Supplementary Information for

Heated Aeration for Nitrite-Oxidizing Bacteria (NOB) Control in Anammox-Integrated Membrane-Aerated Biofilm Reactors (MABR)

Natalia Shiu, Hui Guo, and Younggy Kim*

Department of Civil Engineering, McMaster University, Hamilton, ON, Canada L8S 4L7
*Corresponding Author: younggy@mcmaster.ca

S1. Preliminary MABR Results

The preliminary Anammox-MABR system consist of 6 membranes, each 50 cm long. Three different temperature aeration conditions were monitored: 20 °C, 40 °C, 60 °C. From these results, it was concluded that the residence time of the air through the membrane was too short and may significantly contribute to the high dissolved oxygen in the bulk solution (Figure S1b) which should be taken into consideration for future reactors.

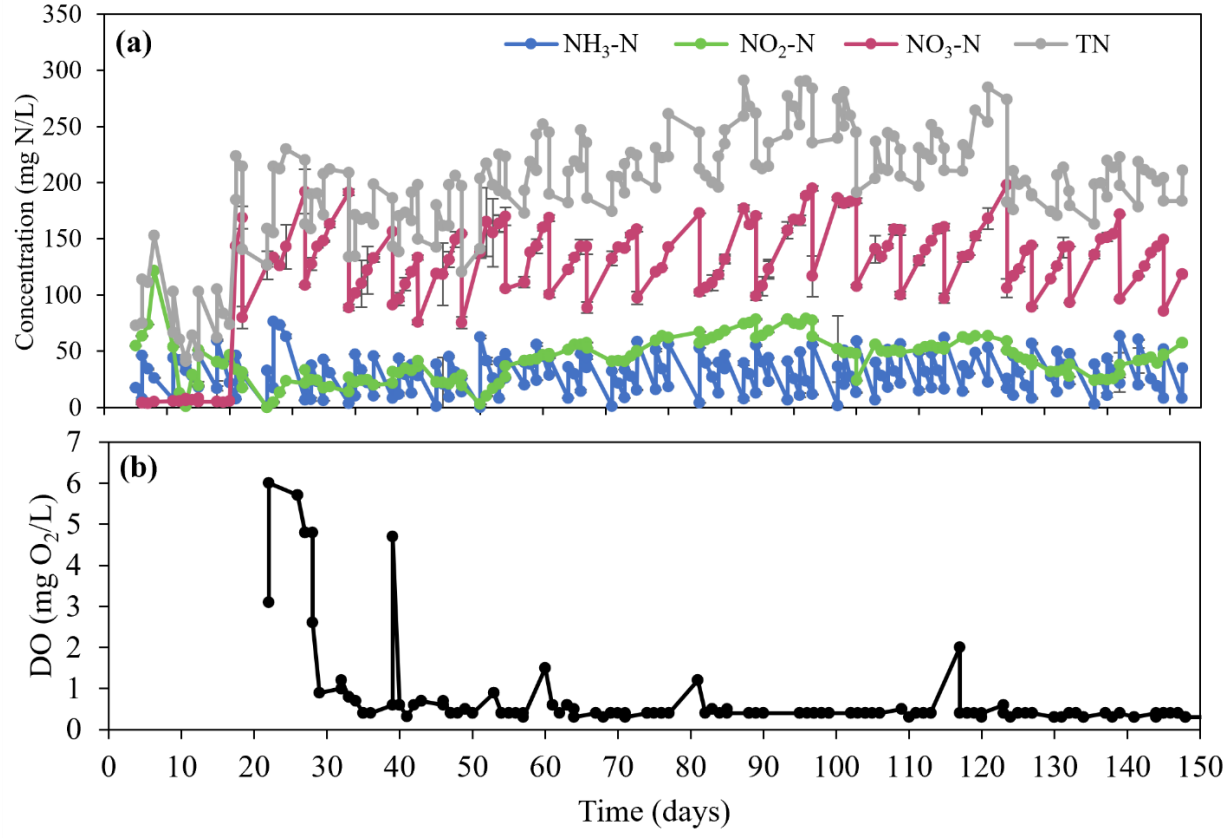

Figure S1. (a) Nitrogen concentrations and **(b)** dissolved oxygen concentrations for the preliminary Anammox–MABR.

Table S1. Effluent air linear velocity and residence time (τ_{air}) for the membranes in the preliminary Anammox–MABR.

Condition	Effluent Linear Velocity (cm/s)	Residence Time, τ_{air} (s)
20 °C – A	7.04	0.14
20 °C – B	2.17	0.04
40 °C – A	3.79	0.08
40 °C – B	8.66	0.17
60 °C – A	8.66	0.17
60 °C – B	1.62	0.03

S2. Nitrogen Removal Mass Balance Equations

By deriving mass balance equations (Eq. S1-S3) from the theoretical biological reactions and molar ratios, the estimated daily nitrogen removal (mg N/L·d) from AOB, Anammox bacteria, and NOB can be described as x_1, x_2, x_3, respectively (Eq. S4-S6).

$$\Delta NH_4 = -x_1 - x_2 \quad \text{(Eq. S1)}$$

$$\Delta NO_2 = x_1 - 1.32x_2 - x_3 \quad \text{(Eq. S2)}$$

$$\Delta NO_3 = x_3 \quad \text{(Eq. S3)}$$

$$x_1 = \left[\frac{-1.32\Delta NH_4 + \Delta NO_2 + \Delta NO_3}{2.32}\right] \quad \text{(Eq. S4)}$$

$$x_2 = \frac{-\Delta NH_4 - \Delta NO_2 - \Delta NO_3}{2.32} \quad \text{(Eq. S5)}$$

$$x_3 = \Delta NO_3 \quad \text{(Eq. S6)}$$

S3. 16S rRNA Amplicon Sequencing

The genomic DNA from the biofilm membrane samples was extracted using the DNA isolation method as described in Stearns et al., 2015 [46]. The samples were further processed for purification using the MagMAX Express 96-Deep Well Magnetic Particle Processor with the MagMAX DNA Multi-Sample Kit (Applied Biosystems, USA). The purified DNA was used to

amplify the V4 region of the 16S rRNA gene by conducting PCR using adapted primers as described by Bartram et al., 2011 [47]. The primers were modified to 515f (GTG YCA GCM GCC GCG GTAA) and 806r (GGA CTA CNV GGG TWT CTA AT). The PCR protocol for the 515f/906r primer pair with the denaturing, annealing, and extension times and temperature can be described in Table S2. The PCR components include 50 ng of DNA templates with 1U of Taq, 1× buffer, 1.5 mM MgCl2, 0.4 mg/mL BSA, 0.2 mM dNTPs, and 5 pmol of each primer. The PCR products were checked by gel electrophoresis and the positive amplicons were sent for sequencing. All the amplicons were visually normalized based on the band intensity on the gel and pooled. The final pool was cleaned using the Promega ProNex Size-Selective Purification System (Promega, USA).

Table S2. PCR protocol for 16S amplicon sequencing used in this study.

Primer	Hold	Denaturation	Annealing	Extension*	Hold
515f	94 °C	94 °C	50 °C	72 °C	72 °C
806r	5 min	30 s	30 s	30 s	10 min

*After extension, repeat denaturation-annealing-extension steps 35×.

DNA sequence reads were filtered and trimmed based on the quality of the reads using Cutadapt with a minimum quality score of 30 and a minimum read length of 100 bp according to Martin, 2011 [48], then the amplicon sequence variants were resolved from the trimmed raw reads by DADA2 according to Callahan et al., 2016 [49]. Finally, bimeras were removed and taxonomy was assigned using the RDP classifier against the SILVA database version 1.3.8.

The genomic DNA extraction, PCR amplification, and sequencing with the Illumina MiSeq platform (paired-end reads, 2 ×300 base pairs [bp]) was conducted at the Farncombe Institute (McMaster University, Hamilton, ON, Canada).

16S amplicon sequencing analysis was conducted for the biofilm samples from each membrane for the two temperature conditions (20 °C and 60 °C) and for the bulk solution in the Anammox-MABR system. The microbial species were then assigned to a target bacterial group of interest: AOB, Anammox bacteria, NOB, denitrifying bacteria, and others.

S4. qPCR Standard Curves

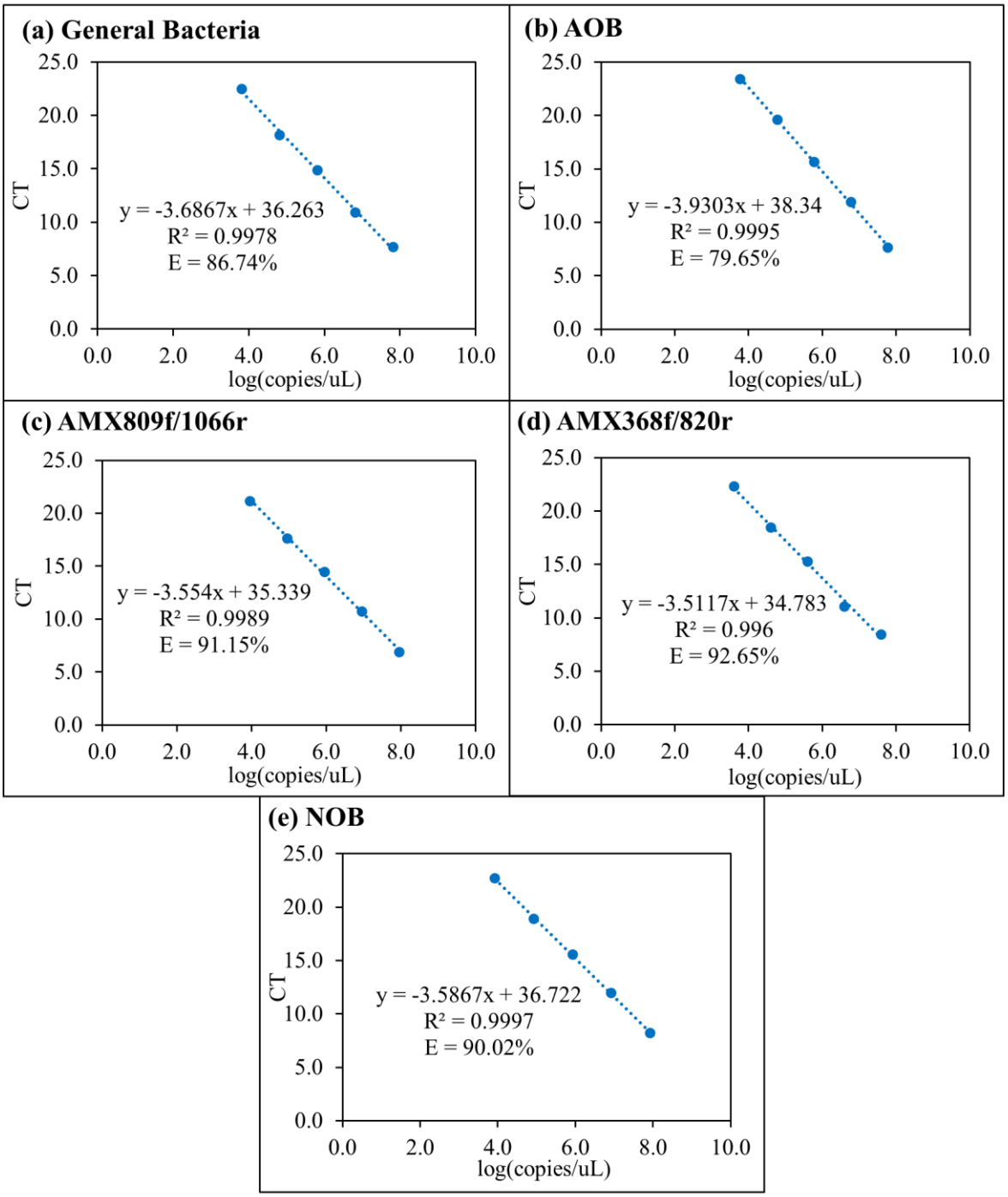

Figure S2. Standard curves constructed from qPCR methods for each primer pair for **(a)** General Bacteria, **(b)** AOB, **(c)** Anammox bacteria (AMX809f/1066r), **(d)** Anammox bacteria (AMX368f/820r), and **(e)** NOB.

S5. Anammox-MABR Performance – Other key operational parameters

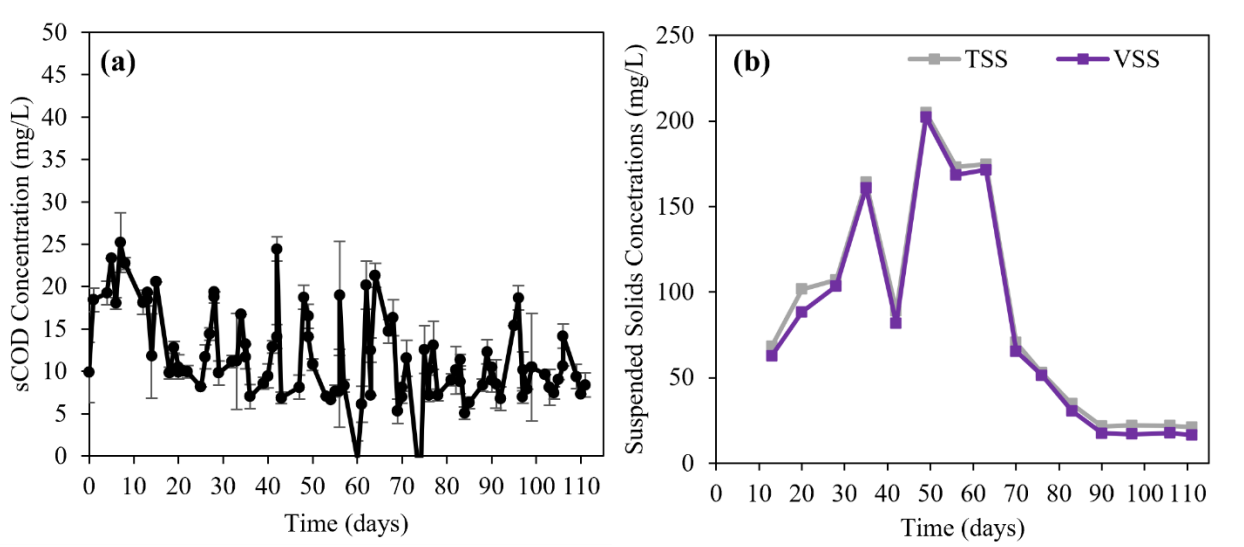

Figure S3. (a) sCOD and **(b)** Suspended Solids concentrations for Anammox–MABR performance throughout 111 days operational period.

S6. Oxygen Transfer through Membranes

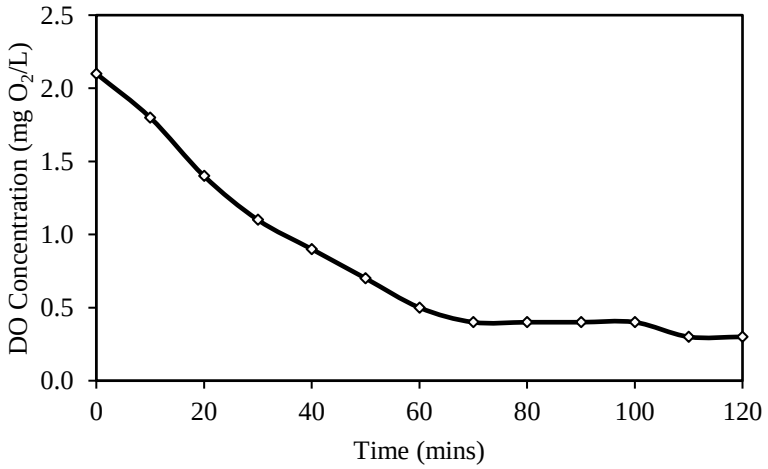

Figure S4. Dissolved oxygen concentrations for MABR system with no aeration conducted on Day 35 for 120 mins.