



16S rRNA Gene Amplicon Sequencing of Microbiota in Polybutylene Succinate Adipate-Packed Denitrification Reactors Used for Water Treatment of Land-Based Recirculating Aquaculture Systems

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ABSTRACT Information on the microbiota in polybutylene succinate adipate (PBSA)-packed denitrification reactors is limited. Here, we provide 439,817 high-quality reads of the 16S rRNA gene sequences of microbiota in PBSA-packed denitrification reactors used for land-based recirculating aquaculture. The predominant microorganisms belonged to the following families: *Nocardiaceae*, *Chitinophagaceae*, *Xanthobacteraceae*, *Burkholderiaceae*, *Rhodocyclaceae*, *Pseudomonadaceae*, *Rhodanobacteraceae*, and *Xanthomonadaceae*.

Biodegradable plastics act as carriers for microorganism adhesion and as electron donors in denitrification reactors for removing nitrate from land-based recirculating aquaculture systems (RAS) (1–4). Biofilms form around the biodegradable plastics packed in the denitrification reactors and contribute to biological denitrification (3, 4). However, limited information regarding the microbiota adhering to the biodegradable plastics, particularly to polybutylene succinate adipate (PBSA), is available. Here, we provide 16S rRNA gene amplicon profiles of the microbiota attached to PBSA to achieve efficient nitrate removal in PBSA-packed denitrification reactors for water treatment in RAS.

PBSA-packed denitrification reactors installed in three RAS tanks (250 liters) (established in Eniwa, Japan, in 2018) discharged breeding water with a nitrate concentration of <60 mg-N-liter⁻¹ after 93 days of operation. The three reactors were loaded with PBSA pellets (Bio-PBS; Mitsubishi Chemical Corporation, Tokyo, Japan) and continuously supplied with breeding water (Table 1). Despite the difference in feeding frequency, each RAS tank bred 20 rainbow trout (average weight per fish, 92.5 ± 1.4 g) (Table 1). The sludge samples adhering to PBSA pellets were collected with the pellets from each reactor. DNA from the samples was extracted with a bead-beating method using 1-mm zirconia beads (5) and then purified according to a previous method (5). The V4 region of the 16S rRNA gene was amplified using Blend *Taq* polymerase (Toyobo, Osaka, Japan) and the 515F/806R primer set (6), with the following PCR conditions: 25 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s. Sequencing libraries of PCR products for each sample were prepared with three biological replicates, and sequences were determined using a MiSeq instrument and a MiSeq reagent kit v2 (2 × 300 bp; Illumina, San Diego, CA, USA) at the Bioengineering Lab. Co., Ltd. (Kanagawa, Japan). Low-quality sequences were removed using Sickle

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TABLE 1 Summary of 16S rRNA gene amplicon profiles of microbiota in bioreactors^a

Analysis measure	Data by sample:		
	SKMS_3 denitrification reactor	SKMS_5 denitrification reactor	SKMS_6 denitrification reactor
SRA run no.	DRR186126–DRR186128	DRR186123–DRR186125	DRR186120–DRR186122
Reactor vol (liters)	0.78	2.34	2.34
PBSA amt (kg)	1	3	3
Feeding frequency	Twice per day	Twice in 2 days	Twice per day
Breeding water temp (°C)	14–16	14–16	14–16
Sampling date	2 October 2018	2 October 2018	2 October 2018
Diversity			
Estimated sample coverage	0.99	0.99	0.99
No. of OTUs	356.3	673.0	901.7
Shannon diversity	2.80	4.18	3.94
Simpson diversity	0.70	0.82	0.78
Chao1 estimator	662.8	1,129.8	1,475.7
ACE ^b estimator	653.5	1,181.9	1,459.1
No. of high-quality reads	92,893	133,017	213,907

^a Polybutylene succinate adipate (PBSA)-packed denitrification reactors.

^b ACE, abundance-based coverage estimator.

v1.33 with the following parameters: -q 20 and -l 40 (7). High-quality paired-end reads were merged using PEAR v0.9.10 with default settings (8), and then sequences were selected by SeqKit v0.8.0 with the parameters -m 245 and -M 260 (9). Selection of operational taxonomic units (OTUs) and assignment of appropriate taxa were performed using a closed-reference OTU-picking script in QIIME v1.9.1 (10) and the SILVA database (release 132) with 97% identity (11). Relative abundances of representative OTUs (>1%), summed at the family level, are shown in Fig. 1, and the indices to assess diversity were calculated with all of the selected high-quality reads for samples by QIIME v1.9.1 (11) (Table 1).

A total of 439,817 high-quality reads were finally obtained from the three analyzed samples. Prominent microorganisms were classified into the families *Nocardiaceae*, *Chitinophagaceae*, *Xanthobacteraceae*, *Burkholderiaceae*, *Rhodocyclaceae*, *Pseudomonadaceae*, *Rhodanobacteraceae*, and *Xanthomonadaceae*. These data provide basic microbial information to achieve efficient operation of the PBSA denitrification reactor for water treatment of land-based RAS.

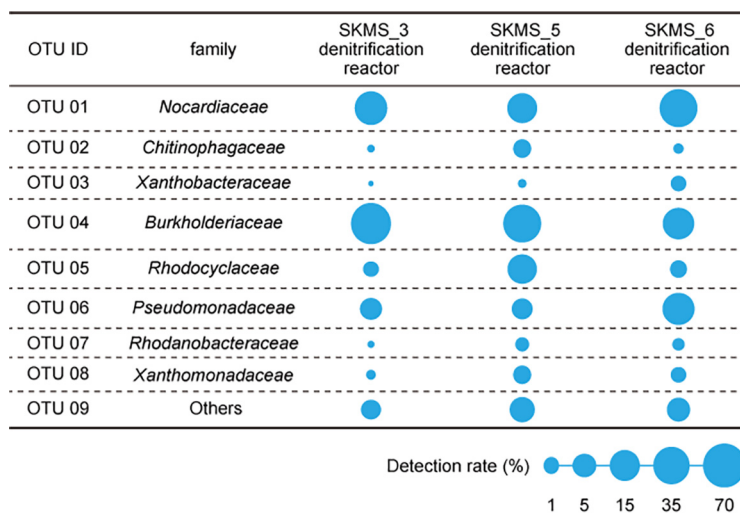


FIG 1 Relative abundances of microbiota in the three PBSA-packed denitrification reactors after 93 days of operation. Relative abundances (>1%) of the microbiota at the family level are displayed in a bubble plot.

Data availability. The 16S rRNA gene sequence data set has been deposited in the NCBI Sequence Read Archive (SRA) under accession number [DRP005307](https://www.ncbi.nlm.nih.gov/sra/DRP005307) and SRA run accession numbers [DRR186120](https://www.ncbi.nlm.nih.gov/sra/DRR186120) to [DRR186128](https://www.ncbi.nlm.nih.gov/sra/DRR186128).

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REFERENCES

1. Boley A, Müller W-R, Haider G. 2000. Biodegradable polymers as solid substrate and biofilm carrier for denitrification in recirculated aquaculture systems. *Aquac Eng* 22:75–85. [https://doi.org/10.1016/S0144-8609\(00\)00033-9](https://doi.org/10.1016/S0144-8609(00)00033-9).
2. Luo G, Xu G, Gao J, Tan H. 2016. Effect of dissolved oxygen on nitrate removal using polycaprolactone as an organic carbon source and biofilm carrier in fixed-film denitrifying reactors. *J Environ Sci (China)* 43: 147–152. <https://doi.org/10.1016/j.jes.2015.10.022>.
3. Deng Y-L, Ruan Y-J, Zhu S-M, Guo X-S, Han Z-Y, Ye Z-Y, Liu G, Shi M-M. 2017. The impact of DO and salinity on microbial community in poly(butylene succinate) denitrification reactors for recirculating aquaculture system wastewater treatment. *AMB Express* 7:113. <https://doi.org/10.1186/s13568-017-0412-3>.
4. Liu D, Li J, Li C, Deng Y, Zhang Z, Ye Z, Zhu S. 2018. Poly(butylene succinate)/bamboo powder blends as solid-phase carbon source and biofilm carrier for denitrifying biofilters treating wastewater from recirculating aquaculture system. *Sci Rep* 8:3289. <https://doi.org/10.1038/s41598-018-21702-5>.
5. Yamada T, Sekiguchi Y, Imachi H, Kamagata Y, Ohashi A, Harada H. 2005. Diversity, localization, and physiological properties of filamentous microbes belonging to *Chloroflexi* subphylum I in mesophilic and thermophilic methanogenic sludge granules. *Appl Environ Microbiol* 71: 7493–7503. <https://doi.org/10.1128/AEM.71.11.7493-7503.2005>.
6. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci U S A* 108:4516–4522. <https://doi.org/10.1073/pnas.1000080107>.
7. Joshi NA, Fass JN. 2011. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files (version 1.33). <https://github.com/najoshi/sickle>.
8. Zhang J, Kobert K, Flouri T, Stamatakis A. 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 30:614–620. <https://doi.org/10.1093/bioinformatics/btt593>.
9. Shen W, Le S, Li Y, Hu F. 2016. SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. *PLoS One* 11:e0163962. <https://doi.org/10.1371/journal.pone.0163962>.
10. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JJ, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7:335–336. <https://doi.org/10.1038/nmeth.f.303>.
11. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and Web-based tools. *Nucleic Acids Res* 41:D590–D596. <https://doi.org/10.1093/nar/gks1219>.