Science of the Total Environment xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

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

Nitrate-rich agricultural runoff treatment by *Vallisneria*-sulfur based mixotrophic denitrification process

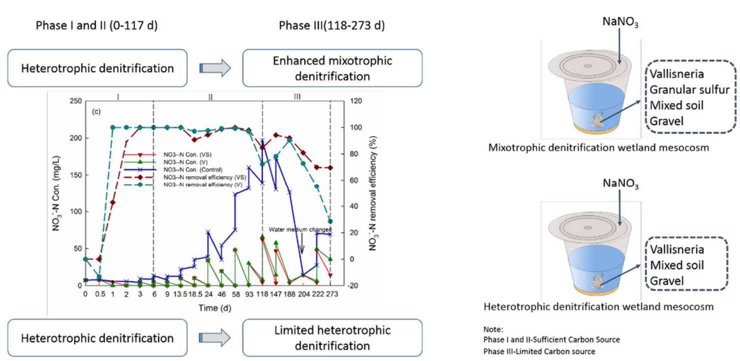
Qianyu Hang [a,b], Haiyan Wang [a,b,*], Zhaosheng Chu [a,c,**], Zeying Hou [a,c], Yuexi Zhou [a,b], Chunmei Li [a,d]

[a] *State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, No. 8 Da Yang Fang, Anwai, Chaoyang District, Beijing 100012, PR China*
[b] *Research Center for Water Pollution Control Technology, Chinese Research Academy of Environmental Sciences, No. 8 Da Yang Fang, Anwai, Chaoyang District, Beijing 100012, PR China*
[c] *Research Center of Lake Eco-Environments, Chinese Research Academy of Environmental Sciences, No. 8 Da Yang Fang, Anwai, Chaoyang District, Beijing 100012, PR China*
[d] *Lanzhou University, Lanzhou 730000, PR China*

HIGHLIGHTS

- A *Vallisneria*-sulfur mixotrophic denitrification process is developed.
- The feasibility and mechanism of the process are studied for 273-day operation.
- Average NO_3^--N removal efficiency and denitrification rate were 97.7% and 1.5 g NO_3^--N m^{-3} d^{-1} at steady state.
- No additional alkalinity supplement is needed in the mixotrophic denitrification.
- *Vallisneria* decomposition and denitrifying functional genes are also analyzed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 October 2016
Received in revised form 6 February 2017
Accepted 7 February 2017
Available online xxxx

Editor: D. Barcelo

Keywords:
Vallisneria decomposition
Sulfur
Mixotrophic denitrification
Agricultural runoff

ABSTRACT

Vallisneria-sulfur based mixotrophic denitrification (VSMD) process was put forward for the treatment of nitrate-rich agricultural runoff with low COD/TN (C/N) ratio in free water surface constructed wetland mesocosms, whose feasibility and mechanism were thoroughly studied through 273-day operation. The results showed that the average NO_3^--N removal efficiency and denitrification rate of VSMD mesocosms were 97.7% and 1.5 g NO_3^--N m^{-3} d^{-1} under 5.0 or higher C/N ratio conditions in phase II (7– 117 d), which were similar with those of *Vallisneria* packed heterotrophic denitrification (VHD) mesocosms. However, VSMD mesocosms with 2.0 average C/N ratio in phase III (118–273 d) were more stable and efficient than VHD mesocosms. More than 49.4 mg NO_3^--N was reduced by VSMD mesocosms than that by VHD mesocosms throughout the operation. NO_2^--N accumulation in phase I (0– 6 d) had no influence on denitrification performance of VSMD mesocosms. In phase II and III, effluent COD, NH_4^+-N and NO_2^--N could meet the Class II standard of *Environmental quality for surface water* (GB3838-2002) if the experiment was carried out in batch mode. pH in VSMD mesocosms fluctuated between 7.0 and 8.9 throughout the operation without any pH buffer. The abundance of three denitrifying genes coding for nitrate (*narG*), nitrite (*nirS*), and nitrous oxide (*nosZ*) reductases in bottom soil and mixture from litter bags was quantified. VSMD could supply more favorable circumstances for the growth of denitrificans

* Correspondence to: H. Wang, Research Center for Water Pollution Control Technology, Chinese Research Academy of Environmental Sciences, No. 8 Da Yang Fang, Anwai, Chaoyang District, Beijing 100012, PR China.
** Correspondence to: Z. Chu, Research Center of Lake Eco-Environments, Chinese Research Academy of Environmental Sciences, No. 8 Da Yang Fang, Anwai, Chaoyang District, Beijing 100012, PR China.
E-mail addresses: wanghy@craes.org.cn (H. Wang), chuzs@craes.org.cn (Z. Chu).

http://dx.doi.org/10.1016/j.scitotenv.2017.02.069

Please cite this article as: Hang, Q., et al., Nitrate-rich agricultural runoff treatment by *Vallisneria*-sulfur based mixotrophic denitrification process, Sci Total Environ (2017), http://dx.doi.org/10.1016/j.scitotenv.2017.02.069

containing *narG* ($3.1 \times 10^8 \pm 7.9 \times 10^7$ copies g^{-1} $mixture^{-1}$) and *nirS* ($2.1 \times 10^8 \pm 2.0 \times 10^6$ copies g^{-1} $mixture^{-1}$) in litter bags than VHD, i.e., $8.7 \times 10^7 \pm 1.4 \times 10^7$ and $1.4 \times 10^8 \pm 1.5 \times 10^7$ copies g^{-1} $mixture^{-1}$ for *narG* and *nirS* respectively. Sulfur addition in VSMD mesocosms might increase the abundance of denitrificans containing *narG* and *nirS*, thus led to better denitrification performance.

1. Introduction

The removal of nitrogen from agricultural runoff with high nitrate and low COD/TN (C/N) ratio is difficult due to insufficient organic carbon availability for heterotrophic denitrificans, and free water surface constructed wetlands are frequently used (Mander et al., 2014).

Microbial denitrification, occurring either heterotrophically or autotrophically, is generally considered to be the main nitrate removal mechanism in constructed wetlands (Vymazal et al., 2006). In the last few decades, sulfur-limestone based autotrophic denitrification or simultaneous mixotrophic denitrification processes have been widely recommended for the economical treatment of wastewater with low C/N ratios, and such processes have high denitrification efficiency and consistent pH (Della Rocca et al., 2006; Oh et al., 2003; Zhang and Lampe, 1999).

However, due to Ca^{2+} dissolution in the sulfur-limestone based autotrophic denitrification process, the increased hardness and sulfate in the effluent has hampered its wide utilization (Sahinkaya and Dursun, 2012). As for the mixotrophic denitrification process, frequently applied carbon sources for heterotrophic denitrification such as methanol, glucose, sodium acetate, etc. are costly due to their continuous dosing. Moreover, unwanted chemical organic residuals need to be further treated. Alternatively, various plant biomass were used as carbon sources for heterotrophic denitrification, and high nitrate removal could be attained in the biomass-added constructed wetlands (Hang et al., 2016). Submerged macrophytes could provide special niches for biofilms favoring the growth of denitrificans (Eriksson and Weisner, 1999). Since *Vallisneria* is widely distributed at Erhai littoral zone, it was selected as plant carbon source in this study. Sulfur is also a popular electron donor for autotrophic denitrification with several advantages as cost-effectiveness, non-toxicity, water-insolubility, wide availability, etc. (Zhang and Lampe, 1999).

Therefore, from economic and environmental points of view, a simultaneous *Vallisneria*-sulfur based mixotrophic denitrification (VSMD) process was developed in this study for the treatment of nitrate-rich agricultural runoff, in which heterotrophic and autotrophic denitrification processes complemented each other.

Dissolved organic matter (DOM) is an important fraction in the organic component release of plant biomass, which is a key indicator for denitrification performance. The three-dimensional excitation-emission matrix (EEM) fluorescence spectrophotometer is a good tool for the assessment of DOM characteristics (Chen et al., 2003; Sheng and Yu, 2006; Yu et al., 2010). Biological denitrification process of NO_3^--N reduction to N_2 consists of four consecutive reaction steps, and nitrate reductase (*narG*), nitrite reductase (*nirS*) and nitrous-oxide reductase (*nosZ*) genes are considered to be the pivotal functional genes involved. The reduction of NO_3^--N to NO_2^--N can be catalyzed by membrane-bound nitrate reductase, which is encoded by *narG* gene. The *nirS* gene presents the cytochrome cd1-containing nitrite reductase, which is widely distributed (Chen et al., 2014b). The *nosZ* gene expresses nitrous oxide reductase, which catalyzes the reduction of N_2O to N_2.

This study was to verify the feasibility of VSMD process in free water surface constructed wetland mesocosms for the treatment of high nitrate and low C/N ratio agricultural runoff through 273-day operation, and the denitrification performance and nutrients release pattern were also extensively studied. The DOM was analyzed by EEM fluorescence PARAFAC method (Stedmon and Bro, 2008). The characteristic of denitrifying functional genes including *narG*, *nirS*, and *nosZ* genes was evaluated by Q-PCR (Chon et al., 2011).

2. Materials and methods

2.1. Materials and synthetic agricultural runoff preparation

Samples of aboveground *Vallisneria* and soil from wetland and paddy-field were all collected along Erhai littoral zone (25°36′–25°58′ N, 100°06′–100°18′ E) in August 2014. Soil samples from wetland and paddy-field were mixed homogeneously in 1:1 mass ratio, air-dried to constant mass, milled and screened through 100-mesh sieve. *Vallisneria* samples were cleaned, cut into 1– 2 cm pieces and oven-dried at 40 °C to constant mass. Granular sulfur (1– 2 cm) and gravel (1– 2 cm) were bought from local companies.

Synthetic agricultural runoff fed to the mesocosms was prepared from tap water, whose composition was as follows (per 50 l): 1.457 g sodium nitrate, 0.057 g sodium nitrite, 0.023 g ammonia chloride, 0.241 g potassium dihydrogen phosphate and 0.419 g glucose. The NH_4^+-N, NO_3^--N, NO_2^--N, total inorganic nitrogen (TIN), TP and COD of the synthetic wastewater were about 0.5, 7.4, 1.2, 9.1, 1.2 and 3.2 mg L^{-1}, respectively. The pH was 8.3.

2.2. Experimental design

Mesocosm is an experimental ecosystem to simulate the natural conditions, which can reduce the natural variability in consideration of ecosystem structural richness and specific portions (Giesy et al., 1983). Six lab-scale free water surface constructed wetland mesocosms including 2 VSMD mesocosms (packed with *Vallisneria* pieces and sulfur), 2 *Vallisneria* packed heterotrophic denitrification (VHD) mesocosms (packed with only *Vallisneria* pieces) and 2 control mesocosms were fed with 2.0 L synthetic agricultural runoff to simulate the micro-decomposition circumstance of wetlands. The synthetic agricultural runoff was stripped by nitrogen gas to obtain anoxic conditions at the beginning of the experiment. Each mesocosm had a duplicate. The diagram of the experimental mesocosm was shown in Fig. 1. Mesocosms were made by 5 L plastic buckets (0.17 m bottom inner

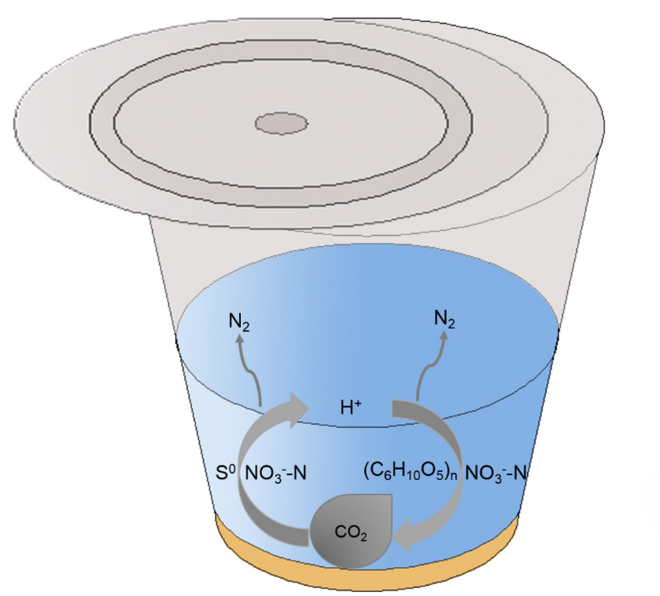


Fig. 1. The diagram of the experimental set-up.

Please cite this article as: Hang, Q., et al., Nitrate-rich agricultural runoff treatment by *Vallisneria*-sulfur based mixotrophic denitrification process, Sci Total Environ (2017), http://dx.doi.org/10.1016/j.scitotenv.2017.02.069