Chapter 29
Sulfur Cycling in Constructed Wetlands

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Abstract Constructed wetlands (CWs) have been successfully employed in both mining and domestic wastewater applications, yet the fundamental processes responsible for treatment are poorly quantified. Sulfur is common in CW influent streams and is highly reactive, redox-sensitive, and microbially active; therefore, it plays an important role in both desirable and deleterious processes in CWs. In this chapter we review the major sulfur transformations likely occurring in CWs, their interactions with other important processes, and their role in the treatment process. We also present two case studies on the influence of sulfate-reducing bacteria and sulfur-oxidizing bacteria on the performance of CW systems designed to treat mining-contaminated and municipal wastewater, respectively. In both cases there is a feedback between these microbial consortia and other microbes responsible for treatment. A better understanding of the important sulfur transformations in CWs will lead to better design and more confident performance expectations.

Keywords Bacteria, chemical precipitation, mining, sulfate, sulfide, treatment wetlands

29.1 Introduction

Constructed wetlands (CWs) have been successfully employed to treat a wide range of municipal, industrial, and mining wastewaters. In addition to their low operational costs, an attractive feature of CWs is their versatility in treating waste streams which

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may vary significantly in organic carbon and nutrient loading. Constructed wetlands are adaptable to organic carbon removal from high strength (copiotrophic) domestic and municipal wastes as well as contaminant removal from mesotrophic and oligotrophic waste streams, such as nutrient removal in agricultural runoff and acid neutralization and precipitation of metals from acid mine drainage (Kadlec & Knight, 1996). This versatility is, at least in part, attributable to the wide range of bacterial niches present in CWs. Oxidation-reduction (redox) state is known to vary spatially along the flow path, with distance from the air–water interface, and/or distance from plant tissues, especially roots (Kadlec & Knight, 1996; Allen *et al.*, 2002; Garcia *et al.*, 2003). Spatial gradients lead to a variety of ecologically distinct zones which may be inhabited by obligate aerobic microorganisms, facultative anaerobes, iron- and manganese-oxidizing organisms, sulfate-reducing bacteria, methane-producing bacteria, and fermentative organisms. The extent to which members of these groups thrive is dependant on characteristics of the wastewater, temperature, season, wetland plant phenology, and wetland design.

Gaining a comprehensive understanding of the interplay between the biotic and abiotic reactions in wetlands has been a major challenge in CW engineering and operation. The success of the traditional "black box" approach to CW operation is testament to the above-mentioned innate versatility of these systems, yet a broader understanding of microscale wetland processes would clearly assist their design. Research over the past decade has shed light on the complexity of wetlands microbiology and geochemistry, yet, many questions remain. Several recent investigators have identified sulfur-related processes within wetlands as both poorly understood and of prime importance in advancing a broader understanding of wetland function (Whitmire & Hamilton, 2005; Wiessner *et al.*, 2005). Sulfur can occur in four valence states, $-2(H_2S)$, $0(S^0)$, $+2(S_2O_3^{2-})$ and $+6(SO_4^{2-})$; thus, it is reactive under both oxidized and reduced conditions as well as in both biotic and abiotic settings. It can be an electron donor or electron acceptor in energy-producing microbial reactions and reacts with virtually all metals (except gold and platinum) to form metal sulfides. Sulfur is also a macronutrient for microbial and plant growth. It is typically abundant in CW influent streams, including municipal and industrial wastewater and especially, acid rock drainage. Sulfur's high reactivity, redox sensitivity, and microbial activity, when combined with the range of conditions found in many CWs, leads to complex geomicrobial interactions. These interactions are often an integral part of the treatment process, e.g., metals removal; may inhibit a desired process, e.g. plugging due to precipitate formation; and at the least are indicators of dominant pathways for, and/or the relative importance of other important removal mechanisms.

In this chapter, we review the various biologically catalyzed and abiotic sulfur fate and transport pathways that are likely active within CWs. To keep application as broad as possible, we do not specify the type of CWs, e.g., free water surface (FWS) or subsurface flow (SF), nor do we specify the type of wetland, scales, and other considerations – such as influent water chemistry, macrophytic plant species selection, temperature, and season – that will influence the magnitudes of the sulfur transformations that might occur in a specific CW. Finally, we present two case examples of sulfur transformations in operating and model CWs.

29.2 Conceptual Model of Sulfur Fate and Transport in Constructed Wetlands

Influent sulfur to wetlands is typically in the form of sulfate in oxidized environments and sulfide in reduced environments, although other sulfur compounds representing intermediate valence states, including thiosulfate, other polythionates, and organic sulfur can also occur in CW influent. Sulfate is highly soluble under all temperature and pH conditions, whereas sulfide solubility is pH-dependant. Sulfide solubility increases tenfold between pH 6–8. At acidic pH (<6) sulfide will be present as H_2S, which has a much lower solubility than the deprotonated form, HS^-, which predominates above neutral pH ($pK_a = 7.04$) (Stumm & Morgan, 1996). Lower pH systems thus have the propensity to offgas H_2S, with the accompanying rotten-egg odor, whereas pH-neutral and above systems maintain higher sulfide concentrations in solution.

In well-aerated systems, or systems with relatively low levels of assimilable organic carbon (AOC), the entire water column (in the case of FWS) and the upper reaches of the sediment strata or sediment within the plant rhizosphere (in the case of FWS and SF) may be aerobic. Sulfate concentrations will be lowered under these conditions mainly through abiotic mineral precipitation (such as gypsum, $CaSO_4$) or biological assimilation into plant or microbial tissue (Fig. 29.1). Bacterial assimilation of sulfur occurs through the reduction of sulfate to the amino acid cysteine ($C_3H_7NO_2S$), thus creating organic sulfur (Le Faou *et al.*, 1990).

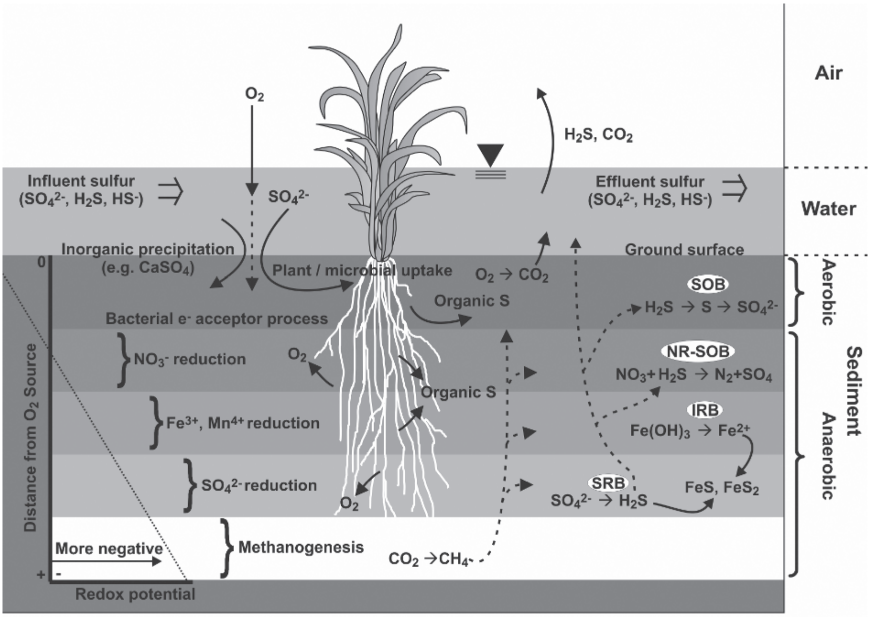

Fig. 29.1 Major biotic and abiotic sulfur transformations in constructed wetlands and their relation to redox potential

Sulfide dissolved in influent water or diffusing from anoxic sediment strata may be subject to oxidation in more aerobic zones through either abiotic processes or via reactions catalyzed by sulfur-oxidizing bacteria (SOB). Under anoxic conditions, sulfide may also precipitate with metals to form metal sulfides, such as iron sulfide (FeS). By far the most important biotic reactions influencing sulfur are those catalyzed by dissimilatory sulfate-reducing bacteria (SRB), which generate energy from the transfer of electrons from organic substrates to sulfate, thereby reducing it to sulfide. Sulfide thus generated may precipitate (as above), diffuse into the water column and offgas, or be oxidized back to sulfate through biotic or abiotic reactions. A variety of biogenic organic sulfur compounds may also exist in equilibrium in the aqueous phase or in transition to the vapor phase. These compounds may be produced by either sediment-associated microbial activity, or as a result of plant activity.

Microbially catalyzed reactions in CW sediments occur in zones which reflect the sequential consumption of electron acceptors based on the energy available from each process. Oxygen diffusing into sediments is rapidly consumed by aerobic or facultative organisms, as it has the highest available energy of potential electron acceptors. In anoxic sediments, nitrate (if present) is often the next most energetic electron acceptor, followed by mineral phase ferric iron or manganese (Mn^{+4}), sulfate, and methanogenesis. The conceptual location of these electron acceptor zones in relation to an oxygen source (atmosphere and/or plant roots) is shown in Fig. 29.1. In all but the most oligotrophic wetlands environments, AOC is present at concentrations sufficient to deplete available dissolved oxygen and overwhelm the oxygen supply rate, leading to the sequential consumption of the less-energetic electron acceptors. Sulfate reduction has been found to be the dominant terminal electron acceptor process in high organic carbon-containing groundwater and open water systems (Wiedemeier *et al*., 1999), as well as in low-AOC systems containing high concentrations of sulfate (D'Hondt *et al*., 2002). The abundance of both AOC and sulfate in many CW environments therefore suggests that sulfate reduction will likely account for a large proportion of the total microbial activity. The fate and transport of influent sulfur in a CW depends on the interplay between these bacterially catalyzed reactions, which are discussed in detail below.

29.2.1 Bacterially Catalyzed Sulfur Reactions

29.2.1.1 Bacterial Sulfate Reduction

Sulfur participates in a variety of bacterially catalyzed oxidation and reduction reactions which may impact its mobility and fate in CWs. The most familiar of these is the dissimilatory sulfate-reduction reaction catalyzed by SRB in anoxic water and sediment. SRB gain energy by coupling the oxidation of organic compounds or H_2 to sulfate reduction and liberate inorganic carbon and sulfide (H_2S, HS^-, or S^{2-}, depending on pH), as the primary end products of organic matter mineralization (Megonikal *et al.*, 2004). The process is of major importance in wetlands due to the propensity of produced sulfide to form insoluble metal sulfides. Dissimilatory sulfate reduction is thought to be among the oldest metabolic processes of life on Earth and is found among a wide range of both gram-positive and gram-negative bacterial genera. SRB are a phylogenetically diverse group of δ-Proteobacteria encompassing over 20 genera and utilizing a range of organic electron donors, including H_2, volatile fatty acids (VFAs), and some primary alcohols. SRB are divided into two main groups: (1) incomplete oxidizers (*Desulfovibrio*, *Desulfomicrobium*), which utilize VFAs such as pyruvate, formate, and butyrate and produce acetate; and (2) complete oxidizers (*Desulfobacter, Desulfobacterium*), which utilize fatty acids, including acetate, and produce carbon dioxide (Widdel, 1988). SRB were once thought to be capable of growth using only sulfate as the electron acceptor, and only in the absence of dissolved oxygen. Recent research has shown that some SRB are capable of growth using more energetic electron acceptors, particularly nitrate (Itoh *et al.*, 2002; Lopez-Cortes *et al.*, 2006). In addition, some SRB have been shown to both tolerate low concentrations of oxygen and possess mechanisms for oxygen detoxification (Vasconcelos & McKenzie, 2000). Such attributes generally insure the survival of SRB populations in sediments that are periodically (or seasonally) exposed to oxygen or other electron acceptors.

The catabolic bacterial sulfate-reduction reaction generates one mole of sulfide per mole of sulfate utilized, as illustrated in the following stoichiometry with acetate as the electron donor:

$$CH_3COO^- + H^+ + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$$

SRB activity also results in the generation of alkalinity, which may raise the pH of acidic systems. It is important to note that sulfate reduction does not occur in isolation, but in concert with other microbial reactions (Fig. 29.1), including fermentation and methanogenesis. The use of organic acids as electron donors by SRB implies a close relationship between SRB activity and the activity of fermentative organisms which generate VFAs as a product of metabolism. These reactions create a highly reducing environment, which in sediments may accumulate reduced inorganic species such as Fe^{2+}, Mn^{2+}, NH_4^+, and CH_4, in addition to sulfide and bicarbonate. Biogenic sulfide may undergo further biotic and abiotic reactions with these compounds.

Dissimilatory sulfate reduction can account for half or more of the total organic carbon mineralization in many environments (Jørgensen, 1982). In freshwater environments, SO_4^{2-} reduction can account for a significant portion of anaerobic mineralization processes, and in some instances can be the dominant pathway (Bak & Pfennig, 1991; Urban *et al.*, 1994; Holmer & Storkholm, 2001). In wetlands that do not have significant dissolved metal concentrations or iron-containing sediments, biogenic sulfide production may exceed the capacity of the precipitation mechanisms mentioned above and diffuse out of anoxic sediments into either oxygen-containing sediments or the water column. Under these conditions, H_2S may offgas to the atmosphere, or more commonly, reoxidize back to thiosulfate

(S_2O_3) or sulfate through reaction with ferric iron, manganese dioxide, nitrate, or oxygen. Sulfide can also be oxidized back to elemental sulfur or sulfate by chemolithotrophic bacteria under aerobic conditions (Nelson *et al.*, 1986). Sulfur-oxidizing bacteria (SOB) are typically active in a relatively narrow ecological zone where oxygen diffusing in one direction occurs concurrently with sulfide diffusing in the other, and rates of bacterial sulfur oxidation are highest where oxygen concentrations are limited. Where oxygen is plentiful, abiotic sulfide oxidation accounts for the majority of reoxidation to sulfate.

29.2.1.2 Bacterial Iron Reduction

Ferric oxyhydroxide minerals are very common in wetland sediments, and ferrous iron (Fe^{2+}) results from bacterial reduction of these minerals under anoxic conditions. Solution phase Fe^{2+} reacts rapidly with biogenic sulfide to form amorphous iron(II) monosulfide (FeS), which typically precipitates as a black solid. Newly formed FeS is noncrystalline and thus does not have a repeating structure; however, amorphous FeS further reacts with reduced sulfur species to form more sulfur-enriched crystalline solids, such as greigite (Fe_3S_4) and, ultimately, pyrite (FeS_2). While newly formed FeS is subject to re-dissolution under some conditions (such as low pH), the evolved crystalline solids are more thermodynamically stable and resistant to dissolution (Sweeney & Kaplan, 1973). Immobilization of precipitated metal sulfides is an important metal- and sulfur-removal process in CWs and other bioreactor systems designed to treat metal-rich wastewater. Furthermore, rapid consumption of biogenic sulfide via FeS precipitation acts as a detoxification mechanism to prevent sulfide accumulation and toxicity to SRB (Reis *et al.*, 1992), thereby allowing further sulfate consumption.

29.2.1.3 Bacterial Manganese Reduction

Like ferrous iron, solution-phase divalent manganese results from the bacterial reduction of manganese minerals, such as manganese dioxide (MnO_2), under anoxic conditions. Because manganese sulfides are highly soluble, Mn(II) typically does not precipitate as MnS. However, where SRB are active, MnO_2 may react directly with H_2S to form solution phase Mn^{2+} and elemental sulfur (S^0). Sulfur may then undergo bacterial disproportionation to sulfide and sulfate (Thamdrup *et al.*, 1993). Sulfide may then be reoxidized to S to repeat this cycle, and sulfate may be reduced by SRB or diffuse into the water column.

29.2.1.4 Bacterial Nitrate Reduction

As the next most energetic electron acceptor after oxygen, nitrate is usually rapidly consumed by heterotrophic nitrate-reducing bacteria (NRB) in anoxic zones of CWs.

The first step in nitrate reduction produces nitrite (NO_2^-), which is actively inhibitory to SRB (Sturman *et al.*, 1999). Nitrite is then typically further reduced to nitric oxide (NO), nitrous oxide (N_2O), and ultimately to di-nitrogen (N_2). Where biogenic sulfide diffusing from the SRB-active zone is present concurrently with nitrate, chemolithotrophic bacteria can couple the reduction of nitrate with the oxidation of sulfide, as shown below.

$$5HS^- + 8NO_3^- \rightarrow 5\ SO_4^{2-} + 4N_2 + 3OH^- + H_2O$$

These so-called nitrate-reducing – sulfur-oxidizing bacteria (NR-SOB) are typified by members of the genus *Thiomicrospira*, and their activity has been noted to inhibit SRB through the production of the intermediate species nitrite (NO_2^-) during nitrate reduction (Haveman *et al.*, 2005). Therefore, in a CW, NR-SOB bacteria would likely be located in anoxic regions near SRB activity, but not concurrent with them. Sulfate produced by NR-SOB would either reenter the water column or diffuse into the SRB-active zone.

29.2.1.5 Bacterial Sulfur Oxidation

In the presence of available electron acceptors, sulfide, elemental S, thiosulfate, and tetrathionate are oxidized by both chemical and biological pathways (Wainwright, 1984; Paul & Clark, 1996):

$$SH^- \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$$

Sulfur oxidizing bacteria include primarily chemolithotrophic genera, but also phototrophic genera. Photosynthetic SOB couple the oxidation of reduced sulfur (H_2S, S^{2-}, S^0) with CO_2 reduction. They typically occupy anaerobic zones where light penetrates and sulfide is abundant, and accumulate elemental sulfur. So-called purple sulfur bacteria (Thiorhodaceae; e.g., *Chromatium*) generally deposit sulfur internally, whereas green sulfur bacteria (Chlorobacteriaceae, e.g., *Chlorobium*) accumulate sulfur extracellularly. In both cases, accumulated sulfur may be further oxidized to sulfate under conditions of sulfide limitation (Madigan *et al.*, 2000; Wetzel, 2001). Both forms are commonly found in mud and stagnant waters containing H_2S and exposed to light. They reoxidize H_2S, coming from lower anaerobic layers. They require light as an energy source and H_2S as an electron donor in the photosynthetic reduction of CO_2 (Trudinger, 1979; Paul & Clark, 1996; Wetzel, 2001).

Aerobic chemolithotrophic SOB can catalyze the oxidation of reduced sulfur to sulfate where sulfide and oxygen occur concurrently. Also known as colorless sulfur bacteria, these genera are most commonly associated with acidic conditions, such as would be associated with mine waste, but some genera are capable of growth under neutral pH conditions as well. The most common SOB genera in low pH mine waste streams are *Acidithiobacillus*, *Acidiphilium*, and *Sulfobacillus* where they catalyze the transformation of thiosulfate ($S_2O_3^{2-}$), elemental sulfur (S^0), or polysulfide (H_2S_n) from the immediate vicinity of active pyrite (or other metal sulfide) dissolution (Johnson, 1998; Fowler & Crundwell, 1999). Many acidophilic SOB are also capable of iron oxidation, and some species are also capable of heterotrophic growth utilizing organic carbon sources in addition to CO_2 (Johnson, 1998).

In neutral streams chemolithotrophic SOB typically occupy microaerophilic zones where they catalyze the oxidation of H_2S to sulfate. *Beggiatoa*, a long filamentous gliding bacterium, and *Thiothrix* are common bacteria that oxidize H_2S with deposition of sulfur intracellularly (Kowallik & Pringsheim, 1966; Shively, 1974; Strohl & Larkin, 1978). Colorless sulfur bacteria of the genus *Beggiatoa* are among the largest and most conspicuous of all bacteria. In nature, the filaments grow only where both H_2S and O_2 are present (Jørgensen, 1977; Kuenen & Beudeker, 1982). Since H_2S is not stable in oxic waters due to autocatalytic oxidation by O_2, the habitat of *Beggiatoa* is restricted to the transition zone between oxic and anoxic environments where O_2 and H_2S are continuously supplied by diffusion along opposite gradients. Where these gradients are steep, *Beggiatoa* and other types of colorless sulfur bacteria may form white patches of dense cell masses (Jørgensen, 1977; Whitcomb *et al.*, 1989). Oxidation of sulfide to sulfate, via S^0 intermediate, was described for *Beggiatoa* more than 100 years ago by Winogradsky (1887, 1888). These so-called gradient organisms (Konhauser, 2007) occupy a relatively narrow zone of low dissolved oxygen, taking advantage of the energy available in reduced sulfur before it can diffuse into more oxidized zones where sulfide is more likely to be oxidized abiotically. Because the zone of sulfide and oxygen overlap may vary temporally, many colorless sulfur bacteria are capable of storing partially oxidized sulfur (in the form of elemental sulfur) intracellularly, thereby insuring a source of sulfur if sulfide becomes limiting. In the wetlands context, it has been observed that *Beggiatoa* growing in association with plant roots serves to detoxify sulfide in the root zone, utilizing oxygen exuded by wetlands plants (Joshi & Hollis, 1977).

29.2.1.6 Methanogenesis (CH_4)

Methane is produced under anaerobic conditions through the activity of methanogenic bacteria. Methanogens utilize hydrogen and CO_2 (and in some cases simple organic molecules) as substrates to form methane. Methanogenic respiration yields the least energy of the common electron acceptor processes ($O_2 > NO_3^- > Mn^{4+}, Fe^{3+} > SO_4^{2-} >$ methanogenesis) and therefore methanogenesis typically occurs in sediment strata most isolated from atmospheric or dissolved oxygen. Methane is a highly energetic compound, of course, and may be utilized by SRB (in symbiotic association with some *Archaea*) or other heterotrophic bacteria as it diffuses away from methanogenic activity following production (Niewöhner *et al.*, 1998).

29.3 Sulfur Transformations in Constructed Wetlands for Mining Applications

Mining wastewater is typically high in dissolved metals and sulfate, and can range in pH from highly acidic (pH 1–3) to circumneutral, depending on the mineralogy of the mine and buffering capacity of subsequently encountered rock. Recognizing that this chemistry can be ideal for SRB activity and the subsequent precipitation of dissolved metals with the produced sulfide (provided organic carbon is available), CWs have been successfully employed to treat mine wastewater since the 1980s. However, evidence suggests that rates of sulfate reduction in wetlands are extremely variable and depend on many factors including pH, redox potential, type and quantity of available organic matter, and the ratio of organic carbon to sulfur (Westrich & Berner, 1988; Webb *et al.*, 1998; Lyew & Sheppard, 1999). Because SRB activity is essential to successful metals removal in CWs, the wetland should be designed to provide: (1) anaerobic conditions, (2) adequate organic carbon for SRB growth, and (3) some means of preventing sediment plugging that could result from the precipitation of metal sulfide solids. Maintaining adequate permeability to insure proper treatment is largely an engineering challenge, and is accomplished through either periodic solids removal or adequate initial treatment volume to insure the necessary life-span.

As noted in Section 29.2, SRB can survive periodic exposure to oxidized conditions, but will not actively reduce sulfate unless more energetic electron acceptors are absent. Since wetland plants add organic carbon necessary for consumption of more energetic electron acceptors (such as oxygen, nitrate, and ferric iron), but also oxygen, the most favorable electron acceptor, their effect on CW redox potential and microbial processes is important, site-specific, and poorly understood (Stein & Hook, 2005). To insure that energetically more-favorable electron acceptors do not overwhelm the desired SRB activity, dissolved organic carbon is usually added to the CW system (Lloyd *et al.*, 2004). However, the quantity required is likely influenced by specific influent chemistry, plant species selection, temperature, and season.

Utilizing a year-long cycle of varying temperature simulating seasonal variation under greenhouse conditions, Stein *et al.* (2007) compared the influence of two plant species (and unplanted CW) and two influent organic carbon concentrations on redox potential, sulfate reduction, and subsequent zinc precipitation. Results indicated that temperature, season, and plant species had significant interacting affects on redox potential, quantity of sulfate utilized, and the relative influence of sulfate reduction on organic carbon utilization. At identical organic carbon concentrations, redox potential was universally lowest and sulfate reduction was typically highest in unplanted CW, indicating that the net influence of plants is inhibitory for sulfate reduction. Across all plant treatments, sulfate reduction was least at 4°C in winter, but winter inhibition was greater in the planted CW, especially those planted with bulrush (*Schoenoplectus acutus*), which also displayed increased winter redox levels indicating oxygen was being utilized over sulfate for removal of organic carbon.

Higher influent organic carbon concentrations in bulrush treatments increased sulfate reduction in all seasons and dampened the observed increase in redox during winter. Similar patterns of zinc removal were observed; but variation due to temperature, season, and plant species was typically dampened.

The above-mentioned results clearly demonstrate that plant species selection and season can influence sulfate reduction in CWs by influencing root-zone oxygen release (Stein *et al.*, 2007). Because utilization of the influent organic carbon (as measured by chemical oxygen demand, COD) was virtually complete, regardless of temperature, season, and plant species – variation in sulfate removal is an indication of the competition between aerobic heterotrophs, methanogens and SRB in CW systems (no other electron acceptors were present). Results reinforce conclusions of previous studies (Callaway & King, 1996; Moog & Brüggemann, 1998) that roots of some plant species (but not others) release oxygen in winter. Increased winter oxygen availability increases aerobic respiration over other less-favorable metabolic pathways including sulfate reduction (Allen *et al.*, 2002; Stein & Hook, 2005). Thus, the quantity of organic carbon required to optimize a CW for sulfate reduction and the removal of dissolved metals will vary depending on operating temperature, season, and plant species (Fig. 29.2).

An unreported result of the above study was the evidence of purple photosynthetic SOB growing on the inside walls of the clear influent tubing. Some sulfate reduction occurred in the holding tanks and, due to the presence of sunlight in the connecting lines, these bacteria were able to utilize the produced sulfide and available organic carbon for growth. It is unknown whether these bacteria produced

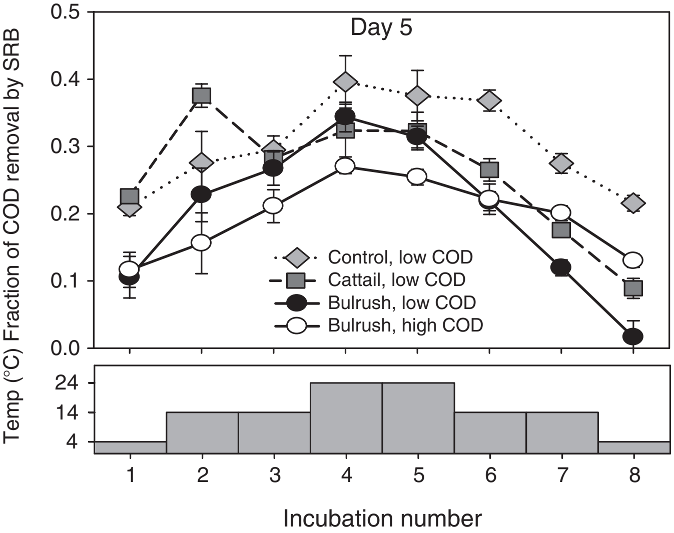

Fig. 29.2 Seasonal variation in the fraction of assimilable organic carbon removal attributable to the activity of sulfur-reducing bacteria (From Stein *et al.*, 2007. With permission from Elsevier)

elemental sulfur or if the sulfide was complexly oxidized to sulfate and then available for sulfate reduction once again in the CW, but their existence indicates that sulfur can cycle between oxidized, reduced, and back to oxidized states over relatively short spatial and temporal scales.

29.4 Sulfur Transformations in Constructed Wetlands for Domestic Wastewater Applications

Wastewater from domestic sources is rich in organic carbon and typically has sulfur concentrations 5–20 mg l^{-1} higher than the original water source, which can regionally have widely varying sulfur concentrations (Crites & Tchobanoglous, 1998). Significant industrial inputs can increase CW influent sulfur concentrations even more; thus, sulfur cycling can be an important component in domestic wastewater treatment CWs. As with mining applications, the most important biologically catalyzed sulfur transformation is sulfate reduction by SRB as the copious organic carbon concentrations typically overwhelm any oxygen supply and transfer mechanisms. Hook *et al*. (2003) observed that temperature, season, and plant species effects on sulfate reduction and redox potential at domestic wastewater influent concentrations were similar to, but often even more dramatic than, those subsequently observed at mining wastewater concentrations (Stein *et al*., 2007). Thus interactions between plant-mediated oxygen transfer and SRB activity may be more dramatic in CWs treating domestic wastewater.

Vymazal and Kröpfelová (2005) noted that a few CWs for domestic wastewater treatment in the Czech Republic precipitated what proved to be elemental sulfur within the effluent conduits and/or immediately upon contact with the receiving stream (Fig. 29.3), but most seemingly similar CWs did not. Treatment plant operators and local inhabitants equated the presence of these deposits with CW failure despite good performance for traditional parameters such as suspended solids and BOD_5 which met the discharge limits. Presumably the elemental sulfur deposits are evidence of SOB activity at the anoxic–oxic transition at the tail end of the CWs; however, there was no visual evidence of photosynthetic SOB anywhere in the system and the deposits appear to be formed extracellularly, while most chemolithotrophic SOB deposit sulfur intracellularly. The formation could be an abiotic process of unknown type. Regardless, the necessary requirement for elemental sulfur deposition is the reduction of influent sulfate to sulfide within the CW bed by SRB activity and consequent oxidation of sulfide upon release to oxic conditions.

A collection of limited water-quality data (Table 29.1) has not revealed a method to successfully predict the formation of white elemental sulfur patches in the CW effluent. The initial assumption was that systems with large reductions in sulfate concentration might lead to sulfur deposition due to high concentrations of effluent sulfide. However, the data revealed that at some systems with substantial sulfate concentration reductions (Chmelná, Břehov), white patches do not occur while massive patches occur even in systems with a very little evidence of sulfate reduction

Fig. 29.3 White patches of elemental sulfur in a stream receiving the outflow from the constructed wetland Mořina (Photo Jan Vymazal)

(Obecnice, Trhové Dušníky). Perhaps the sulfide produced in locations such as Chmelná and Břehov was retained in the CW by precipitation of metal sulfides (or outgassed at low pH), but this would not explain why some systems with poor sulfate reduction (Obecnice, Trhové Dušníky) created elemental sulfur deposits.

Table 29.1 Data from horizontal flow constructed wetlands treating municipal sewage in the Czech Republic

Locality	BOD_5 in (kg ha^{-1} day^{-1})	BOD_5 out (mg l^{-1})	BOD_5 removal (%)	NH_4-N removal (%)	Total N removal (%)	SO_4 removal (%)	Elemental sulfur deposits
Chlístovice	6	4	25	41	46	12	NO
Onšov	7	6	46	37	26	1	NO
Doksy	9	6	84	66	44	2	NO
Mořina 2002	16	4	90	28	35	28	NO
Chmelná	26	5	76	17	21	49	NO
Obecnice	27	11	93	28	33	29	MASSIVE
Břehov	30	11	78	33	35	56	NO
Mořina 2003–2004	36	45	68	15	17	55	MASSIVE
Koloděje	54	10	93	35	38	23	NO
Čistá	59	7	81	17	21	15	NO
Ondřejov	73	11	92	15	20	29	MILD
Trhové Dušníky	145	56	92	50	51	20	MASSIVE

In general, elemental sulfur precipitation occurred in systems with higher organic loads, but not all heavily loaded systems exhibited the formation of elemental sulfur. There is also a mild correlation between sulfur deposits and higher outflow BOD_5 concentrations, but in Obecnice massive deposition occurred with BOD_5 concentration as low as 11 mg l^{-1}. More detailed water-quality monitoring and/or microbial assays in the vicinity of the sulfur deposits will be required to determine the cause of their formation.

29.5 Conclusions

Sulfur transformations play an important role in many biogeochemical reactions occurring in CWs. Most important of these is the reduction of sulfate to sulfide, catalyzed by the ubiquitous SRB. The subsequent precipitation of metal sulfides in systems with high dissolved metal concentrations makes this the dominant mechanism for removal of metals in CW treating mining wastewater. Because most CWs treating domestic wastewater have high concentrations of assimilable organic carbon, thereby making the CWs largely anaerobic, SRB activity is also likely an important mechanism for organic carbon removal in these systems. However, oxygen release by plants under some conditions can interfere with the activity of SRB.

There is clear evidence that SOB are also active in CWs. These bacteria are most likely active at the oxic–anoxic interface and can cycle sulfide back to sulfate which can be subsequently utilized by SRB and/or can lead to deposits of elemental sulfur in the exit region of the CWs. A better understanding of sulfur transformations in CWs, and their spatial and temporal variation, will shed light on the relative magnitudes of the microbially catalyzed reactions occurring in CWs that are important in both domestic and mining applications. Also, better understanding sulfur cycling in CWs is necessary to explain the occasional formation of unwanted sulfur deposits at discharge points.

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