Electronic supplementary information

1. DNA extraction

After reaching stable operation, sludge samples taken from $AAMA_{1\#}$ and $AAMA_{2\#}$ were collected for DNA extraction. Before DNA extraction, 1-ml combined sludge samples were centrifuged at 8000g to remove supernatant, corresponding to 0.25 g pellet was collected for use. In this study, total genomic DNA was extracted using a Power-Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) according to the manufacturer's instructions. For examining the quantity and quality of the extracted DNA of the sludge samples, a Beckman DU800 spectrophotometer was applied by set its absorbance at 260 and 280 nm.

2. High-throughput pyrosequencing of 16S rRNA gene

The V1-V3 region of the bacteria 16S ribosomal RNA gene were amplified by polymerase chain reaction（95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min) by using primers 27F 5'-(CGTATCGCCTCCCTCGCGCCATCAG-3' 5'-AGAGTTTGATCCTGGCTCAG)-3' and 533R 5'- (CTATGCGCCTTGCCAGCCCGCTCAG- 3' -MID tags-5'-ATTACCGCGGCTGCTGGCA)-3'. PCR reactions were performed in a 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.

3. 454 pyrosequencing and processing of pyrosequencing data

After purification using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) and quantification using QuantiFluor™ -ST (Promega, U.S.), a mixture of amplicons was used for pyrosequencing on a Roche 454 GS FLX+ Titanium platform (Roche 454 Life Sciences, Branford, CT, U.S.) according to standard protocols.

Pyrosequencing produced 10267 ($AAMA_{1\#}$-sample) and 10006 ($AAMA_{2\#}$-sample) high-quality V1-V3 tags of the 16S rRNA gene with an average length of 455 bp. The resulting sequences were processed using QIIME (version 1.17). After removing sequences with average quality score <20 over a 50 bp sliding window and sequences shorter than 200bp, with homopolymers longer than six nucleotides, and containing ambiguous base calls or incorrect primer sequences, a total of 10267 ($AAMA_{1\#}$-sample) and 10006 ($AAMA_{2\#}$-sample) high-quality sequences were produced with an average length of 455 bp per sequence. Operational Taxonomic Units (OTUs) were clustered with 97% similarity cutoff using UPARSE（version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU115)16S rRNA database using confidence threshold of 70%.[2]

4. Biodiversity analysis and phylogenetic classification

The obtained sequences were clustered into operational taxonomic units (OTUs) by setting a 0.03 distance level (97% similarity) or 0.05 distance limit (95% similarity) using the MOTHUR program. In this study, the sequences were phylogenetically allocated down to the phylum, class and genus levels using the MOTHUR program (http://www.mothur.org/wiki/Main_Page). For a given phylogenetic group, the relative abundance was set as the number of sequences affiliated with that group which is divided by the total number of sequences.[3] As shown in Figs. 2, 3, 4, phyla, class, and genus with relative abundance lower than 0.5 % were defined as "Others".

References

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3 J. N. Jia, Y. Tang, B. F. Liu, D. Wu, N. Q. Ren, D. F. Xing, *Bioresource Technol.*, 2013, **144**, 94-99.