Chemosphere 205 (2018) 308–316

Contents lists available at ScienceDirect

Chemosphere

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

Long-term impact of a tetracycline concentration gradient on the bacterial resistance in anaerobic-aerobic sequential bioreactors

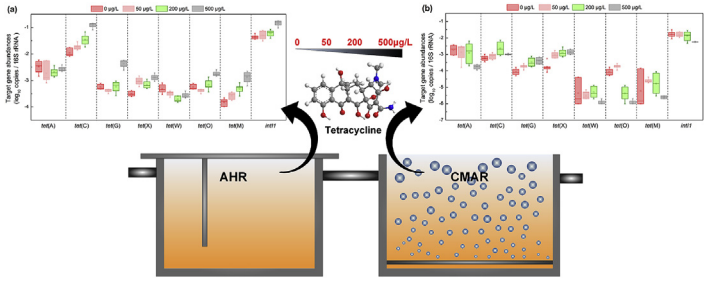
Pin Gao [a, *, 1], Wenli Xu [a], Xiaohui Ruan [a], Yajie Qian [a], Gang Xue [a], Hanzhong Jia [b, **]

[a] College of Environmental Science and Engineering, Donghua University, Shanghai 201620, China
[b] State Key Laboratory of Soil Erosion and Dryland Farming on Loess Plateau, Institute of Soil and Water Conservation, College of Resources and Environment, Northwest A & F University, Yangling 712100, China

HIGHLIGHTS

- AAS bioreactors showed promise for reducing tetracycline, *tet* and *intI1* genes.
- A tetracycline level gradient selected for bacterial resistance in an anaerobic environment.
- The abundance of *tet*(X) was largely unaffected by the AAS treatment.
- *IntI1* played a crucial role in horizontal dissemination of *tet* genes in the AAS.
- Tetracycline and *intI1* were the main factors affecting *tet* genes in the AAS.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 January 2018
Received in revised form
17 April 2018
Accepted 21 April 2018
Available online 23 April 2018

Handling Editor: A. Adalberto Noyola

Keywords:
Tetracycline
Antibiotic resistance gene
Integrase gene *intI1*
Anaerobic-aerobic sequential bioreactor
Concentration gradient

ABSTRACT

Wastewater treatment systems are considered as hotspots for release of antibiotic resistance genes (ARGs) into the environment. Anaerobic-aerobic sequential (AAS) bioreactors now are intensively used for wastewater treatment worldwide. However, the occurrence of ARGs in wastewater treatment systems exposed to low-level (i.e., sub-inhibitory) antibiotic is poorly known. Here, we studied the distribution patterns of seven tetracycline resistance genes (*tet* genes) including *tet*(A), *tet*(C), *tet*(G), *tet*(X), *tet*(M), *tet*(O), and *tet*(W), as well as one mobile element [class 1 integron (*intI1*)] in AAS bioreactors under exposure to tetracycline from 50 μg/L to 500 μg/L. Additionally, effect on the removal performance of nutrients and tetracycline in both anaerobic and aerobic units was also investigated. A tetracycline concentration gradient selected for bacterial resistance in the anaerobic reactor, with the exception of *tet*(A) and *tet*(W), and the tetracycline removal deteriorated by 47%. However, the abundance of *tet* and *intI1* genes reduced in the subsequent aerobic unit, and the removal of tetracycline, soluble COD, and NH_4^+-N maintained at average efficiencies of 91%, 90%, and 93%, respectively. The level of *tet*(X) was largely unaffected by AAS treatment. It is notable that *intI1* genes probably played a crucial role on the horizontal dissemination of *tet* genes. The tetracycline levels and *intI1* genes appear to be the primary factors influencing the occurrence of *tet* genes in AAS bioreactors. Nonetheless, AAS treatments still show promise for reducing antibiotics, ARGs and mobile elements without affecting nutrient removal, and need further research for practical applications.

* Corresponding author. 2999 North Renmin Road, Songjiang District, Shanghai 201620, China.
** Corresponding author.
E-mail addresses: pingao@dhu.edu.cn (P. Gao), jiahz@ms.xjb.ac.cn (H. Jia).
[1] Present address: Department of Microbiology and Biochemistry, Rutgers University, New Brunswick, NJ 08901, USA.

https://doi.org/10.1016/j.chemosphere.2018.04.135
0045-6535/

1. Introduction

Antibiotics have been widely used in both human and veterinary medicine. Since these active compounds are not totally metabolized by humans and animals, and are not completely removed in wastewater treatment systems (Michael et al., 2013), they are frequently found in a multitude of environmental compartments (Petrie et al., 2015; Zhang et al., 2015). There is growing evidence that antibiotic residues are in part linked to the propagation of antibiotic resistance, and cause a vast enrichment of resistance genes (Gao et al., 2012a; Lehtinen et al., 2017; Levin-Reisman et al., 2017; Qiao et al., 2018). The rapid evolution and spread of antibiotic resistance is currently an emerging concern to public health owing to the increasing occurrence and dissemination in clinical pathogenic bacteria (Martinez, 2008; Zhu et al., 2017a).

Tetracycline is one of the most extensively used antibiotics globally. More than 5900 tons of tetracycline antibiotics were sold and distributed for use in livestock in the U.S. in 2012 (USFDA, 2014), while approximately 12,000 tons were used in China in 2013 (Zhang et al., 2015). Numerous studies have reported that a large fraction of tetracycline residues were discharged in unchanged and/or active forms to wastewater at concentrations from ng/L to μg/L (i.e., domestic and animal wastewater) (Gao et al., 2012b; Wei et al., 2011), and even at mg/L levels (i.e., pharmaceutical wastewater) (Larsson et al., 2007). Nevertheless, little is known regarding their related ecological effects on the underlying processes responsible for resistance expansion in natural environments (Gu, 2014).

The consequences of tetracycline antibiotics on microbial processes for nutrient cycles or non-target microorganisms are still not well-documented (Roose-Amsaleg and Laverman, 2016). Although exposure to high tetracycline levels has been studied and reported to cause acute toxicity and to select for resistant strains (Blake et al., 2003; Cetrcioglu et al., 2013; Rysz et al., 2013; Shi et al., 2011), unknowns regarding long-term exposure to low-dose (i.e., sub-inhibitory) tetracycline antibiotic remain. Recently, Zhang et al. (2014) reported that antibiotic resistance response increased with increasing tetracycline levels (from 25 μg/L to 125 μg/L). Additionally, several studies demonstrated that tetracycline at μg/L level exerted selection pressures on the evolution of microbial communities and enrichment of tetracycline resistance genes (*tet* genes) in anaerobic or aerobic sequencing batch reactors (SBRs) (Aydin et al., 2015a; Zhang et al., 2013). For example, Matos et al. (2014) found that the bacterial community shifted with exposure to 50 μg/L of tetracycline in sequencing batch biofilm reactors, but the organic compound biodegradation and nitrification activity were not affected. The combined effect of antibiotic mixtures containing tetracycline on performance of anaerobic SBRs was investigated by Aydin et al. (2015d). The majority of studies have generally focused on the effect of tetracycline or mixtures on nitrifying microorganisms due to their sensitivity to various toxic substances, and SBRs were commonly used as model systems owing to their simplicity, operation convenience, and small footprint. SBRs are particularly attractive for treating small wastewater flows (Jafarinejad, 2017). By contrast, anaerobic-aerobic sequential (AAS) bioreactors are more extensively employed in industrial and municipal wastewater treatment because of reduced energy use, low capital cost, and possible biogas production (Chan et al., 2009; Gao et al., 2016; Kassab et al., 2010). A recent study suggested that the AAS system appeared to be superior to other treatment opinions for mitigating the dissemination of antibiotic resistance genes (ARGs) in addition to have lower energy consumption and better nutrient removal rates (Christgen et al., 2015). However, it is not yet known how AAS reactors affect the fate and distribution behavior of ARGs under long-term exposure to low-level of antibiotics.

The main objective of this study was to investigate the chronic impact of a tetracycline concentration gradient on the distribution patterns of *tet* genes, including three efflux pump genes [*tet*(A), *tet*(C), *tet*(G)], three ribosomal protection genes [*tet*(M), *tet*(O), *tet*(W)], and one enzymatic modification gene [*tet*(X)], as well as integrase gene of class 1 integron (*intI1*) in AAS bioreactors treating synthetic domestic wastewater. Tetracycline was selected because it has been widely used and frequently detected in wastewater (Michael et al., 2013). Also, the nutrient removal performance, such as soluble chemical oxygen demand (sCOD) and ammonia nitrogen (NH_4^+-N), and tetracycline removal was evaluated.

2. Materials and methods

2.1. Reactor setup and operation

Reactor configurations are shown in Fig. S1. The initial AAS system included an anaerobic hybrid reactor (AHR) and a completely mixed aerobic reactor (CMAR). The constituents of the synthetic wastewater contained: 206.4 mg/L glucose, 256.0 mg/L $CH_3COONa \cdot 3H_2O$, 35.4 mg/L $(NH_4)_2SO_4$, 11.1 mg/L K_2HPO_4, 14.2 mg/L $CaCl_2$, 0.21 mg/L $MgSO_4 \cdot 7H_2O$, 0.225 mg/L $FeCl_3$, 0.018 mg/L $MnCl_2 \cdot 4H_2O$, 0.023 mg/L H_3BO_3, 0.018 mg/L $ZnSO_4 \cdot 7H_2O$, 0.005 mg/L $CuSO_4 \cdot 5H_2O$, 1.5 mg/L EDTA, and 0.027 mg/L KI. The final wastewater characteristics were as follows (mean ± standard errors): sCOD = 415 ± 21 mg/L, NH_4^+-N = 7.5 ± 0.4 mg/L, and pH = 7.3 ± 0.1. Wastewater was fed using peristaltic pumps (Huxi HL-5, Shanghai, China) into the AHR and CMAR successively. Effluent from the CMAR was pumped into a sedimentation tank for sludge-liquid separation.

The AHR was packed with fixed fiber media. The reactor had a working volume of 12 L, and was operated at a hydraulic retention time (HRT) of 8 h. The sludge recirculation flow ratio for the AHR reactor was 100%. Wastewater influent was introduced to the AHR unit and mixed via hydraulic agitation. The dissolved oxygen (DO) level was measured below 0.1 mg/L. The operation temperature was maintained at 35 ± 0.8 °C and pH was controlled in the range of 7.0–7.4.

The CMAR had a working volume of 15 L, and was operated at 10 h HRT. Air was introduced using aerated pipes to maintain the dissolved oxygen (DO) concentration between 3.5 and 4.5 mg/L, and also to provide mixing. The CMAR was maintained at room temperature of 20 ± 1 °C.

The operation of the AHR and CMAR system included a start-up period of around 40 days for acclimation and establishment of a steady state in accordance to the sCOD and NH_4^+-N removal. Then, to investigate the tolerance and resistance selection of the microbial communities, the AAS was operated over a duration of 87 days under steady-state conditions in a sequence of three stages with gradually increasing tetracycline doses from 50 μg/L to 500 μg/L. During the first stage (stage I) of days 41–72, the influent tetracycline dose was maintained at 50 μg/L. At stage II (days of 73–112), the tetracycline dose was increased to 200 μg/L, and further to 500 μg/L at stage III (days 113–127). The tetracycline stock solution was prepared in methanol and stored in the aluminum foiled 100 mL Kimax bottle.

2.2. Sample collection and DNA extraction

For each sampling event, duplicate wastewater effluent samples from both AHR and CMAR were collected and processed for DNA extraction. Approximately 500 mL each of the effluent samples from both units were filtered through a 0.22 μm nitrocellulose membrane, and the trapped biomass was collected to extract genomic DNA using the TIANamp Soil DNA extraction kit (TIANGEN,