



Changes of quinolone resistance genes and their relations with microbial profiles during vermicomposting of municipal excess sludge



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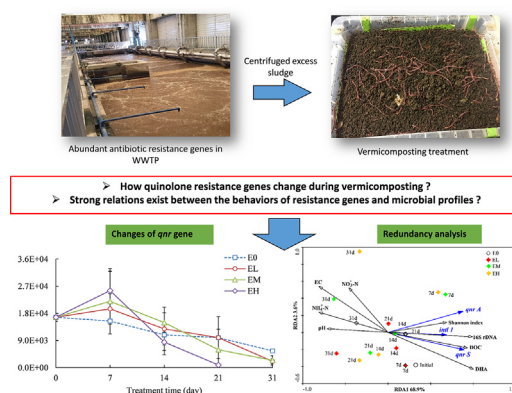
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HIGHLIGHTS

- *qnr* A, S genes and microbial profiles were determined during the vermicomposting of municipal excess sludge.
- Abundance of *qnr* A and *qnr* S genes were significantly attenuated by 85.6–100% and 92.3–95.3%, respectively.
- Vermicomposting promoted removal of quinolone resistance genes via enhancing decreases of bacterial abundance and activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Antibiotic resistance genes abundant in municipal excess sludge reduce the agricultural value of vermicompost. However, little attention has been paid on the fate and behavior of the problem-causing agents in vermicomposting. In this study, the fate and behavior of quinolone resistance genes in excess activated sludge during vermicomposting were studied with reactors introduced with *Eisenia fetida* for three different densities. The substrate pile without earthworms was operated as control in parallel. The results showed that earthworms could significantly reduce the absolute abundance of quinolone resistance genes in the excess sludge, with a reduction ratio of 85.6–100% for *qnr* A and 92.3–95.3% for *qnr* S, respectively ($p < 0.05$). For microbial profiles, both the dehydrogenase activity and the abundance of microbes (16S rDNA) revealed a distinct decreasing trend after 7 days from the start of the experiment; however, the bacterial diversity in the final products seemed to be enriched with the emergence of the uncultured *Flavobacteriales* bacterium and uncultured *Anaerolineaceae* bacterium. Redundancy analysis revealed clearly that the *qnr* genes had positive correlations with the targeted indexes of microbial profiles, with the correlations with the bacterial abundance and dehydrogenase activity being more statistically significant than the bacterial diversity ($p < 0.05$). The results of this study suggested that earthworms could promote the attenuation of quinolone resistance genes in the excess sludge through

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lowering the bacterial abundance and activity, and the promotion effect could be enhanced by increasing the density of earthworms.

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1. Introduction

The discharge of large amounts of pharmaceuticals into the environment has caused increases of bacterial resistance, one of the most concerned health threats in the world (UNEP, 2017). Anthropogenic compounds like antibiotics contained in wastewater can hardly be eliminated in biological wastewater treatment process. According to the documented report (Mao et al., 2015), many of the antibiotics are transferred from wastewater to the excess sludge, a major byproduct of the municipal wastewater treatment plants, and the abundant and diverse microorganisms in the sludge could facilitate the formation and spread of resistant bacteria. Consequently, excess sludge is becoming the hotspot and reservoir for the existence of antibiotic resistance genes (Stalder et al., 2012; Kaplan et al., 2013; Rizzo et al., 2013; Xu et al., 2015) and also for the spread of the genes to water and soil environments, thus causing adversary impacts on the environments and agricultural activities. Among the reported antibiotic resistance genes in excess sludge, quinolone resistance genes (*qnr A* and *qnr S*) have been widely detected due to the overuse of quinolones (Jia et al., 2012; Kaplan et al., 2013; Xu et al., 2015). Therefore, it is necessary to take proper measures to eliminate the harmful effects and risks posed by the quinolone resistance genes existed in excess sludge.

Biological treatment methods for sludge, such as anaerobic digestion, aerobic composting and biological drying, generally reveal a better performance in stabilizing excess sludge. Also, they may demonstrate significant influences on the fate and behavior of antibiotic resistance genes during the treatment process. For instance, the study of bio-dry for sludge showed that many antibiotic resistance genes could be attenuated significantly by improving the aeration strategy. However, there are also many genes, like *ermF*, *sullI* and *tetX* genes, whose concentrations can get increased due to the enrichment of bio-copper (Zhang et al., 2016). Su et al. (2015) found that the abundance and diversity of resistance genes as well as the integrase genes increased significantly after the composting treatment of excess sludge. Comparatively, anaerobic digestion could significantly lower the abundance of tetracycline resistance genes in excess sludge, whereas, aerobic digestion showed only a slight effectiveness in eliminating the resistance genes (Diehl and Lapara, 2010). Overall, these approaches have demonstrated their impacts on the fate of antibiotic resistance genes in excess sludge, depending on the types and abundance of the genes as well as the composition of microbial communities.

Vermicomposting is a traditional biochemical method for treating organic wastes through the joint action of earthworms and microorganisms (Bhat et al., 2013). Many types of organic wastes such as agricultural waste (Sharma and Garg, 2018), livestock manure (Monroy et al., 2009), fruit and vegetable wastes (Huang et al., 2017) as well as excess sludge (Suthar and Singh, 2008; Fu et al., 2015; Villar et al., 2016) have been studied for treatment by vermicomposting. As a green technology, vermicomposting is effective for treatment of excess sludge, and so far, many studies focusing mainly on the treatment efficiency under different conditions have been conducted, together with the studies for evaluation of the agricultural value of the final products when used as bio-fertilizers (Fu et al., 2015; Varma et al., 2015; Nigussie et al., 2016; Villar et al., 2016; Malińska et al., 2017; Bhat et al., 2018). However, there are few studies that have attempted to evaluate the fate and behavior of antibiotic resistance genes in vermicomposting treatment of excess sludge.

It is well known that even in vermicomposting, microorganisms still involve as the main driving force for stabilization of organic wastes (Sen

and Chandra, 2009; Ravindran et al., 2015). Earthworms could modify the bacterial abundance and diversity (Fu et al., 2015; Villar et al., 2016) and, at the same time, their competition for carbon source could also reduce the growth and activity of microorganisms. The direct consumption of microorganisms by earthworms also adds to the reduction of microbial biomass at the end of the treatment of sludge (Aira and Domínguez, 2008; Villar et al., 2016). Accordingly, when the fate and behavior of antibiotic resistance genes in vermicomposting of excess sludge are concerned, specific attention should be paid to the microorganisms involved in the treatment, including their activity, abundance and diversity, although the involvement and effects of such factors as temperature, aeration, earthworm's metabolic products (cast and mucus) are also important.

In this study, targeting on *qnr A* and *qnr S*, two well-detected quinolone resistance genes in excess sludge, the changes of the resistance genes in vermicomposting treatment of excess sludge were investigated through vermicomposting experiment with reactors added with three different densities of earthworms. Meanwhile, by analyzing the time profiles of the microbial activity, bacterial abundance and diversity, the changes of microbial profiles in the vermicomposting treatment were also evaluated. Moreover, based on the results of redundancy analysis, for which the environmental factors (including pH, electrical conductivity, dissolved organic carbon, ammonium and nitrate) and the integrase gene that transmits resistance genes were also used as variables, the relationship of the quinolone resistance genes with the microbial profiles during vermicomposting treatment of excess sludge was also discussed.

2. Materials and methods

2.1. Earthworm and sludge

Eisenia fetida, a common epigeic earthworm species used for vermicomposting, was purchased from Agricultural Management Institute, Co. Ltd., Japan. Earthworms with an individual weight of 0.3–0.4 g were selected for use in the experiment. Before inoculating into excess sludge, all earthworms were washed with sterilized water and then cleaned with wet filter papers inside petri dishes. Filter papers were replaced every 8 h until no intestinal excrement was observed (normally two days were required).

Excess sludge was collected from a wastewater treatment plant that treats municipal wastewater from Gifu-city, Japan using the conventional anaerobic/aerobic biological treatment process. In order to reduce water content, the collected fresh sludge was immediately centrifuged at 3500 rpm for 10 min after transported to the laboratory. The settled sludge after centrifuging was spread out under room temperatures (Fig. S1 in Supplementary material) and was turned over every 8 h for further reduction of the water content. This took two days for the sludge to reach the expected water content of about 85% for direct use as the substrate for vermicomposting treatment. The physicochemical properties of the sludge after reduction of the water content are shown in Table 1.

2.2. Vermicomposting process

Vermicomposting treatments and the control treatment (treatment without earthworms) were established simultaneously. Plastic bins with a size of 22 cm × 15 cm × 9 cm for each were used as the reactors. As for the vermicomposting treatments, earthworms with designated

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