WATER RESEARCH 43 (2009) 1801–1809

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Review

Ammonia-oxidizing archaea involved in nitrogen removal

Jia You[a], *Atreyee Das*[a], *Elizabeth M. Dolan*[b], *Zhiqiang Hu*[a,*]

[a]*Department of Civil and Environmental Engineering, University of Missouri, E2509 Lafferre Hall, Columbia, MO 65211, United States*
[b]*School of Natural Resources, University of Missouri, Columbia, MO 65211, United States*

ARTICLE INFO

Article history:
Received 30 July 2008
Received in revised form
16 January 2009
Accepted 21 January 2009

Keywords:
Nitrogen cycle
Ammonia oxidation
Nutrient removal
Ammonia-oxidizing archaea
Ammonia-oxidizing bacteria

ABSTRACT

Ammonia oxidation is critical to global nitrogen cycling and is often thought to be driven only by ammonia-oxidizing bacteria. The recent finding of new ammonia-oxidizing organisms belonging to the archaeal domain challenges this perception. Two major microbial groups are now believed to be involved in ammonia oxidation: chemolithotrophic ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). *Candidatus* ''*Nitrosopumilus maritimus*'', the first isolated ammonia-oxidizing archaeon from a tropical marine aquarium tank, representative of the ubiquitous marine group 1 *Crenarchaeota*, contains putative genes for all three subunits (*amoA*, *amoB*, and *amoC*) of ammonia monooxygenase, the key enzyme responsible for ammonia oxidation. In this article, important concepts of the nitrogen cycle, ammonia oxidation processes, ammonia-oxidizing organisms, and their physiology are described. AOA are found to thrive in various habitats including hot/thermal springs, marine and fresh waters, soils, and wastewater treatment systems, where they may outnumber their counterpart, AOB. Various molecular tools have been applied to study AOB and AOA and determine their abundance and community structure changes from natural and engineered systems. The presence of AOA in activated sludge opens new opportunities for elucidating its role of ammonia removal in wastewater treatment plants and wetlands. Several significant questions related to AOA research have been raised to evoke reader involvement for broadening future studies.

Contents

1. Introduction 1802
  1.1. Ammonia-oxidizing organisms 1802
    1.1.1. Ammonia-oxidizing bacteria (AOB) 1802
    1.1.2. Ammonia-oxidizing archaea (AOA) 1803
2. Ammonia-oxidizing archaea in natural habitats 1803
  2.1. Thermophilic AOA from hot/thermal springs 1803
  2.2. AOA discovered in marine and estuarine systems 1804
  2.3. AOA in soil/terrestrial ecosystems 1804
  2.4. AOA in engineered systems 1805
  2.5. AOA detection by molecular techniques 1805

---

* *Corresponding author*. Tel.: +1 573 884 0497; fax: +1 573 882 4784.
E-mail address: huzh@missouri.edu (Z. Hu).

0043-1354/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2009.01.016

3. Research prospective of AOA in nitrogen removal 1805
4. Question 1. What fraction of ammonia oxidation in wetlands or WWTPs is due to AOA versus AOB? What are their activities? 1806
5. Question 2. Are there any nitrite-oxidizing archaea (NOA) similar to NOB involved in nitrification? 1806
6. Question 3. How do environmental factors change the AOA or AOB compositions of microbial communities and affect effluent water quality? 1806
7. Question 4. Are AOA also responsible for denitrification? 1806
8. Question 5. Will the bioprocess models be modified to incorporate AOA in N removal? 1807
9. Conclusions 1807
Acknowledgements 1807
References 1807

1. Introduction

The global nitrogen cycle describes the transformation of nitrogen gases and nitrogen-containing compounds on the earth. It consists of mainly microbial-driven processes, including assimilation, ammonification, nitrification, denitrification, nitrogen fixation, and anaerobic ammonia oxidation (Fig. 1). In engineered systems, such as wastewater treatment plants (WWTPs) and constructed wetlands, the coupled nitrification and denitrification processes are considered the major mechanism of nitrogen removal. Anaerobic ammonia oxidation, referred to as anammox by bacterial communities of *Planctomycetes* (Strous et al., 1999), can be of importance in the environment in some cases (Jetten et al., 1998; Sliekers et al., 2002).

Nitrification involves ammonia oxidation to convert ammonia (NH_3) to nitrite (NO_2^-) by ammonia-oxidizing organisms and nitrite oxidation to convert nitrite to nitrate (NO_3^-) by nitrite-oxidizing organisms. Ammonia oxidation is often the rate-limiting step of nitrification in a wide variety of environments, and therefore critical to wastewater nitrogen removal and global N cycling (Kowalchuk and Stephen, 2001; Hu et al., 2003; Choi and Hu, 2008). Reactions of ammonia oxidation include the following:

$$NH_3 + O_2 + 2e^- + 2H^+ \rightarrow NH_2OH + H_2O \tag{1}$$

$$NH_2OH + 1/2O_2 \rightarrow NO_2^- + 3H^+ + 2e^- \tag{2}$$

$$2NH_3 + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 2H^+ \tag{3}$$

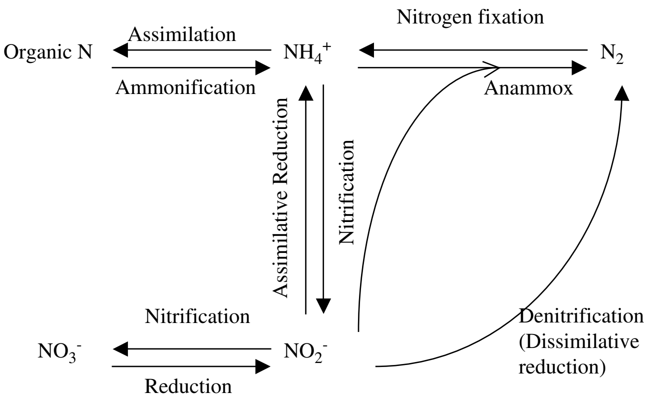

Fig. 1 – Schematic of microbial nitrogen cycle.

While ammonia-oxidizing bacteria (AOB) are considered critical in nitrification, recently molecular biological studies demonstrate that members of the kingdom *Crenarchaeota*, within the archaeal domain, play an important role in nitrification in soils and aquatic systems (Könneke et al., 2005; Hansel et al., 2008; Tourna et al., 2008). Könneke and colleagues isolated a new strain of marine ammonia-oxidizing archaea (AOA), *Candidatus* "*N. maritimus*", which contains putative genes for all three subunits (*amoA*, *amoB*, and *amoC*) of ammonia monooxygenase, the enzyme responsible for ammonia oxidation (Könneke et al., 2005). *Candidatus* "*Cenarchaeum symbiosum*" is also shown to harbor the genes for ammonia oxidation, although a pure culture is currently not available (Hallam et al., 2006a,b). These archaeal species may contribute substantially to ammonia oxidation in marine and terrestrial environments (Horner-Devine and Martiny, 2008).

The objective of this review is to assess the current state of the science of AOA, evaluate the role of AOA in nutrient removal, and make recommendations for future research of AOA, particularly in engineered systems. Nutrient contamination remains a serious environmental issue and a threat to sustainable development worldwide. As the world faces stringent nutrient discharge regulations, a critical review on AOA and their bacterial counterparts would be beneficial to the increasing awareness of researchers of the complexity of biological nitrogen removal and nitrogen cycling in the environment.

1.1. *Ammonia-oxidizing organisms*

Increasing evidence shows that there are two major microbial groups involved in ammonia oxidation: ammonia-oxidizing bacteria and ammonia-oxidizing archaea. While there are heterotrophic ammonia oxidizers (Otte et al., 1999), chemolithotrophic AOB were long thought to be responsible for ammonia oxidization. The recent discovery of AOA in natural and engineered systems demonstrates the role of AOA in nutrient removal and global N cycling.

1.1.1. *Ammonia-oxidizing bacteria (AOB)*

Ammonia-oxidizing bacteria utilize reduced nitrogen (e.g., ammonia) as an energy source, carbon dioxide as a carbon source, and molecular oxygen as an electron acceptor. These chemolithotrophic AOB commonly belong to the *Beta-* and *Gammaproteobacteria*, including *Nitrosomonas* (Beta), *Nitrosospira* (Beta), and *Nitrosococcus* (Gamma) (Madigan et al., 2000).