Wetlands and Aquatic Processes

Temperature and Wetland Plant Species Effects on Wastewater Treatment and Root Zone Oxidation

Winthrop C. Allen,* Paul B. Hook, Joel A. Biederman, and Otto R. Stein

ABSTRACT

Constructed wetlands are widely used for wastewater treatment, but there is little information on processes affecting their performance in cold climates, effects of plants on seasonal performance, or plant selection for cold regions. We evaluated the effects of three plant species on seasonal removal of dissolved organic matter (OM) (measured by chemical oxygen demand and dissolved organic carbon) and root zone oxidation status (measured by redox potential [Eh] and sulfate [SO_4^{2-}]) in subsurface-flow wetland (SSW) microcosms. A series of 20-d incubations of simulated wastewater was conducted during a 28-mo greenhouse study at temperatures from 4 to 24°C. Presence and species of plants strongly affected seasonal differences in OM removal and root zone oxidation. All plants enhanced OM removal compared with unplanted controls, but plant effects and differences among species were much greater at 4°C, during dormancy, than at 24°C, during the growing season. Low temperatures were associated with decreased OM removal in unplanted controls and broadleaf cattail (*Typha latifolia* L.) microcosms and with increased removal in beaked sedge (*Carex rostrata* Stokes) and hardstem bulrush [*Schoenoplectus acutus* (Muhl. ex Bigelow) A. & D. Löve var. *acutus*] microcosms. Differences in OM removal corresponded to species' apparent abilities to increase root zone oxygen supply. Sedge and bulrush significantly raised Eh values and SO_4^{2-} concentrations, particularly at 4°C. These results add to evidence that SSWs can be effective in cold climates and suggest that plant species selection may be especially important to optimizing SSW performance in cold climates.

Subsurface-flow wetlands (SSWs) are widely used in wastewater treatment systems, but design guidelines for cold climates, which we define as climates with temperatures near or below freezing over extended periods, are not well tested. The USEPA (1993) conducted a SSW technology assessment and identified high-priority research topics including (i) temperature and seasonal effects on wastewater treatment, (ii) the role of plants in providing oxygen for root zone processes, and (iii) investigation of additional plant species suited for use in treatment wetlands. A better understanding of temperature effects and their possible seasonal interactions with plant species may be particularly important to optimizing design and management of SSWs in cold climates.

W.C. Allen, CH2M HILL, 555 South Flower Street, Suite 3550, Los Angeles, CA 90071. P.B. Hook, Dep. of Land Resources and Environmental Sciences, Montana State Univ., P.O. Box 173120, Bozeman, MT 59717-3120. J.A. Biederman and O.R. Stein, Dep. of Civil Engineering and Center for Biofilm Engineering, Montana State Univ., P.O. Box 173900, Bozeman, MT 59717-3900. Journal Series no. 2001-10 of the Montana Agricultural Experiment Station, Montana State University-Bozeman. Received 29 Mar. 2001. *Corresponding author (wallen@ch2m.com).

Published in J. Environ. Qual. 31:1010–1016 (2002).

Reviews of operational data for full-scale systems indicate that SSWs can meet effluent criteria in cold climates, and that temperature effects on removal of organic matter (OM) may be less than expected (Kadlec and Knight, 1996; USEPA, 2000). The lack of significant temperature effects on OM removal in full-scale SSWs has been attributed to sedimentation, temperature adaptation of microbes, variation in decomposition rates, and thermal buffering by plant litter, snow, and ground heat, but mechanistic explanations remain speculative (Kadlec and Knight, 1996; Wittgren and Maehlum, 1997). It is also possible that seasonal differences in oxygen release from plant roots may contribute to the lack of temperature response; however, the relative contribution of plant oxygen transport to wastewater treatment remains controversial. Some wetland designers assume that plant oxygen transport is significant (e.g., Campbell and Ogden, 1999; DeBusk and DeBusk, 2001), while others dismiss it as negligible compared with oxygen demand and undependable due to seasonal senescence (USEPA, 2000).

Wetland plants are known to transport oxygen into their roots to support aerobic respiration and to oxidize phytotoxic reduced compounds (Fe^{2+}, Mn^{2+}, S^{2-}) in the rhizosphere. Some wetland plants can release enough oxygen into the root zone to support aerobic microbial activity (Reddy et al., 1989b; Bodelier et al., 1996; Armstrong et al., 1990), and this may sometimes represent as much as 90% of the total oxygen entering a wetland substrate (Reddy et al., 1989a). Quantification of oxygen flux from entire root systems has been complicated by species and seasonal differences, spatial heterogeneity, and other measurement issues such as oxygen demand of the root zone solution and root to solution volume (Bedford et al., 1991; Sorrell and Armstrong, 1994). Plants' capacity to supply oxygen to the root zone varies among species due to differences in vascular tissues, metabolism, and root distribution (Gersberg et al., 1986; Steinberg and Coonrod, 1994; Jackson and Armstrong, 1999). Because root and rhizome respiration consumes most oxygen that diffuses through plant shoots and oxygen demand for root and rhizome respiration declines with temperature, the potential for plants to release oxygen into the root zone may increase during cold periods (Howes and Teal, 1994; Callaway and King, 1996).

Abbreviations: COD, chemical oxygen demand; DO, dissolved oxygen; DOC, dissolved organic carbon; OM, organic matter; SSW, subsurface-flow wetland.

Based on the temperature and species effects on oxygen release reported in the ecological literature, we hypothesized that (i) plants would modify the effects of temperature on root zone oxidation and wastewater treatment in SSWs and (ii) plant effects on temperature responses would vary among species. We tested these hypotheses in a relatively long-term (28-mo) greenhouse experiment using batch incubations in subsurface-flow wetland microcosms at temperatures from 4 to 24°C. In this paper, we contrast results for incubations at the maximum and minimum temperatures (August 1998 and January 1999), which illustrate major differences between the growing season and winter.

MATERIALS AND METHODS

A controlled-temperature greenhouse experiment using subsurface-flow wetland microcosms ("columns") was conducted at Montana State University in Bozeman, Montana (45°40′ N, 111°03′ W; 1490 m elev.) from April 1997 through July 1999. A series of 20-d incubations of simulated wastewater was conducted over 20 mo at temperatures ranging from 4 to 24°C (Fig. 1). Hourly temperatures fluctuated around the set thermostat temperatures, but the temperatures shown in Fig. 1 represent average daily greenhouse temperatures. Supplemental lighting was not used; cumulative daily net solar radiation ranged from 1 to 8 MJ m^{-2} d^{-1} and was about 25% of locally recorded net solar radiation throughout the year (Towler, 1999). Net solar radiation was relatively low as a fabric light filter was employed to improve temperature control. Relative humidity ranged from 30 to 70%, with no seasonal pattern. The combination of greenhouse temperatures and natural light patterns was sufficient to support robust plant growth and to induce seasonal cycles of plant dormancy and growth.

Thirty-two columns (eight replicates per treatment) were constructed from polyvinyl chloride (PVC) pipe (60 cm in height × 20 cm in diameter) and filled to a depth of 50 cm with washed pea-gravel (0.3–1.3 cm in diameter). The local alluvial gravel was derived from noncalcareous rock of igneous and metamorphic origin. Porosity was 0.27; pore volume was 4.3 L and did not differ significantly among treatments or with time throughout the experiment. Access tubes (1.1-cm-i.d. PVC) for permanently installed platinum redox electrodes and solution sampling tubes (0.3-cm-i.d. vinyl tubing) were installed from above with openings at 5-, 15-, and 30-cm depths (Fig. 2). The tops of redox electrode access tubes were sealed with a rubber stopper. Water level was automatically maintained at the gravel surface by replacing evaporative losses with dechlorinated tap water (at greenhouse temperature) added to the bottom of the columns. Each column functioned as an independent batch reactor.

Mature sedge, bulrush, and cattail plants were collected locally in March and April 1997 and were dormant or had minimal new growth. Rhizomes were washed free of sediments and planted in the gravel-filled columns. Columns were filled with a standard nutrient solution (50 mg L^{-1} Peter's 20–10–20 GP; The Scotts Company, Marysville, OH) from April 1997 to September 1997 and with simulated wastewater starting in October 1997. Following preliminary incubations in November and December 1997, standardized incubations were conducted from January 1998 through July 1999 (see Allen, 1999 for details). This paper reports results for August 1998 (24°C) and January 1999 (4°C) only, when the experimental units were 16 and 21 mo old, respectively, and had been receiving wastewater for 11 and 16 mo.

A synthetic wastewater simulating secondary domestic effluent was mixed from sucrose, hydrolyzed meat protein, and inorganic nutrient and metal salts to ensure consistent, known composition for all incubations (Allen, 1999). Mean influent concentrations of the main constituents were measured to be 151 mg organic C L^{-1} (chemical oxygen demand [COD] = 470 mg L^{-1}), 44 mg N L^{-1} (27 mg amino N L^{-1} [TN persulfate digestion; 0–150 mg L^{-1} test, Hach Company, Loveland, CO], 17 mg NH_4–N L^{-1} [modified Berthelot method]), 8 mg PO_4–P L^{-1} (Dionex [Sunnyvale, CA] Model DX-500), and 14 mg SO_4–S L^{-1}. Columns were gravity-drained and filled with fresh, synthetic wastewater 3 d before each incubation, and then again at the start of each incubation. Dilution of the influent wastewater attributed to water retained in the porous media was determined to be ≤5% using a bromide tracer, and showed no significant difference among plant treatments.

Calculations were made to determine the significance of dissolved oxygen (DO) inputs associated with water added to replace evaporative losses. Evaporative losses were greater

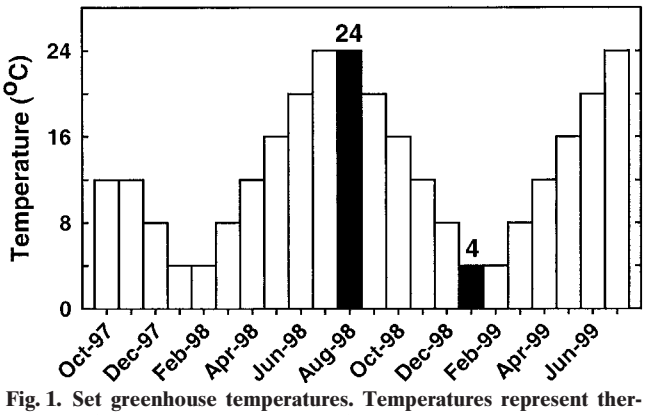

Fig. 1. Set greenhouse temperatures. Temperatures represent thermostatically set, mean daily operating temperatures. Data collected during August 1998 and January 1999 are discussed in this paper.

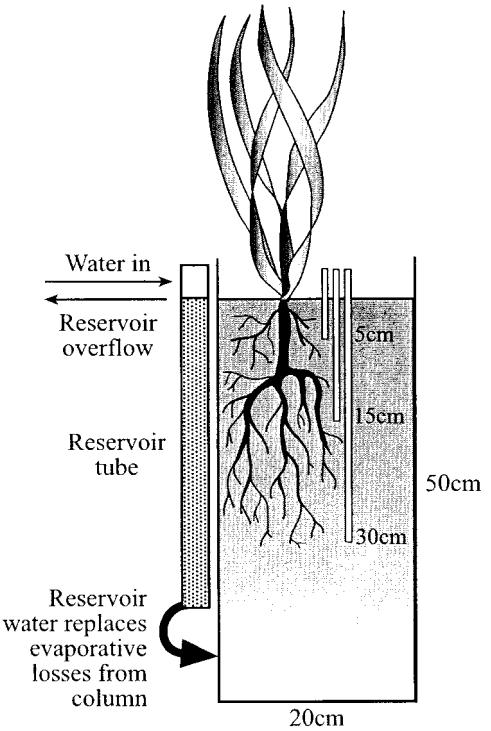

Fig. 2. Schematic of column design and water delivery system. Dechlorinated tap water was continually supplied to the reservoir tube.

for planted columns than for unplanted controls, but did not vary among species. The maximum evapotranspiration (ET) rate for planted columns at 24°C was measured to be 0.7 L d^{-1} $column^{-1}$. Assuming an oxygen solubility of 8.5 mg O_2 L^{-1}, this corresponded to an input of approximately 6 mg O_2 d^{-1} $column^{-1}$ or 1% of the influent COD per day. The maximum ET rate for planted columns at 4°C was less than 0.4 L d^{-1} $column^{-1}$. Assuming an oxygen solubility of 13 mg O_2 L^{-1}, this corresponded to an input of less than 5 mg O_2 d^{-1} $column^{-1}$ or 1% of the influent COD per day.

Column solution samples were collected with a 60-mL syringe after three sampling tube volumes (approximately 25 mL) had been withdrawn and discarded. Solution samples collected from 5-, 15-, and 30-cm depths during preliminary incubations showed no measurable vertical gradients for COD, dissolved organic carbon (DOC), or SO_4^{2-}. Samples were subsequently collected from 15 cm only. Samples were collected from four replicates immediately after filling columns and on Days 1, 3, 6, 9, 14, and 20 of each incubation. Samples were analyzed immediately for chemical oxygen demand (COD; 0–1500 mg L^{-1} test; Hach Company), then filtered to sterilize (0.22-μm cellulose acetate filter), stored in sterile test tubes at 2°C, and analyzed for SO_4^{2-} using ion chromatography (Dionex Model DX-500). Two replicates were also sampled (i) every 4 h for the first 16 h, (ii) at 32 and 48 h, and (iii) on Days 3, 6, 9, 14, and 20 for additional dissolved organic carbon (DOC) analyses. These samples were filtered (0.20-μm nylon filter), acidified with 20% H_3PO_4, and analyzed using a Dohrmann DC-80 carbon analyzer (Xertex Corp., Santa Clara, CA).

Two flow-through cells, one housing a standard O_2 electrode (Yellow Springs Instruments [Yellow Springs, OH] Model 5739) and the other a flat-surface pH electrode, were operated in series to measure DO and pH when collecting samples for COD, DOC, and SO_4^{2-} analyses. As described above, one solution sample was collected using a 60-mL syringe. After aliquots for laboratory analyses were removed, approximately 10 pore volumes (40 mL) of the original solution sample were passed through the flow cells and DO and pH readings were recorded. The DO and pH probes were checked against reference solutions before and after sampling.

Platinum redox electrodes (Faulkner et al., 1989) were permanently installed in all 32 columns at 5-, 15-, and 30-cm depths, connected to a computer via a multiplexer, and read automatically every 4 h. Columns were connected by a salt bridge with two saturated calomel reference electrodes located centrally (Veneman and Pickering, 1983). Redox potential (Eh) was estimated from measured electrode potential by adding 244 mV (Stumm and Morgan, 1996). Because pH was consistently circumneutral (average 6.8 with and 7.0 without plants) and the effect of temperature on measured potential is relatively small (Stumm and Morgan, 1996), Eh was not corrected for pH or temperature. Redox probes were checked periodically against a ferrous–ferric standard solution (Light, 1972).

Data were analyzed by repeated measures analysis of variance, with time and depth (for Eh) as within-subjects factors. Data for both temperatures and all incubation times, plant treatments, and depths were first analyzed together. Because statistical interactions among factors were common, separate analyses were then performed for different temperatures, times, treatments, or depths as needed to clarify results. Analyses were conducted using SAS Version 6.12 (SAS Institute, 1996). All differences reported below were statistically significant at $p = 0.05$. There were four replicates for COD, SO_4^{2-}, DO, and pH, and two for DOC. There were eight replicates for Eh normally, but only five for cattail and controls in August 1998.

RESULTS

Temperature significantly affected COD and DOC removal and the two indicators of root zone oxidation status, Eh and SO_4^{2-} (Fig. 3 and 4). However, presence and species of plants also significantly influenced these variables, in some cases negating the effects of temperature. Compared with unplanted controls, the effects of plants were greater at low than high temperatures. Differences among plant species were also much larger at low temperatures. Concentrations of DOC and COD were strongly correlated (COD = 3.0 × DOC + 16.8, $R^2 = 0.96$, $n = 124$), and patterns of DOC removal closely paralleled those for COD; therefore, only data for COD are presented graphically.

Effects of temperature on COD and DOC removal depended on plant treatment and varied through time (temperature × treatment and temperature × time interactions significant). In unplanted controls, COD and DOC removal were significantly less at 4 than 24°C (Fig. 3). Cold also tended to reduce COD and DOC removal in cattail columns, but differences between 4 and 24°C were not significant. In sedge and bulrush columns, COD and DOC removal was relatively complete at both temperatures, but removal was more rapid at 4 than 24°C. Plants did not significantly affect COD and DOC removal at 24°C. At 4°C, all plant species enhanced COD removal compared with controls, and removal was greater in sedge and bulrush columns than in cattail columns.

At both temperatures, COD and DOC removal was initially rapid, slowed over time, and asymptotically approached a relatively persistent residual level. Temperature and plant effects developed rapidly after the start of incubations. After 4 h at 24°C, DOC removal averaged 40% for all treatments; at 4°C, 4-h removal averaged 41% for controls and cattail but 58% for sedge and bulrush, though only sedge and controls differed significantly. After 24 h at 24°C, COD removal averaged 52% and DOC removal averaged 59%, with no significant differences among treatments. At 4°C, COD re-

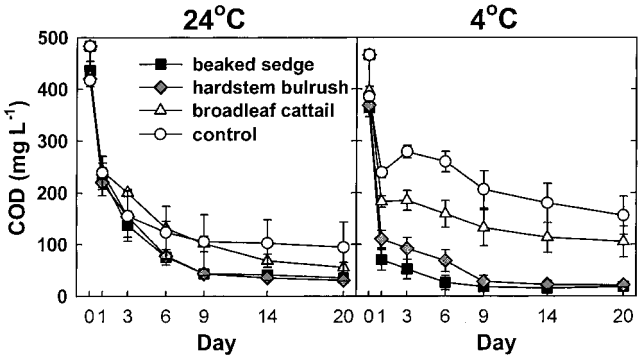

Fig. 3. Chemical oxygen demand (COD) concentrations during incubations at 24 and 4°C. Symbols and bars represent means of four replicates ± one standard error. The two sets of symbols for Day 0 represent influent wastewater and samples taken from columns immediately after filling with fresh wastewater.

moval over 24 h was significantly less in control and cattail columns (55%) than in sedge and bulrush columns (80%); DOC removal was significantly lower in controls (50%) than sedge and bulrush columns (80%).

The greater COD removal for sedge and bulrush at 4°C generally continued throughout time within each incubation. Differences were greatest on Days 3 and 6 and then diminished somewhat (Fig. 3). Final COD removal was relatively complete for sedge and bulrush at both temperatures (93–96%). At 24°C final COD removal for cattail and controls was also relatively complete (89 and 81%, respectively). However, at 4°C final removal was 77% for cattail columns and 67% for unplanted controls.

The two indicators of root zone oxidation status, Eh and SO_4^{2-} concentration, were affected significantly by plant treatment, temperature, and incubation time, and Eh sometimes varied significantly with depth (Fig. 4). Patterns of Eh and SO_4^{2-} concentration were consistent with those for COD and DOC removal: plant effects on Eh and SO_4^{2-} were greater at low temperatures, and sedge and bulrush enhanced root zone oxidation. Dissolved oxygen was consistently below 1 mg L^{-1} and did not vary with temperature or plant treatment.

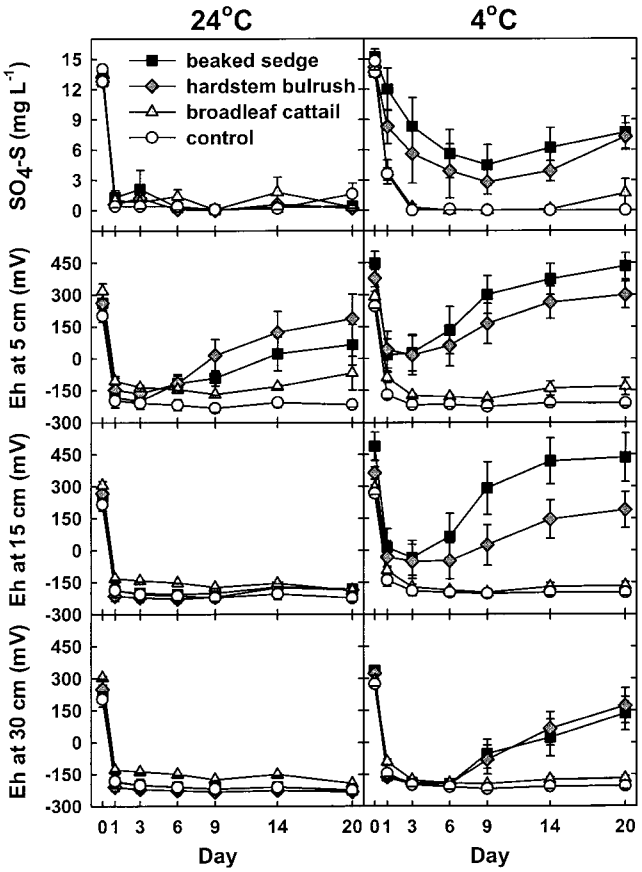

Fig. 4. Sulfate concentration and redox potential (Eh) during incubations at 24 and 4°C. Symbols represent means of four replicates for SO_4^{2-} and eight replicates for Eh (five for broadleaf cattail and controls at 24°C). Error bars represent ± one standard error. The two sets of symbols for SO_4^{2-} on Day 0 represent influent wastewater and samples taken from columns immediately after filling with fresh wastewater.

Averaged across all depths and times at 4°C, Eh values were significantly higher in sedge and bulrush columns than in cattail and control columns (Fig. 4). In contrast, when averaged across all depths and times at 24°C, Eh did not differ significantly among treatments. At 24°C, Eh values were higher in sedge and bulrush columns than cattail and control columns at the 5-cm depth, but not at 15 or 30 cm. In sedge and bulrush columns, Eh was higher at 4 than 24°C at all depths, but in cattail and control columns, Eh remained low at both temperatures.

Redox potentials and sulfate concentrations were high immediately after filling the columns with fresh wastewater and generally decreased rapidly within 24 h (Fig. 4). At 4°C and all depths, Eh decreased from an initial range of approximately +250 to +450 mV to a range of approximately −170 to +40 mV within 24 h. In cattail and control columns, Eh reached a minimum of approximately −220 to −170 mV by Day 3 and remained near this level for the rest of the incubation. In contrast, Eh in sedge and bulrush columns reached a minimum of approximately −200 to +20 mV by Day 3, but values increased continuously after Days 3 to 6 and reached final values of approximately +140 to +430 mV, which were approximately 340 to 640 mV greater than in controls. As depth increased, minimum Eh values in sedge and bulrush columns decreased and persisted longer, and the final values decreased. Plant species effects on Eh at 4°C were significant except during the time period 8 to 48 h.

Plant species and depth effects on Eh were far less pronounced at 24°C (Fig. 4). No plant effects were observed at 15 and 30 cm, and values were approximately −230 to −130 mV from Day 1 through Day 20. At 5 cm, however, plant species effects were similar to those observed at 4°C, but weaker. Again, Eh approached minimum values in 1 d in all columns, but in bulrush and sedge columns Eh values increased after 3 to 6 d, while values remained low in cattail and control columns. Unlike at 4°C, the increase was more pronounced in bulrush than sedge.

As with COD, DOC, and Eh, plant effects on sulfate concentration were more pronounced at low temperature (Fig. 4). Across all times at 4°C, plant species influenced SO_4^{2-} concentrations significantly, with higher values in sedge and bulrush columns than in cattail and control columns. At 24°C, SO_4^{2-} concentrations were uniformly low and were not influenced significantly by plants. Sulfate concentrations in sedge and bulrush columns were higher at 4 than 24°C, but temperature did not affect SO_4^{2-} concentrations in cattail and control columns.

DISCUSSION

In subsurface-flow wetlands (SSWs), influent dissolved organic matter is believed to be removed primarily by anaerobic microbial metabolism, with some aerobic metabolism near roots and at the gravel bed surface (USEPA, 1993, 2000). Because microbial metabolism generally decreases with decreasing temperature, wet-

land design criteria transferred from conventional wastewater treatment engineering have generally assumed the same temperature dependency (Reed et al., 1995; Campbell and Ogden, 1999). The cold-season decline in dissolved OM removal (measured by COD and DOC) we observed for unplanted controls, and to a lesser extent cattail columns, reflected this. However, greater OM removal in sedge and bulrush columns at low temperature supports the conclusion of many researchers, as summarized in Kadlec and Knight (1996), that low temperatures need not decrease OM removal in operational SSWs. In fact, our results indicate that factors that enhance electron acceptor availability or root zone oxidation status can be at least as important as temperature in controlling OM removal. The most rapid and extensive OM removal was associated with the highest observed Eh values and SO_4^{2-} concentrations, in sedge and bulrush columns at 4°C. In cattail and control columns, Eh values and SO_4^{2-} concentrations were not affected by temperature and OM removal was depressed in winter.

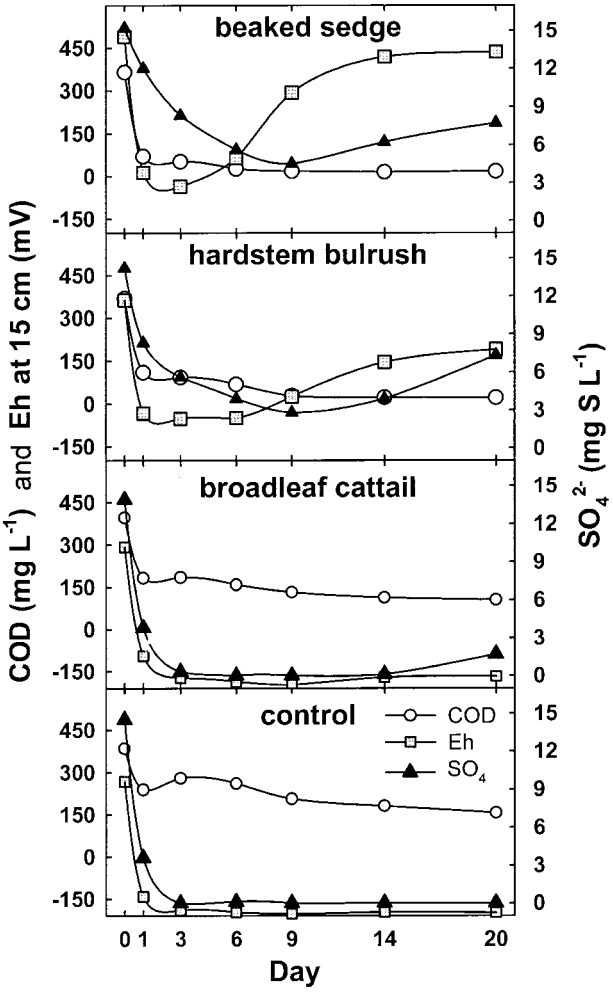

Fig. 5. Chemical oxygen demand (COD) and root zone oxidation status during incubations at 4°C. Mean values from Fig. 3 and 4 are combined to show consistencies among temporal patterns of COD, Eh, and SO_4^{2-}. Collectively, these results suggest that increased COD removal with beaked sedge and hardstem bulrush at low temperature reflects their greater ability to oxidize the root zone compared with broadleaf cattail and unplanted controls.

Greater dissolved OM removal and increased root zone oxidation for sedge and bulrush columns at low temperature were potentially due to both an increase in oxygen flux from plant roots and a decrease in root zone decomposition processes that produce dissolved OM, but the absence of this temperature response in cattail and control columns suggests that oxygen flux from sedge and bulrush root systems was significant. Our indirect indicators of root zone oxygen supply, Eh and SO_4^{2-} in the bulk solution, represent the net effect of plants and microbes on root zone oxidation status, and should not be confused with measurements of actual oxygen flux from roots (Bedford et al., 1991; Steinberg and Coonrod, 1994; Jespersen et al., 1998). Nonetheless, other studies have suggested greater potential for plants to supply oxygen for root zone processes at low than high temperatures (Gries et al., 1990; Howes and Teal, 1994; Callaway and King, 1996). Oxygen consumption by root respiration, which varies seasonally with temperature and plant growth, appears to be the major variable influencing root zone oxygen supply (Howes and Teal, 1994). In our study, plant effects on Eh and SO_4^{2-} concentrations generally developed when oxygen consumption by plants and microbes was probably lowest; that is, at low temperature, as described above, or in later days of 24°C incubations, after most dissolved OM had been consumed. Studying a reed bed system, Griffin et al. (1999) reported indirect evidence for seasonal differences in root zone oxidation; sulfide odor was a problem during summer but not during winter at low temperatures. Sulfate is converted to sulfide by sulfate-reducing bacteria only after oxygen and other more thermodynamically favorable electron acceptors have been depleted.

We interpret the relationships among changes in COD, Eh, and SO_4^{2-} during low-temperature incubations as reflecting a strong connection between total oxygen demand and root zone oxidation status (Fig. 5). For all planted and unplanted columns, the majority of COD removal at 4°C occurred within 24 h and was associated with a rapid reduction in Eh values and SO_4^{2-} concentrations. However, removal of COD was more rapid in sedge and bulrush columns than in cattail and control columns even though the associated decreases in Eh values and SO_4^{2-} concentrations were less pronounced, suggesting greater oxygen supply to the bulk solution, presumably due to oxygen flux from plant roots. In cattail and control columns, COD depletion slowed after 24 h and Eh values and SO_4^{2-} concentrations remained low, indicating there was insufficient oxygen supply to meet all respiratory demands. In sedge and bulrush columns, COD was largely depleted after 3 to 6 d; thereafter, Eh values rose steadily. Sulfate concentrations began to rise after Day 9, indicating that oxygen supply may have exceeded respiratory demands and was sufficient for some sulfide oxidation. Similar to other findings that measurement of oxidation around root tips is influenced by the reducing capacity of the soil (Flessa and Fischer, 1992), there appeared to be a threshold of low dissolved OM (in this case COD equal to approximately 30 mg L^{-1} for sedge and bulrush) that had to be reached before oxygen flux from plant roots could be

expressed as an increase in Eh of the bulk solution. This threshold was reached in sedge and bulrush columns at 4°C and to a more limited extent at 24°C, but it was not reached in cattail columns. Seasonal patterns of OM removal, Eh, and SO_4^{2-} over the entire study period were consistent with these interpretations (Hook et al., 2002).

Our inference that species' differences in OM removal and root zone oxidation reflected differences in oxygen release from roots is consistent with observations by other researchers. More iron oxide accumulated on roots of sedge than cattail growing together in an Ontario, Canada wetland (Crowder and MacFie, 1986). Better performance of bulrush than cattail with respect to ammonium removal in a California wetland was attributed to better nitrification (Gersberg et al., 1986), consistent with greater oxygen release reported by Bedford et al. (1991). Variation in oxygen flux from roots of different plant species results from differences in aerenchyma development, permeability of root surfaces, oxygen transport mechanisms, and metabolic pathways (Reddy et al., 1989a; Jackson and Armstrong, 1999), and differences in root densities and depth distributions (Gersberg et al., 1986; Campbell and Ogden, 1999; Moorhead and Reddy, 1988). While not evaluated in our study, these characteristics offer possible reasons for increased OM removal and root zone oxidation in sedge and bulrush columns than in cattail columns.

Our results, together with evidence from field studies (Kadlec and Knight, 1996; Smith et al., 1997; Wittgren and Maehlum, 1997) and recent research on arctic and alpine soil microbiology (Brooks et al., 1996; Schmidt et al., 1999), reinforce doubts about two common assumptions in SSW design: (i) that biological treatment processes are insignificant at temperatures near freezing, and (ii) that plants have a minimal role in treatment processes. There is substantial evidence that even at temperatures near and below 0°C soil microbial processes can be significant and regulated by factors other than temperature, such as organic matter quantity and quality, electron acceptor availability, or nutrient availability (Brooks et al., 1996; Schmidt et al., 1999). In operational SSWs, water temperatures often remain above freezing even when air temperatures are well below 0°C for long periods (Kadlec and Knight, 1996; Smith et al., 1997). Our microcosm results suggest that effective organic matter removal in SSWs is possible at temperatures near freezing, depending on the presence and species of plants. Somewhat surprisingly, differences in OM removal and root zone oxidation among species were greatest at low temperatures, suggesting a key role for plants especially during dormancy.

Reviews that question the importance of plant selection to SSW performance (USEPA, 2000) may reflect emphasis on continuous high organic matter loading and warm temperatures in comparative studies, as well as the greater complexity and lack of statistical replication in operational treatment wetlands. The controlled environments used here were not intended to recreate realistic winter conditions of operational SSWs, and the relatively small size of our experimental units may have accentuated root and shoot density and, therefore, altered plant effects. Nonetheless, our experiments represented a wide range of temperatures and OM levels (due to depletion over time within incubations), achieved a degree of control and replication not feasible in the field, and allowed for careful evaluation of the relative performance of species tested. The success, especially in winter, of sedge, which is much less researched than bulrush or cattail, points to the need to investigate additional plant species and to quantify the role of oxygen flux from root systems in operational cold-climate SSWs.

ACKNOWLEDGMENTS

We thank David Shanight and Kate Riley for many hours of sample collection and analysis. Funding was provided by: USDA-NRI Competitive Grants Program, Award 96-35102-3837; Bureau of Reclamation, Agreement 97-FG-60-09030; Montana University Water Resources Center and the USGS Water Resource Research Program; USEPA Science to Achieve Results (STAR) Fellowship Program; and Montana Agricultural Experiment Station, Projects MONB00127 and MONB00199.

REFERENCES

Allen, W.C. 1999. Seasonal plant effects on wastewater treatment and root-zone oxidation in model wetlands. M.S. thesis. Montana State Univ., Bozeman.

Armstrong, W., J. Armstrong, and P.M. Beckett. 1990. Measurement and modeling of oxygen release from roots of *Phragmites australis*. p. 41–51. *In* P.F. Cooper and B.C. Findlater (ed.) Constructed wetlands in water pollution control. Pergamon Press, Oxford.

Bedford, B.L., D.R. Bouldin, and B.D. Beliveau. 1991. Net oxygen and carbon-dioxide balances in solutions bathing roots of wetland plants. J. Ecol. 79:943–959.

Bodelier, P.L.E., J.A. Libochant, C.W.P.M. Blom, and H.J. Laanbroek. 1996. Dynamics of nitrification and denitrification in root-oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats. Appl. Environ. Microbiol. 62:4100–4107.

Brooks, P.D., S.K. Schmidt, and M.W. Williams. 1996. Winter production of CO_2 and N_2O from alpine tundra: Environmental controls and relationship to inter-system C and N fluxes. Oecologia 110: 403–413.

Callaway, R.M., and L. King. 1996. Temperature-driven variation in substrate oxygenation and the balance of competition and facilitation. Ecology 77:1189–1195.

Campbell, C.S., and M.H. Ogden. 1999. Constructed wetlands in the sustainable landscape. John Wiley & Sons, New York.

Crowder, A.A., and S.M. MacFie. 1986. Seasonal deposition of ferric hydroxide plaque on roots of wetland plants. Can. J. Bot. 64: 2120–2124.

DeBusk, T.A., and W.F. DeBusk. 2001. Wetlands for water treatment. p. 241–279. *In* D.M. Kent (ed.) Applied wetlands science and technology. 2nd ed. Lewis Publ., Boca Raton, FL.

Faulkner, S.P., W.H. Patrick, Jr., and R.P. Gambrell. 1989. Field techniques for measuring wetland soil parameters. Soil Sci. Soc. Am. J. 53:883–890.

Flessa, H., and W.R. Fischer. 1992. Plant-induced changes in the redox potentials of rice rhizospheres. Plant Soil 143:55–60.

Gersberg, R.M., B.V. Elkins, S.R. Lyon, and C.R. Goldman. 1986. Role of aquatic plants in wastewater treatment by artificial wetlands. Water Res. 20:363–368.

Gries, C., L. Kappen, and R. Losch. 1990. Mechanism of flood tolerance in reed, *Phragmites australis* (Cav.) Trin. ex Steudel. New Phytol. 114:589–593.

Griffin, P., P. Jennings, and E. Bowman. 1999. Advanced nitrogen removal by rotating biological contactors, recycle, and constructed wetlands. Water Sci. Technol. 40:383–390.

Hook, P.B., O.R. Stein, W.C. Allen, and J.A. Biederman. 2002. Plant species effects on seasonal performance patterns in model subsurface wetlands. *In* Ü. Mander and P.D. Jenssen (ed.) Constructed

wetlands for wastewater treatment in cold climate areas. WIT Press, Ashurst, UK (in press).
Howes, B.L., and J.M. Teal. 1994. Oxygen loss from *Spartina alterniflora* and its relationship to salt marsh oxygen balance. Oecologia 97:431–438.
Jackson, M.B., and W. Armstrong. 1999. Formation of aerenchyma and the process of plant ventilation in relation to soil flooding and submergence. Plant Biol. 1:274–287.
Jespersen, D.N., B.N. Sorrell, and H. Brix. 1998. Growth and root oxygen release by *Typha latifolia* and its effects on sediment methanogenesis. Aquatic Bot. 61:165–180.
Kadlec, R.H., and R.L. Knight. 1996. Treatment wetlands. Lewis Publ., Boca Raton, FL.
Light, T.S. 1972. Standard solution for redox potential measurements. Anal. Chem. 44:1038–1039.
Moorhead, K.K., and K.R. Reddy. 1988. Oxygen transport through selected aquatic macrophytes. J. Environ. Qual. 17:138–142.
Reddy, K.R., E.M. D'Angelo, and T.A. DeBusk. 1989a. Oxygen transport through aquatic macrophytes: The role in wastewater treatment. J. Environ. Qual. 19:261–267.
Reddy, K.R., W.H. Patrick, Jr., and C.W. Lindau. 1989b. Nitrification–denitrification at the plant root–sediment interface in wetlands. Limnol. Oceanogr. 34:1004–1013.
Reed, S.C., R.W. Crites, and J.E. Middlebroooks. 1995. Natural systems for waste management and treatment. 2nd ed. McGraw–Hill, New York.
SAS Institute. 1996. SAS Version 6.12. SAS Inst., Cary, NC.
Schmidt, S.K., S. Jonason, and A. Michelsen. 1999. Mineralization and microbial immobilization of N and P in arctic soils in relation to season, temperature, and nutrient amendment. Appl. Soil Ecol. 11:147–160.
Smith, I.D., G.N. Bis, E.R. Lemon, and L.R. Rozema. 1997. A thermal analysis of a sub-surface, vertical flow constructed wetland. Water Sci. Technol. 35:55–62.
Sorrell, B.K., and W. Armstrong. 1994. On the difficulties of measuring oxygen release by root systems of wetland plants. J. Ecol. 82: 177–183.
Steinberg, S.L., and H.S. Coonrod. 1994. Oxidation of the root zone by aquatic plants growing in gravel–nutrient solution culture. J. Environ. Qual. 23:907–913.
Stumm, W., and J.J. Morgan. 1996. Aquatic chemistry: Chemical equilibria and rates in natural waters. John Wiley & Sons, New York.
Towler, B.W. 1999. Evapotranspiration crop coefficients for two constructed wetland macrophytes: Cattail and bulrush. M.S. thesis. Montana State Univ., Bozeman.
USEPA. 1993. Subsurface flow constructed wetlands for wastewater treatment: A technology assessment. EPA/832/R/93/001. USEPA, Office of Water, Washington, DC.
USEPA. 2000. Manual: Constructed wetlands treatment of municipal wastewaters. EPA/625/R-99/010. USEPA, Natl. Risk Management Res. Lab., Office of Res. and Development, Cincinnati, OH.
Veneman, P.L.M., and E.W. Pickering. 1983. Salt bridge for field redox potential measurements. Commun. Soil Sci. Plant Anal. 14: 669–677.
Wittgren, H.B., and T. Maehlum. 1997. Wastewater treatment wetlands in cold climates. Water Sci. Technol. 35:45–53.