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Elucidation of microbial nitrogen-transformation mechanisms in activated sludge by comprehensive evaluation of nitrogen-transformation activity

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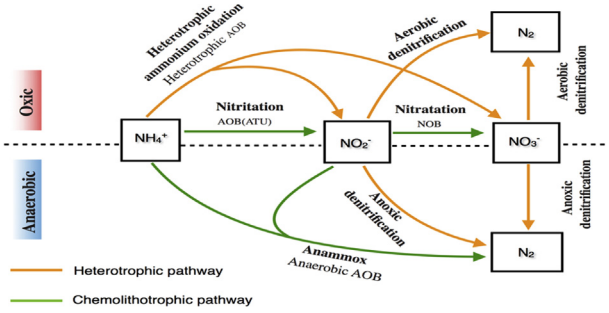
HIGHLIGHTS

- It developed a simple and reliable method to differentiate various N-transformation activities.
- Optimization of S_0/X_0 ratios reduced N-transformation activity determination errors.
- The oxygen environment and assay media effectively differentiate N-transformation activities.
- ATU addition can differentiate chemolithotrophic from heterotrophic ammonia oxidization.
- Comprehensive activity evaluation elucidated major microbial N-transformation mechanisms.

GRAPHICAL ABSTRACT

Based on the classic nitrification and denitrification activity tests, this study developed a simple, rapid and reliable method to individually determine and compare the comprehensive N-transformation activity characteristics of activated sludge by a combination of oxic/anaerobic conditions, assay media, and inhibitor addition (only ATU).



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ABSTRACT

Using prepared nitrifying sludge, anaerobic ammonia oxidization (anammox) sludge and two heterotrophic ammonia oxidization bacterial (AOB) species as inocula, this study elucidated the effect of oxygen conditions, assay media, and selective metabolic inhibitors on various microbial nitrogen (N)-transformation activities including aerobic chemolithotrophic ammonia and nitrite oxidization, aerobic heterotrophic ammonia oxidization, anammox, and aerobic and anoxic denitrification. The oxygen conditions and assay media effectively differentiated among almost all ammonia removal pathways except for separating aerobic chemolithotrophic ammonia oxidization from aerobic heterotrophic ammonia oxidization. A final allylthiourea concentration of 10 $mg \cdot L^{-1}$ was optimal for accurate determination of aerobic heterotrophic ammonia oxidization activity in the presence of aerobic chemolithotrophic AOB. Finally, this study developed a simple and reliable method to individually determine and compare the comprehensive N-transformation activity characteristics of several activated sludge samples from different origins, and to elucidate the major microbial N-transformation mechanisms for ammonia removal and N_2 production.

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1. Introduction

Biological nitrogen (N) removal (BNR) is widely used in wastewater treatment plants (WWTP) worldwide as a means to control eutrophication in natural waterbodies (Zhu et al., 2008). The conventional BNR process, called the anoxic/oxic (A/O) process, includes a combination of ammonia oxidation to nitrite (i.e. nitritation) and nitrate (i.e. nitratation) by aerobic chemolithoautotrophic nitrifying microorganisms under oxic conditions, and denitrification that reduces these nitrification products to nitrogen gas (N_2) by a large diversity of facultative chemoorganoheterotrophic denitrifying microorganisms under anoxic conditions (Hallin et al., 2005). The aerobic chemolithoautotrophic nitrifying microorganisms include two phylogenetically different groups: (i) ammonia-oxidizing bacteria (AOB) in the β and γ subclasses of proteobacteria, along with ammonia-oxidizing archaea (AOA), and (ii) nitrite-oxidizing bacteria (NOB) (Zhu et al., 2008). AOB are widely considered to be more competitive than AOA in activated sludge, and thus may function as the main contributors to chemolithotrophic ammonia oxidation (Gao et al., 2014). In the last two decades, several novel N-transformation mechanisms, including aerobic heterotrophic ammonium oxidation, aerobic denitrification, and chemolithoautotrophic anaerobic ammonia oxidation (anammox) have drastically altered our understanding of the possible N-transformation pathways occurring in wastewater treatment systems (Dapena-Mora et al., 2007). AOB that undertake aerobic heterotrophic ammonium oxidation are widely distributed across more than 10 genera, including *Pseudomonas*, *Alcaligenes, Bacillus*, *Thiosphaera*, *Comamonas*, *Microvirgula* and *Rhodococcus* (Chen et al., 2012; Li et al., 2015). In most cases, the heterotrophic AOB species can simultaneously carry out both aerobic heterotrophic ammonium oxidation and aerobic denitrification reactions, e.g. *Pseudomonas stutzeri*, *Alcaligenes faecalis*, and *P. putida* (Chen et al., 2012; Li et al., 2015). On the other hand, the reported anaerobic AOB that can perform chemolithoautotrophic anammox are limited to a few genera: *Candidatus Brocadia*, *C. Kuenenia*, *C. Scalindua* (Dapena-Mora et al., 2007), *C. Anammoxoglobus,* and *C. Jettenia* (Li et al., 2010). Fig. 1 describes the possible microbial N-transformation pathways occurring in BNR units, in which ammonia oxidation and N_2 production are two key steps in N removal from wastewater. There appear to be remarkable differences in oxygen and nutrient requirements among the three main pathways of ammonia removal (aerobic chemolithotrophic and heterotrophic ammonia oxidation, and anammox) and N_2 production (anammox, aerobic denitrification, and anoxic denitrification).

These newly discovered microbial N-transformation mechanisms have triggered the rapid development and industrial application of novel BNR processes, including simultaneous nitrification and denitrification (Sliekers et al., 2002), oxygen-limited nitrification and denitrification (Schmidt et al., 2003), and anammox (Strous et al., 1999). Because these complex microbial N-transformation mechanisms occur simultaneously in a BNR unit, a better understanding of the structure of N-transformation microbial communities and the relative contribution of different N-transformation pathways is often essential to control, improve, and optimize BNR processes. In recent decades, the rapid development of modern molecular biological techniques has enabled better elucidation of the presence, distribution, and population dynamics of AOB (Hallin et al., 2005), NOB (Ge et al., 2014), AOA (You et al., 2009), and anaerobic AOB (Schmidt et al., 2003). However, the broad phylogenetic differences among heterotrophic AOB, and aerobic and anoxic denitrifying bacterial species make it difficult to develop a genomic approach that can clarify the structure and quantity of those microbial communities in activated sludge, and these difficulties undoubtedly influence the investigations into the structure of different microbial communities involved in N-transformation. Furthermore, the relative contributions of various N-transformation pathways have still not been thoroughly explored using structure analysis of the N-transforming microbial community.

Quantification of aerobic chemolithotrophic ammonia/nitrite oxidation and anoxic denitrification rates in activated sludge have long been considered simple and reliable tools that are valuable for the measurement of potential aerobic chemolithotrophic ammonia/nitrite oxidation (PAO and PNO) and potential anoxic denitrification (PAnD) activities (Moussa et al., 2003), providing essential input data for modeling and controlling AOB, NOB, and anoxic denitrifying bacteria. Quantities of AOB and NOB are correlated with PAO and PNO activities (Cebron et al., 2003), and PAO and PNO activities can therefore be used as indices presenting the sizes of active AOB and NOB populations in environmental samples (Ke et al., 2013; Kurola et al., 2005). Based on the classic PAO, PNO,

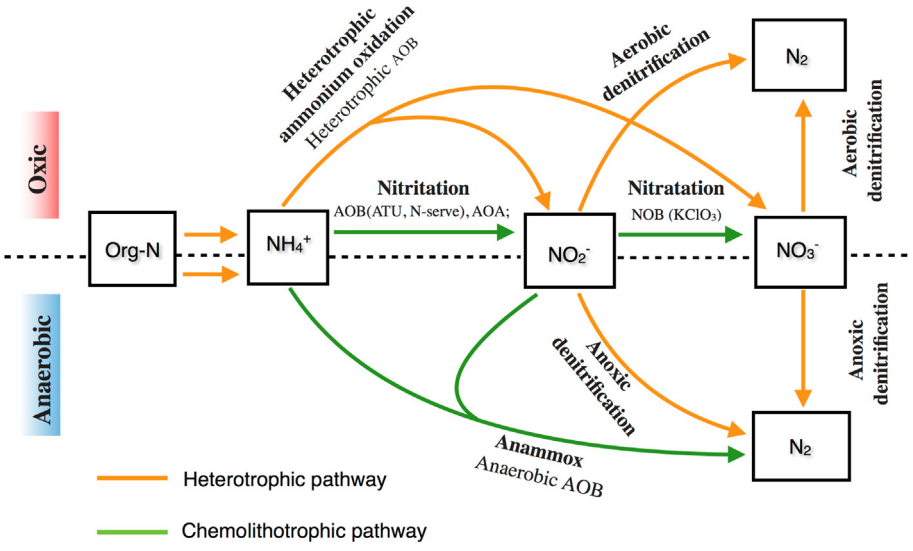

Fig. 1. Possible microbial nitrogen (N) transformation pathways occurring in biological nitrogen removal systems. Anammox: anaerobic ammonium oxidation; AOA: ammonia-oxidizing archaea; AOB: ammonia-oxidizing bacteria; NOB: nitrite-oxidizing bacteria; ATU: allylthiourea, an inhibitor of ammonia monooxygenase; N-Serve: nitrapyrin, a specific inhibitor of chemolithotrophic ammonium oxidizers; $KClO_3$: a specific inhibitor of chemolithotrophic nitrite oxidizers.

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