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Short communication

# Treatment of rich ammonia nitrogen wastewater with polyvinyl alcohol immobilized nitrifier biofortified constructed wetlands

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#### ABSTRACT

Polyvinyl alcohol (PVA) immobilized nitrifier was adopted to enhance the treatment of wastewater containing rich ammonia nitrogen (NH<sub>4</sub>-N) in constructed wetlands (CWs). NH<sub>4</sub>-N removal in immobilized nitrifier biofortified constructed wetlands (INB-CWs) was positively correlated with the dosage of PVA pellets. INB-CWs planted with *Canna indica* L. exhibited removal efficiencies of 46% NH<sub>4</sub>-N, 43% total nitrogen and 65% COD, which are significant higher than control. INB-CWs outperformed the unplanted and non-immobilized constructed wetland or nitrifier suspension system. The ideal reaction conditions for treating synthetic wastewater containing 200 mg L<sup>-1</sup> NH<sub>4</sub>-N were obtained as follows: pH, 8.5; dissolved oxygen, 4.0 mg L<sup>-1</sup>; temperature, 30 °C and PVA pellets dosage, 60 g L<sup>-1</sup>. In addition, degradation of PVA pellets accelerated nitrification. Results indicated that the PVA immobilized nitrifier had great potential applications for treating wastewater containing rich ammonia nitrogen in CWs.

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#### 1. Introduction

Untreated wastewater contains rich ammonia nitrogen (NH<sub>4</sub>-N) which consequently causes eutrophication and eco-toxicity to aquatic species due to the depletion of dissolved oxygen in receiving water (Tak et al., 2015; Obaja et al., 2003). In the last decades, constructed wetlands (CWs) have shown good performance in treating a variety of wastewater (Vymazal, 2005, 2013; Wang et al., 2016). The advantages of CWs include moderate costs, low energy consumption and less maintenance requirements (Cronk, 1996; Vymazal, 2011). CWs are appropriate to remove nitrogenous compounds (Vymazal, 2005; Ding et al., 2012). Nitrogen removal in CWs primarily depends on microbial activities associated with processes of nitrification and denitrification (Cui et al., 2013; Martin and Moshiri, 1994). Nitrification, which is a biological oxidation of ammonia to nitrite followed by the oxidation of the nitrite to nitrate process, is considered to be the primary rate-limiting step

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http://dx.doi.org/10.1016/j.ecoleng.2016.05.078 0925-8574/© 2016 Elsevier B.V. All rights reserved. for nitrogen removal in CWs (Tanner et al., 2002). Nitrifier as key microbial in CWs, determined the nitrification reaction under aerobic conditions affecting the biological nitrogen removal efficiency (Vymazal, 2013; Ding et al., 2012). The amount of nitrifier was usually positively correlated with the NH<sub>4</sub>-N removal. However, slow growth and survival rate usually cause low nitrifier abundance in CWs, significantly limit the NH<sub>4</sub>-N oxidation in CWs.

In the study, in order to enhance the microbial abundance of nitrifier in CWs, immobilization technology was adopted to load nitrifier into media internal pores. Thus, nitrification can be enhanced by the high nitrifier concentration in the media. Polyvinyl alcohol (PVA) as a gel matrix was used to immobilize nitrifier by repeated freezing and thawing (Cao et al., 2002; Chang et al., 2005). To our knowledge, the feasibility and application of immobilization technology in CWs, especially immobilization of concentrated nitrifier in CWs has seldom been reported. The main objectives of this paper were oriented as: i) discuss the effects of parameter variations on degradation efficiency of immobilized nitrifier and obtain optimal conditions for efficient nitrification; ii) evaluate the performance of four wetland systems and ascertain the utility of immobilized nitrifier as a viable technique for efficient NH<sub>4</sub>-N removal in CWs.









Fig. 1. Effects of temperature (a), pH (b), DO (c) and pellet dosage (d) on NH<sub>4</sub>-N removal efficiency.

#### 2. Materials and methods

#### 2.1. Isolation of nitrifier

In this study, the activated sludge collected from Songjiang Municipal Wastewater Treatment Plant (Shanghai) was utilized for nitrifier separation and cultivation. The ingredients of culture solution (mg L<sup>-1</sup>) composed of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 800; Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 250; NaCl, 98; KCl, 45; MgSO<sub>4</sub>·7H<sub>2</sub>O, 30; CaCl<sub>2</sub>·2H<sub>2</sub>O, 50 and NaHCO<sub>3</sub>, 1000. After 3-month incubation in laboratory, the concentration and amount of nitrifier were about 2760 mg L<sup>-1</sup> and 7.5 × 10<sup>9</sup> L<sup>-1</sup> (MPN), respectively (McCrady, 1915; Moaledj, 1986; Manju et al., 2009).

#### 2.2. Immobilization

Suspensions of nitrifier cells were centrifuged at 3000 rpm for 15 min, thereafter, washed with normal saline and centrifuged for twice. Concentrated cells were added to the solution containing 10% PVA and 2% sodium alginate (SA). Then, the mixture was stirred evenly. After agitation and calcification for 30 min, the mixture was dropped into 4% of CaCl<sub>2</sub> solution with an injector to obtain the pellets with the diameter of 2–3 mm. The obtained pellets were washed thoroughly with distilled water after frozen in a refrigerator overnight at 4 °C.

### 2.3. Morphological observation by scanning electron microscopy (SEM)

The surface morphological characteristics of the immobilized pellets were examined by scanning electron microscopy (SEM) (S-4800, Hitachi, Japan). The collected samples were rinsed with  $0.1 \text{ mol } \text{L}^{-1}$  phosphate buffer three times, fixed with 2.5% glutaraldehyde solution for 12 h at 5 °C. Subsequently, the samples were washed and dehydrated in a graded series of ethanol solution (30%, 50%, 70%, 80%, 90% and 100%). The dewatered samples were

dried by the critical point method and prepared for SEM observation.

#### 2.4. Batch experiment

Batch experiments were conducted to inspect characteristics of immobilized pellets for the subsequent application in CWs. Experiments were carried out in a bubble-column bioreactor of 1000 mL working volume with heating jacket. To determine the effects of temperature, pH, dissolved oxygen (DO) and pellet dosage on NH<sub>4</sub>-N removal by the immobilized nitrifier in pellets, the following conditions were adopted: temperature of 15-45°C, pH of 5.0–9.5, DO concentration of 1.0–6.0 mg  $L^{-1}$  and pellet dosage of 10–90 g L<sup>-1</sup>. During this set of experiments, only one parameter was modified at a time. 0.1 mol L<sup>-1</sup> HCl/NaOH buffer solution was used to adjust pH. DO concentration was controlled by adjusting the air flow rate. 800 mL of synthetic wastewater was added into the reactor. The parameters of synthetic wastewater  $(mgL^{-1})$  were set as follows: NH<sub>4</sub>-N,  $200 \pm 6.7$ ; COD,  $800 \pm 10.3$ ; total nitrogen (TN),  $204 \pm 5.2$ . The reactor was operated intermittently with hydraulic retention time (HRT) of 5 d.

#### 2.5. Experimental setup

The experiments were performed for 6 months (April to September in 2015) on campus of Donghua University, Shanghai. Twelve polyethylene plastic reactors ( $0.57 \text{ m} \times 0.4 \text{ m} \times 0.35 \text{ m}$ ), used as microcosm CWs, were filled with approximately uniform quartz sand with the height of 0.27 m ( $\Phi$  3–6 mm). The reactors including the control were divided into 4 groups (System A–C and D) and each group contained 3 replicates. The four systems were operated under different conditions. System A (control) was not planted while Systems B–D were planted with *Canna indica* L. transferred from Shanghai Chenshan Botanical Garden. Each reactor contained two macrophytes. Before the experiment, Hogland's solution was fed for cultivation. The formal experiment was performed after one month of stable plant growth, during which

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