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Characterization of *Thauera*-dominated hydrogen-oxidizing autotrophic denitrifying microbial communities by using high-throughput sequencing

Yanping Mao, Yu Xia, Tong Zhang *

Environmental Biotechnology Laboratory, Department of Civil Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong

HIGHLIGHTS

- Firstly reported a *Thauera*-dominated hydrogenotrophic denitrifying consortium.
- *Thauera* were enriched into the dominant population from different seed sludges.
- Enriched denitrifying cultures were investigated by High-throughput sequencing.
- Enriched cultures achieved comparable nitrogen removal rates with *P. denitrificans*.

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ABSTRACT

The present study, for the first time, reported a *Thauera*-dominated hydrogen-oxidizing autotrophic denitrifying microbial community enriched from different seed sludges including activated sludge and anaerobic digestion sludge. After 244 days enrichment, nitrogen removal rates reached up to 0.2 mg N/mg VSS/d which were comparable to that of the model organism *Paracoccus denitrificans* under the same conditions. Furthermore, high-throughput sequencing was applied to characterize and compare the seed sludges and enriched cultures. Operational taxonomic units (OTU)-based analysis (97% similarity cutoff) of total 280,000 16S rRNA gene V6 region sequences from 7 sludge samples (40,000 sequences per sample) revealed that the microbial diversity decreased after the enrichment, indicated by OTU numbers drop of 55–60%. *Thauera* species in the class of β-*Proteobacteria* were enriched into the dominant populations with relative abundances of 47–62%, regardless of seed sludge sources.

1. Introduction

Hydrogen-oxidizing autotrophic denitrification is an effective biological process to remove nitrogen (N) from nitrate-contaminated organic-limited water systems such as groundwater and drinking water since it has unique advantages over heterotrophic denitrification, including less sludge generation and no external organic substrate requirement (Mansell and Schroeder, 2002; Smith et al., 2005; Sunger and Bose, 2009).

Autotrophic denitrification could be conducted by some bacteria through using hydrogen as the electron donor, inorganic carbon as the carbon source and nitrate/nitrite as the electron acceptor in the absence of oxygen (Mateju et al., 1992). Most of the reported hydrogen-oxidizing denitrifiers belong to the phylum of *Proteobacteria*, including *Paracoccus denitrificans* in the class of α-*Proteobacteria* (Szekeres et al., 2002; Vasiliadou et al., 2006b), *Hydrogenophaga* sp. (Zhang et al., 2009), *Rhodocyclus* sp. (Smith et al., 2005) and *Alcaligenes* sp. (Ho et al., 2001; Sunger and Bose, 2009) in β-*Proteobacteria*, and *Acinetobacter* sp. (Vasiliadou et al., 2006b), *Aeromonas* sp., *Pseudomonas* sp. and *Shewanella* sp. (Liessens et al., 1992) in γ-*Proteobacteria*.

So far the reported information about hydrogen-oxidizing autotrophic denitrifiers is still very limited. In order to enrich hydrogenotrophic denitrifiers, different types of seed sludges including activated sludge (AS) and anaerobic digestion sludge (ADS) from three sewage treatment plants (STPs) in Hong Kong were inoculated in different batch reactors respectively. Then the enriched cultures were compared to the autotrophic denitrification model microorganism (*P. denitrificans*) regarding to their denitrifying rates.

Advanced high-throughput sequencing (or next generation sequencing) can generate huge amounts of DNA sequences, and have been used for analysis of mixed cultures from sewage treatment plants (Zhang et al., 2011; Hu et al., 2012), nitrification reactors (Ye et al., 2011), cellulose degradation reactors (Xia et al., 2012), and etc. Applications of this technology revealed that the clone library used before was far from enough to reflect the whole

* Corresponding author. Tel.: +852 28578551; fax: +852 25595337.
E-mail address: zhangt@hku.hk (T. Zhang).

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