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Sulfate removal and sulfur transformation in constructed wetlands: The roles of filling material and plant biomass

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ABSTRACT

Sulfate in effluent is a challenging issue for wastewater reuse around the world. In this study, sulfur (S) removal and transformation in five batch constructed wetlands (CWs) treating secondary effluent were investigated. The results showed that the presence of the plant cattail (*Typha latifolia*) had little effect on sulfate removal, while the carbon-rich litter it generated greatly improved sulfate removal, but with limited sulfide accumulation in the pore-water. After sulfate removal, most of the S was deposited with the valence states S (-II) and S (0) on the iron-rich gravel surface, and acid volatile sulfide was the main S sink in the litter-added CWs. High-throughput pyrosequencing revealed that sulfate-reducing bacteria (i.e. *Desulfobacter*) and sulfide-oxidizing bacteria (i.e. *Thiobacillus*) were dominant in the litter-added CWs, which led to a sustainable S cycle between sulfate and sulfide. Overall, this study suggests that recycling plant litter and iron-rich filling material in CWs gives an opportunity to utilize the S in the wastewater as both an electron acceptor for sulfate reduction and as an electron donor for nitrate reduction coupled with sulfide oxidation. This leads to the simultaneous removal of sulfate, nitrate, and organics without discharging toxic sulfide into the receiving water body.

1. Introduction

Sulfate is a common contaminant of wastewater, and is not usually considered a health concern, but it can, under some circumstances, cause diarrhea. However, sulfate reduction may produce hydrogen sulfide (H_2S) and organic sulfur (S) compounds, which normally cause aesthetic problems (taste, color and/or odor) in the wastewater and the effluent-dominated river. Moreover, H_2S can cause serious corrosion to water pipes during the transportation of reused water and/or phytotoxicity to plants during irrigation (EPA, 2004). Therefore, the removal of sulfate in the effluent from wastewater treatment plants (WWTP) with minimum H_2S accumulation is of great importance to wastewater reuse around the world.

Constructed wetlands (CWs) are widely used as a tertiary treatment to polish the WWTP effluent for wastewater reuse due to their low implementation costs, simple operation, and efficient removal of effluent contaminants (Greenway, 2004; Jasper et al., 2014). CWs act as an eco-buffer zone between the WWTP and receiving waters, and could become promising artificial ecosystems for odor control in effluent-dominated rivers if the majority of the S could be immobilized or dissipated in CWs beds. Sulfur transformation in CWs has become increasingly important in recent years due to the high S reduction and oxidation activities shown in wetlands (Baldwin and Mitchell, 2012; Wu et al., 2013). In subsurface flow constructed wetlands (SSF CWs), the relatively low redox condition provides a high thermodynamic potential for sulfate reduction. However, the amount of internal carbon from the rhizosphere and external carbon from secondary effluent are not enough to drive significant sulfate reductions in CWs (Stein et al., 2007). Plant litter is one of the most abundant carbon sinks in wetlands (500–2000 g C m^{-2} yr^{-1}) (Hume et al., 2002). While, the structure of SSF CWs prevents aboveground plant litter from reaching the subsurface water and inhibits the carbon release from

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plant litter. Therefore, recycling the carbon in plant litter could be a low cost and sustainable way to enhance sulfate reduction in CWs. Chen et al. (2014a) showed that plant litter greatly stimulated sulfate reduction in CWs through the on-site production of carbon sources such as carbohydrate and volatile fatty acids. However, as far as can be ascertained, there have not been any studies on the effect of plant litter on S transformation in CWs treating secondary effluent.

Sulfide is considered to be the main product of sulfate reduction, and can severely inhibit ammonium/carbon removal and plant photosynthesis, which decreases the treatment efficiency of CWs. Sulfide detoxification can be achieved when CWs are supplied/filled with metal-enriched substrates, because sulfide can precipitate along with heavy metals (i.e. iron, zinc) (Stein et al., 2007; Wu et al., 2012). Wiessner et al. (2010) calculated that nearly half the sulfate-S was immobilized inside CWs. However, the amount and speciation of the immobilized S (solid-phase) is often unknown. Acid volatile sulfide (AVS) is considered to be the main component in the solid-phase S, and it is a complex and variable component that includes diverse reduced S forms (e.g. FeS, Fe_3S_4, and FeS_2) (Rickard and Morse, 2005). At present, AVS detection relies on the application of acid-based extraction methods, which are relatively efficient, but do not detect 100% of the AVS since not all of the Fe_xS_y can be fully extracted. X-ray photoelectron spectros-copy (XPS) has emerged as an element-sensitive technique for describing the speciation and distribution of S at the microscale in recent years (Sun et al., 2009). Despite the wide use of acid-based AVS extraction and S speciation identification by XPS (Baldwin and Mitchell, 2012; Johnston et al., 2014), very few studies have investigated the solid-phase S in the filling material of CWs. Therefore, there has not been complete elucidation of the S species distribution and related S transformations in CWs.

Apart from the precipitation of sulfide with metals, oxidation is another effective method of avoiding sulfide accumulation. Oxygen released from plant roots and the atmosphere oxidizes harmful sulfide to harmless forms (e.g. elemental S and sulfate) in CWs (Faulwetter et al., 2009). Previous studies have suggested that 41%–90% of the reduced S was re-oxidized by root-mediated oxygen in planted wetlands (Wiessner et al., 2010; Wu et al., 2011). Wu et al. (2011) further demonstrated the multiple S transformations (i.e. sulfide re-oxidation) in CWs using the ^{34}S isotope approach. Apart from oxygen, nitrate in the influent can also easily drive sulfide and elemental S oxidation to sulfate in the organic-rich wetlands (Krishnakumar and Manilal, 1999; Londry and Suflita, 1999). Chemical and microbial oxidation are the main sulfide oxidation processes in CWs (Wu et al., 2013). Bacterial sulfur oxidation is mainly driven by S oxidizing bacteria (SOB), and sulfide is oxidized to sulfur (or sulfate) using oxygen or nitrate as electron acceptors (Faulwetter et al., 2009). At present, information on the SOB community in CWs is incomplete due to the inefficient detection of species that are present at low levels (Hallberg and Johnson, 2005; Nicomrat et al., 2006). Therefore, a sensitive and comprehensive detection method for S-related bacteria based on next-generation sequencing is urgently needed to improve understanding of the mechanism underlying microbial S oxidation in CWs.

In this study, S transformation was characterized in five iron-rich media containing CWs with or without cattail (*Typha latifolia*) and externally added carbon sources. The objectives were to (1) study the effects of plants and plant litter as carbon sources on sulfate removal, sulfide accumulation, and intermediate-S formation. (2) quantify the solid AVS and the multi-valence distribution of S in the iron-rich gravel; (3) quantify S species distribution and elucidate S transformation; and (4) characterize the structures of SRB/SOB communities in CWs.

2. Materials and methods

2.1. Design and operation of the SSF CW

Five sequencing batch SSF CW microcosms, each with a bulk volume of 0.045 m^3 (length: 0.3 m, width: 0.3 m, height: 0.5 m) and a pore volume of 12 L, were set up in this study. Five systems: an unplanted control (W0), two litter-added microcosms (W1: 100 g; W2: 200 g), a planted microcosm (W3: 22 plants m^{-2}) and a planted plus litter added microcosm (W4: 100 g litter, 22 plants m^{-2}), were established. All the microcosms were filled with iron-rich gravel (φ 8–13 mm, porosity = 0.4, iron content 4.7%, w/w) up to a height of 40 cm. The water level was adjusted to be 5 cm below the gravel bed surface. Two (W3 and W4) CWs were planted with cattail (*Typha latifolia*). The wetland microcosms have been located in an air-conditioned greenhouse at a temperature of 25 ± 1 °C since 2005. Prior to the start of the experiment, the five microcosms were fed, in batches, with a modified secondary effluent for 6 months pre-incubation in order to establish the plant shoots and microorganisms. Then, cattail litter (1–1.5 cm lengths) was added to the W1, W2, and W4 microcosms as the carbon source to drive sulfate reduction. The cattail litter was homogeneously mixed with gravel, and the mixed media were compacted with a tamping rod at 5 cm increments during loading and filled the microcosms to a height of 40 cm.

2.2. Batch experiment

The batch experiment began after a 6 month pre-incubation. The wetland microcosms were fed with the secondary effluent from a neighboring WWTP, and the characteristics of the wetland influent were seen in Table S1. Influent was introduced into the microcosm from the top and gravity drained from the bottom. The microcosm was operated in batch mode with five days for each batch (HRT = 5 d). Feeding, reaction and draining was designed as illustrated in Fig. 1. Briefly, each batch started with a feeding stage (1 h), followed by a reaction stage (118 h), and terminated with a draining stage (1 h). All the treatments (W0–W4) were triplicated and the duration of the batch experiment was 100 d, which included 20 batches. Water samples were taken from each microcosm and each batch. A 100 mL syringe was used to collect water samples at 5, 20, and 30 cm depths from the central sampling pipe. Only water samples taken from 20 cm depth were reported because no vertical gradients in the water chemistry were observed in the preliminary experiment and in previous experiments with the same microcosms (Wen et al., 2010).

2.3. S-based autotrophic denitrification kinetic tests

The autotrophic denitrification kinetic tests were carried out according to Chen et al. (2014b). Briefly, 1000 g of gravel was taken from W0–W4 before batch 20 and respectively transferred to 1 L serum bottles (S0–S4). After a 10 d pre-incubation period (removal of the original nitrate, sulfate, and endogenous organic matters inside the cell), nitrate (10 mg L^{-1} NO_3^--N) was added to the serum bottles, which were then incubated in an anaerobic environment (25 °C) for five days. Nitrate and sulfate concentrations were measured every 12 h. There was no organic carbon in the feeding water, so nitrate loss in the serum bottles could be mainly attributed to autotrophic denitrification.

2.4. Aqueous-phase methods

Five 50 mL water samples, withdrawn at the appropriate time intervals, were membrane-filtered (0.22 μm) and immediately