Our results highlight several practical considerations for the use of constructed wetlands for sulfate reduction and subsequent metal precipitation in cold climates. The effect of plants appears to be inhibitory for sulfate reduction, especially during periods of plant dormancy in winter. The magnitude of the inhibitory effect is linked to the ability of plants to raise redox potential in winter; bulrush had less sulfate reduction and higher redox potentials than cattail, which had less sulfate reduction and higher redox potentials than gravel alone. Plants can provide both oxygen, which should decrease SRB activity, and organic carbon, which should increase SRB activity, but their net effect was to reduce sulfate reduction throughout the year, and especially in winter. With the possible exception of the high-COD bulrush columns in summer, the quantity of COD appeared to limit sulfate reduction even though COD was supplied at 2 or 4

times that required to completely utilize all available sulfate. Therefore, organic carbon must be supplied at a rate well in excess of that predicted by stoichiometry if sulfide production is a goal. Our results indicate that sulfate reduction might be optimized by varying the influent COD load by season, adding less in summer and more in winter to counteract the effects of seasonally varying oxygen supply. Practically, SRB activity might be maximized by a continuous input of a soluble, labile organic carbon source to a deep wetland with a large anaerobic zone beneath the slightly oxygenated root zone. This would create a large wetland volume for sulfate reduction while simultaneously providing an aesthetically pleasing surface cover and an aerobic, vegetated cap which could minimize release of anaerobic respiration by-products.

Our results also shed light on the influence of plant species and seasonal dynamics on the relative contributions of various microbial consortia to removal of influent COD. Stoichiometrically, the maximum amount of observed COD removal attributable to sulfate reducing bacteria occurred in summer when influent COD was 267 mg/L: approximately 40% in unplanted columns, 30% in cattail and 25% in bulrush columns. In winter, the SRB contribution decreased to approximately 25% in unplanted, 15% in cattail, and virtually zero in low-COD bulrush columns. Doubling COD in bulrush columns increased the relative contribution of SRB to approximately 15% in winter. In unplanted columns, the influence of aerobic bacteria was probably minimal, at least during the summer, so that the difference between planted and unplanted columns reflected the influence of plant-mediated oxygen transfer on the relative contribution of aerobic bacteria to COD removal. In planted columns, aerobic respiration was less than the combined effect of SRB and MPB in summer but may have been the dominant COD removal mechanism in winter. Future detailed measurements of microbial activity, by either direct microbial community assay techniques or by measuring metabolic by-products, are required to definitively quantify the relative contributions of competing microbial consortia.

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