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Effects of residual organics in municipal wastewater on hydrogenotrophic denitrifying microbial communities

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ABSTRACT

Hydrogenotrophic denitrification is promising for tertiary nitrogen removal from municipal wastewater. To reveal the influence of residual organics in municipal wastewater on hydrogenotrophic denitrifiers, we adopted high-throughput 16S rRNA gene amplicon sequencing to examine microbial communities in hydrogenotrophic denitrification enrichments. Using effluent from a municipal wastewater treatment plant as water source, COD, nitrate and pH were controlled the same except for a gradient of biodegradable carbon (*i.e.*, primary effluent (PE), secondary effluent (SE), or combined primary and secondary effluent (CE)). Inorganic synthetic water (IW) was used as a control. *Hydrogenophaga*, a major facultative autotroph, accounted for 17.1%, 5.3%, 32.7% and 12.9% of the sequences in PE, CE, SE and IW, respectively, implicating that *Hydrogenophaga* grew well with or without organics. *Thauera*, which contains likely obligate autotrophic denitrifiers, appeared to be the most dominant genera (23.6%) in IW and accounted for 2.5%, 4.6% and 8.9% in PE, CE and SE, respectively. *Thermomonas*, which is related to heterotrophic denitrification, accounted for 4.2% and 7.9% in PE and CE fed with a higher content of labile organics, respectively. In contrast, *Thermomonas* was not detected in IW and accounted for only 0.6% in SE. Our results suggest that *Thermomonas* are more competitive than *Thauera* in hydrogenotrophic denitrification with biodegradable organics. Moreover, facultative autotrophic denitrifiers, *Hydrogenophaga*, are accommodating to residual organic in effluent wastewater, thus we propose that hydrogenotrophic denitrification is amenable for tertiary nitrogen removal.

Introduction

Nitrogen pollution is a major contributing nutrient to eutrophication and may pose potential hazards to humans, livestock and the environment (Breisha and Winter, 2010; Ghafari et al., 2008). Total nitrogen (TN) control has become one of the most challenging and important targets of wastewater treatment plants (WWTPs). In recent years, the standard for TN control

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has become increasingly rigid, particularly for water reuse purposes (Zhao et al., 2013). However, nitrate removal by conventional secondary treatment in WWTPs can hardly fulfil the strict TN control standard (Boltz et al., 2012). Thus, tertiary nitrogen removal units are needed to remove nitrate in the secondary effluent (SE) by applying the principle of heterotrophic denitrification, which utilizes organic carbon sources as electron donors for microbial reduction of nitrate. However, the SE from WWTPs contains insufficient biodegradable organics; hence, dosing with additional organics, such as methanol, ethanol and acetate, is implemented to provide sufficient amounts of carbon source for heterotrophic denitrification (Breisha and Winter, 2010). However, in heterotrophic denitrification, nitrite can accumulate which is toxic to humans, if carbon sources are depleted. In contrary, excessive organic residue may remain if organics are overdosed (Her and Huang, 1995).

As an alternative to heterotrophic denitrification, hydrogenotrophic denitrification has elicited much attention. It involves the autotrophic metabolism of microorganisms, which uses hydrogen as inorganic electron donors and inorganic carbon (carbon dioxide or bicarbonate) as carbon sources (Karanasios et al., 2010). This process is environment friendly because hydrogen is non-toxic and the byproduct is water. In addition, autotrophic metabolism generates less biomass compared to heterotrophic denitrification, and the cost of excess sludge treatment can be saved (Ghafari et al., 2008). Thus, hydrogenotrophic denitrification has attracted the interest of many researchers, especially in the fields of nitrate removal from drinking water (Lee and Rittmann, 2002; Park and Yoo, 2009). However, only a few studies have investigated the application of hydrogenotrophic denitrification in tertiary nitrogen removal from wastewater, which focused on process performance and gas diffusion (Celmer et al., 2008; Li et al., 2013).

Primary wastewater treatment focuses on the removal of suspended solids using physical and chemical technologies and the remaining effluent contains relatively high content of labile organics. Secondary treatment aims to remove most biodegradable organics by applying biological technologies so effluent contains organic residues with low biodegradability (Tchobanoglous et al., 2002). When investigating autotrophic denitrification under diffident C/N ratios by using methanol or acetate as organic carbons, some researchers reported that organics can enhance heterotrophic denitrification and thus improve nitrate removal efficiency in simultaneously heterotrophic and autotrophic denitrification (Kiskira et al., in press; Zhao et al., 2012). Along with the increasing C/N ratio, the proportion of heterotrophic denitrifying bacteria increases and that of autotrophic denitrifying bacteria decreases (Hao et al., in press). For industrial application of hydrogenotrophic denitrification in tertiary wastewater treatment, it is important to reveal whether residual organics in effluents of wastewater can stimulate the growth of heterotrophic bacteria in hydrogenotrophic denitrifying process. Moreover, given the fluctuation of organic loading in municipal wastewater, it is also crucial to reveal the competition of facultative autotrophic, obligate autotrophic and heterotrophic denitrifiers in hydrogenotrophic denitrification. The understanding of microbial community can facilitate good controls of facultative autotrophic denitrifiers *versus* heterotrophic denitrifiers common in WWTPs, thus improve the stable and highly efficient performance of hydrogenotrophic denitrification.

High-throughput sequencing of the 16S rRNA gene is an efficient technology to obtain in-depth information of microbial communities (MacLean et al., 2009). It has been utilized in a few recent studies (Chen et al., 2015; Mao et al., 2013) to reveal the microorganisms in autotrophic denitrification process with inorganic synthetic water (IW). However, no study has investigated the influence of residual organics in municipal wastewater effluents on hydrogenotrophic denitrifier communities. In this study, microbial communities in hydrogenotrophic denitrification enrichments cultivated in three types of effluents from a WWTP (*i.e.*, primary effluent (PE; effluent from the primary sedimentation tank), SE, or 1:1 combination of municipal primary and SEs (CE)) were examined by sequencing, with IW serving as a control. All of the key parameters (COD, nitrate concentration, pH, *etc.*) were maintained identical except for biodegradable carbon proportion. The aim is to reveal the diversity of hydrogenotrophic denitrifiers, and to resolve the influence of labile and low-biodegradable organics on hydrogenotrophic denitrifier community. The results can provide microbiological support to determine the feasibility of hydrogenotrophic denitrification in tertiary nitrogen removal from municipal wastewater.

1. Materials and methods

1.1. *Hydrogenotrophic denitrifying enrichments*

Hydrogenotrophic denitrifiers were cultivated in four 1000 mL serum bottles with tight caps. The apparatus and research flow are shown in Fig. 1. Three hundred fifty millilitres of medium was placed in each bottle, and the headspace was filled with hydrogen gas. To determine the effects of residual organics on hydrogenotrophic denitrification, three effluent sources (PE, CE, and SE) from Xiaojiahe (XJH) WWTP in Beijing and one inorganic control (IW) were used. According to the effluent analysis in XJH WWTP during the experiment, the average COD concentration in PE was 220 mg/L and that in SE was 40 mg/L. The value of BOD_5/COD, which represents the biodegradability of wastewater, was 0.45 in PE, and only 0.05 in SE. Thus BOD_5/COD was calculated as 0.25 in CE according to the 1:1 volume rate of primary and SEs. For all effluent types, media COD was adjusted to the same concentration of 40 mg/L by dilution with deionized water, and NO_3^-–N was adjusted to 30 ± 1.0 mg/L by adding KNO_3. In each serum bottle, H_2 was excess for hydrogenotrophic denitrification of 30 mg/L NO_3^-–N. Since heterotrophic denitrifiers rely on high organic carbon demand, with COD/N ratio above 3–5 (Lee et al., 2001), the organic carbon source of 40 mg/L COD is insufficient for heterotrophic denitrification. Therefore, the competition of autotrophic and heterotrophic denitrifiers is expected in serum bottles fed with PE, CE and SE. As a control, the medium for IW only included KNO_3 (NO_3^-–N concentration of 30.0 ± 1.0 mg/L). $NaHCO_3$ (500 mg/L) was used as the inorganic substrate for all cultures. A microelement concentrated solution (1 mL/L), which was added to all substrate to support microbial growth, was prepared in advance as

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