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# Minimization of n2o Emission through Intermittent Aeration in a Sequencing Batch Reactor (Sbr): Main Behavior and Mechanism

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## Supplementary Materials

### Procedures of qPCR amplification

The qPCR amplification was performed in a 20-μL reaction mixture, which consisted of 2.0-μL template DNA, 10-μL SYBR Premix Ex Taq and 0.5-μL forward and reverse primers for each sample [1]. The PCR procedure contained 15-min enzyme activation and initial denaturation at 95 °C, followed by 40 cycles of 30-s denaturing at 95 °C, 30-s annealing at 58 °C and 45-s extension at 72 °C. The specificity of PCR products was confirmed by dissociation curve, then visualized using 1.2% agarose gel electrophoresis. Standard curves were generated using standard plasmids containing corresponding genes and parallelly performed with DNA. The target gene copies in the unit volume template were calculated through the standard curve.

**Table 1.** Primer sets of qPCR amplification used in this study.

| Target Gene | Primer Name | Primer Sequence (5′-3′) | Amplified Length | References |
|---|---|---|---|---|
| Comammox-*amoA* | *comamoA* AF | AGGNGAYTGGGAYT TCTGG | 436 | [1] |
| | *comamoA* 3SR | CCGVACATACATRA AGCCCAT | | |
| AOB-*amoA* | *amoA* 1F | GGGGTTTCTACTGGT GGT | 491 | [2] |
| | *amoA* 2R | CCCCTCKGSAAAGC CTTCTTC | | |
| AOA-*amoA* | *archamoA* 19F | ATGGTCTGGYTWAG ACG | 629 | [3] |
| | *archamoA* 616R | GCCATCCABCKRTA NGTCCA | | |

## References

1. Wang, M.; Huang, G.; Zhao, Z.; Dang, C.; Liu, W.; Zheng, M. Newly designed primer pair revealed dominant and diverse comammox amoA gene in full-scale wastewater treatment plants. *Bioresour. Technol.* **2018**, *270*, 580–587, doi:10.1016/j.biortech.2018.09.089.
2. Rotthauwe, J.H.; Witzel, K.P.; Liesack, W. The ammonia monooxygenase structural gene amoa as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl. Environ. Microbiol.* **1997**, *63*, 4704–4712, doi:10.1128/aem.63.12.4704-4712.1997.
3. Pester, M.; Rattei, T.; Flechl, S.; Gröngröft, A.; Richter, A.; Overmann, J.; Reinhold-Hurek, B.; Loy, A.; Wagner, M. AmoA-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of amoA genes from soils of four different geographic regions. *Environ. Microbiol.* **2012**, *14*, 525–539, doi:10.1111/j.1462-2920.2011.02666.x.