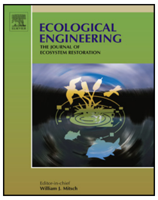
Ecological Engineering 97 (2016) 363–369

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



Effect of vegetation on nitrogen removal and ammonia volatilization from wetland microcosms

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ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form 20 July 2016
Accepted 5 October 2016

Keywords:
Ammonia nitrogen
Nitrification
Denitrification
Plant uptake
N mass balance

ABSTRACT

Constructed wetlands are increasingly used to control nitrogen (N) pollution diffusion from agricultural source to downstream water. In order to compare N removal efficiencies for swine wastewater treatment, three wetland microcosms vegetated with *Myriophyllum aquaticum* (Myriophyllum), *Alternanthera philoxeroides* (Alternanthera), or without vegetation (Control) were constructed in an agricultural area of southern China. Meanwhile, the key environmental factor and N removal pathway were investigated among the three microcosms. The results showed that, after 30 days incubation, 96.4% of TN was removed in Myriophyllum microcosm, significantly higher than 74.2% and 47.3% in Alternanthera and Control microcosms ($p < 0.05$). Plant N accumulation was significantly higher in *M. aquaticum* than *A. philoxeroides* ($p < 0.05$). The analysis of Pearson's correlation revealed that the NH_4^+-N concentrations were negatively correlated with DO concentrations and E_h values. Microbial nitrification-denitrification was the dominant N removal pathway in the three microcosms, accounting for 66.9–80.5% of the TN removal. Furthermore, NH_3 volatilization in Control was significantly higher than those in Myriophyllum and Alternanthera microcosms ($p < 0.05$). These findings suggested that plant vegetation in constructed wetlands (e.g., *M. aquaticum*) improved N removal efficiency in treating swine wastewater and additionally reduced NH_3 volatilization.

1. Introduction

Swine wastewater from animal production is characterized by high concentrations of ammonia nitrogen (NH_4^+-N) and organic matter. Water pollution has arouse widespread concerns because the random discharge of swine wastewater leads to deterioration of water quality in streams, rivers, and lakes (Cheng et al., 2002; Dong and Reddy, 2010; Ryu and Lee, 2010). To effectively control pollution diffusion from the sources of animal breeding into downstream watersheds, alternative control strategies, e.g., membrane bioreactors, sequencing batch reactors, and constructed wetlands, are typically applied to treat swine wastewater (Kishida et al., 2004; Zhang et al., 2015). Compared with other alternatives, constructed wetlands have been recognized as an effective and low-cost technology for swine wastewater treatment (Forbes et al., 2010; Liu et al., 2016).

The constructed wetlands consist of aquatic plants, substrates, microbes, and fauna, where numerous physicochemical and biological reactions occur in the process of wastewater treatment. In wetland systems, the pathways that contribute to N removal mainly include microbial nitrification-denitrification, ammonia (NH_3) volatilization, plant uptake, and soil sorption (Lu et al., 2009; Vymazal, 2007). Microbial nitrification and denitrification is considered to be the dominant pathway for N removal (Kløve et al., 2005). For nitrification, NH_4^+-N is first oxidized to nitrite (NO_2^--N) by ammonia-oxidizing bacteria (AOB) or ammonium-oxidizing archaea (AOA); then, the conversion of NO_2^--N to nitrate (NO_3^--N) is stimulated by nitrite-oxidizing bacteria (NOB) (Wu et al., 2009). Denitrification is performed by different archaea or bacteria, where NO_3^--N is reduced to nitric oxide (NO), nitrous oxide (N_2O), or nitrogen (N_2) (Rodriguez-Caballero et al., 2013). The generations of

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http://dx.doi.org/10.1016/j.ecoleng.2016.10.021
0925-8574/

gas species are affected by many factors such as pH, oxygen availability, soil moisture content, C and N contents and so on (Ludwig et al., 2001).

NH_3 volatilization also plays an important role in N removal for swine wastewater treatment, and it is a reversible physicochemical process, mainly affected by temperature, light conditions, wind speed, and the water pH value (Lin et al., 2012). NH_3 volatilization from animal wastes was serious, accounting for 80% of the total NH_3 emissions in European and American countries (Blanes-Vidal and Nadimi, 2011). In addition, NH_3 volatilization could contribute 12% to N removal in the process of treating swine wastewater (Forbes et al., 2010). NH_3 is an undesirable gas and its deposition may lead to the acidification of water and soil. However, less attention had been paid to the effect of plant vegetation in constructed wetlands on NH_3 volatilization.

Aquatic plant is an important biological component in the constructed wetland (Brix, 1994; Silvan et al., 2004). The plant itself can assimilate inorganic N from water or soil to composite the tissue compounds (Vymazal, 2007). In addition, the roots of plants can not only secrete oxygen and organic carbon into the wetland systems, but also provide favorable inhabits for microorganisms (Cronk, 1996; Liu et al., 2012). Obviously, the N removal efficiencies are affected by plant species because different plants have variation in N uptake capacities and radial oxygen loss (ROL) (Lai et al., 2011). In constructing wetlands, the understandings of N removal characteristics for different plants can provide important information for the optimized design of wetland systems. *Alternanthera philoxeroides* is perennial emergent plant and classified as invasive species, with characteristics of fast growth, strong adaptability, and high tolerance of pollutants (Zuo et al., 2012). *Myriophyllum aquaticum* grows well in nutrient-rich water and has a high potential to uptake N from wastewater (Zhang et al., 2016). However, for treating swine wastewater, comparisons of N removal in wetlands vegetated with *M. aquaticum* and *A. philoxeroides* were rarely reported.

In this study, *M. aquaticum* and *A. philoxeroides* were planted in wetland microcosms; environmental factors, dissolved N concentrations, N contents in plants and soils, and NH_3 volatilization were investigated in the process of treating swine wastewater. The ultimate objectives were to: (i) evaluate the effect of plant vegetation on N removal and NH_3 volatilization, (ii) analyze the relationship between N concentrations and environmental factors, and (iii) identify the dominant N removal pathway via the N mass balance method.

2. Materials and methods

2.1. Experimental plant, soil, and wastewater

M. aquaticum and *A. philoxeroides*, transplanted from a natural drainage ditch, were used as experimental plants. All the seedlings for the experiment were well selected, with similar age and size. Prior the experiment, the two plant species were pre-incubated in low N-loaded swine water for five days. The substrate soil was collected from a vegetable field, with a soil pH of 6.3, total nitrogen (TN) of $1.4\,\mathrm{g\,N\,kg^{-1}}$, soil organic carbon of $18.7\,\mathrm{g\,C\,kg^{-1}}$, and sand, silt, and clay contents of 41.3%, 35.3%, and 23.4%, respectively. Swine wastewater for this study was collected from a pig farm, with NH_4^+-N of $157.8\,\mathrm{mg\,L^{-1}}$, NO_3^--N of $0.2\,\mathrm{mg\,L^{-1}}$, TN of $213.5\,\mathrm{mg\,L^{-1}}$, TP of $18.5\,\mathrm{mg\,L^{-1}}$, and COD of $932.5\,\mathrm{mg\,L^{-1}}$.

2.2. Experimental setup

The wetland microcosms consisted of 9 square organic glass tanks, each tank 50 cm length, 40 cm width, and 50 cm depth. 10 kg of fresh upland soil mixed evenly was filled into each tank as the substrate layers. After washing with distilled water, approximately 500 g of fresh biomass for each plant species was inserted into the soil layer of one tank. Three types of wetland microcosms were set up: wetland microcosms vegetated with *M. aquaticum* (Myriophyllum) and *A. philoxeroides* (Alternanthera) and non-vegetated wetland microcosms (Control). All the wetland microcosms were randomly arranged in triplicate.

The experiment was carried out from June 15th to July 15th, 2014, at the Changsha Research Station for Agricultural & Environmental Monitoring, Hunan Province, China (28°32′N, 113°19′E). During the incubation period, air temperatures in the field covered a range of 22.7–35.8 °C. Above the wetland microcosms, a euphotic shelter with a height of 3 m was built to keep out rain. The study swine wastewater, collected from an anaerobic lagoon, was first filtered with filter membranes to avoid the algae and impurities. Then 30 L of swine wastewater was added to each tank, keeping the water depth at 15 cm. During the whole monitoring period, the water depth was maintained at 15 cm by replenishing evaporation losses with distilled water.

2.3. Water sampling and analysis

On days 0, 1, 4, 7, 10, 14, 18, 22, 26, and 30, 150 mL of wastewater were sampled at each wetland microcosm by a 50 mL syringe. By filtering through 0.45 μm membranes, the dissolved NH_4^+-N and NO_3^--N concentrations were analyzed by an automatic flow-injection analyzer (Fia-star 5000, Foss Tecator, Sweden). For analysis of TN, the wastewater was first digested in solutions of alkaline potassium persulfate, and then the oxidized NO_3^--N was measured via the automatic flow-injection analyzer (Fia-star 5000, Foss Tecator, Sweden). At each wetland microcosm, the water quality parameters of pH, DO, E_h, and temperature (T) were determined *in situ* by a portable multi-parameter meter (SG68-ELK, Mettler-Toledo, Switzerland).

2.4. Plant sampling and analysis

Before the experiment, the initial biomass for *M. aquaticum* and *A. philoxeroides* were first weighed, and part of the plants was selected for analysis of initial TN content. At the end of the experiment, *M. aquaticum* and *A. philoxeroides* were all collected from the tanks, with the fresh biomass weighed immediately. The fresh plant samples were first oven-dried at 105 °C for 30 min, and then kept at 70 °C until the dried plant tissues obtained constant weight. The dried plant materials were ground into power by a grinding machine and filtered through a 1 mm mesh sieve. To measure TN contents in plant tissues, the plant powers were digested in a H_2SO_4-H_2O_2 solution, with the transformed NH_4^+-N analyzed by the automatic flow injection analyzer (Fia-star 5000, Foss Tecator, Sweden).

2.5. Soil sampling and analysis

At each microcosm, about 150 g of soil was collected on days 0, 7, 14, and 30. Each sample was mixed evenly, with impurities removed from the soil. To measure the water content of soil, part of the fresh soil was oven-dried at 105 °C for 48 h. Soil NH_4^+-N and NO_3^--N contents were extracted by a 2 M KCl solution and measured via the Fia-star 5000 flow injection analyzer. TN contents in the soil samples were determined by the semi-micro Kjeldahl digestion method.

2.6. Measurement of NH_3 volatilization

On days 1, 4, 7, 10, 14, 18, 22, 26, and 30, NH_3 volatilization was collected for four times at 8:00–10:00 a.m. and 3:00–5:00 p.m.