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Deciphering the factors influencing the discrepant fate of antibiotic resistance genes in sludge and water phases during municipal wastewater treatment

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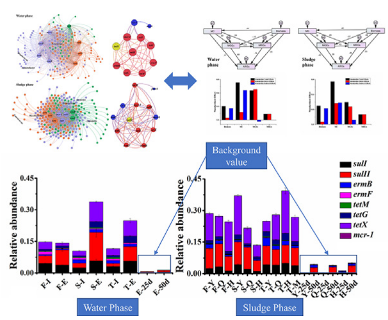
GRAPHICAL ABSTRACT



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ABSTRACT

The discrepant fate of antibiotic resistance genes (ARGs) in sludge and water phases was investigated in a municipal wastewater treatment plant, and a lab-scale A^2O-MBR was operated to provide background value of ARGs. The influencing factors of ARGs including microbial community, co-selection from heavy metals, biomass and horizontal gene transfer were concerned. Results showed that iA^2O (inversed A^2O) showed better ARGs reduction, and longer SRT (sludge retention time) increased ARGs relative abundance while reduced the gene copies of ARGs in the effluent, but significantly increased the ARGs in sludge phase. Compared to background value, the most enriched ARG was *tetX* in water phase, while it was *intI1* in sludge phase. There existed higher abundance of multi-resistant bacteria in sludge phase, and microbial community determined the fate of ARGs in both water and sludge phase, while the direct effects from horizontal gene transfer should not be overlooked especially in water phase.

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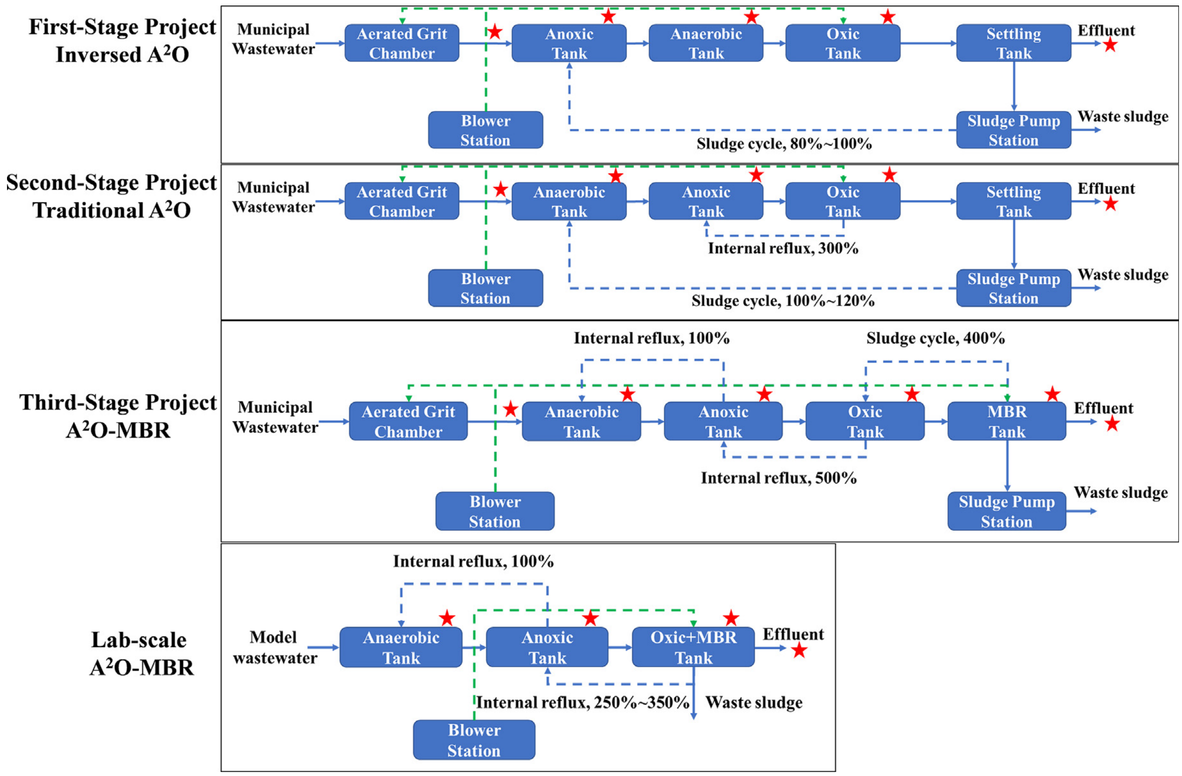

Fig. 1. Process flow diagrams and sampling points (★) in this study.

1. Introduction

Antibiotic resistance genes (ARGs), the culprit of bacterial resistance to antibiotics, are gradually considered to be an emerging pollutant in the environment (Pruden et al., 2013, 2006). Human overuse of antibiotics, which are released in the form of municipal sewage , contributes to the problem, and thus, municipal wastewater treatment plants (WWTPs) have become one of the most important reservoirs of ARGs (Auguet et al., 2017; Nnadozie et al., 2017; Zhang and Zhang, 2011). However, WWTPs are designed to remove nutrient substances such as organic carbons, nitrogen and phosphorous excluding the ARGs and antibiotics (Rafraf et al., 2016; Su et al., 2017). It is even indicated that the conditions in WWTPs are favorable for the proliferation of antibiotic-resistant bacteria (ARB) as well as the mobilization of ARGs (Luo et al., 2014), but the fate of ARGs could differ depending on process configuration and operating conditions (Bougnom and Piddock, 2017; Bouki et al., 2013; Mao et al., 2015; Tang et al., 2016).

Anaerobic-anoxic–oxic (A^2O) is the commonly used process in WWTPs, and it is estimated that half of WWTPs adopt A^2O process in China (Peng et al., 2006). Generally, there exist two kinds of A^2O process, traditional A^2O (Anaerobic → anoxic → oxic, tA^2O) and inversed A^2O (Anoxic → anaerobic → oxic, iA^2O), and iA^2O process is designed to accomplish better nitrogen and phosphorous removal (Qi et al., 2012). The membrane technology is generally adopted following the A^2O process due to the well-recognized advantages, such as better and stable effluent quality, less excess sludge production and flexible installation (Yang et al., 2016). The comparison of these processes on the removal of traditional contaminants has been widely investigated (Zhang et al., 2011), whereas there is little information on the comparative analysis of ARGs fate. Besides, the background value of ARGs without selective pressure from antibiotics in these processes also need to be answered, which could guide the ARGs reduction in WWTPs.

Furthermore, operational parameters have significant impacts on the efficiency of these biological processes, and one of the critical operational parameters is the sludge retention time (SRT) which represents the mean residence time of microorganisms in the biological reactors (Neyestani et al., 2017). Potentially due to more abundant biomass and/or shifts in microbial community structure, longer SRT have been shown to better reduction of total organic carbon and trace organic compound concentrations including antibiotics (Gerrity et al., 2013; Vuono et al., 2016). Nonetheless, studies that directly evaluate the relationship between SRT and the prevalence of ARGs are limited (Neyestani et al., 2017).

It is generally considered that residual sludge contributed higher amounts of ARGs into the environment than the effluent in WWTPs (Mao et al., 2015; Munir et al., 2011), and there existed significantly different microbiome in the sludge phase and water phase. The mechanisms of ARGs proliferation in water phase and sludge phase could be divergent, but such information is limited. Besides, there existed many factors influencing the ARGs distribution, e.g., environmental variables, horizontal gene transfer (HGT) through mobile genetic elements (MGEs), co-selection from heavy metals, microbial community composition and biomass, but which dominated the ARGs distribution varied from the environments (Mao et al., 2015; Zhu et al., 2017). It would be helpful to make clear the separate contribution to the ARGs distribution in the two phases.

Thus, the fate of ARGs in the sludge and water phases in a full-scale WWTP consisting of iA^2O, tA^2O and A^2O-MBR (membrane bioreactor) was investigated, and a lab-scale A^2O-MBR treating model municipal wastewater was established to elucidate the background value of ARGs at different SRTs. The aims of this study were to 1) compare the occurrence of ARGs in sludge and water phases between process configurations; 2) figure out the potential background value of ARGs in the WWTPs; 3) clarify the main contributors to the ARGs distribution in sludge and water phases, respectively.