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Effects of plant biomass on nitrogen transformation in subsurface-batch constructed wetlands: A stable isotope and mass balance assessment

Yi Chen [a,b], Yue Wen [a,*], Qi Zhou [a], Jan Vymazal [b]

[a] Key Laboratory of Yangtze Water Environment of Ministry of the State Education, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, PR China

[b] Department of Landscape Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, 16521 Prague 6, Czech Republic

ARTICLE INFO

Article history:
Received 25 January 2014
Received in revised form
28 May 2014
Accepted 11 June 2014
Available online 20 June 2014

Keywords:
Subsurface-batch constructed wetlands
Typha latifolia
Nitrogen
Nitrogen stable isotopes
Denitrification

ABSTRACT

Nitrate is commonly found in the influent of subsurface-batch constructed wetlands (SSB CWs) used for tertiary wastewater treatments. To understand the effects of plants and the litter on nitrate removal, as well as on nitrogen transformation in SSB CWs, six laboratory-scale SSB CW microcosms were set up in duplicate and were operated as batch systems with hydraulic residence time (HRT) of 5d. The presence of *Typha latifolia* enhanced nitrate removal in SSB CWs, and the N removed by plant uptake was mainly stored in above-ground biomass. *Typha* litter addition greatly improved nitrate removal in SSB CWs through continuous input of labile organic carbon, and calculated enrichment factors (ε) were between −12.1‰—−13.9‰ from the nitrogen stable isotope analysis, suggesting that denitrification plays a dominant role in the N removal. Most significantly, simultaneous sulfur-based autotrophic and heterotrophic denitrification was observed in CWs. Finally, mass balance showed that denitrification, sedimentation burial and plant uptake respectively contributed 54%–94%, 1%–46% and 7.5%–14.3% to the N removal in CWs.

1. Introduction

Excessive nitrogen (N) and phosphorus discharged into water can cause serious ecological problems such as eutrophication of lakes and rivers. It was reported that eutrophication due to N pollution is serious in some lakes and reservoirs (Yang et al., 2008). Conventional biological N removal processes such as Modified Ludzack-Ettinger process (MLE) have been widely used around the world, however it is still difficult to meet the stringent discharge standard released by the environmental protection agency in many countries without further treatment.

Constructed wetlands (CWs) are suitable for wastewater treatment due to their simple operation and low implementation costs. Subsurface flow constructed wetlands (SSF CWs) provide suitable redox environment for denitrification, which involves the anoxic biological conversion of NO_3^- to N_2 (IWA, 2000; Kadlec and Wallace, 2008). However, the influent wastewater carbon is mostly oxidized in the aeration processes (Leverenz et al., 2010) and the internal carbon from rhizosphere is insufficient for denitrification (Kuschk et al.,

* *Corresponding author*. Tel./fax: +86 21 65982697.
E-mail addresses: weny@tongji.edu.cn, wenyue@vip.163.com (Y. Wen).
http://dx.doi.org/10.1016/j.watres.2014.06.015
0043-1354/

2003). Therefore, an alternative carbon source is needed to drive the denitrification. Various carbon sources were used to improve the denitrification performance in carbon-limited wetlands, including glucose, fructose, soils and plant biomass (Davidsson and Stahl, 2000; Hume et al., 2002a; Lu et al., 2009; Lin et al., 2002). Among the various carbon sources, plant biomass could be used as an alternative carbon source because of the low costs, renewable biomass and wide availability (Wen et al., 2010).

Wetland plant litter has been reported to accumulate at a rate of 500–2000 g C m^{-2} $year^{-1}$ in a mature wetland (Hume et al., 2002b), and it could serve as a convenient carbon source for treating the nitrate-dominated wastewater. However, the SSF CWs are only marginally successful at removing nitrate from wastewater because the gravel layer prevents the aboveground plant litter from reaching the water and inhibits the carbon release from plant litter (IWA, 2000). Thus, numerous studies have added plant litter and mixed them with gravel, in order to enhance bacterial denitrification in CWs. Ingersoll and Baker (1998) demonstrated the feasibility of using *Typha* litters as an organic carbon source for nitrate removal in CWs. Wen et al. (2010) further found that there were strong positive correlations between available carbon sources and nitrate removal rates ($R^2 = 0.93$), and labile carbon ratio and nitrate removal rates ($R^2 = 0.99$). Fleming-Singer and Horne (2002) showed that the denitrification occurred mainly in the plant litter, and the denitrification rate constant for the plant litter treatment is 1.75 times of that for the sediment-only treatment. Despite higher nitrate removal efficiency can be achieved in the litter added CWs, the relative contribution of denitrification and other pathways in the N removal in CWs is still not fully known.

Estimating the N removal via denitrification in CWs is usually difficult (Groffman et al., 2006). The measurements of N_2 production and acetylene block are two typical approaches for estimating the denitrification, but they may underestimate denitrification because of the inhibition of nitrification as a function of partial acetylene blockage (Yu et al., 2008). As a tool to discern pathways and rates of N transformations, stable isotope analyses have been found increasingly applied in recent years (Lund et al., 2000; Reinhardt et al., 2006; Wunderlich et al., 2012). Denitrification leads to significant isotope enrichment of ^{15}N in residual nitrate coupled to a depletion of ^{15}N in products because the microbes discriminate against the heavy N isotope. Estimation of N isotope fractionation is an important approach for assess microbial denitrification, and N isotope fractionation in surface-flow constructed wetlands (SF CWs) was previously observed in previous studies (Lund et al., 2000; Reinhardt et al., 2006). However, little is known about N isotope fractionation in SSF CWs as well as the influence of carbon sources on nitrate isotope fractionation during microbial denitrification. Furthermore, very few study achieved complete N mass balances in CWs used to treat municipal wastewaters as tertiary treatment stage.

The objectives of this study were: (1) investigate the effects of plant and plant litter on nitrate removal. (2) estimate the contribution of denitrification to N removal using stable nitrate isotope. (3) assess the contribution of different pathways (denitrification, sedimentation, and plant uptake) to the N removal.

2. Materials and methods

2.1. Source of plant litter and treatment

In this study, cattail (*Typha latifolia*) litter was used as the carbon source to drive denitrification. *Typha* litter was collected from wetland microcosms in our laboratory in November, 2010. After collection, *Typha* litter was cleaned, washed using neutral detergent, cut into 1–1.5cm lengths, and dried at 40 °C to a constant mass before being finally preserved in a moisture free container at room temperature (20 °C).

2.2. Design and operation of the SSF CW

Twelve sequencing batch SSF CW microcosms (6 sets × 2 replicates), 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. Six types of systems: unplanted control (W1: no plants), litter added microcosms (W2: 100 g; W3: 200 g), planted microcosms (W4: 22 plants m^{-2}; W5: 44 plants m^{-2}) and planted plus litter added microcosms (W6: 100 g litter, 22 plants m^{-2}) were established. All the microcosms were filled with gravel (ϕ 8–13 mm, porosity: 0.4) and three of them (W4, W5 and W6) were planted with *T. latifolia*. The wetland microcosms were located in an air-conditioned greenhouse at a temperature of 25 ± 1 °C. Details of the wetland microcosm design have been given in our previous studies (Chen et al., 2014, 2011; Wen et al., 2010).

Before the start of the experiment, the microcosms were fed in batches with modified secondary effluent for 6 months until microorganisms were well established. *Typha* litter was added to the W2, W3 and W6 microcosms after a 6 month acclimatization period. *Typha* litter was homogeneously mixed with gravel, and the details of litter loading have been given by Chen et al. (2011). *Typha* seedlings (25 cm in height) were planted in W4, W5 and W6 microcosms after the addition of cattail litters. The experiment started one month after the planting. The wetland microcosms were fed with secondary effluent from a neighboring wastewater treatment plant (WWTP), and the characteristics of the influent are shown in Table 1. The twelve microcosms operated as batch systems, which were filled with wastewater at the start of each batch and were gravity drained within 1 h prior to the next batch. Each batch was held for 5 days (HRT = 5 d), and there were a total of 18 batches (90 d). Before each batch, the feeding water was flushed with pure N_2 for 10 min to remove the residual Cl_2, which could injure microorganisms and plants in CWs. Water samples were collected from each microcosm and each batch every day. As no vertical gradients in the water chemistry were observed, a syringe and peristaltic pump was used to collect water samples at a depth of 20 cm from the center sampling pipe.

2.3. Sulfur-based autotrophic denitrification kinetic tests

Before batch 18, 1000 g gravel was collected from W1–W6 and respectively transferred to 1L serum bottles (S1–S6). The gravel was pre-incubated with no stirring for 10 d in