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Comparative network analysis revealing the mechanisms of antibiotic resistance genes removal by leachate recirculation under different hydraulic loadings

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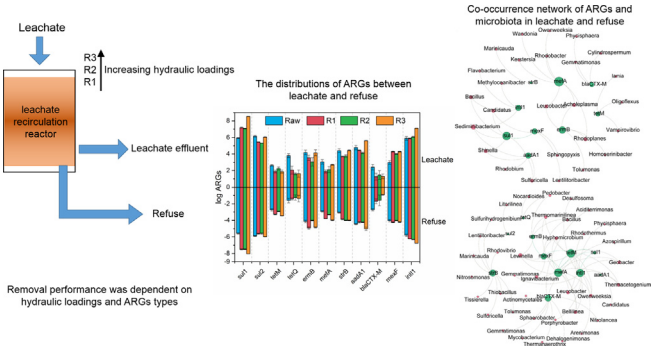
HIGHLIGHTS

- ARGs removal was affected by hydraulic loadings and ARGs types.
- Higher hydraulic loadings decreased ARGs adsorption and degradation capacities.
- More genera were closely correlated with ARGs occurrence in refuse than leachate.
- Taxonomic category other than diversity of host genera shaped the ARGs removal.
- Refuse had stable resistome and community structure despite varied conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

The wide dissemination of antibiotic resistance is a pervasive global health threat, and landfill leachate has been an important hotspot of antibiotic resistance genes (ARGs). This study aimed to investigate the removal performance and mechanism of ARGs from leachate under different hydraulic loadings. ARGs removal efficiencies were dependent on hydraulic loadings and ARGs types other than operating time, and reactors operated with hydraulic loadings of 25 and 50 $L \cdot m^{-3} \cdot d^{-1}$ exhibited greater removal potential than 100 $L \cdot m^{-3} \cdot d^{-1}$. ARGs removal patterns varied from different subtypes, for genes *sul*2, *tet*Q, *aad*A1 and $bla_{CTX\text{-}M}$ were eliminated from both leachate and refuse, and *tet*M, *erm*B, *mef*A, and *str*B were removed from leachate but enriched in refuse. Under different hydraulic loadings, bacterial communities shift shaped ARGs fates in leachate, but refuse had more stable antibiotic resistome and community structure. The topology comparison analysis of co-occurrence network suggested a closer hosting relationship between ARGs and genera in refuse than leachate. Furthermore, taxonomic category of host bacteria other than diversity of host genera determined the ARGs removal, and the ARGs harbored in phyla Cyanobacteria, Tenericutes and Acidobacteria were more likely to be removed. These findings can potentially foster the understanding of ARGs removal mechanism in biological treatment processes under different operating conditions.

1. Introduction

Extended and excessive use of antibiotics for clinical, animal farming, and agricultural applications has caused the continuous antibiotic release into environments and subsequently raised the widespread

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occurrence of antibiotic resistance in environmental and human commensal microbes (Levin-Reisman et al., 2017; Sommer et al., 2009). The increasing prevalence of antibiotic resistance genes (ARGs) has become a serious threat to public health, and the property of horizontal gene transfer (HGT) across bacteria further accelerated ARGs dissemination (Martínez et al., 2015). Until now, ARGs and antibiotic resistance bacteria (ARB) have been widely found in natural and engineered ecosystems such as soil, surface water, and wastewater treatment plants (Guo et al., 2017; Pruden, 2014; Udikovic-Kolic et al., 2014). Among these environment matrices, landfill is strongly affected by human activities for sanitary landfill is the most common method of municipal solid wastes disposal. Due to the anthropogenic activities, discarded pharmaceuticals and personal care products caused the increased residual antibiotics in landfill, which further prompt ARGs dissemination in the landfill system. The mobile leachate, being generated during the landfilling process, would be an important hotspot for dissemination of ARB and ARGs (Graham et al., 2011; Wu et al., 2015). Therefore, to reduce the potential risk to adjacent environmental matrices, extensive attention should be paid on the ARGs profile during leachate treatment.

Landfill leachate treatment was a challenge due to the high and variable concentrations of dissolved solids, organics, heavy metals and xenobiotic organics, and conventional processes (such as activated sludge treatment and anaerobic digestion) exhibited limited potential for leachate treatment due to the low biodegradability. Several leachate treatment strategies were used to deal with leachate, including aerobic/anaerobic biological process, chemical oxidation, adsorption and membrane process. Compared to other chemical and physical methods, leachate recirculation has been largely used in the past decades as a prevailing alternative leachate management option due to a range of manipulation and management purposes such as the least expensive option available (Renou et al., 2008). Our previous publication had proved that leachate recirculation by the use of aged refuse could serve as a feasible and effective alternative for nutrient pollutants and ARGs removal from leachate (Su et al., 2017), but the removal patterns and mechanism were still intangible. The dissemination of ARGs were considered to be affected by a variety of factors, such as nutrient element, heavy metal, and antibiotics (Li et al., 2017; Wu et al., 2015; Zhang et al., 2016c). In biological process, operating parameters played crucial roles in the conventional and emerging pollutants removal. For example, wetland substrates, plant species and hydraulic loadings determined the ARGs removal performances in constructed wetlands (Chen et al., 2016a; Chen et al., 2016b). Hydraulic loadings were crucial parameters influencing the operating status of bioprocesses, as well as pollutants removal and microbial community (Li et al., 2008; Xie et al., 2012). The current information regarding the effects of hydraulic loadings on ARGs removal is limited. Therefore, further study is required to understand the elimination performance of ARGs in leachate under different hydraulic loadings, especially considering the fluctuant flux of landfill leachate.

Concerning the potential ecological risk of ARGs, recent studies have focused on the removal of ARGs from different environmental matrices. For wastewater, ARGs reductions of 1–3 and 0.6–1.2 orders of magnitude were observed for the advanced treatment processes and constructed wetland respectively (Chen and Zhang, 2013). Previous studies have investigated the removal of ARGs in waste sludge, by pH control, microwave pretreatment, bio-drying and addition of natural zeolite (Huang et al., 2017; Tong et al., 2016; Zhang et al., 2016a; Zhang et al., 2016b). Despite the effective removal of ARGs, the mechanism of ARGs elimination by biological processes remained ambiguous for the driving factors determining ARGs dissemination were uncertain. For example, bacterial community was usually considered to drive the ARGs behaviors (Jia et al., 2015), but others found the disagreement between ARB and ARGs profiles for complete removal of ARB but incomplete removal of ARGs was observed in wastewater treatment (Lamba and Ahammad, 2017). In biological treatment processes, complex conditions and diverse microbiota might shape the ARGs by several pathways. Absorption might be responsible for the removal of ARGs in wetlands (Chen et al., 2016a). In leachate recirculation system, aged refuse was rich in a great diversity of microbiota including pathogenic and functional bacteria, and also had high adsorption capacity of pollutants (Chai and Zhao, 2006). In addition, the ARGs harbored in refuse and leachate might exchange each other, and causing the ARGs spread into environments. However, seldom publications had investigated the ARGs removal by analyzing the relationship between effluent and solid matrix. Given these facts, the mechanism of ARGs removal from leachate should be revealed from various aspects based on the properties of refuse.

In the present study, lab-scale bioreactors were constructed by the use of aged refuse to simulate leachate recirculation, and the removal efficiencies of ARGs under various hydraulic loadings were evaluated. The distributions of ARGs in leachate and refuse were investigated and the similarity/difference of ARG compositions among different leachate and refuse samples was analyzed using non-metric multidimensional scaling (NMDS) analysis. The microbial community shift driven by hydraulic loadings was conducted with principal co-ordinates analysis (PCoA), and comparative co-occurrence network analysis between ARGs and bacteria in leachate and refuse were used to reveal the removal mechanism of ARGs. The outcome will provide important information for the optimization of ARGs removal from leachate and the elimination mechanisms of biological treatment processes.

2. Materials and methods

2.1. Operation of simulated leachate recirculation bioreactor of lab-scale

To simulate the leachate recirculation treatment, the cylindrical refuse bioreactors with available volume of 2 L were built in the lab, and a layer of gravel was placed at the bottom to ensure good drainage. Prior to fill the reactors, aged refuse from Shanghai Laogang Landfill was shredded into particles. The characteristics of aged refuse used in this study were as follow: volatile suspended solids percent of 9–14%, moisture content of 14–17%, and density of 1.48–1.79 g/cm^3. The stabilized leachate (>10 years) from Shanghai Laogang Landfill was fed to the reactors, and the characteristics were determined according to literatures (Xie et al., 2010; Xu et al., 2016) and shown in Table S1. The reactors were operated at three different loadings 25, 50 and 100 $L \cdot m^{-3} \cdot d^{-1}$, and the operational condition was kept at 20 ± 2 °C. The leachate recirculation of field reactors was generally operated at 25 $L \cdot m^{-3} \cdot d^{-1}$, which was set as the regular hydraulic loading, and 50 and 100 $L \cdot m^{-3} \cdot d^{-1}$ were used for simulating shock loadings.

For water quality analysis, the samplings were conducted every 4 days, and the filtrate was gotten by 0.45 μm membrane (NCM, Whatman, UK). After the reactors run stably for 30 days, the samples were taken every two weeks for heavy metal and ARGs detections. The operational performance of nutrient elements removal was shown in Fig. S1 (Supplementary material). The measurement of heavy metal was conducted in a inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Thermo Scientific-iCAP6300, Waltham, MA), and the procedure was according to previous publication (Wu et al., 2015).

2.2. DNA extraction from samples and ARGs quantification

Total DNA was extracted from leachate and refuse using a PowerSoil DNA Isolation kit (MoBio, USA) according to the manufacturer's instruction. The concentration of DNA was measured using a SMA4000 Spectrophotometer (Merinton, China), and then the extracted DNA was stored at −20 °C until use.

According to the survey, antibiotics sulfonamides, tetracyclines, macrolides, and β-lactams shared >60% of the total usage, and also were frequently detected in the environments (Zhang et al., 2015), so the corresponding ARGs were investigated in the current study. The