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Bacterial community and nitrate removal by simultaneous heterotrophic and autotrophic denitrification in a bioelectrochemically-assisted constructed wetland

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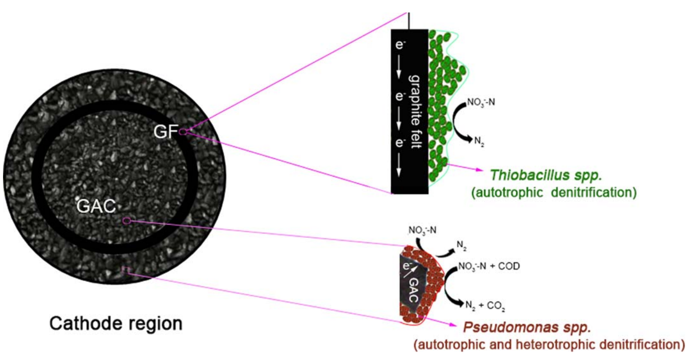
GRAPHICAL ABSTRACT



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ABSTRACT

To enhance nitrate removal in constructed wetlands (CWs), a bioelectrochemically-assisted CW (BECW) integrating a three-dimensional biofilm-electrode reactor (3D-BER) into the CW was evaluated for the effectiveness of combined autotrophic and heterotrophic denitrification in the presence of organic matter and applied current. The effects of COD/N ratios on nitrate removal were investigated, and the bacterial communities in the granular active carbon (GAC) and graphite felt (GF) in the reactor's cathode region were compared. The highest NO_3^--N and TN removal efficiencies of 91.3 ± 7.2% and 68.8 ± 7.9% were obtained at the COD/N ratio of 5. According to the results of high-throughput sequencing analysis, sample GAC was enriched with a high abundance of *Pseudomonas* (17.29%) capable of autotrophic and heterotrophic denitrification, whereas autotrophic bacteria *Thiobacillus* (43.94%) was predominant in sample GF. The synergy between heterotrophic and autotrophic denitrification bacteria is believed to cause the high and stable nitrogen removal performance.

1. Introduction

Owing to uncontrolled discharge of wastewater and intensive use of fertilizers in agriculture, nitrate pollution in water resource has become a serious threat to human health and water ecosystem. Intake of high nitrate concentration in drinking water may be linked to infant methemoglobinemia and bladder cancer (Weyer et al., 2001). The discharge of wastewater containing excessive nitrate levels into aquatic

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systems (e.g., lakes, rivers or seas) can lead to adverse effects associated with eutrophication, including algal blooms, changes in biodiversity and bottom anoxia (Maier et al., 2009). Extensive research has been done on the development of technologies for eliminating nitrate from wastewater and water. Physicochemical methods are not economically feasible for large applications because of high cost and the need for waste brine disposal (Park & Yoo, 2009). As an alternative, biological denitrification is considered the most promising approach for nitrate removal because of its effective performance and low cost.

Constructed wetlands (CWs) have become increasingly and extensively used as an alternative treatment method for nitrate-rich water and wastewater (Almeida et al., 2017), owing to their favorable purification efficiency, low cost, simple operation and maintenance, and environmental friendliness. Nitrate nitrogen (NO_3^--N) in CWs is removed mainly by conventional heterotrophic denitrification (Zhi & Ji, 2014), in which organic carbon compounds are combined with electron acceptors (NO_3^-) to yield oxidized carbon (CO_2), a reduced product (N_2), and energy. Thus, it is often difficult to maintain high nitrate removal efficiency when organic carbon in wastewater is insufficient. To date, numerous studies have been undertaken to enhance denitrification in CWs when treating low chemical organic demand to nitrogen (COD/N) ratio wastewaters, which mainly include looking for cheap and abundantly available alternative carbon sources (e.g., various plant materials) (Hang et al., 2016) and incorporating autotrophic denitrification into CWs (Park et al., 2015; Song et al., 2016a). However, directly adding plant carbon source into CWs is increasingly unfavorable because of its low effective utilization compared to soluble substrates (e.g., methanol), need for optimization of plant biomass dosage and dosing position, as well as potential risk of secondary pollution resulting from excessive organic carbon (Hang et al., 2016). Autotrophic denitrification can be attained by using a variety of inorganic reduced compounds (e.g., sulfur-reduced compounds, ferrous iron, and hydrogen gas) as electron donors to reduce nitrate (Park & Yoo, 2009; Song et al., 2016a; Xu et al., 2016). Contrary to heterotrophic denitrification, autotrophic denitrification has the unique advantages of lower cost and risk of adding organic carbon compounds, as well as effectively mitigating the clogging problem of CWs (Xu et al., 2016). Thus, some newly intensified CWs associated with various autotrophic denitrification processes (e.g., sulfur-based and hydrogenotrophic denitrification), have been receiving more attention in recent years (Song et al., 2016a; Xu et al., 2016).

Biofilm-electrode reactors (BERs) are one of the most extensively studied autotrophic denitrification technologies owing to their advantages of harmless products, lack of need to add reduced inorganic compounds, and precise control of the electron donor (Park & Yoo, 2009). In a BER, hydrogen gas is produced by water electrolysis, which is used as an electron donor for autotrophic denitrification by denitrifying bacteria immobilized on the cathode surface. In particular, the novel three-dimensional BER (3D-BER) was developed with the objective to improve denitrification performance and reduce electrical energy consumption. In a 3D-BER, granular activated carbon (GAC) or GAC mixed with other particulate matters is introduced and packed in a common two-dimensional BER (2D-BER) to act simultaneously as a third bipolar electrode and a biocarrier, which provides a large surface area for biomass growth and attachment as well as makes high H_2 production possible (Hao et al., 2016). Previous studies of 2D-BER or 3D-BER technologies have mainly focused on the optimization of reactor design and operating factors (Capua et al., 2015; Mousavi et al., 2012); a few studies had revealed the information of associated bacterial community. Moreover, the studies of bacterial community in 3D-BERs only focused on the 3D electrode (Hao et al., 2016), the comparative analysis of the bacterial community enriched on the 3D electrode (e.g., GAC or AC) and the conventional 2D electrode (e.g., graphite felt (GF), carbon rod, or graphite plate) is lacking. Recently, a preliminary study of a bioelectrochemically-assisted constructed wetland (BECW) integrating a 3D-BER into a CW, constructed by using GAC as the 3D electrode and GF as the 2D electrode both in the anode and cathode regions, was presented (Xu et al., 2017). Completely autotrophic denitrification (78.92 ± 3.12% of nitrate removal) was obtained in the BECW system with an applied current of 15 mA. However, in fact, most of the low COD/N ratio wastewaters still contain some organic carbon, such as some domestic sewage and secondary domestic/municipal wastewater. The presence of organic matters in wastewaters would reduce the consumption of electrical energy and enhance the denitrification behavior, as well as influence the distributions of denitrifying bacteria in the 2D and 3D electrodes.

Thus, the objective of this study was to investigate the effect of COD/N ratios on BECW nitrate removal performance under the mixotrophic condition of organic matter and applied current. Moreover, to elucidate the denitrification mechanism and optimize reactor performance further, the bacterial community attached to GAC and GF biofilms in the cathode region were compared using high-throughput 16S rDNA pyrosequencing.

2. Materials and methods

2.1. BECW reactors set-up and operation

As illustrated in Fig. 1, the three BECW reactors consisted of polyvinyl chloride (PVC) columns (700 mm height, 160 mm in diameter), a 25 L holding bucket, and a peristaltic pump connected to inlet pipes at the bottom of the reactors. The constructional details and start-up procedures of the reactors have been described in a previous study (Xu et al., 2017). Briefly, the reactors were alternately filled with gravel media (particle size of 8–16 mm) and GAC (diameter 3–5 mm) from the bottom upward: gravel (100 mm in depth), GAC (100 mm in depth), gravel (100 mm in depth), GAC (100 mm in depth), followed by the gravel planted with *Canna indica* var. *flava* (200 mm in depth). The upper layer filled with GAC acted as the cathode region for autohydrogenotrophic denitrification, whereas the lower GAC layer acted as the anode region. In addition, GFs (300 mm length × 100 mm width × 6 mm thickness) were inserted into the randomly packed GAC to collect or release electrons. To induce cathode reaction, a constant current of 15 mA was applied to the circuit by connecting the positive pole of a DC power supply (LongWei PS-305DM, Shenzhen, China) to the anode, and the negative pole to the cathode.

At the beginning of autotrophic denitrifier enrichment, each reactor

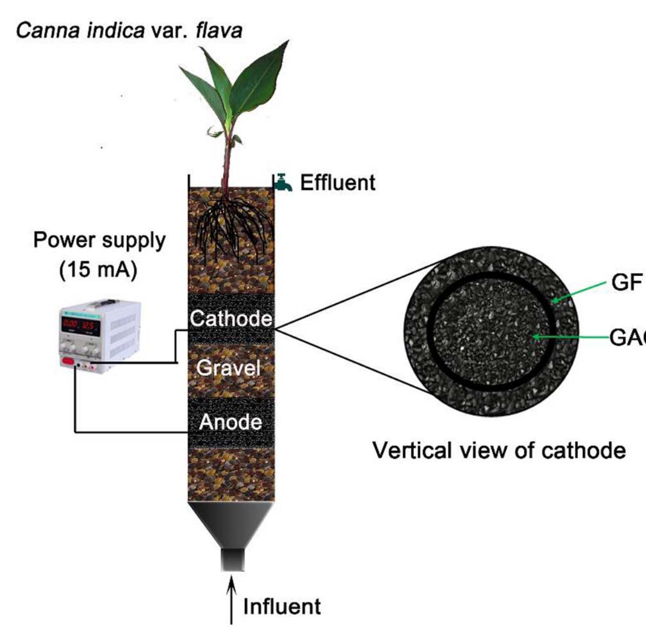

Fig. 1. Schematic representation of BECWs.