Bioresource Technology 186 (2015) 163–172

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Microbial structures and community functions of anaerobic sludge in six full-scale wastewater treatment plants as revealed by 454 high-throughput pyrosequencing

Duntao Shu [a], Yanling He [b,*], Hong Yue [c], Qingyi Wang [d]

[a] Center for Mitochondrial Biology and Medicine, The Key Laboratory of Biomedical Information Engineering of the Ministry of Education, School of Life Science and Technology and Frontier Institute of Science and Technology, Xi'an Jiaotong University, 710049 Shaanxi, China
[b] School of Human Settlements & Civil Engineering, Xi'an Jiaotong University, 710049 Shaanxi, China
[c] State Key Laboratory of Crop Stress Biology in Arid Areas, College of Agronomy and Yangling Branch of China Wheat Improvement Center, Northwest A&F University, Yangling, 712100 Shaanxi, China
[d] School of Chemical Engineering & Technology, Xi'an Jiaotong University, 710049 Shaanxi, China

HIGHLIGHTS

- Microbial communities were investigated by 454 pyrosequencing and qPCR.
- AOA and anammox bacteria may be heterotrophic or mixotrophic.
- AOA and AOB showed partially beneficial cooperation with anammox genes.
- Coexistence of anammox, denitrification and DNRA was confirmed in WWTPs.
- Operational parameters have highly effects on the microbial communities.

ARTICLE INFO

Article history:
Received 19 January 2015
Received in revised form 12 March 2015
Accepted 14 March 2015
Available online 19 March 2015

Keywords:
Anaerobic sludge
Pyrosequencing
Microbial community structures
Quantitative real-time PCR (qPCR)
Redundancy analysis

ABSTRACT

The microbial communities and abundance in anaerobic sludge from 4 industrial and 2 municipal wastewater treatment plants were investigated using 454 pyrosequencing technology in this study. A total of 5482–8692 high-quality reads of 16S rRNA V3-V5 regions were obtained. Taxonomic analysis using QIIME and RDP classifier found that *Proteobacteria*, *Bacteroidetes*, *Chloroflexi* and *Firmicutes* were the most abundant phyla in these samples. Furthermore, real-time PCR was used to validate the absolute abundance of these 16S rRNAs and some functional genes, including total bacteria, anammox bacteria, NOB (*Nitrobacter*, *Nitrospira*), AOA amoA, AOB amoA, nosZ, nirS, nirK, narG, napA, nrfA, mcrA and dsrA. Multivariate linear regression analysis indicated that AOA might be mixotrophic. Finally, redundancy analysis was used to reveal the relationships between operation parameters and microbial communities. Results showed that the coexistence of anammox, denitrification and DNRA could be useful for the simultaneous removal of nitrogen and organic matter.

1. Introduction

Biological treatment has been widely applied in municipal and industrial wastewater treatment plants (WWTPs) due to its high efficiency and low cost. As the major technology for energy, nutrients and water recovery from various types of wastewater, anaerobic wastewater treatment accounted for about 80% of wastewater treatment systems in all over the world (Lettinga et al., 1997). It is energy-efficient, cost-effective and self-sustaining. Although this technology has been around for over 100 years, the microbial influence on the development and function of anaerobic sludge still remains unclear at present. Therefore, better understanding of the microbial structure and functional genes of anaerobic sludge in WWTPs will not only be beneficial to elucidate the mechanisms of nitrogen and organic matter removal, but also help to enhance the performance and operational stability of anaerobic bioreactors.

Anaerobic treatment which simultaneously eliminate nitrogen, remove carbon and recover energy, was generally considered as the key technology for sustainable management of wastewater (Kumar and Lin, 2010). It has been demonstrated that the complex biological processes and catalytic mechanism were present in

* Corresponding author. Tel.: +86 13891939065; fax: +86 029 83395128.
E-mail addresses: D.T.Shu_xjtu@outlook.com, heyl@mail.xjtu.edu.cn (Y. He).

http://dx.doi.org/10.1016/j.biortech.2015.03.072
0960-8524/

anaerobic treatment. The nitrogen removal processes included aerobic ammonium oxidation (AOB), archaea ammonium oxidation (AOA), anaerobic ammonium oxidation (anammox), nitrite oxidation, anaerobic denitrification and heterotrophic nitrification, as well as dissimilatory nitrate reduction to ammonium (DNRA) (Graham et al., 2014; Ji et al., 2013), while the organic matter degradation processes included anaerobic methane formation, sulfate reduction and so on. These processes were mainly catalyzed by different 16S rRNAs and functional genes, such as anammox 16S rRNA, nitrite oxidation bacteria (NOB) 16S rRNA, ammonia monooxygenase (*amo*A), archaea ammonia monooxygenase (*amo*A), periplasmic (napA), membrane-bound nitrate reductase (narG), nitrous oxide reductase (nosZ), copper-containing nitrite reductase (nirK), nitrite reductase (nirS), dissimilatory nitrate reductase (nrfA), methyl coenzyme-M reductase (mcrA) and sulfate reductase (mcrA) (Nakamura et al., 2009; Wang et al., 2014). However, the exact mechanisms they interact with the ecological systems remained unknown.

Various methods have been used to investigate the microbial structures in anaerobic sludge, including cloning library of 16S rRNA genes, denaturing gradient gel electrophoresis (DGGE) analysis and fluorescence in situ hybridization as well as 454-pyrosequencing (Ye and Zhang, 2013). But these methods have been mostly focused on microbial composition. With the development of the second generation sequencing recently, some studies which employed high-throughput pyrosequencing to explore microorganisms in WWTPs were also reported. However these studies only concentrated on the microbial structures in the single WWTPs. Furthermore, most of these approaches have been used only in anaerobic digestion sludge, while anaerobic flocculent sludge and granule sludge are few. Links between microbial community functional structures and operational variation are still lacking. Therefore, it is worthwhile to investigate microbial composition, signature populations, functional structures and their linkages to environmental factors.

In this study, 454 high-throughput sequencing was conducted to investigate the bacterial diversity and abundance in six anaerobic sludge samples from various WWTPs. Clustering analysis, weighted UniFrac distance metrics, and PCoA analysis were used to assess the similarity or difference of various samples, and to identify the signature microbial species in different anaerobic samples as well as the core populations shared by them. Then, qPCR was used to quantify 16S rRNA gene copy numbers of total bacteria, anammox bacteria and NOB (*Nitrobacter*, *Nitrospira*). The absolute abundance of functional genes, including AOA *amo*A, AOB *amo*A, nosZ, nirS, nirK, narG, napA, nrfA, mcrA and dsrA, were also quantified in all six samples. Based on the qPCR results, multivariate linear regression including Pearson correlation coefficients and Spearmans' rank correlation coefficient (SRCC) were applied to evaluate the ecological correlations between genes across various species. Multivariate statistical analysis (redundancy analysis, RDA) was used to investigate the relationship between operational parameters and microbial community structures as well as the specific genes.

2. Methods

2.1. Description of WWTPs and sample collection

Samples of anaerobic sludge were taken from six full-scale WWTPs in Shaanxi, China. The descriptions of these WWTPs were summarized in Table 1. These WWTPs differed mainly in their influent characteristics, especially their COD and ammonium concentrations. The plants GW, QX, FX, and WF were industrial WWTPs, while SW and SK were municipal ones. Upflow anaerobic sludge blanket process (UASB) plus Orbal oxidation ditch was applied in the plant GW for treating starch wastewater. The plant QX was equipped with the Upflow anaerobic sludge blanket process plus biological contact oxidation for juice removal. The plants FX and WF operated with UASB plus modified Carrousel process for treating pulp and paper wastewater. The plants SW and SK treated municipal wastewater and campus domestic sewage through the anaerobic/anoxic/oxic (A/A/O) process. The sludge samples were collected from anaerobic tanks in the plants SW and SK. For anaerobic granules, they were collected from UASB reactors in the plants GW, QX, FX, and WF. In total, 18 samples were obtained from the six WWTPs. After samples collection, they were immediately fixed in 50% (v/v) ethanol aqueous solution and then stored at −80 °C until DNA extraction.

2.2. DNA extraction and PCR amplification

Approximately 0.5 g of wet sludge sample pellet was used for DNA extraction with the E.Z.N.A.® Soil DNA kits D5625-01 (Omega, USA) according to the manufacturer's instructions. Extracted DNA concentrations were determined through Nanodrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, USA). Additionally, extracted genomic DNA was detected by 1.5% agarose gel electrophoresis and stored at −20 °C until use.

For gene libraries construction, DNA from the 18 samples were amplified by PCR using primer set 357F (5′-CCTACGGGAGGC AGCAG-3′) and 926R (5′-CCGTCAATTCMTTTRAGT-3′) (Bai et al., 2012) for the V3–V5 regions of the 16S rRNAs. The 25 μl PCR mixture contained 5 μl of 5Q × 5 buffer, 5 μl of 5 × GC enhancer, 2 μl of dNTP (2.5 mM), 4 μl of DNA (2 ng/μl), 1 μl of each primer (10 μM), and 0.25 μl Q5 DNA polymerase (Q5™ High-Fidelity DNA Polymerase, NEB, USA). The PCR protocol were as follow: 98 °C for 5 min, followed by 28 cycles at 98 °C for 30 s, 55 °C for 45 s, 72 °C for 1 min and a final extension at 72 °C for 5 min. For minimizing the PCR-induced recombination, the PCR amplifications were performed in the log-linear phase as determined by real-time PCR. The fused forward and reserve primers include a 10-nucleotide barcode modified with 5′ Adaptor-A and 5′ Adaptor-B (454 Life Sciences), respectively.

2.3. 454 High-throughput 16S rRNA pyrosequencing

After amplification, the PCR products were purified using AMPure Beads (Beckman Coulter, USA). The DNA concentration of purified products was measured with PicoGreen dsDNA Assay Kit (Thermo Scientific, USA) on the TBS-380 Fluorometer (Turner Biosystems, CA, USA). The amplicons from triplicate anaerobic sludge samples were polled with equal amounts and subjected to emulsion PCR before sequencing. Then, the mixture of six amplicons was used for pyrosequencing on the Roche 454 GS-FLX Titanium sequencer (Roche 454 Life Sciences, Branford, CT, USA) at the Personalbio (Shanghai Personal Biotechnology, Co., Ltd., Shanghai, China). All the raw reads have been archived at NCBI Sequence Read Archive (SRA) database with accession number of SRR1765717.

2.4. Sequence analysis and phylogenetic classification

After pyrosequencing, all the raw reads were analyzed using QIIME standard pipeline (Caporaso et al., 2010), to trim off the low quality reads, adaptors, barcodes and primers. Then the sequences containing any ambiguous base calls (Ns) or more than six homopolymer runs ('N'), as well as the sequences shorter than 50 bp or longer than 1000 bp were also removed.

The remaining 16S rRNA sequences from pyrosequencing in this study were clustered into OTUs using UCLUST, with setting 0.03