

*Submitted to Applied and Environmental Microbiology*

Supporting information for:

**Halophilic anaerobic oxidation of methane coupled to nitrite reduction by  
marine NC10 bacteria**

He Zhanfei, Geng Sha, Cai Chaoyang, Liu Shuai, Liu Yan, Pan Yawei, Lou Liping,  
Zheng Ping, Xu Xinhua, Hu Baolan\*

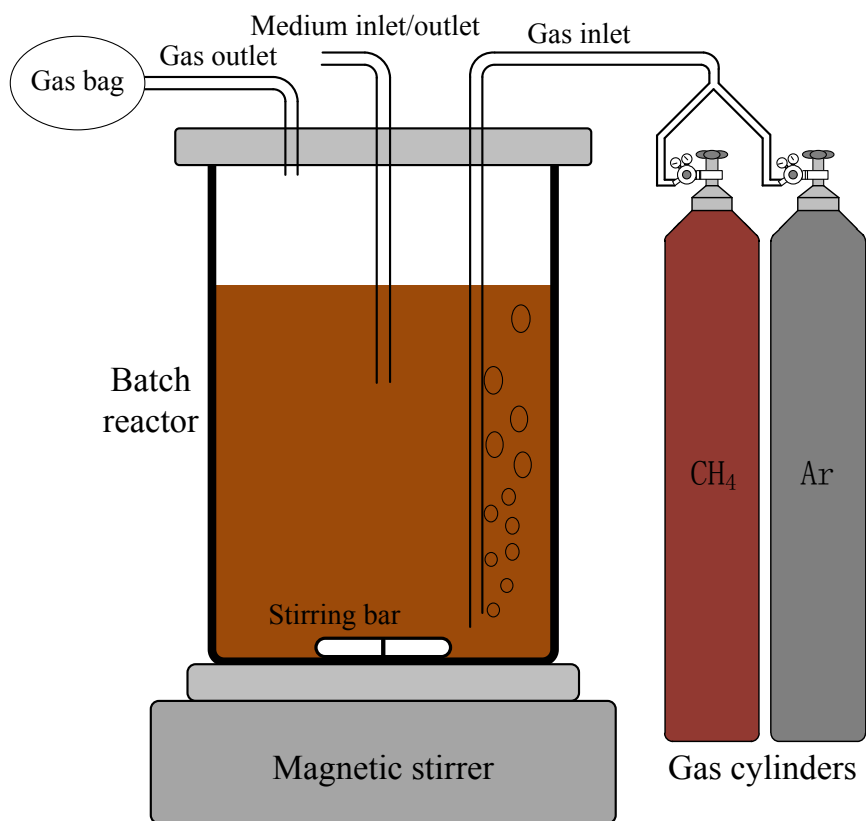
*Department of Environmental Engineering, Zhejiang University, Hangzhou 310058,  
China*

\* Corresponding author:

*Email address: blhu@zju.edu.cn*

*Tel: 0086 0571 88982340; Fax: 0086 0571 88982340*

1. Schematic diagram of the sequencing batch bioreactor



**Fig. S1** Schematic diagram of the sequencing batch bioreactor in this work.

## 2. The primers used in PCR amplification

**Table S1.** PCR primers used in this work.

Primers	Sequences (5'-3')	References
202F	GACCAAAGGGGGCGAGCG	(1)
1545R	CAKAAAGGAGGTGATCC	(2)
qP1F	GGGCTTGACATCCCACGAACCTG	(1)
qP1R	CGCCTTCCTCCAGCTTGACGC	(1)
qP2R	CTCAGCGACTTCGAGTACAG	(1)
A189_b	GGNGACTGGGACTTYTGG	(3)
cmo682	AAAYCCGGCRAAGAACGA	(3)
cmo182	TCACGTTGACGCCGATCC	(3)
cmo568	GCACATACCCATCCCCATC	(3)

### 3. Calculation of methane concentration in saline water

By definition, the Bunsen solubility coefficient can be expressed as follows:

$$\beta = \frac{V_{gas,STP}}{V_{liquid}} \quad (S1),$$

Where  $\beta$  is the Bunsen solubility coefficient and expressed as ml CH<sub>4</sub> (STP) dissolved in 1 ml H<sub>2</sub>O [-];  $V_{gas,STP}$  is the normalized volume of gas absorbed in liquid [ml]; and  $V_{liquid}$  is the volume of liquid [ml].

The ideal gas equation,  $PV = nRT$ , can be substituted into Eq. S1, and it gives

$$\beta = \frac{nRT}{P_{STP}V_{liquid}} = \frac{RT}{P_{STP}}c_{STP} \quad (S2),$$

Where  $n$  is the amount of gas substance [mole];  $R$  is the universal gas constant [8.3145 J·K<sup>-1</sup>·mol<sup>-1</sup>];  $T$  is temperature in Kelvin degrees [K];  $P_{STP}$  is the standard pressure [101.325 kPa];  $c_{STP}$  is gas solubility in liquid at standard pressure [mol·L<sup>-1</sup>].

In dilute solutions, the gas solubility is in direct proportion to its pressure in headspace, so the gas solubility is

$$c = c_{STP} \frac{P}{P_{STP}} \quad (S3),$$

Where  $c$  is gas solubility [mol·L<sup>-1</sup>] and  $P$  is gas pressure in headspace [kPa].

Based on Eq. S2 and Eq. S3, the correlation between gas solubility and pressure can be given as

$$c = \frac{\beta}{RT} P \quad (S4).$$

The Bunsen solubility coefficient can also be expressed as a function of temperature and salinity as follows (4):

$$\ln(\beta) = A_1 + A_2 \frac{100}{T} + A_3 \ln\left(\frac{T}{100}\right) + S \left[ B_1 + B_2 \frac{T}{100} + B_3 \left(\frac{T}{100}\right)^2 \right] \quad (\text{S5}),$$

Where  $S$  is salinity in parts per thousand [‰] and  $A_1$ ,  $A_2$ ,  $A_3$ ,  $B_1$ ,  $B_2$ , and  $B_3$  are constants [-] and their values are -67.1962, 99.1624, 27.9015, -0.072909, 0.041674, and -0.0064603, respectively, calculated by Yamamoto et al. <sup>4</sup>.

In this work, the temperature is 298.15 K and the salinity is 20.5 ‰, and the Bunsen solubility coefficient is calculated to be 0.02784. Finally, Eq. S4 can be simplified into

$$c[\mu\text{M}] = 11.23P[\text{kPa}] \quad (\text{S6}).$$

## References

1. **Ettwig KF, van Alen T, van de Pas-Schoonen KT, Jetten MS, Strous M.** 2009. Enrichment and molecular detection of denitrifying methanotrophic bacteria of the NC10 phylum. *Applied and environmental microbiology* **75**:3656-3662.
2. **Juretschko S, Timmermann G, Schmid M, Schleifer K, Pommerening-Roser A, Koops H, Wagner M.** 1998. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitrosococcus mobilis* and *Nitrospira*-like bacteria as dominant populations. *Appl Environ Microbiol* **64**:3042-3051.
3. **Luesken FA, Zhu B, van Alen TA, Butler MK, Diaz MR, Song B, Op den Camp HJ, Jetten MS, Ettwig KF.** 2011. pmoA Primers for detection of anaerobic methanotrophs. *Appl Environ Microbiol* **77**:3877-3880.
4. **Yamamoto S, Alcauskas J, B., Crozier T, E.** 1976. Solubility of methane in distilled water and seawater. *J Chem Eng Data* **21**:78-80.