Nitrogen removal performance and microbial diversity of bioreactor packed with cellulosic carriers in recirculating aquaculture system

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Abstract

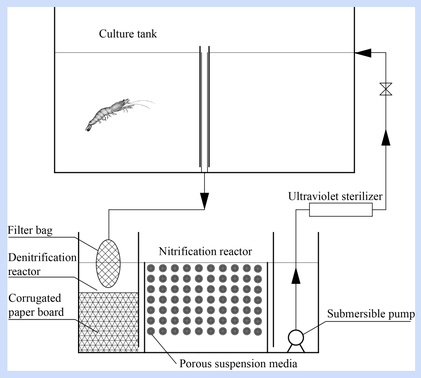
In this research, a solid-phase denitrification reactor (SPDR) packed with corrugated paperboard was introduced into the recirculating aquaculture system (RAS) of *Litopenaeus vannamei* for nitrate removal. Results showed that 82.88% of the nitrogen was removed from the culture water with the denitrification rate of 0.74 mg/(L·day) and the concentration of nitrate was controlled at 2.31 ± 1.00 mg/L. High throughput sequencing analysis showed that the corrugated paperboard was degraded by *Lachnospiraceae*, *Saprospiraceae*, *Exiguobacterium*, *Mesoflavibacter*, etc. to provide carbon source for denitrifying bacteria, including *Rhizobiaceae*, *Rhodobacteraceae*, *Bacillus*, *Nitratireductor*, *Sphingobium*, *Marinicella*, *Desulfovibrio*, *Marinobacter*, and *Labrenzia*. The abundance of the functional genes *amoA*, *nirK*, and *nosZ* indicated that nitrogen can be efficiently converted into N_2 in the SPDR.

Introduction

Nitrate is a common nitrogen species in aquaculture, especially in recirculating aquaculture systems (RAS). The key treatment in RAS is the nitrification process, where ammonia and nitrite are converted to nitrate. The concentration of nitrate in RAS can reach 500 mg/L. Although nitrate is generally considered of low toxicity, high concentrations of nitrate also harm farming organism. Denitrification provides an alternative method for nitrate removal in RAS with a variety of carbon sources supplemented as electron donors. However, due to the high cost of construction with sophisticated equipment, high operating costs, and complex operating skills, traditional denitrification has been rarely used in RAS. Solid phase substrates for denitrification could potentially provide a more balanced carbon supply in RAS and represent an alternative approach to remove nitrate from wastewater. In this study, a solid-phase denitrification reactor packed with corrugated paperboard was introduced into the RAS of *Litopenaeus vannamei* for degrading nitrate.

Materials and methods

- Nitrogen budget
- Microbial community analysis
  - Bacterial community analysis based on the V3–V4 regions of 16S rRNA
  - Community analysis of denitrifying bacteria based on nirK/nirS
- Stable isotope dilution method

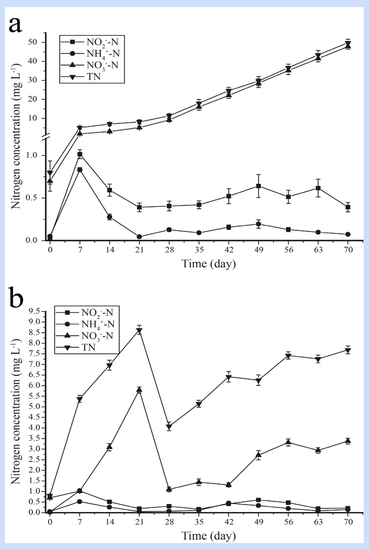


$$m_{NO3\ produced} = \frac{M0-M}{t} \times \frac{\log_{10}(H0M/HM0)}{\log_{10}(M0/M)},\ m \neq i$$

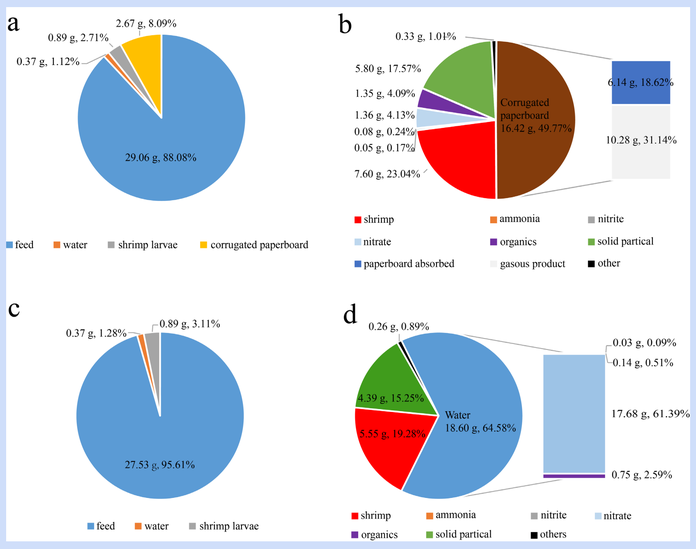
$$m_{NO3\ consumed} = \frac{M0-M}{t} \times \frac{\log_{10}(H0/H)}{\log_{10}(M0/M)},\ m \neq i$$

$m_{NO3produced}$ = mass of nitrate produced per unit time per unit mass of water (nitrification rate); $m_{NO3consumed}$ = mass of nitrate consumed per unit time per unit mass of water (denitrification rate); *H0* = mass of heavy atoms per unit mass of water at time *t* = 0; *M0* = mass of heavy and light atoms per unit mass of water at time *t* = 0; *H* = mass of heavy atoms per unit mass of water at time *t* = t; *M* = mass of heavy and light atoms per unit mass of water at time *t* = t; *t* = time lapsed between measurements for *H0*, *M0,* and *H*, *M*.

Results



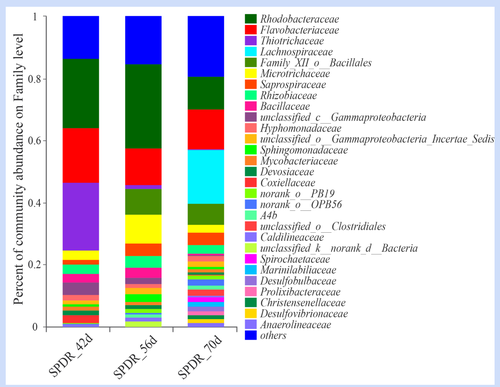
Variation of ammonia, nitrite, nitrate, and TN in the control (a) and experimental system (b).



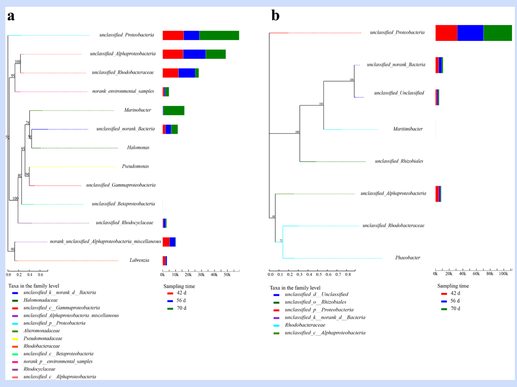
Nitrogen budget of the RAS for *L. vannamei*. a, nitrogen input of the SDS. b, nitrogen output of the SDS. c, nitrogen input of the control. d, nitrogen output of the control.

Results

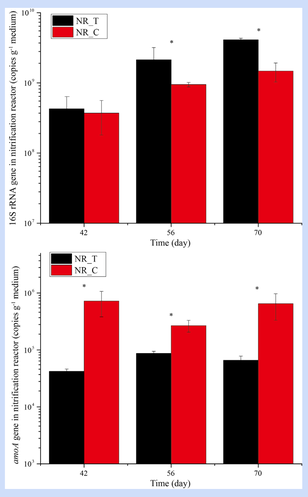
Nitrogen biogeochemistry based on the $^{15}NO_3^-$-N in the SDS. ■ relative abundance of ^{15}N in NO_2^--N and NO_3^--N, ● concentration of NO_2^--N and NO_3^--N, ▲ cumulative production rate of NO_2^--N and NO_3^--N, ▼ cumulative consumption rate of NO_2^--N and NO_3^--N.



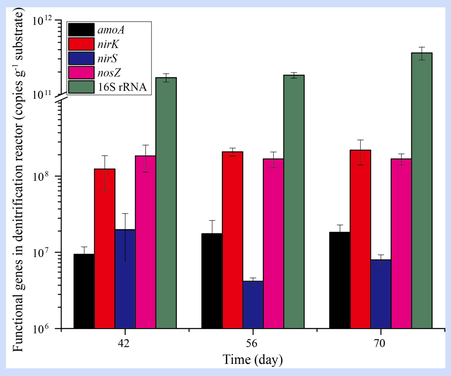
The bacterial community in the SPDR at phylum (a) and family (b) level based on 16S rRNA.



The phylogenetic tree of the bacterial community in the SPDR based on the *nirS* (a) and *nirK* (b) gene at genus level.



The abundance of 16S rRNA gene (a) and *amoA* gene (b) in the nitrification reactor. NR_T was the nitrification reactor in the treated system. NR_C was the nitrification reactor in the control system. * indicate the sifnificant difference ($p < 0.05$) between NR_T and NR_C.



The abundance of *amoA*, *nirS*, *nirK*, *nosZ* and 16S rRNA gene in the SPDR.

Conclusions

- The SPDR packed with corrugated paperboard can remove >80% of the nitrogen in the culture water, which was proved to be an effective method for nitrogen removal in RAS.
- The bacterial community of the SPDR contains abundant nitrogen functional genes, such as *amoA*, *nirK* and *nosZ*, which can convert nitrogen into N_2 with a rate of 11.00 g(m^3 medium·d).
- *Rhizobiaceae*, *Rhodobacteraceae*, *Bacillus*, *Nitratireductor*, *Sphingobium*, *Marinicella*, *Desulfovibrio*, *Marinobacter*, *Labrenzia* were key players in denitrification. Meanwhile, *Lachnospiraceae*, *Saprospiraceae*, *Exiguobacterium*, and *Mesoflavibacter* were the predominant taxa in substrate degradation.

Acknowledgements

This study was financially supported by the National Key Research and Development Program of China (No. 2019YFD0900403, 2018YFD0900702); the National Natural Science Foundation of China (31873039); the China Agriculture Research System (No. CARS-48); the Aoshan Innovation Project of Qingdao National Laboratory for Marine Science and Technology (No. 2015ASKJ02); and the Projects of International Exchange and Cooperation in Agriculture, Ministry of Agriculture and Rural Affairs of China-Science, Technology and Innovation Cooperation in Aquaculture with Tropical Countries.

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