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High efficiency of inorganic nitrogen removal by integrating biofilm-electrode with constructed wetland: Autotrophic denitrifying bacteria analysis

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HIGHLIGHTS

- The inorganic nitrogen removal and microbial species in CW-BER and CW were compared.
- Removal efficiency of NH_3-N and NO_3-N have been significantly promoted in CW-BER.
- Nitrate removal in CW-BER decreased with current intensity increased to 15–20 mA.
- Relative abundance of *Thiobacillus* in CW-BER was apparently higher than that of CW.

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ABSTRACT

The constructed wetland coupled with biofilm-electrode reactor (CW-BER) is a novel technology to treat wastewater with a relatively high level of total inorganic nitrogen (TIN) concentration. The main objective of this study is to investigate the effects of C/Ns, TIN concentrations, current intensities, and pH on the removal of nitrogen in CW-BER; a control system (CW) was also constructed and operated with similar influent conditions. Results indicated that the current, inorganic carbon source and hydrogen generated by the micro-electric field could significantly improve the inorganic nitrogen removal with in CW-BER, and the enhancement of average removal rate on NH_3-N, NO_3-N, and TIN was approximately maintained at 5–28%, 5–26%, and 3–24%, respectively. The appropriate operation conditions were I = 10 mA and pH = 7.5 in CW-BER. In addition, high-throughput sequencing analysis implied that the CW-BER reactor has been improved with the relative abundance of autotrophic denitrifying bacteria (*Thiobacillus* sp.).

1. Introduction

In wastewater from poultry industries and livestock, the pollutant loads of ammonia and nitrate generally ranged in a high level (400–500 mg L^{-1}). The mixture wastewater of these contaminants and domestic usually detected with a total nitrogen (TIN) concentration varied from 45 to 100 mg L^{-1}, which contributes to the increased aquatic eutrophication and biota toxicity in receiving rivers or lakes (Sotres et al., 2016; Wang et al., 2016). Ammonia (NH_3-N), nitrate (NO_3-N), and nitrite (NO_2-N) are the common forms of nitrogen in the wastewater. The rising in nitrogen concentration in water bodies has been regarded as a serious hazard for animal, human and health (Fitzgerald et al., 2015; Kim et al., 2016). Hence, focuses on the enhancement of nitrogen removal has intensified for decades. It is generally believed that nitrification and denitrification are the main process of nitrogen removal. Previous studies indicated that pollutants in wastewater such as ammonia, nitrate could be removed by physical, biochemical and the combination of physical and biochemical processes (Vymazal and Kroepfelova, 2011). Nevertheless, these methods have the disadvantage of heterotrophic denitrification process which might be inhibited by the limitation of carbon source.

As an easy maintenance and cost-effective method, constructed wetland (CW) is a feasible technology for wastewater treatment

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(Allende et al., 2014; Doherty et al., 2015; Vymazal and Kroepfelova, 2015). CW has the advantages of aerobic and anoxic condition that are widely distributed in the upper and lower part, which could be used to promote the occurrence of simultaneous nitrification and denitrification process (Vymazal, 2011). However, the removal efficiency of total nitrogen in CW usually ranged from 40% to 70%, which is mainly caused by lack of carbon source in denitrification process. In addition, microbial community structure, especially nitrobacteria and denitrifying bacteria, could be easily affected by in variation of operation conditions such as pH, oxygen, temperature, etc (Mietto et al., 2015). *Dechloromonas*, *Nitrosomonas, Desulfobulbus*, *Pseudomonas, Flavobacterium*, *Propionivibrio,* and *Geobacter* have been reported nitrogen-removal microorganisms in activated sludge or biofilm in previous studies (Fitzgerald et al., 2015; Gao et al., 2016; Kim et al., 2007; Lu et al., 2015; Sotres et al., 2016; Wang et al., 2016). To improve the nitrogen removal, one of the possible solution is using novel technology to select nitrobacteria and denitrifying bacteria as the predominant microorganisms in CW.

Electrochemical system, especially biofilm-electrode reactor (BER), offer a feasible technology for nutrient removal while at the same time acclimating microbial community compositions. In BER systems, production of H_2 by water electrolysis could be used as electrons in denitrification; the coexistence autotrophic bacteria and denitrifying bacteria can be immobilized on the surface of the cathode by micro-electric field (Mousavi et al., 2012; Park et al., 2006). Moreover, the inorganic carbon source (CO_2) generated by anode electrode material (carbon) could serve as pH buffer in BER (Hao et al., 2013). Notably, organic carbon source is also employed to strengthen the process of denitrification in BER (Feng et al., 2013). Furthermore, the nitrogen removal also significantly affected by the current intensity in the BER system. When current ranged from 0 to 20 mA, the nitrogen removal efficiency had apparently positive correlation with current intensity (Sakakibara et al., 1994). However, when electric current intensity exceeds 0.029 mA cm^{-2}, the denitrification efficiency has been inhibited by a plenty of hydrogen production (Sakakibara et al., 1994). In addition, in our previous study, the hybrid of CW and BER has been connected in a series way to treat the wastewater with a high concentration of inorganic nitrogen, the results implied that the hybrid of the two technology could significantly promote the removal efficiency of inorganic nitrogen (He et al., 2016a). Considering that the enhancement footprint of the above process, in this study, using of BER coupled with CW reactors in operated with a suitable current intensity may also reduce the inhibition of carbon source and footprint limitation during the practical application of this technology (Liu et al., 2015).

To date, the combination of BER and CW for wastewater treatment has few reports in the literature. Therefore, in this study, inorganic nitrogen removal efficiency and microbial community structure responses of C/N ratio, pH values, current intensity, and total inorganic nitrogen (TIN) concentration to CW-BER were reported with the aim of implying the autotrophic denitrification process has been improved in CW-BER. Meanwhile, a control system (CW) was also constructed and operated with similar influent conditions. The pollutants removal ratio of CW and CW-BER with different operation conditions were analyzed. At the end of the experiment, high-throughput sequencing analysis was employed to compare the microbial community compositions between the two reactors.

2. Materials and methods

2.1. Experimental apparatus construction

The scheme of CW-BER is shown in Fig. 1. Two containers (59 L × 45 W × 40 H cm; material: polyethylene plastic) were filled with quartz sand (average diameter 1.4 cm; porosity 0.47) and planted with disease-free *Cannas indica L.* (initial stem length 30 ± 4 cm; 3 plants $reactor^{-1}$). Before transplanting, wetland plants were propagated for an acclimation of three weeks. The packed height of media was 32 cm. Peristaltic pump was used to fed the synthetic wastewater on the surface of media; then samples were collected at the bottom of microcosm. In CW-BER system, an adjustable direct current (DC) power was used to from micro-electric field between the anode (graphite robs; 31.0 cm length and 1.0 cm diameter) and cathode (carbon fiber felt; 25.2 cm length and 14.2 cm diameter) electrodes. Each cathode electrode was installed to the inner of plexiglass cylinder. Both control (CW) and CW-BER systems were maintained at indoor conditions with an air-condition, and air temperature ranged from 20 to 25 °C.

2.2. Experiment start-up and operation

After construction, activated sludge (20 L) was collected from Songjiang wastewater treatment plant (Shanghai, China) and diluted with tap water, then inoculated into two reactors with equal volume. During a one-month cultivation and acclimation, synthetic domestic sewage was feed onto the surface of the media with a hydraulic retention time (HRT) of 2 days; meanwhile, the CW-BER system was not applied with electric current. At the end of cultivation, the wastewater treatment performance of CW and CW-BER was stable (effluent pH, nitrate, and COD was 7.3 ± 0.4, 5.4 ± 1.5 mg L^{-1}, 36 ± 3.5 mg L^{-1}, respectively). Synthetic wastewater was prepared by composing of glucose, NH_4Cl, $NaNO_3$, and some trace element ingredients such as $ZnSO_4$, $CuSO_4$, $MnSO_4$, H_3BO_3, and (NH_4) Mo_7O_{24}. The original pH of the synthetic water ranged from 7.2 to 7.8. CW-BER system was operated under different C/Ns, TINs, current intensity, and pH (Table S1); meanwhile, CW was tested for wastewater treatment with the same influents. In this study, the C/N gradients (ammonia: 32 mg L^{-1} and nitrate: 64 mg L^{-1}) was respectively tested for 1:2, 3:4, 1:1, and 2:1. TIN was chosen as 45, 60, 75, and 90 mg L^{-1}. Current intensity (I) was selected as 5, 10, 15, and 20 mA. The pH was tested for 6.5, 7.0, 7.5, and 8.0. During the preparation of synthetic wastewater, phosphate buffer (KH_2PO_4 and Na_2HPO_4) was used to adjust the pH values of influents. Each operational condition was maintained with a stable nitrate removal performance for at least 5 days.

2.3. Sampling and analysis

Samples of influents and effluents were collected with a 250 mL of beaker for wastewater treatment analysis. Dissolved oxygen (DO), temperature, and pH were analyzed by a portable multi-parameter water quality monitoring (HQ40d, HACH, USA). COD, NH_3-N and TIN were measured with an ultraviolet spectrophotometer (DR890, HACH, USA) according to standard methods. NO_2-N was measured using a gas-phase molecular absorption spectrometry (GMA3202, Shanghai Beiyu Analytical Instruments C_O., Ltd., China). NO_3-N was analyzed by ultraviolet spectrophotometer (UV2000, Shanghai Unico Instrument Co., Ltd., China).

2.4. High-throughput sequencing analysis

At the end of experiment, the biofilm sample for cathode electrode was detached from its surface; meanwhile, the biofilm of media (quartz sand) at the same height was also collected. Then, samples were dispersed into a sterile bottle (250 mL) and centrifuged at 200 rpm (12 min). The supernatant was removed during the centrifugation process, and total of pellet in both samples were collected for DNA extraction in duplicate with the OMEGA Soil DNA kit (D5625-01, USA). Polymerase chain reaction (PCR) amplification of hypervariable regions V4 and V5 was performed with