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Evaluating death and activity decay of Anammox bacteria during anaerobic and aerobic starvation

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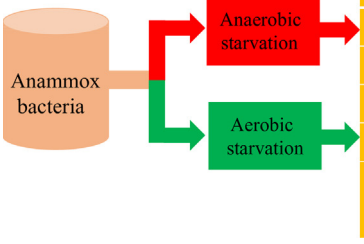
HIGHLIGHTS

- Death and activity decay of Anammox bacteria were distinguished.
- Decay, death and activity decay rates of Anammox bacteria were determined.
- Death was mainly responsible for the decreased Anammox activity during starvation.
- Activity decay played a minor role in the decreased Anammox activity.

GRAPHICAL ABSTRACT



Parameter	Anaerobic starvation	Aerobic starvation
Decay rate (d^{-1})	0.015±0.001	0.028±0.001
Death rate (d^{-1})	0.011±0.001	0.025±0.001
Activity decay rate (d^{-1})	0.004±0.001	0.003±0.001
Contribution of death (%)	73±4	89±4
Contribution of activity decay (%)	27±5	11±5

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ABSTRACT

The decreased activity (i.e. decay) of anaerobic ammonium oxidation (Anammox) bacteria during starvation can be attributed to death (i.e. decrease in the amount of viable bacteria) and activity decay (i.e. decrease in the specific activity of viable bacteria). Although they are crucial for the operation of the Anammox process, they have never been comprehensively investigated. This study for the first time experimentally assessed death and activity decay of the Anammox bacteria during 84 days' starvation stress based on ammonium removal rate, Live/Dead staining and fluorescence in-situ hybridization. The anaerobic and aerobic decay rates of Anammox bacteria were determined as 0.015 ± 0.001 d^{-1} and 0.028 ± 0.001 d^{-1}, respectively, indicating Anammox bacteria would lose their activity more quickly in the aerobic starvation than in the anaerobic starvation. The anaerobic and aerobic death rates of Anammox bacteria were measured at 0.011 ± 0.001 d^{-1} and 0.025 ± 0.001 d^{-1}, respectively, while their anaerobic and aerobic activity decay rates were determined at 0.004 ± 0.001 d^{-1} and 0.003 ± 0.001 d^{-1}, respectively. Further analysis revealed that death accounted for $73 \pm 4\%$ and $89 \pm 5\%$ of the decreased activity of Anammox bacteria during anaerobic and aerobic starvations, and activity decay was only responsible for $27 \pm 4\%$ and $11 \pm 5\%$ of the decreased Anammox activity, respectively, over the same

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starvation periods. These deeply shed light on the response of Anammox bacteria to the starvation stress, which would facilitate operation and optimization of the Anammox process.

1. Introduction

In the wastewater treatment plants (WWTPs), bacteria are frequently exposed to the starvation conditions because of the large fluctuations in the wastewater flow and composition. Under such starvation conditions, bacteria could switch on programmed cell death (Lewis, 2000; Yarmolinsky, 1995), which is a genetically determined cell self-destruction process, to maintain partial bacterial activity. This results in bacteria death (i.e. decrease in the amount of viable bacteria). In addition, bacteria could also adjust their metabolic processes and decrease their maintenance energy requirement under starvation conditions (Boutte and Crosson, 2013; Durfee et al., 2008). In that case, bacteria would be self-controlled via enzymatic regulation instead of being dead, which leads to activity decay (i.e. decrease in the specific activity of viable bacteria). Bacteria death and activity decay collectively contribute to the decreased bacterial activity (i.e. bacteria decay) in the starvation condition (Hao et al., 2009, 2012; van Loosdrecht and Henze, 1999).

Bacteria decay will significantly affect the robustness and performance of WWTPs. Therefore, plenty of researches have been conducted to measure the decay rates of key bacteria/archaea involved in wastewater treatment, such as ammonium-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), heterotrophic bacteria, polyphosphate-accumulating organisms (PAOs), glycogen-accumulating organisms (GAOs) and methanogens (Carvalheria et al., 2014; Hao et al., 2009, 2012; Salem et al., 2006; Vargas et al., 2013). For instance, the decay rates of AOB and NOB were determined as 0.02 d^{-1} and 0.08 d^{-1}, respectively, by Salem et al. (2006). In addition, Hao et al. (2009, 2010a, 2012) also further experimentally evaluated the death and activity decay of AOB, NOB, PAOs, GAOs, heterotrophic bacteria and methanogens.

In addition to the above bacteria/archaea, anaerobic ammonium oxidation (Anammox) bacteria are also of great importance to wastewater treatment (Kartal et al., 2010; Xu et al., 2015). The partial nitritation and Anammox process has revolutionized the way of nitrogen removal (Kartal et al., 2010; Lotti et al., 2015; Xu et al., 2015). In this process, AOB first oxidize about half of the ammonium to nitrite, while the Anammox bacteria subsequently convert nitrite and the remaining ammonium to N_2. Compared to traditional nitrogen removal via nitrification and denitrification, the partial nitritation and Anammox process has lower energy consumption, negligible organic carbon requirement and much less sludge production (Kartal et al., 2010; Lotti et al., 2015; Xu et al., 2015). More than one hundred full-scale Anammox plants have already been operated for the treatment of municipal and industrial wastewaters until now (Lackner et al., 2014). Unfortunately, the aerobic decay rate of Anammox bacteria has never been measured so far although the anaerobic decay rate of Anammox bacteria has been measured by many researchers (Ma et al., 2016; Scaglione et al., 2009; Xing et al., 2016a; Zhang et al., 2015). Determining aerobic decay rate of Anammox bacteria is crucial because the Anammox bacteria could also be exposed to the aerobic starvation condition at a dissolved oxygen (DO) concentration of up to 1.5 mg/L (Lackner et al., 2014). In addition, due to the lack of a suitable approach, previous studies did not differentiate death from activity decay (Scaglione et al., 2009; Xing et al., 2016a; Zhang et al., 2015). Therefore, several questions have been raised. Do the death and activity decay of the Anammox bacteria happen simultaneously or sequentially under starvation conditions? What is the contribution of death and activity decay to the decreased activity of Anammox bacteria? These questions have not been answered so far.

By addressing the above questions, this study aims to experimentally assess death and activity decay of the Anammox bacteria under both anaerobic and aerobic starvation conditions. The enriched Anammox culture was exposed to the anaerobic and aerobic starvation for 84 days, during which Live/Dead staining and fluorescence in-situ hybridization (FISH) were conducted periodically to determine the ratios of viable bacteria and viable Anammox bacteria, respectively. The activity of Anammox bacteria was also monitored over the same period via a series of batch tests. The anaerobic and aerobic decay rates, death rates and activity decay rates of the Anammox bacteria were then determined and assessed by the detailed analysis of the comprehensive experimental data. The contributions of death and activity decay to the decreased Anammox activities were also calculated.

2. Materials and methods

2.1. Cultivation of Anammox bacteria

One pilot-scale up-flow bioreactor with a working volume of 50 L was set up to cultivate Anammox bacteria. The bioreactor was operated at 33 ± 2 °C with a pH of 7.5 ± 0.3 and a DO of approximately 0 mg/L. The bioreactor received synthetic wastewater (sparging N_2 regularly) continuously, which contained 1180 mg $(NH_4)_2SO_4$/L (i.e. 250 mg N/L), 1230 mg $NaNO_2$/L (i.e. 250 mg N/L), 540 mg $NaHCO_3$/L, 32 mg KH_2PO_4/L, 216 mg $CaCl_2 \cdot 2H_2O$/L, 360 mg $MgSO_4 \cdot 7H_2O$/L, 0.018 mg H_3BO_3/L, 0.275 mg $NaMoO_4 \cdot 2H_2O$/L, 0.3 mg $CoCl_2 \cdot 6H_2O$/L, 3.75 mg EDTA/L, 0.24 mg $NiCl_2 \cdot 6H_2O$/L, 0.25 mg $MnCl_2 \cdot 4H_2O$/L, 0.54 mg $ZnSO_4 \cdot 7H_2O$/L and 0.31 mg $CuSO_4 \cdot 5H_2O$/L. The bioreactor was operated for 280 days (hydraulic retention time was 0.5 d) with a total nitrogen removal rate of approximately 630 mg N/L/d and an Anammox bacteria population of around 83% while reaching steady state. AOB were undetectable.

2.2. Starvation tests

3.0 L of Anammox sludge was taken out from the pilot-scale up-flow bioreactor when it reached steady state. Afterwards, the Anammox sludge was washed using the ammonium and nitrite free synthetic wastewater (see Section 2.1) for 5 times until no ammonium or nitrite were detectable. Then, the Anammox sludge was equally transferred into two starvation reactors (i.e. Erlenmeyer flask, 1.5 L each) to initiate the batch-mode starvation tests. One starvation reactor was operated under the anaerobic condition. The other starvation reactor was operated under the aerobic condition with a DO concentration of ~1.5 mg/L, which was the highest DO concentration reported in literature for the Anammox reactor (Lackner et al., 2014). pH in both starvation reactors was at approximately 7.5 ± 0.3 and the temperature was 33 ± 2 °C. The starvation tests lasted for 84 days, during which Live/Dead staining and FISH were conducted regularly. The mixed liquor volatile suspended solids (MLVSS) concentration was also monitored