Journal of Environmental Management 166 (2016) 596–604

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Nitrogen removal and mass balance in newly-formed *Myriophyllum aquaticum* mesocosm during a single 28-day incubation with swine wastewater treatment

Feng Liu [a, b, *, 1], Shunan Zhang [a, c, 1], Yi Wang [a, b], Yong Li [a, b], Runlin Xiao [a, b], Hongfang Li [a, c], Yang He [a, c], Miaomiao Zhang [a, b], Di Wang [a, c], Xi Li [a, b], Jinshui Wu [a, b, **]

[a] Key Laboratory of Agro-ecological Processes in Subtropical Regions, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan, 410125, PR China

[b] Changsha Research Station for Agricultural & Environmental Monitoring, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan, 410125, PR China

[c] Graduate University of Chinese Academy of Sciences, Beijing 100039, PR China

ARTICLE INFO

Article history:
Received 7 March 2015
Received in revised form
10 October 2015
Accepted 9 November 2015
Available online 19 November 2015

Keywords:
Myriophyllum aquaticum
Swine wastewater
Nitrogen removal
Plant uptake
Nitrification and denitrification
Nitrogen mass balance analysis

ABSTRACT

The aim of this research was to assess the applicability of *Myriophyllum* (*M.*) *aquaticum* for swine wastewater treatment. Nitrogen (N) removal processes were investigated in *M. aquaticum* mesocosms with swine wastewater (SW), 50% diluted swine wastewater (50% SW), and two strengths of synthetic wastewater, 200 mg $NH_4^+ - N\ L^{-1}$ (200 $NH_4^+ - N$) and 400 mg $NH_4^+ - N\ L^{-1}$ (400 $NH_4^+ - N$). During a 28-day incubation period, the average $NH_4^+ - N$ and TN removal rates were 99.8% and 94.2% for 50% SW and 99.8% and 93.8% for SW, which were greater than 86.5% and 83.7% for 200 $NH_4^+ - N$, and 73.7% and 74.1% for 400 $NH_4^+ - N$, respectively. A maximum areal total nitrogen (TN) removal rate of 157.8 mg N m^{-2} d^{-1} was found in *M. aquaticum* mesocosms with SW. During the incubation period, the observed dynamics of $NO_3^- - N$ concentrations in water and gene copy numbers of ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), *nirK* and *nirS* in soil unraveled strong nitrification and denitrification processes occurring in *M. aquaticum* mesocosms with swine wastewater. The N mass balance analysis indicated that plant uptake and soil N accumulation accounted for 17.9–42.2% and 18.0–43.8% of the initial TN load, respectively. The coupled nitrification and denitrification process was calculated to account for, on average, 36.8% and 62.8% of TN removal for 50% SW and SW, respectively. These findings demonstrated that the N uptake by *M. aquaticum* contributed to a considerable proportion of N removal. In particular, the activities of ammonia-oxidizing and denitrification microbes responsible for nitrification and denitrification processes in *M. aquaticum* mesocosm accelerated $NH_4^+ - N$ and TN removal from swine wastewater.

1. Introduction

Wastewater from animal operations that contain high concentrations of nutrients, chemical oxygen demand (COD) and suspended particles have caused the degradation of water quality in many rivers and lakes (Schaafsma et al., 2000; Xu and Shen, 2011; Li et al., 2014). Low-cost constructed wetlands (CWs) have been utilized to control pollution from animal wastewater in many sites all over the world (Cronk, 1996; Vymazal, 2011). Surface-flow CWs with macrophytes have shown good performance in treating a variety of wastewater (Vymazal, 2013a). In addition, two types of surface-flow CWs of continuous-marsh and marsh-pond-marsh have been preferred as useful methods for swine wastewater treatment in the U.S. (Poach et al., 2004).

Macrophytes are a key biological component and play an important role in removing N in CWs. Plant growth assimilates

* Corresponding author. Changsha Research Station for Agricultural & Environmental Monitoring, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan, 410125, PR China.

** Corresponding author. Changsha Research Station for Agricultural & Environmental Monitoring, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan, 410125, PR China.

E-mail addresses: liufeng@isa.ac.cn (F. Liu), jswu@isa.ac.cn (J. Wu).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jenvman.2015.11.020

nutrients to form organic compounds such that when the plants are harvested, the N and P are removed from the polluted water bodies (Vymazal, 2007, 2011). Plant vegetation provides a carbon source and a high surface area for the improvement of microbial activities in wetlands (Cronk, 1996). Radial oxygen loss (ROL) creates oxygenation areas adjacent to plant roots, which stimulates aerobic processes such as nitrification (Brix, 1997; Stottmeister et al., 2003). The accumulation of decaying plant litter within the bottom layer of wetlands, which is always anoxic, supports enough bioavailable carbon sources for the denitrification process to occur (Vymazal, 2013b). Hence, plant vegetation effectively increases the microbial transformation of N, thus enhancing N removal (Tanner et al., 1995; Maltais-Landry et al., 2009). Coupled nitrification and denitrification in wetlands contribute to N removal from ammonia-rich animal wastewater (Morgan and Martin, 2008). Ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) are two groups of prokaryotes, which are responsible for a rate-limiting step (the oxidation of ammonia to nitrite) in nitrification (Hou et al., 2013). Analysis of the functional genes of nitrate reductase (*narG*), nitrite reductase (*nirK* and *nirS*), and nitrous oxide reductase (*nosZ*) can provide valuable information on the diversity of denitrifiers in environment samples (Kandeler et al., 2006).

The N removal abilities of CWs depend on plant species, wastewater type, and N loading rates. Plant uptake contributes to N removal rates of 3–47% (Gottschall et al., 2007; Vymazal, 2007). A thorough understanding of the role of plants in CWs can provide important information for the optimization of wetland design. For animal wastewater treatment, the selected plant species should be tolerant of high ammonium concentrations and have high productivity to enable rapid nutrient uptake (Cronk, 1996). *Myriophyllum aquaticum* is a widespread submergent and emergent herb that can grow with stem branches of up to 1 m and that readily grows in tropical regions of the world (Torres Robles et al., 2011). Despite its invasive status in natural water bodies, the risk of *M. aquaticum* spreading to other ecosystems is manageable in CWs. *M. aquaticum* can be planted in a drainage ditch to mitigate agricultural non-point runoff pollution. Study results show that the vigorous growth of *M. aquaticum* contributes to the rapid accumulation of organic carbon in ditch soils, which enhances the soil phosphorus adsorption capacity (Liu et al., 2013). On other way, it is reported that *M. aquaticum* roots have the ability to create oxidized areas and release organic compounds at the root–sediment interface that play important roles in plant metal accumulation (Teuchies et al., 2012). Overall, there is limited available research regarding the use of *M. aquaticum* in improving environmental quality. Meanwhile, the potential of *M. aquaticum* for animal wastewater treatment have been unknown.

To understand the potential of *M. aquaticum* for N removal from wastewater, the main objectives of this study were: (1) to determine the changes in NH_4^+-N, NO_3^--N, and TN concentrations in *M. aquaticum* mesocosms; (2) to investigate the effects of wastewater types with two strengths on the dynamics of the abundance of ammonia-oxidizing and denitrifying functional genes; and (3) to evaluate N removal pathways in *M. aquaticum* mesocosms through a N mass balance analysis.

2. Materials and methods

2.1. The study plant, wastewater and soil

Field observations showed that *M. aquaticum* can grow well in drainage ditches, streams, ponds, and wetlands in subtropical China from March to December when the daily mean temperature is above 10 °C (Liu et al., 2013). *M. aquaticum* used in this study was transplanted from a novel constructed drainage ditch. Swine wastewater for the study was collected from an anaerobic lagoon in a pig-breeding farm, and the soil was sampled from a paddy field, both of which were located within the Changsha Research Station for Agricultural & Environmental Monitoring of the Chinese Academy of Sciences in Hunan Province, P.R. China. The sampled swine wastewater contained 416.8 mg L^{-1} NH_4^+-N, 0.41 mg L^{-1} NO_3^--N, 458.1 mg L^{-1} total nitrogen (TN), 31.6 mg L^{-1} total phosphorus (TP), and 1142.5 mg L^{-1} COD. The soil properties were as follows: TN of 1.86 g kg^{-1}, TP of 0.83 g kg^{-1}, total carbon content of 21.93 g kg^{-1}, a pH value of 6.71 (measured at soil to water solution ratio of 1:2.5 w/v), and a loam texture consisting of 32.6% sand, 41.1% silt, and 26.3% clay.

2.2. Setup of mesocosms

The experimental mesocosms were investigated in a greenhouse from August 14th to September 11th. During the experimental incubation period, air temperatures in the greenhouse were between 18.8 °C and 37.4 °C.

Twelve square plastic tanks with dimensions of 50 cm length × 40 cm width × 50 cm depth were used to prepare *M. aquaticum* mesocosms. Ten kilograms of air-dried paddy soil was added to each tank to produce a soil layer with approximately 5 cm in depth, which were firstly incubated with tap water for 3 days. One hundred plant shoots with a uniform length of 20 cm and a total fresh weight of approximately 90 g were planted into the soil layer of each tank, after which they were incubated with tap water for another 5 days. After the added tap water was completely discharged, *M. aquaticum* mesocosm was used as a batch treatment system with the addition of 15 L of wastewater at one time. To investigate the differences in N removal from different types of wastewater in the *M. aquaticum* mesocosm, two types of wastewater, each with two strengths, were prepared. The sampled swine wastewater from an anaerobic lagoon was used as high-strength swine wastewater (SW). By diluting SW with tap water at a 1:1 (v/v) ratio, the 50% diluted swine wastewater (50% SW) was prepared as low-strength swine wastewater. Based on the NH_4^+-N concentrations in the two different strengths of swine wastewater, two strengths of synthetic wastewater – 200 mg L^{-1} NH_4^+-N(200 NH_4^+-N) and 400 mg L^{-1} NH_4^+-N solution (400 NH_4^+-N)— were prepared with ammonium sulfate. Each wastewater was spiked into *M. aquaticum* mesocosms in triplicates. According to high N removal rates of 98% for NH_4^+-N and 97% for TN obtained in a field-scale integrated *M. aquaticum* wetlands with a hydraulic retention time of about one month (Li et al., 2015), an experimental time of 28 days was used as the incubation period in this study. During the whole experimental period, distilled water was added to replace evaporation losses and maintain a constant water depth of 7.5 cm.

2.3. Sampling and analysis

After the study wastewater was added into *M. aquaticum* mesocosms, 100 mL water samples were collected by a 50 mL syringe on days 0, 1, 3, 7, 10, 14, 17, 22, 25 and 28. The concentrations of NH_4^+-N and NO_3^--N in the wastewater were measured using a fully automated flow-injection system (FIA-star 5000 analyzer, Foss Tecator, Höganäs, Sweden). To analyze TN, water samples were first digested using $K_2S_2O_8$–NaOH solution, and the digested NO_3^--N was measured using the automated flow-injection system. Additionally, the water quality parameters of pH, DO, and temperature (T) were measured using a portable multiple parameter meter (SG68-ELK, Mettler Toledo, Switzerland) at a water depth of 5 cm in *M. aquaticum* mesocosms at 09:00 AM.

By using a cylindrical stainless auger with a diameter of 2 cm,