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A high-throughput sequencing study of bacterial communities in an autohydrogenotrophic denitrifying bio-ceramsite reactor

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ABSTRACT

For the first time, high-throughput sequencing was employed to investigate the microbial communities of the biofilm in an autohydrogenotrophic denitrifying bio-ceramsite reactor. 30,418 and 39,178 bacterial 16S rRNA gene sequences were obtained from biofilm Samples C1 and C2 in the reactor under HRT 16 h, pH 7.0 and HRT 48 h, pH 9.0 conditions. Results showed that the mainly reported autohydrogenotrophic denitrifying phyla *Proteobacteria*, *Firmicutes* and classes *Gammaproteobacteria*, *Alphaproteobacteria*, *Bacilli* were all detected in the reactor, and their high relative abundances demonstrated they played key roles in the autohydrogenotrophic denitrification process, suggesting that this bio-ceramsite reactor presented better denitrification performance. The heatmap analysis illustrated that the largest genus in Sample C1 was *Acinetobacter*, while *Planomicrobium* was the largest genus in Sample C2. In addition, the reported hydrogenotrophic denitrifying genera *Ochrobactrum*, *Paracoccus*, *Pseudomonas*, *Hydrogenophaga*, and *Thauera* were always observed in the reactor, suggesting that this bio-ceramsite reactor exhibited autohydrogenotrophic denitrifying capacity to convert nitrate to nitrogen gas. This work might add some new insights into microbial communities in autohydrogenotrophic denitrification process.

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1. Introduction

Since nitrate pollution in drinking water often causes many serious problems in human health such as gastric cancer and Methemoglobinemia, the World Health Organization stipulates that nitrate concentration in drinking water is 11.30 mg/L [1]. Many chemical and biological methods are carried out to remove nitrate from drinking water [2–4]. Commonly, heterotrophic and autotrophic denitrification technologies are the main biological process for nitrate removal from drinking water. Although heterotrophic denitrification is effective [5], this treatment process always causes excessive biomass and organic carbon in the effluent because this process often needs external carbon source to achieve persistent efficiency. Autohydrogenotrophic denitrification refers to an autotrophic process, in which microorganisms use hydrogen as electron donor, nitrate as electron acceptor, and inorganic carbon for assimilation.

The autohydrogenotrophic denitrification reactions are as follows:

$$NO_3^- + H_2 \rightarrow NO_2^- + H_2O \quad (1)$$

$$NO_2^- + 1.5H_2 \rightarrow 0.5N_2 + H_2O + OH^- \quad (2)$$

Overall reaction:

$$NO_3^- + 2.5H_2 \rightarrow 0.5N_2 + H_2O + OH^- \quad (3)$$

Hydrogenotrophic denitrification attracts many researchers' interests and has been developed as an alternative treatment method to heterotrophic method for nitrate removal in recent years [6–8], because this process is cost-effective, lower biomass, and without organic carbon in the treated water [9,10]. Currently, various kinds of systems such as membrane reactors [6,11], bio-electrochemical systems [12], fluidized-bed reactors [13] and fixed-bed reactors [14] have been studied for hydrogenotrophic denitrification treatment process. Li et al. [15] investigated hydrogenotrophic denitrification for nitrate removal from municipal wastewater using a lab-scale membrane diffusion packed-bed bioreactor, and achieved relatively high denitrifiaction rate. Lee et al. [5] used a lab-scale packed bed reactor (PBR) to investigate hydrogenotrophic denitrification performance, and optimized the hydrogenotrophic process. In the study of Vasiliadou et al. [16], the

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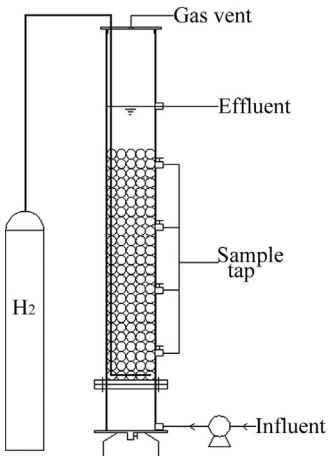

Fig. 1. Schematic of the bio-ceramsite reactor.

kinetics of hydrogenotrophic denitrification process was studied and the kinetic parameters were discussed.

Culture-based and culture-independent methods are the first technologies for analyses of bacterial communities in drinking water treatment process and these classical microbiological methods often achieve unrepresentative results [17]. Recently, clone library [18], microarray [19,20], fluorescent in situ hybridization [21], and real time polymerase chain reaction [22,23] are often employed to evaluate the bacterial communities in activated sludge and biofilms in water treatment process. However, high-throughput sequencing is a more powerful method than the above-mentioned methods for evaluation of microbial communities [24–26] in denitrification process, due to that denitrification process always exhibits extraordinary diversity of microorganisms and high-throughput sequencing method can accomplish this evaluation preferably.

The high-throughput analytical approach pyrosequencing was developed by Roche 454 FLX Titanium platform (Roche, Basel, Switzerland), which uses massively parallel sequencing-by-synthesis method [27] in order to produce larger amounts of DNA reads. This method has been widely applied to evaluate microbial communities in many types of environmental samples such as soil [28], marine water [29], wastewater treatment plant influent [30], and human distal intestine [31]. However, relatively few studies have investigated the microbial communities on biofilm samples especially for hydrogenotrophic denitrification process using high-throughput analytical method. Kwon and coworkers [10,25,32] investigated the microbial communities in activated sludge system and membrane filtration system for water treatment process using high-throughput method and the results represented the bacterial diversity and community structure commendably.

The aim of the present work was to investigate the denitrification performance and analyze the bacterial communities through 454-pyrosequencing, in order to discuss and reveal the relationship between bacterial community structure and nitrate removal performance in the autohydrogenotrophic denitrifying bio-ceramsite reactor. This work would add some new insights into the evaluation of bacterial communities in autohydrogenotrophic denitrification process.

2. Materials and methods

2.1. Reactor operation

A schematic diagram of the autohydrogenotrophic denitrifying bio-ceramisite reactor used in this work is shown in Fig. 1, which was studied in our previous work [33]. The compositions of synthetic influent water and start-up of the bio-ceramsite reactor followed our previous work [33]. Synthetic wastewater contained $NaNO_3$, KH_2PO_4, $NaHCO_3$, piped water, and trace elements contained $ZnCl_2$, $CoCl_2$, $MnSO_4$, $FeSO_4$, $CaCl_2$, etc. Start-up of the bio-ceramsite reactor was initiated by seeding with anaerobic activated sludge which covered the ceramsite. Hydrogen and sodium bicarbonate (inorganic carbon source) were introduced to the reactor. When denitrification rate reached 80% and a compacted biofilm formed on the surface of the ceramsite after 3 weeks, the domestication phase was accomplished.

Table 1
Reactor operation.

Stages	HRT (h)	pH
Stage 1 (day 1–100)	5	6.0
Stage 2 (day 101–200)	16	7.0
Stage 3 (day 201–300)	24	8.0
Stage 4 (day 301–400)	48	9.0

Then the bio-ceramsite reactor was continuously and steadily operated for 400 days. As shown in Table 1, the operation of the reactor was divided into four stages in terms of pH and hydraulic retention time (HRT) because HRT and pH played important roles in the autohydrogenotrophic denitrification process according to our previous study [33] and many other researches [34,35]. The pH of synthetic wastewater was adjusted by adding HCl or NaOH solutions, and HRT was adjusted by regulating influent flow. The nitrate loading, C/N (carbon to nitrate ratio) and temperature were maintained at 30 mg NO_3^--N/L, 1.0 and 25 °C during the whole operation.

2.2. Analysis methods

Total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and nitrite nitrogen (NO_2^--N) were determined according to Standard Methods for the Examination of Water and Wastewater [36]. Nitrogen gas (N_2) and nitrous oxide (N_2O) were measured by an Agilent HP4890D gas chromatography. The pH was measured by a pH meter (PHS-3C, Kexiao Instrument, China). The water temperature was measured by a thermometer (TM827, Zhugongda Instrument, China).

2.3. Microbial community analysis

2.3.1. DNA extraction, PCR amplification and pyrosequencing

The biofilm Samples C1 and C2 were collected from the surfaces on the bottom, middle, and upside of the ceramsite in the reactor from Stage 2 and Stage 4 during the steady operation period on day 150 and day 350 under HRT 16 h, pH 7.0 and HRT 48 h, pH 9.0 conditions, respectively. Bacterial genomic DNA was extracted using the PowerSoil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA) following the manufacturer's instruction. The PCR amplification (Supplementary Materials) used the universal bacteria primers 8F and 533R, which were targeting the V1 and V3 hypervariable regions [26]. The primers was modified by adding a 10-nucleotide barcode (C1: 5′-AGTACTACTA-3′; C2: 5′-CTCGAGTCTC-3′) and corresponding pyrosequencing adaptors. The final sequences of the primers were 8F (5′-AGAGTTTGATCCTGGCTCAG-3′), 533R (5′-TTACCGCGGCTGCTGGCAC-3′). Then the pyrosequencing procedure was according to our previous approach [37].

2.3.2. Sequence analysis

The sequence analysis followed the method (Supplementary Materials) described in our previous study [37]. Operational taxonomic unit (OTU), rarefaction curves and the diversity indices

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