Bioresource Technology 256 (2018) 152–159

Contents lists available at ScienceDirect

Bioresource Technology

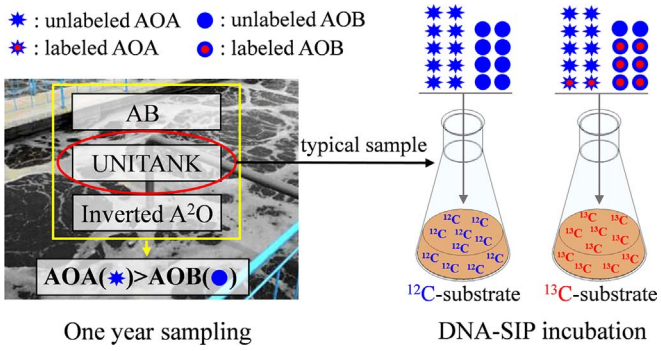
journal homepage: www.elsevier.com/locate/biortech

Ammonia-oxidizing bacteria dominate ammonia oxidation in a full-scale wastewater treatment plant revealed by DNA-based stable isotope probing

Kai-Ling Pan[a], Jing-Feng Gao[a,*], Hong-Yu Li[a], Xiao-Yan Fan[a], Ding-Chang Li[a], Hao Jiang[b]

[a] National Engineering Laboratory for Advanced Municipal Wastewater Treatment and Reuse Technology, College of Environmental and Energy Engineering, Beijing University of Technology, Beijing 100124, China
[b] Research Department of Microbiology, Allwegene Technology Co., Ltd, Beijing 102209, China

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Ammonia-oxidizing archaea
Ammonia-oxidizing bacteria
Seasonality
Treatment process
DNA-based stable isotope probing

ABSTRACT

A full-scale wastewater treatment plant (WWTP) with three separate treatment processes was selected to investigate the effects of seasonality and treatment process on the community structures of ammonia-oxidizing archaea (AOA) and bacteria (AOB). And then DNA-based stable isotope probing (DNA-SIP) was applied to explore the active ammonia oxidizers. The results of high-throughput sequencing indicated that treatment processes varied AOB communities rather than AOA communities. AOA slightly outnumbered AOB in most of the samples, whose abundance was significantly correlated with temperature. DNA-SIP results showed that the majority of AOB *amoA* gene was labeled by ^{13}C-substrate, while just a small amount of AOA *amoA* gene was labeled. As revealed by high-throughput sequencing of heavy DNA, *Nitrosomonadaceae*-like AOB, *Nitrosomonas* sp. NP1, *Nitrosomonas oligotropha* and *Nitrosomonas marina* were the active AOB, and *Nitrososphaera viennensis* dominated the active AOA. The results indicated that AOB, not AOA, dominated active ammonia oxidation in the test WWTP.

1. Introduction

Aerobic ammonia oxidation is the first and rate-limiting step of nitrogen removal in wastewater treatment plants (WWTPs), which is potentially driven by two phylogenetically distinct microorganisms named as ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). Recently, complete ammonia oxidizers (comammox) are detected in a bioreactor (van Kessel et al., 2015), their distribution in full-scale WWTPs attracts considerable attention. The distribution of AOA and AOB in WWTPs has been worldwide studied, such as Thailand

* Corresponding author.
E-mail address: gao.jingfeng@bjut.edu.cn (J.-F. Gao).

https://doi.org/10.1016/j.biortech.2018.02.012
Received 5 December 2017; Received in revised form 31 January 2018; Accepted 2 February 2018
Available online 07 February 2018
0960-8524/

(Kayee et al., 2011), Canada (Sauder et al., 2012) and China (Chen et al., 2017). Whereas the phylogenetic community structures and numerical relationships of AOA and AOB in WWTPs are still in debate. For phylogenetic diversity, several studies found that *Nitrososphaera* cluster is the dominant AOA in WWTPs (Gao et al., 2013; Yan et al., 2016), while *Nitrosopumilus* cluster is the predominant AOA in some WWTPs (Gao et al., 2014). On the other hand, *Nitrosomonas oligotropha*, *Nitrosomonas europaea* and *Nitrosomonas nitrosa* were reported as the prevalent AOB species in different WWTPs (Kayee et al., 2011; Wells et al., 2009; Zhang et al., 2015). For numerical relationships, AOB outnumber AOA in many WWTPs (Gao et al., 2013), but some studies confirmed that the abundance of AOA is higher than AOB (Kayee et al., 2011; Roy et al., 2017). Until now, factors affecting the diversity and abundance of ammonia-oxidizing microorganisms (AOMs) in WWTPs remain unclear.

The stable treatment performance of WWTPs is inevitably challenged by the change of temperature caused by seasonality. As susceptible microorganisms, AOMs tend to be easily influenced by different temperature. The community structure of AOMs has been investigated in different WWTPs as case studies, but little information can be obtained about the seasonal or temporal change of these microorganisms. Wells et al. (2009) found that the diversity of AOB in WWTPs is affected by the seasonal variation. But results of the study investigating AOB in 12 WWTPs through three seasons showed that seasonality varies their abundance instead of diversity (Limpiyakorn et al., 2005). For AOA, their abundance in two WWTPs applied biofilm process is higher in warmer months than colder months, but their communities seem to be insensitive to seasonal variation (Roy et al., 2017). In this study, we investigated the response of AOA and AOB communities to seasonal variation in a full-scale WWTPs over a year.

Many biological treatment processes are applied in engineered WWTPs to realize nitrogen removal. The study of AOMs communities in eight WWTPs applied different treatment processes, such as anoxic/oxic and oxidation ditch, found that different processes do not change numerical relationships of AOA and AOB, but affect the diversity of AOA (Kayee et al., 2011). Whereas Gao et al. (2013) found treatment process plays an important role in shaping AOA and AOB communities. However, sewage characteristics and geographic locations of WWTPs may also contribute the dynamic variation of AOMs community structure (Bai et al., 2012; Zhang et al., 2011). Little comparative studies are available about the significance of treatment process for AOA and AOB communities eliminating the influence of wastewater quality and geographic location.

Since AOA and AOB may assemble different metabolic pathways and own different ammonia oxidation kinetics, their activity and relative contribution to ammonia oxidation are significant to the design and operation of WWTPs. Mußmann et al. (2011) found that AOB can solely respond for nitrification in a refinery WWTP and AOA is not essential to nitrification. But later study found that both AOA and AOB are active in nitrification, which was tested through specific inhibitors (Roy et al., 2017). As the occurrence of AOA and AOB in WWTPs cannot stand for their nitrification activity, it is urgent to explore their relative contribution to ammonia oxidation. DNA-based stable isotope probing (DNA-SIP) is a powerful technique that links microorganisms with their metabolic functions. It can provide an insight into the active microorganisms in nitrification by tracing special substrate (e.g. ^{13}C). Until now, DNA-SIP has been applied to identify active ammonia oxidizers in agricultural soil (Jia and Conrad, 2009), creek ecosystems (Avrahami et al., 2011) and acid soil (Zhang et al., 2012). As so far, DNA-SIP is rarely reported to explore the active AOMs in complex WWTPs. Using DNA-SIP, Gao et al. (2016) determined that AOB strongly contribute to nitrification in WWTP, while AOA may be the active AOMs under 50 mg L^{-1} TiO_2 nanoparticles. However, it is still poorly understood about the activity and relative contribution of AOA and AOB to nitrification in WWTPs.

The key objectives of this study were (1) to investigate the effects of seasonality and treatment process on the diversity and abundance of AOMs in full-scale WWTPs, eliminating the influence of sewage quality and geographic location and (2) to determine the active AOMs responsible for ammonia oxidization in the WWTPs. To achieve these goals, a full-scale WWTP equipped with three separate biological treatment processes was sampled in each month over a year. The diversity and abundance of AOMs were revealed by high-throughput sequencing and quantitative real-time PCR (qPCR), respectively. Then, statistical analyses were applied to further assess the relationships between seasonality and treatment process and AOMs communities. Especially, DNA-SIP combined high-throughput sequencing was applied to determine the active ammonia oxidizers and their relative contribution to nitrification.

2. Materials and methods

2.1. Site description and samples collection

LieDe (LD) WWTP (23.22°N, 113.35°E) locates in Guangzhou city, China, which is equipped with three separate biological treatment processes. Adsorption Biodegradation (AB), UNITANK and anoxic/anaerobic/aerobic (inverted A^2O) processes are employed in 1st, 2nd and 3rd stage construction, named as LD1, LD2 and LD3, respectively. Raw wastewater passed the primary stage is introduced into three biological treatment processes, respectively. Table 1 shows the related parameters of these three processes. Activated sludge was collected from the aeration tanks of LD1, LD2 and LD3 on a routine basis each month for a year period from February 2013 to January 2014. Sludge samples were taken at three different locations at the end of aeration tanks, and the samples from the same treatment process were then mixed as one sample after briefly settling on site. Sludge samples were kept on ice-box and transported to laboratory immediately. After freeze-dried by Labconco Freezone 1 L (Labconco, USA), 0.05–0.10 g dry sludge was used to extract DNA by a FastDNA SPIN kit for soil (Qiagen, CA, USA). Totally, 35 samples were obtained in a year, excepting the sample from LD1 in January for process modification.

2.2. DNA-SIP incubation, isopycnic centrifugation and gradient fractionation

Activated sludge sample from LD2 at July with highest AOA abundance was typically selected for DNA-SIP incubation to investigate the active ammonia oxidizers. Pre-incubation was performed to consume the organic matter and ammonium in the supernatant. Then, time course DNA-SIP experiments were set up in duplicate. DNA-SIP microcosms were performed in 250 mL Erlenmeyer flasks containing pre-incubated activated sludge and synthetic wastewater either with $Na_2{}^{12}CO_3$ or $Na_2{}^{13}CO_3$ (Cambridge Isotope Laboratories, Inc., USA) as sole carbon source. To prepare the inorganic medium for DNA-SIP incubation, synthetic wastewater was provided with $Na_2{}^{13}CO_3$ (0.214 g L^{-1}) or $Na_2{}^{12}CO_3$ (0.212 g L^{-1}), NH_4Cl (0.107 g L^{-1}), NaCl (0.585 g L^{-1}), KH_2PO_4 (0.054 g L^{-1}), KCl (0.075 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (0.147 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.049 g L^{-1}) and 1 mL non-chelated trace element mixture (Cua and Stein, 2011). The microcosms were incubated at 25 °C and shaken at 100 rpm in dark. MLSS, DO, and pH were controlled at 3000 mg L^{-1}, 2 mg L^{-1} and 7.5–8.0 as *in situ*, respectively. When ammonium was degraded completely in the supernatant, one cycle of incubation ended. After briefly settling and supernatant decanting, the activated sludge samples were submerged in new synthetic wastewater to generate new experimental microcosms, respectively. At each cycle of incubation, the concentrations of ammonium, nitrite and nitrate were measured in the influent and effluent according to standard methods (APHA, 2005). At the cycles of three, six and nine, destructive sampling was taken, and the microcosms were frozen at −20 °C after centrifugation for 1 min at 5000 rpm to remove the supernatant.