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Effects of pH and seasonal temperature variation on simultaneous partial nitrification and anammox in free-water surface wetlands

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ABSTRACT

Design considerations to enhance simultaneous partial nitrification and anammox in constructed wetlands are largely unknown. This study examined the effects of pH and seasonal temperature variation on simultaneous partial nitrification and anammox in two free-water surface wetlands. In order to enhance partial nitrification and inhibit nitrite oxidation, furnace slag was placed on the rooting substrate to maintain different pH levels in the wetland water. The wetlands were batch operated for dairy wastewater treatment under oxygen-limited conditions at a cycle time of 7 d. Fluorescence in situ hybridization analysis found that aerobic ammonium oxidizing bacteria and anammox bacteria accounted for 42–73% of the bacterial populations in the wetlands, which was the highest relative abundance of ammonium oxidizing and anammox bacteria in constructed wetlands enhancing simultaneous partial nitrification and anammox. The two wetlands removed total inorganic nitrogen efficiently, 3.36–3.38 $g/m^2/d$ in the warm season with water temperatures at 18.9–24.9 °C and 1.09–1.50 $g/m^2/d$ in the cool season at 13.8–18.9 °C. Plant uptake contributed 2–45% to the total inorganic nitrogen removal in the growing season. A seasonal temperature variation of more than 6 °C would affect simultaneous partial nitrification and anammox significantly. Significant pH effects were identified only when the temperatures were below 18.9 °C. Anammox was the limiting stage of simultaneous partial nitrification and anammox in the wetlands. Water pH should be controlled along with influent ammonium concentration and temperature to avoid toxicity of free ammonia to anammox bacteria.

1. Introduction

Constructed wetlands have been designed to remove nitrogen mainly by nitrification and denitrification. Nitrogen removal from ammonium-rich wastewater in constructed wetlands using the nitrification–heterotrophic denitrification process is usually restricted by limited availability of oxygen and organic carbon (Kadlec and Wallace, 2009; Vymazal, 2007). Nitrification is a two-step oxidation process including aerobic ammonium oxidation to nitrite (partial nitrification) by ammonium oxidizing bacteria (AOB) and nitrite oxidation by nitrite oxidizing bacteria (NOB). Anaerobic ammonium oxidation (anammox) was discovered in the mid-1990s (van de Graaf et al., 1996), which uses nitrite to oxidize ammonium under anaerobic conditions. When partial nitrification and anammox are coupled, only about one half of ammonium needs to be oxidized to nitrite first and anammox bacteria use the remaining ammonium as electron donors for autotrophic denitrification of the nitrite produced (van der Star et al., 2007; Kartal et al., 2010). Compared with the nitrification–denitrification process, the partial nitrification–anammox process requires 60% less of oxygen and eliminates the requirement for organic carbon. The partial nitrification–anammox process is typically used to treat warm wastewater with high ammonium concentrations such as sludge digester liquor and landfill leachate. Partial nitrification and anammox have been coupled for ammonium removal either in two reactors in series or simultaneously in one oxygen-limited reactor (van der Star et al., 2007; Kartal et al., 2010).

Simultaneous partial nitrification and anammox (SNA) has been employed recently in free-water surface wetlands receiving ammonium-rich wastewater under oxygen-limited conditions. Dong and Sun (2007) modified a subsurface flow wetland to enhance SNA in a following free-water surface wetland. Tao and Wang (2009) found that vegetation and basic rooting substrate produced slightly aerobic and basic conditions in free-water surface wetlands, which

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were favorable to SNA. Tao et al. (2011) examined the influence of ammonium loading rate on SNA in free-water surface wetlands.

In single constructed wetlands, NOB compete with AOB for dissolved oxygen and with anammox bacteria for nitrite. Therefore, NOB should be inhibited for enhancement of SNA. Temperatures higher than 30 °C and pH between 7.8 and 8.5 have been used to inhibit NOB and promote AOB in bioreactors (Bae et al., 2001; Hellinga et al., 1998; Park et al., 2007; van der Star et al., 2007). Anammox has an optimal pH of 7.5–8.0 and optimum temperature around 30–40 °C (Strous et al., 1997; van de Graaf et al., 1996; van der Star et al., 2007). Unlike traditional bioreactors, constructed wetlands as natural wastewater treatment systems are subject to seasonal temperature variations. To date, it remains unclear about the effects of pH and seasonal temperature variation on the entire SNA process in single constructed wetlands.

This study tracked nitrogen removal and operational conditions in two pilot-scale free-water surface wetlands enhancing SNA for dairy wastewater treatment. Furnace slag was utilized in the free-water surface wetlands as a low-cost method to buffer pH of wetland water. The main objectives of this study were: 1) to examine the effectiveness of SNA for nitrogen removal from dairy wastewater in free-water surface wetlands; and 2) to evaluate the effects of pH and seasonal temperature variation on SNA in free-water surface wetlands. Microbial community composition and the contribution of plant assimilation to nitrogen removal were also investigated in the wetlands.

2. Materials and methods

2.1. Free-water surface wetlands

Two free-water surface wetlands (CWS and CW) were constructed in a greenhouse in Syracuse, New York, USA on 5 February 2010 as depicted in Fig. 1. The wetlands were rectangular tanks lined with ethylene propylene diene monomer pond liner. Each tank had internal flat dimensions of 0.42 m by 0.45 m. The wetlands had 0.20 m of sandy loam as rooting substrate. Segments of common reed (*Phragmites australis*) roots were transplanted to the wetlands on 15 February and 18 March 2010. Water level in the wetlands was gradually increased until 17 May 2010 while new shoots were developed. Electric arc furnace slag (effective size of 5 mm) was put on the rooting substrate of CWS (5.4 L each) and CW (2.2 L) between 7 June and 12 July 2010 to buffer water pH. More slag was put on the rooting substrate of the two wetlands (1.5 L each) on 31 August 2010 to raise water pH. More slag (0.5 L) was added again to the rooting substrate of CWS on 14 December 2010 to maintain higher pH values.

The free-water surface wetlands were operated by a batch mode with a cycle time of 7 d. The wetlands were discharged through a ball valve 10 mm above the rooting substrate. Dairy wastewater pretreated in partial nitrification–anammox biofilters was fed to the wetlands starting from 5 July 2010. Organic carbon-free, pretreated synthetic wastewater (NH_4Cl dissolved in tap water) was fed to the wetlands to enrich anammox bacteria and AOB during the initial five months before 5 July 2010. Treatment performance and the discussion hereinafter are based on the operational results between 5 July 2010 and 10 March 2011 when dairy wastewater was treated. The free-water surface wetlands were a part of a treatment train to remove the high concentrations of ammonium from the dairy wastewater. The wetland influent was rich in ammonium and had relatively low concentrations of nitrite and nitrate (Fig. 2). The ammonium concentrations in the influent fed to the two wetlands were higher than that (39–266 mg N/L) in the dairy wastewater treated in earlier constructed wetlands (Kadlec and Wallace, 2009; Knight et al., 2000). Hydraulic loading rate and nitrogen loading rate to the wetlands were 22.1 mm/d and 5.9 g/m^2/d, respectively. Because of infestation of common reeds, the wetlands were closed on 10 March 2011.

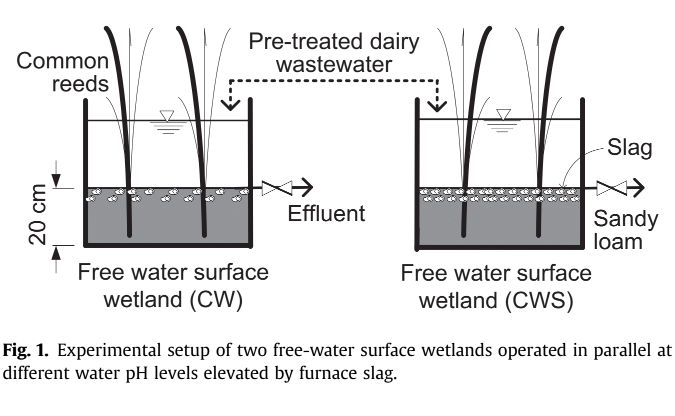

Fig. 1. Experimental setup of two free-water surface wetlands operated in parallel at different water pH levels elevated by furnace slag.

2.2. Field measurements and chemical analyses

Influent and effluent were measured weekly for dissolved oxygen, redox potential, temperature, and pH with portable meters. Influent and effluent volumes were measured with a graduated cylinder. Influent and effluent samples were analyzed weekly with a QuickChem 8500 series automatic flow injection analyzer (Lachat Instruments, Loveland, CO, USA) using the phenolate method for ammonium and hydrazine reduction method for nitrate and nitrite (APHA et al., 1998). NH_4^+–N concentration determined with the phenolate method represents the total ammonia concentration (NH_4^+–N + NH_3–N) in the water samples. Total inorganic nitrogen (TIN) was calculated as the sum of NH_4^+–N, NO_2^-–N and NO_3^-–N. Free ammonia concentration was calculated with measured total ammonia concentration, temperature and pH (Camargo Valero and Mara, 2010). Nitrogen was not measured in January and early February 2011 due to logistical reasons. COD was determined occasionally, following Standard Method 5220 (APHA et al., 1998).

Plant average height, stem diameter and stem number were recorded to estimate the growth of plant biomass when a significant change was observed. Different sizes of stems (total 14) were randomly sampled from the wetlands on 7 October 2010. After

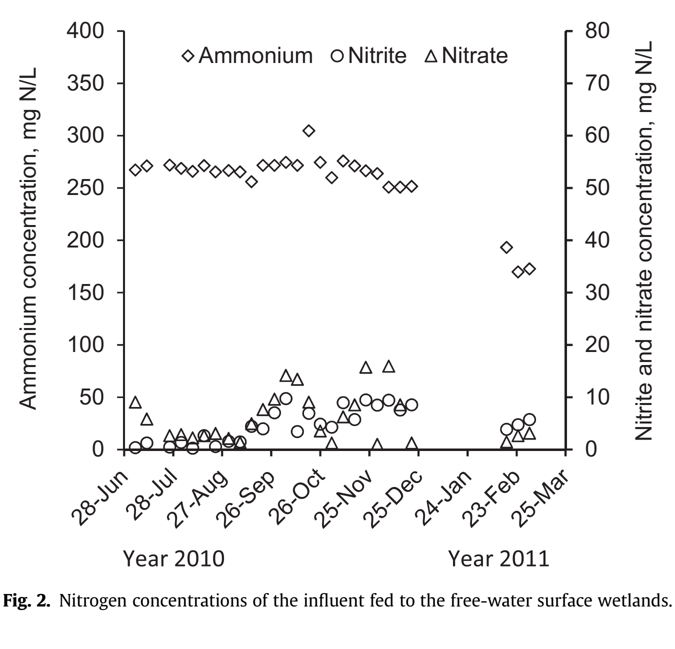

Fig. 2. Nitrogen concentrations of the influent fed to the free-water surface wetlands.