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Modeling of the interaction among aerobic ammonium-oxidizing archaea/bacteria and anaerobic ammonium-oxidizing bacteria

Yuting Pan [a,b], Bing-Jie Ni [b,*], Yiwen Liu [b], Jianhua Guo [b,*]

[a] Department of Environmental Science and Engineering, School of Architecture and Environment, Sichuan University, Chengdu, Sichuan 610065, China
[b] Advanced Water Management Centre, The University of Queensland, St Lucia, QLD 4072, Australia

HIGHLIGHTS

- A model gives the first insights on the role of AOA in Nitritation/Anammox system.
- AOA would outcompete AOB under low ammonium concentration.
- AOA are better partners to Anammox in treating low strength nitrogen sewage.

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ABSTRACT

Biological nitrogen removal by using a co-culture of Anammox bacteria, ammonia-oxidizing archaea (AOA) and (ammonia-oxidizing bacteria) AOB microorganisms in a sequencing batch reactor (SBR) has previously been demonstrated experimentally. In this work, a mathematical model is developed to describe the microbial interaction among AOA, AOB and Anammox bacteria in the single-stage SBR and provide the first insights on the key role of AOA in such system. In this model, AOA and AOB jointly convert ammonium to nitrite partially, which provides electron acceptors to Anammox bacteria to oxidize the remaining ammonium forming dinitrogen gas. The model is successfully calibrated and validated using the long-term (around 350 days) dynamic experimental data from the SBR system, as well as two independent batch tests at different operational stages of the SBR. The model satisfactorily describes the nitrogen conversion data from the system. Modeling results show that AOA would outcompete AOB under low ammonium concentration and low dissolved oxygen conditions due to the revealed higher NH_4^+ affinity ($K_{NH_4}^{AOA}$ of 0.06 g N m^{-3}) and thus higher ammonia oxidation rate under oxygen-limited conditions, indicating that AOA could be a better partner to Anammox bacteria compared to AOB when treating low strength nitrogen sewage. The developed model could also predict and distinguish the different contributions of AOA and AOB to overall aerobic ammonia oxidizing potential, with more than 50% of ammonia oxidation being mediated by AOB at initial stage (~300 days) and AOA being responsible for up to 90% of the ammonium removal afterwards. The results suggest AOA coupled with Anammox could provide new possibilities for biological nitrogen removal from low strength ammonium wastewater.

1. Introduction

Anaerobic ammonium oxidation (Anammox) process is an attractive biological nitrogen removal (BNR) process, as it does not require organic carbon or aeration and produces much less sludge than via the traditional nitrification/denitrification route (Kartal et al., 2010) and therefore would dramatically reduce operational costs of up to 90% of wastewater treatment plants (WWTPs) (Jetten et al., 2001).

The Anammox process requires simultaneous supply of NH_4^+ and NO_2^-. While the source of NH_4^+ is commonly presented in the wastewater influent, NO_2^- is usually obtained through nitritation process carried out by ammonia-oxidizing bacteria (AOB) (Joss et al., 2011). Therefore, an Anammox process has to be preceded by a partial nitritation step, which can be realized in one single reactor (one-stage Anammox process) or by using two separate reactors (two-stage Anammox process) (Van Hulle et al., 2010). In two-stage system, one of the key challenges is to obtain a stable, Anammox-suited feeding from the nitritation system, i.e. with the molar ratio between NH_4^+ and NO_2^- being 1:1.32. A few

* Corresponding authors.
E-mail addresses: b.ni@uq.edu.au (B.-J. Ni), j.guo@awmc.uq.edu.au (J. Guo).

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strategies were developed to provide the proper feeding, however, they usually require particular influent characteristics (e.g. high ammonium concentrations, approximately equal molar ratio between ammonium and bicarbonate (Jetten et al., 2001; Fux et al., 2002)), or unique growth conditions (e.g. at a high temperature of around 35 °C (Van Hulle et al., 2007)).The application of a one-stage system has certain advantages compared to two-stage system, e.g. higher volumetric nitrogen removal rate, lower construction footprint, almost no nitrite accumulation and considerable simplification of reactor control and operation (e.g., no pH control) (Van Hulle et al., 2010). However, difficulties with dissolved oxygen (DO) regulation and incomplete nitrogen removal treating high loaded wastewaters has been reported (Hao et al., 2002a).

To date, most of the nitritation-Anammox technologies have been applied in the side-stream wastewater treatment, while the main-stream application is still under development. The key challenges in applying combined nitritation-Anammox in the mainstream WWTPs include achieving stable nitritation and low effluent nitrogen concentration (Lotti et al., 2014). In addition, the coordination of the activity of AOB and Anammox bacteria remained a challenge, since a high DO concentration might inhibit the activity of Anammox bacteria while a low DO would reduce the activity of AOB.

The discovery of ammonia-oxidizing archaea (AOA) provides a potential solution to the problem (Francis et al., 2005; Könneke et al., 2005). AOA often thrive at DO levels of 0.1 g DO m^{-3} and can achieve higher ammonia oxidation rate compared to AOB under oxygen-limited conditions (You et al., 2009; Park et al., 2006). In addition, AOA have been reported to have a high NH_4^+ affinity (Erguder et al., 2009), which might be beneficial for achieving a lower effluent ammonia concentration (e.g. 1 g N m^{-3}). Therefore, AOA are likely a better partner with Anammox bacteria than AOB when treating the low strength mainstream in WWTPs. As a matter of fact, in natural ecosystems such as marine or soil, substantial experiments have proven AOA often dominant over AOB and provide nitrite and also create anoxic microenvironments for Anammox bacteria by consuming microenvironment oxygen (Lam et al., 2007; Mosier and Francis, 2008). It has also been shown that the co-culture of AOA an Anammox bacteria is ubiquitous in various natural environments (Erguder et al., 2009). It might be worthwhile to investigate whether cooperation between AOA and Anammox occurs within, or might benefit treatment of very dilute waste streams.

A study investigating the cooperation between AOA and Anammox bacteria was reported by Yan et al. (2012). An enriched Anammox bacterium *Candidatus* 'Scalindua profunda' and *Nitrosopumilus maritimus* (one typical AOA species) was co-cultured together. *Nitrosomonas*-like AOB was an indigenous component of *Candidatus* 'S. profunda'-dominated Anammox enrichment cultures, 1% of the total microbial community (Yan et al., 2012). Therefore, the system provides an opportunity to evaluate the interactions between AOA, AOB and Anammox bacteria.

Up to now, a number of models was developed to describe the microbial interactions for N-removal via partial nitritation through AOB and Anammox (Ni et al., 2014; Hao et al., 2002b; Gut et al., 2007). However, to our knowledge, no models are available to discuss the competition and cooperation among AOA, AOB and Anammox, due to AOA being a newly discovered microorganisms capable of converting ammonium to nitrite. In addition, there is no reports of co-culturing of AOA and Anammox bacteria treating real wastewater water at the moment to our knowledge. Although AOA and AOB are both aerobic ammonium oxidizers, they possess distinct kinetic features (such as their affinities to ammonium), which needs to be further revealed. Mathematical models provide a powerful tool for gaining an in-depth understanding of the processes and also strongly support the design and optimization of biological treatment systems (Henze et al., 2000). The objective of this work was to develop a mathematical model to describe the interactions of AOA, AOB and Anammox microorganisms in the SBR system reported by Yan et al. (2012). The established model was calibrated and validated using the experimental data from both the long-term operation (around one year) at different operational stages of the SBR and the batch tests. It is expected that the developed model would provide support for further development of a more efficient nitrogen removal process driven by the co-culture of AOA and Anammox microorganisms.

2. Material and methods

2.1. Model development

In the system investigated, *Candidatus* 'S. profunda' bacteria (an Anammox species), *N. maritimus* AOA and *Nitrosomonas*-like AOB were co-cultured together (Yan et al., 2012). Therefore, it is likely that three microorganisms have both mutualistic and competing interactions, with AOA and AOB oxidizing NH_4^+ to nitrite to feed Anammox, while all of them would compete for NH_4^+ as substrate (Yan et al., 2012), as illustrated in Fig. 1. To investigate the potential interactions among them, the model developed in this work incorporated AOA, AOB, and Anammox processes. Bioconversion processes related to nitrite oxidizing bacteria (NOB) and heterotrophic bacteria were also included to describe the possible oxidation of nitrite to nitrate by NOB and the potential heterotrophic growth under aerobic and anoxic conditions.

The reactions of AOB, Anammox bacteria, NOB and heterotrophic bacteria have been extensively studied previously, therefore, the model developed in this work have adopted widely accepted reactions for these processes. However, little effort has been dedicated to modeling the reactions of AOA. In this study, we modeled conversion of NH_4^+ to NO_2^- by AOA as one-step reaction, with the reactions similar as one-step reaction of AOB (Eq. (1)), but with different kinetic feature (Table S4).

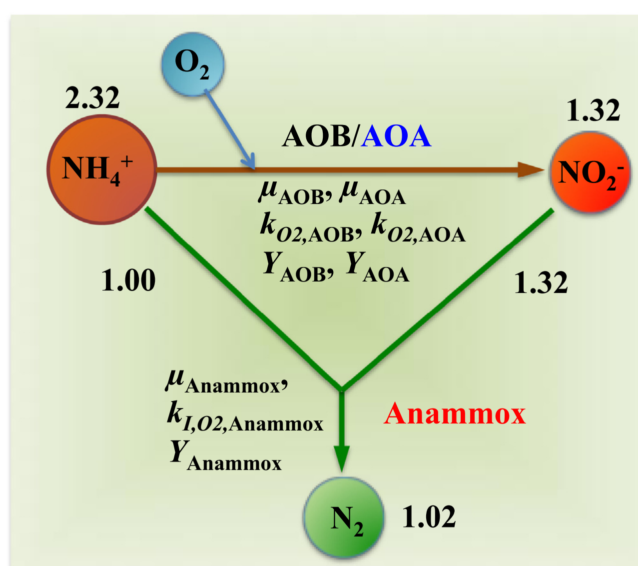

Fig. 1. A conceptual model for the interactions among AOA, AOB and Anammox. (AOA and AOB oxidizing NH_4^+ to nitrite to feed Anammox, while all of them would compete for NH_4^+ as substrate. The competition between AOA and AOB depends on their stoichiometric and kinetic parameters.)