Granular Sludge Coupling Nitrate/Nitrite Dependent Anaerobic Methane Oxidation with Anammox: from Proof-of-Concept to High Rate Nitrogen Removal

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Supporting Information

Number of Pages: 6

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Feeding medium

The compositions of medium were as follows: KH_2PO_4, 0.075 g L^{-1}; $CaCl_2·2H_2O$, 0.3 g L^{-1}; $MgSO_4·7H_2O$, 0.2 g L^{-1}; acidic trace element solution, 0.5 mL L^{-1}, alkaline trace element solution, 0.2 mL L^{-1}. The compositions of acidic trace element solution and alkaline trace element solution were described in Table S1 and S2.

Table S1. The compositions of acidic trace element solution per liter.

component	quality(g)
$FeSO_4·7H_2O$	5.560
$ZnSO_4·7H_2O$	0.068
$CoCl_2·6H_2O$	0.120
$MnCl_2·4H_2O$	0.500
$CuSO_4$	1.600
$NiCl_2·6H_2O$	0.095
H_3BO_3	0.014
HCL	3.65

Table S2. The compositions of alkaline trace element solution per liter.

component	Quality(g)
SeO_2	0.067
$Na_2WO_4·2H_2O$	0.050
Na_2MoO_4	0.242
NaOH	0.4

Inoculum reactor

A sequencing batch reactor (SBR) with a working volume of 2 L was operated at 35 ℃ to cultivate a co-culture of n-DAMO and Anammox microorganisms. Concentrated stock solution of nitrate and ammonium was pulse injected into the reactor to maintain the nitrate and ammonium concentrations. A mixed gas of 95% CH_4 and 5% CO_2 was flushed in the reactor to supply methane for n-DAMO microorganism. 200 mL flocs from the parent reactor and 200 mL anaerobic granular sludge treating paper mill wastewater were transferred to MGSR-I as inoculum. Upon inoculation, the parent reactor reached a nitrate and ammonium removal rate of approximately 60 mg N L^{-1} d^{-1} and 50 mg N L^{-1} d^{-1}. The biomass concentration of the biomass was about 3 g VSS L^{-1}. The high throughput analysis result showed that the parent reactor was dominated by *Candidatus Methanoperedens* and *Candidatus Kuenenia*, with a relative abundance of 37.3 % and 8.8 %, respectively.

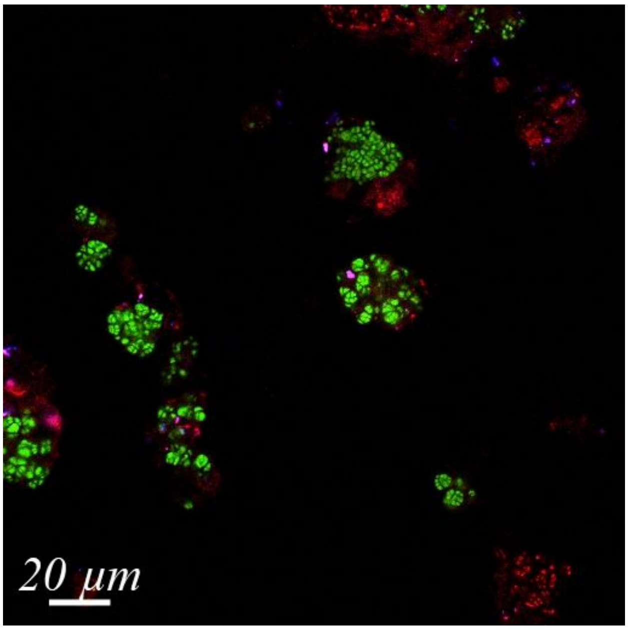

Figure S1. FISH image of the inoculum flocs hybridized with specific probes: S-*-DARCH-0872-a-A-18 for n-DAMO archaea (Green), S-*-NC10-1162-a-A-18 for n-DAMO Bacteria (blue), and S-*-Amx-820-a-A-18 for Anammox bacteria (red).

Determination of biological reaction rates

Based on mass balance and Reaction S1-S3, theoretical nitrate available for n-DAMO archaea ($rNO_3^-{}_{\text{available for n-DAMO archaea}}$) and nitrate produced by Anammox bacteria ($rNO_3^-{}_{\text{Anammox}}$) are calculated as follows:

$$NH_4^+ + 1.32NO_2^- \rightarrow 1.02N_2 + 0.26NO_3^- \quad \text{(S1)}$$

$$CH_4 + 2.67NO_2^- + 2.67H^+ \rightarrow CO_2 + 1.33N_2 + 3.33H_2O \quad \text{(S2)}$$

$$CH_4 + 4NO_3^- \rightarrow CO_2 + 4NO_2^- + 2H_2O \quad \text{(S3)}$$

Nitrate production by anammox:

$$rNO_3^-{}_{\text{Anammox}} = 0.26*rNH_4^+ \quad \text{(S4)}$$

Nitrite consumption by anammox:

$$rNO_2^-{}_{\text{Anammox}} = 1.32*rNH_4^+$$

Theoretical nitrate available for n-DAMO archaea:

$$rNO_3^-{}_{\text{available for n-DAMO archaea}} = rNO_3^-{}_{\text{loading}} + rNO_3^-{}_{\text{Anammox}} \quad \text{(S5)}$$

Nitrate reduction rate by n-DAMO archaea:

$$rNO_3^-{}_{\text{n-DAMO archaea}} = rNO_3^-{}_{\text{loading}} + rNO_3^-{}_{\text{Anammox}} - rNO_3^- \quad \text{(S6)}$$

Nitrite production rate by n-DAMO archaea:

$$rNO_2^-{}_{\text{n-DAMO archaea}} = rNO_3^-{}_{\text{n-DAMO archaea}} \quad \text{(S7)}$$

Nitrite consumption of n-DAMO bacteria:

$$rNO_2^-{}_{\text{n-DAMO bacteria}} = rNO_2^- + rNO_3^-{}_{\text{Anammox}} - rNO_2^-{}_{\text{Anammox}} \quad \text{(S8)}$$

Determination of methane oxidation rates

Based on mass balance and Reaction S1-S3, methane oxidation rates are calculated as follows:

Methane oxidation rate by n-DAMO archaea:

$$rCH_4{}_{\text{n-DAMO archaea}} = 0.286 * rNO_3^-{}_{\text{n-DAMO archaea}} \quad \text{(S9)}$$

Methane oxidation rate by n-DAMO bacteria:

$$rCH_{4\ \text{n-DAMO bacteria}} = 0.428 * rNO_2^-{}_{\text{n-DAMO bacteria}} \tag{S10}$$

Total methane oxidation rate:

$$rCH_4 = rCH_{4\ \text{n-DAMO archaea}} + rCH_{4\ \text{n-DAMO bacteria}} \tag{S11}$$

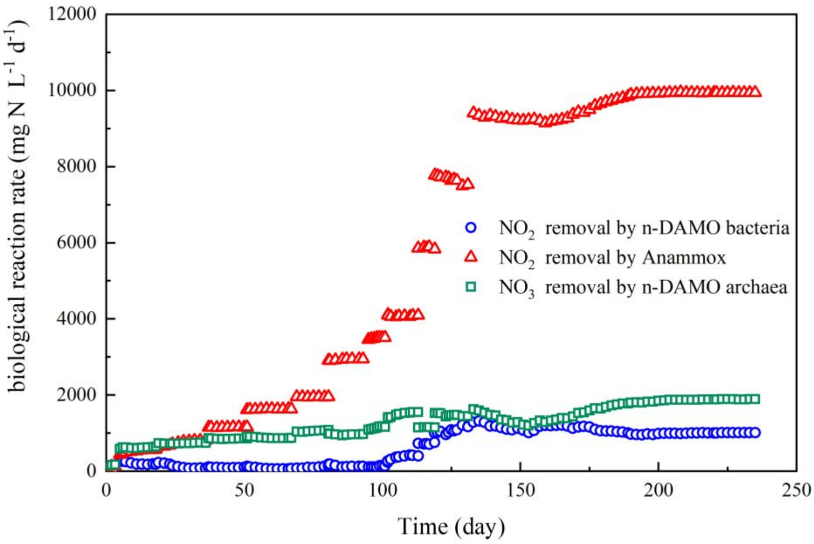

Figure S2. Biological reaction rate of the MGSR-II during 234 days' continuous operation.

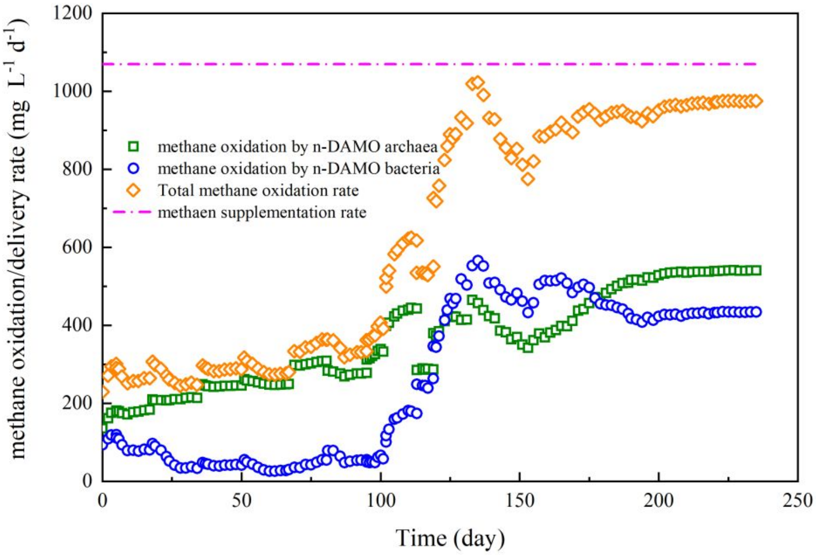

Figure S3. Methane oxidation rate and methane supplementation rate of the MGSR-II during 234 days' continuous operation.

Methane mass balance

The CH_4 flux of membrane module was determined based on the previous method[1], and the methane delivery capacity at 100 kPa was 32.5 g m^{-2} d^{-1}. With the membrane surface area/reactor volume ratio of 33 m^2 m^{-3}, the CH_4 delivery rate to the reactor is 1070 mg L^{-1} d^{-1}. At the final steady stage of MGSR-II, the effluent dissolved methane concentration and the methane utilization efficiency was determined. Based on the calculation above, CH_4 oxidation rate was about 975 mg L^{-1} d^{-1}, including 541 mg L^{-1} d^{-1} by n-DAMO archaea and 434 mg L^{-1} d^{-1} by n-DAMO bacteria. Thus, the CH_4 utilization efficiency was 91.1%. The dissolved methane in effluent could hardly be detected, which may be caused by the high methane utilization efficiency."

Reference

1. Bandara, W. M. K. R. T. W.; Satoh, H.; Sasakawa, M.; Nakahara, Y.; Takahashi, M.; Okabe, S., Removal of residual dissolved methane gas in an upflow anaerobic sludge blanket reactor treating low-strength wastewater at low temperature with degassing membrane. *Water Research* **2011,** *45*, (11), 3533-3540.