

Pollution Characteristics of Typical Antibiotic Resistance Genes in Sludge Treatment Wetlands

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Abstract. The in-situ static box method was used to conduct a comparative experiment on sludge treatment wetlands (STWs). STW1 had ventilation structure, without reeds and STW2 had ventilation and was planted with reeds. The absolute abundance of sulfonamide, tetracycline and macrolide antibiotic resistance genes (ARGs) in the two STWs were analyzed, and the paper discussed pollution characteristics of typical antibiotic resistance genes in the two STWs. The results showed that three ARGs, sul1 (sulfonamides), tetC (tetracyclines), ermF (macrolides), were detected in STW1 and STW2. The concentration of arginine was sul1 > tetC > ermF. The concentration level of ARGs in STW1 and STW2 was lower in bottom layer than that of surface layer sludge. The removal efficiency of ARGs in the same system was tetC > sul1 > ermF, and the removal efficiency of surface sludge and bottom sludge in different systems was STW2 > STW1. Planting wetland plants in the STW can promote the removal of ARGs.

1 Introduction

As a new type of pollutant, antibiotic resistance genes (ARGs) is persistent in environmental media, and can be transferred and transmitted among different bacterial populations through gene level transfer [1]. The development and spread of microbial drug resistance will be an important public-health issue. Although the pollution sources of ARGs are diversified and complex, one of the main reasons for its rapid spread in the environment is the antibiotic residues of man-made wastes. The resistance selection pressure of manure agriculture to soil microorganisms and horizontal gene transfer make the diffusion and transfer of resistance genes continue [2]. Human production and life style have changed the distribution and concentration of resistance genes in sediment, soil and other environmental media [3,4]. The main sources and pathways of resistance genes in soil environment are shown in Fig.1. The wastewater from medical treatment, industry, aquaculture and daily life is the main medium for the transmission of resistance genes in the environment. Some resistance genes enter the sewage treatment plant in the form of parent or metabolites and transfer to the soil environment with the way of sludge farming, and some are directly discharged into the environment. Even if the resistant bacteria can be effectively killed and wiped out, when the DNA released into the environment combines with humus and other substances, it will escape the vicious circle of accounting enzyme degradation and cause resistance gene pollution [5]. The removal of resistance genes in existing sludge treatment processes and sewage treatment technologies is limited, and the vast majority

of resistance genes enter the soil environment through agricultural utilization, thus causing pollution to plants. A large number of resistance genes in the environment pose a threat to human health through enrichment and amplification of the food chain.

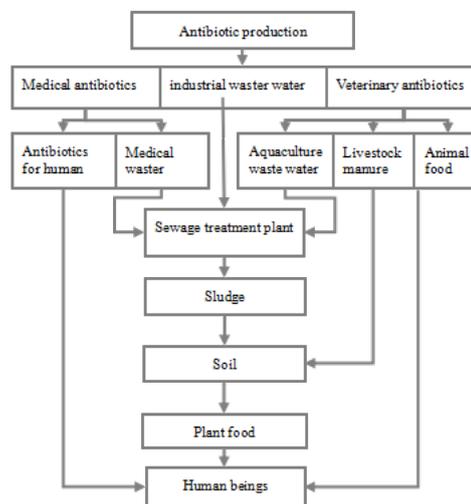


Fig.1. Transmission of antibiotic resistance genes in soil

Sludge treatment wetlands (STWs) is a kind of sludge resource utilization technology which uses constructed wetland to dewater and stabilize the excess sludge of urban sewage treatment plant [6]. STWs has the advantage of low investment is without chemical agent, and the final product after treatment can be used in agriculture [7]. In this study, fluorescence quantitative PCR was used to determine the distribution and variation

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of typical resistance genes in different systems in sludge treatment wetlands. The distribution differences of resistance genes in different sludge treatment wetlands analysis, and the dynamic succession law of resistance gene distribution with time and environmental factors in sludge treatment wetlands was explored. Further strengthen the understanding of the mechanism, design, optimization and management of STWs, provide a scientific basis for the establishment of resistance gene safety evaluation and early warning system in soil ecological environment, and have important theoretical and practical significance in ecological environment protection and human health protection.

2 Materials and methods

2.1 Wetlands system and sampling

The current research system was located in the Dakai WWTP, Dalian, China, and the remaining sludge after the sewage treatment by the activated sludge method was stabilized. The treatment line consists of two sludge pumps, a cloth sludge distribution tank as pretreatment, three vertical subsurface flow (VF) constructed wetlands (3.0m × 1.0m × 1.3m (L × B × H)) in parallel, and finally drainage system. Except for the basement layer, the three systems were totally different in structure (only STW1 and STW2 were analyzed in this study); STW1 and STW2 had ventilation structure, and STW2 and STW3 was planted with reeds.

The sampling time was from April to November, evenly distributed in spring, summer, autumn and winter. The sludge of STW1, STW2 and bottom sludge were collected and mixed to reduce the error of single sampling. The sludge samples were stored in a low-temperature ice box and transferred to the laboratory for ARGs detection at -80 °C.

2.2 Antibiotic resistance genes detection

The genomic DNA of sludge samples was extracted by FastDNA® Soil Rotation Kit, and the concentration and content of DNA were measured by gel electrophoresis and spectrophotometer. The absolute abundance of sulfonamides resistance genes, tetracyclines resistance genes and macrolide resistance genes (sul1, tetC, ermF) was determined by qPCR.

3 Results and discussion

3.1. Distribution of three antibiotic resistance genes in sludge treated wetlands

In order to study the pollution characteristics of ARGs in STWs, we analyzed the absolute abundance of three ARGs (sul1, tetC, ermF) during sludge stabilization in the resting period in STWs, as shown in Fig.2. Generally, three kinds of ARGs were detected in STW1, STW2 surface sludge and bottom sludge, among the ARGs, sul1 showed the highest concentrations, followed

by tetC, and the abundance of ermF was lower in two STWs. This is consistent with previous research results. Ma et al. Analyzed that the concentration of sul1 is the highest among ARGs in sludge stabilization period 5, followed by tetC, sul2, TetA and ermB [8].

According to the results of Fig.2., the absolute abundance of ARGs in surface sludge was higher than that in bottom sludge at the same period in STW1 and STW2. It may be that although the ventilation pipe was set up, the oxygen content in the bottom sludge was always lower than that in the surface sludge, and the hypoxia environment prevents the growth of some bacteria, thus promoting the reduction of ARGs. This is consistent with the study that Miller et al. Anaerobic environment can reduce the abundance of resistance genes to a certain extent [9]. In both STW1 and STW2, ARGs decreased the most from June to September, which was due to the higher system temperature in summer, and consistent with previous studies. High temperature can promote the removal of ARGs [10].

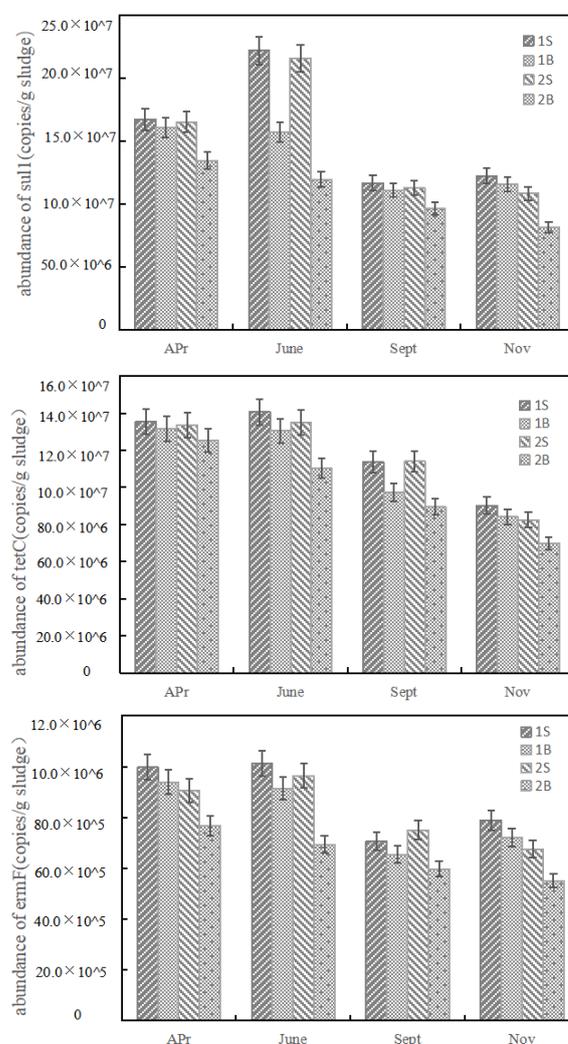


Fig.2. Changes of sul1, tetC and ermF ARGs in two STWs

The highest values of the three ARGs appeared in the surface sludge were 22.2×10^7 copies / g, 14.1×10^7 copies / g, 10.1×10^6 copies / g (sul1, tetC, ermF). If

ARGs in excess sludge is not reduced and removed, ARGs will be enriched in excess sludge and brought into soil and other environment with sludge resource utilization.

3.2 Removal efficiency of three antibiotic resistant genes in sludge treated wetlands

In this study, three kinds of ARGs in the two sludge treatment wetlands decreased significantly, indicating that the sludge treatment wetlands can effectively remove ARGs from the excess sludge of the sewage treatment plant. This is consistent with the fact that Wang and Shi can reduce the resistance genes of macrolides and quinolones in wetlands treated with sludge [11, 12]. The removal rates of three kinds of ARGs (sul1, tetC, ermF) in STW1 and STW2 in the stable period of one year are shown in Fig.3. In the surface and bottom sludge of STW1 and STW2, the removal rate of tetracycline resistance gene (tetC) was the highest, followed by sulfonamide resistance gene (sul1) to macrolide resistance gene (ermF) was the lowest.

This is similar to Cristina et al's study on the removal of antagonistic genes in two vertical flow wetlands, which showed that the average removal rates of sul2 and ermB are 33-97% and 9-99% respectively [13]. STW2 has a high removal rate of STW1 to three kinds of ARGs (sul1, tetC, ermF) in surface sludge and bottom sludge. This reason was that STW2 planted wetland plants, and reed can actively absorb ARGs in sludge. Vaccag G et al. It has been verified that plant roots can absorb and retain microorganisms in sludge to a certain extent, thereby reducing the number of microorganisms in stabilized sludge and effectively reducing ARGs in stabilized sludge [14]. Zhang et al. analyzed the microbial DGGE profile diversity in STW2 sludge samples and found that the microbial diversity in Reed was higher than that in sludge [15]. In addition, Wang et al used Illumina-MiSeq sequencing technology to analyze the STWs microbial community. The results showed that the plant growth in STWs could promote the diversity and richness of bacteria, and improve the removal rate of organic matter and nutrients in the system [16].

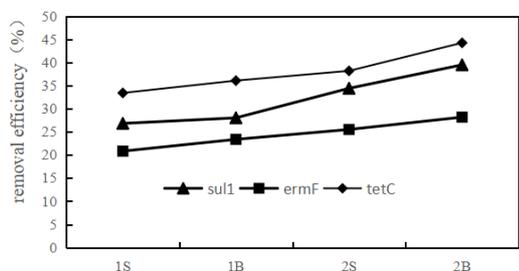


Fig.3. Removal efficiency of sul1, tetC and ermF ARGs in two STWs

In conclusion, STWs has a significant removal effect on three kinds of ARGs: sul1 (sulfonamides), tetC (tetracyclines) and ermF (macrolides), and planting wetland plants can remove ARGs in the excess sludge of

sewage treatment plants more effectively. In this paper, the pollution characteristics of resistance genes in stable period were explored, and the distribution and removal of resistance genes in load period should be studied in the future.

4. Conclusions

By comparing the pollution characteristics of three typical resistance genes of STW1 and STW2 in different periods, it was found that the concentration of arginine of sul1, tetC and ermF; in STWs was sul1 > tetC > ermF, ARGs in STW1 and STW2, and the enrichment degree of sul1, tetC and ermF; in surface sludge was higher than that in bottom sludge. In the same system, the removal rates of three kinds of ARGs are tetC > sul1 > ermF. In different systems, the removal rate of ARGs is the highest in the bottom sludge of STW2, followed by the surface sludge of STW2 and the bottom sludge of STW1, and the removal rate of surface sludge of STW1 is the lowest, that is, the removal rate of both surface sludge and bottom sludge is STW1 < STW2. Therefore, in SWTs, planting wetland plant Reed can promote the reduction of resistance genes in the process of sludge stabilization.

Acknowledgements

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