

PAPER • OPEN ACCESS

Review on the removal of antibiotic resistance genes from livestock manure by composting

To cite this article: Shiyu Xie *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **237** 052010

View the [article online](#) for updates and enhancements.



IOP | ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the **collection** - download the first chapter of every title for free.

Review on the removal of antibiotic resistance genes from livestock manure by composting

Shiyu Xie, Nan Wu*, Jia Tian, Xiangmeng Liu, Shuangshuang Wu, Qiuxia Mo, Shuai Lu

College of Engineering and Technology, Tianjin Agricultural University, Tianjin 300384, China

Corresponding author's e-mail address: nwu@tjau.edu.cn

Abstract: In recent years, due to the abuse of antibiotics in livestock industry, the antibiotic resistance genes (ARGs) are induced to produce in animals. ARGs are excreted into the environment with the feces, and contaminate the soil and groundwater through horizontal gene transfer (HGT). This in turn causes ARGs contamination. Composting, as an excellent traditional method of recycling manure, has a positive effect on the removal of ARGs from the environment. This article focuses on the removal effects of livestock manure composting (including aerobic composting and anaerobic composting) on ARGs, and then evaluates and prospects the removal of ARGs during manure composting.

1. Introduction

Recently, the large-scale use of medical antibiotics, agricultural antibiotics, veterinary antibiotics and industrial antibiotics not only causes environmental antibiotic pollution and induces the generation of resistant organisms and resistance genes, but also promotes the transformation, migration and spread of ARGs in the environment, and then threatens human health through the food chain.

Composting is a way of using various plant residues (crop straws, weeds, leaves, peats, garbage and other wastes) as the main raw materials, mixed with manure and organic fertilizer produced by composting with various microorganisms. By controlling the moisture of compost, oxygen content, pH, C/N ratio, temperature, etc., the plant organic residues can be mineralized, humified and sanitized to transform various complex organic nutrients into soluble nutrients and humus. At the same time, high temperature (60~70 °C) generated during composting is used to kill the germs, eggs and weed seeds brought by the raw materials, and to achieve the purpose of harmlessness. Many recent studies have also pointed out that compost can effectively remove ARGs from the environment.

This paper focuses on the removal of ARGs in the environment during livestock manure composting (including aerobic composting and anaerobic composting), and evaluates and prospects for the removal of ARGs from manure compost.

2. Pollution status of residual antibiotic resistance

As early as in 2004, American scientists Rysz and Alvarez proposed ARGs as a new type of environmental pollutant [1]. In 2006, Prudent et al. [2] found that there were ARGs in the river sediments, irrigation canals, dairy farms and sewage treatment plants, etc. in North Colorado, USA. On 29 January 2018, the surveillance data on antibiotic resistance first released by World Health Organization (WHO) revealed that high levels of resistance to a number of serious bacterial infections existed in both high-income and low-income countries. WHO's new Global Antimicrobial Resistance



Testing System (GLASS) revealed the widespread occurrence of antibiotic resistance among the 500,000 people with suspected bacterial infections across 22 countries [3].

3. Removal of antibiotic resistance genes in livestock manure composting

The effect of composting on the removal of antibiotic resistance in livestock manure is summarized according to other scholars' research, as shown in Table 1.

Table 1. The removal effect of composting technology on AGRs

Gene	Removal effect	Description	References
<i>tetG</i> , <i>tetM</i> , <i>tetX</i> , <i>sul1</i> , <i>sul2</i> , <i>ermB</i> , <i>ermF</i> , <i>ereA</i> , <i>meA</i> , <i>intI1</i>	0.21-1.34 logs decreased	Anaerobic fermentation of pig manure	Sui et al. [4]
<i>ermA</i> , <i>ermB</i> , <i>blaTEM</i> , <i>blaCTX</i> , <i>blaSHV</i> , <i>qnrA</i> , <i>qnrS</i>	0.3-2.0 logs decreased	Pig manure composting	Zheng et al.[5]
<i>tetC</i> , <i>tetM</i> , <i>tetO</i>	0.3-0.9 logs decreased		
<i>ermC</i>	Unchanged		
<i>ermF</i>	Increased		
<i>sul1</i> , <i>sul2</i> , <i>dfrA1</i> , <i>dfrA7</i> , <i>tetQ</i> , <i>tetW</i> , <i>tetC</i> , <i>tetG</i> , <i>tetZ</i> , <i>gyrA</i> , <i>parC</i>	Below detection limit after 42 days	Pig manure composting	Selvam et al. [6]
<i>tetW</i> , <i>sul2</i> , <i>gyrA</i> , <i>intI1</i> , <i>intI2</i>	0.27-1.45 logs decreased	Anaerobic fermentation of cow manure at low temperature	Sun et al.[7]
<i>tetC</i> , <i>tetM</i> , <i>tetQ</i> , <i>tetX</i> , <i>sul1</i>	0.23-3.63 logs increased		
<i>tetW</i> , <i>sul1</i> , <i>sul2</i> , <i>gyrA</i> , <i>intI1</i> , <i>intI2</i>	0.21-1.51 logs decreased	Anaerobic fermentation of cow manure at medium temperature	
<i>tetC</i> , <i>tetM</i> , <i>tetQ</i> , <i>tetX</i>	0.22-0.94 logs increased		
<i>tetM</i> , <i>tetW</i> , <i>tetX</i> , <i>sul1</i> , <i>sul2</i> , <i>gyrA</i> , <i>intI1</i> , <i>intI2</i>	0.11-1.60 logs decreased	Anaerobic fermentation of cow manure at high temperature	
<i>tetC</i>	1.07 logs increased		

3.1. Aerobic composting

Aerobic composting refers to the process of absorbing, decomposing and oxidizing degradable organic matter by aerobic microorganisms in the environment under aerobic conditions, thereby creating a high temperature environment and killing pathogenic bacteria to achieve the purpose of stabilizing organic matter.

The composting temperature mainly has influence on the growth and reproduction of microbial flora [4], which plays a decisive role in the rate of composting reaction. Zheng et al. [5] found that high temperature could effectively reduce the abundance of β -lactam resistance genes and quinolone resistance genes, and the removal rates of *blaTEM*, *blaSHV* and *qnrS* all reached 98% or more when studying the effect of high temperature on the ARGs of pig manure during composting. Gou et al. [6] found that high temperature had a certain reduction effect on tetracycline resistance genes in pig manure. In the three experiments, i.e., pig manure + corn straw, pig manure + corn straw + tetracycline antibiotics (10 mg·kg⁻¹ of TC, OTC, CTC respectively), pig manure + corn straw + tetracycline antibiotics (50 mg·kg⁻¹ of TC, OTC, CTC respectively), the abundances of target genes (*tetA*, *tetC*, *tetG*, *tetM*, *tetQ*, *tetW*) in antibiotics-added groups were generally higher than those in the control group, and the relative abundances of target genes in the low-dose group (except *tetW*) were generally higher than those in the high-dose group. Yu et al. [7] found that 10 kinds of ARGs (*tetA*,

tetC, *tetG*, *tetM*, *tetO*, *tetP*, *tetQ*, *tetS*, *tetT*, *tetW*) were greatly reduced, especially that *tetM*, *tetO*, *tetP*, *tetQ*, *tetS*, *tetT*, *tetW* were below the detection limit in more than 60% of all pig manure compost samples.

In addition, pH is one of the important factors in maintaining an efficient degradation of antibiotics in the environment. Wu et al. [8] found that when pH was 5 and 9, the bacterial concentrations were 2.7×10^9 CFU/mL and 3.8×10^9 CFU/mL, respectively. When the pH was 7, the concentration was 6.6×10^9 CFU/mL and the degradation rate now was the highest which reached 70.68%. Knapp et al. [9] found that the relative abundances of most ARGs, such as *blaCTX* and *ermB*, were significantly correlated with pH values. The high pH environment facilitated the spread of resistance genes, but the effects of pH on different resistance genes were distinct. Sundberg et al. found that when the pH was lower than 6, the respiration rates of microbial were greatly reduced; when the pH was too high, the volatilization of ammonia and nitrogen generation during composting accelerated, resulting in a decrease in the quality of the organic fertilizer [10].

3.2. Anaerobic composting

Anaerobic digestion is the process to decompose organic matter and produce biogas under the disintegration of anaerobic microorganisms. Anaerobic digestion can be used to remove antibiotics contained in the compost and reduce the abundance of ARGs.

The data show that temperature is one of the key factors in anaerobic fermentation, and the changes of temperature can cause variations in ARGs and microbial community succession. For example, moderate temperature anaerobic fermentation can significantly reduce the proportion of cefazolin resistant bacteria [11] and abundances of ARGs, such as *ermB*, *aphA2* and *blaTEM-1* [12]. However, the conclusions of temperature on the effect of anaerobic composting are diverse. Ghosh et al. [13] and Diehl and LaPara [14] found that high temperature was more advantageous than medium temperature, which was more favorable to the removal of ARGs. Ma et al. [15] found that high temperature was more conducive to the reduction of *ermB*, *ermF*, *tetO*, *tetW* abundance compared with the medium temperature, but not as good as the effect of removing other ARGs and *int II* under medium temperature treatment. In addition, Zhang et al. [16] studied the effects of high temperature and medium temperature anaerobic fermentation on reducing ARGs through macro genomic sequencing, and found that temperature had no significant effect on the total abundance of ARGs. Sun et al. [17] studied the effects of low temperature (20 °C), medium temperature (35 °C) and high temperature (55 °C) on the changes of ARGs during anaerobic fermentation of cattle manure. After high temperature anaerobic fermentation, the absolute abundances of 8 ARGs in 10 kinds of ARGs decreased, and 5 ARGs reduced 1.0 log or more. Combined with the data from low and moderate temperature anaerobic fermentation, high-temperature anaerobic fermentation had the greatest effect on removal of ARGs. In terms of relative abundance, the reduction effect showed as high temperature < intermediate temperature < low temperature.

Miller et al. [18] found that ARGs levels in anaerobic fermentation systems were directly related to the survival of antibiotic-resistant bacteria. Liu et al. [19] showed that the microbial community changed greatly during the first two weeks of anaerobic fermentation. After the system was stable, *Methanomicrobiales* got the highest abundance, followed by *Methanobacteriales* and *Methanococcales*. Sui et al. [20] studied the change of microbial community structure in anaerobic membrane bioreactor through T-RFLP method. When the membrane pressure climbed slowly, the dominant bacteria on the surface of the membrane were *Owenweeksia hongkonggensis* and *Raoultella*. When the membrane pressure raised steadily, the dominant bacteria were *Delftia acidovorans* and *Halothiobacillus neapolitanus*. Sun et al. found a similar phenomenon. With the anaerobic fermentation carried forward, the microbial community structures of three groups all had changed which were under different temperatures [17].

Studies have shown that heavy metal resistance genes and ARGs can coexist on the same genetic elements [21]. Heavy metals and antibiotics may express co-resistance or cross-resistance [22]. Graham [23] found a significant positive correlation between the concentration of Zn and the

abundance of *tetM*, *tetQ*, and *tetW* in river sediments. Similarly, Zhang et al. [24] also found a significant positive correlation between the content of As and the abundance of *tetM* and *tetQ* during sludge biological drying procedure. At the same time, Berg et al. [25] found that the proportions of ampicillin, p-aminobenzene sulfonamide, and multidrug resistant (≥ 3 kinds of drugs) microorganisms significantly increased after adding $108 \text{ mg} \cdot \text{kg}^{-1}$ of Cu to the soil, compared with the control group.

4. Conclusions

Composting is a means of effectively reducing or even eliminating ARGs from livestock manure. The higher temperature generated during the composting process can eliminate substances such as antibiotic-resistant microorganisms, and also degrade the antibiotic residues in livestock manure, thereby reducing the damage to the ecological environment and the human health, and achieving the harmless disposal of feces. In addition, by controlling external factors of composting, such as pH, temperature, C/N ratio, the effect of degrading antibiotics and removing resistance genes can be improved, making the composting an effective way to purify the soil environment.

Acknowledgments

This research was financially supported by National Natural Science Foundation of China (No. 21607114), National Undergraduate Training Programs for Innovation and Entrepreneurship (No. 201810061206), and Natural Science Foundation of Tianjin (No. 16JCQNJC08200).

References

- [1] Rysz, M., Alvarez, P.J. (2004) Amplification and attrnuation of tetracycline resistance in soil bacteria: aquifer column experiments. *Water Research*, 38: 3705-3712.
- [2] Pruden, A., Pei, R., Storteboom, H., et al. (2006) Antibiotic resistance genes as emerging contaminants: Studies in northern Colorado. *Environmental Science and Technology*, 40: 7445-7450.
- [3] World Health Organization (WHO) /Media centre.WHO.(2018) High levels of antibiotic resistance found worldwide, new data shows.<http://www.who.int/mediacentre/news/releases/2018/antibiotic-resistance-found/zh/>
- [4] Sui, Q.W., Zhang, J.Y., Chen, M.X., et al. (2016) Distribution of antibiotic resistance genes (ARGs) in anaerobic digestion and land application of swine wastewater. *Environmental Pollution*, 6: 751-759.
- [5] Zheng, N.G. (2016) Effects of high temperature composting process on antibiotic resistance genes from pig manure. *ACTA Science Circumstance*, 37: 1986-1992.
- [6] Selvam, A., Zhao, Z.Y., Wong, J.W.C. (2012) Composting of swine manure spiked with sulfadiazine, chlortetracycline and ciprofloxacin. *Bioresource Technology*, 126: 412-417.
- [7] Sun, W., Qian, X., Gu, J., et al. (2016) Mechanism and effect of temperature on variations in antibiotic resistance genes during anaerobic digestion of dairy manure. *Scientific Report*, 6: 30237.
- [8] Li, G.X. (2003) Research progress on composting and compost additives for solid waste. *Journal of agro-environment science*, 2: 252-256.
- [9] Gou, C.L. (2017) Effects of high temperature compost on tetracycline antibiotics and resistance genes in pig manure. *Journal of environmental science*, 37: 1454-1460.
- [10] Yu, Z.T., Michel, F.C., Hansen, G., et al. (2005) Development and application of real-time PCR assays for quantification of genes encoding tetracycline resistance. *Applied and Environmental Microbiology*, 71: 6926-6933.
- [11] Wu, X.L. (2018) Isolation and Degradation Characteristics of a Efficient Tetracycline-degrading Strain. *Biotechnology Bulletin*, 34: 172-178.
- [12] Knapp, C.W., McCluskey, S.M., Singh, B.K., et al. (2011) Antibiotic resistance gene abundance correlate with metal and geochemical conditions in archived Scottish soils. *PLOS One*, 11: e27300.

- [13] Sundberg, C., Smårs, S., Jönsson, H. (2004) Low pH as an inhibiting factor in the transition from mesophilic to thermophilic phase in composting. *Bioresource Technology*, 95: 145-150.
- [14] Beneragama, N., Moriya, Y., Yamashiro, T., et al. (2013) The survival of cefazolin-resistant bacteria in mesophilic co-digestion of dairy manure and waste milk. *Waste Management & Research*, 31: 843-848.
- [15] Resende, J.A., Silva, V.L., Otenio, M.H., et al. (2014) Dynamics of antibiotic resistance genes and presence of putative pathogens during ambient temperature anaerobic digestion. *Journal of Applied Microbiology*, 17: 1689-1699.
- [16] Ghosh, S., Ramsden, S.J., LaPara, T.M. (2009) The role of anaerobic digestion in controlling the release of tetracycline resistance genes and class 1 integrons from municipal wastewater treatment plants. *Applied Microbiology and Biotechnology*, 84: 791-796.
- [17] Diehl, D.L., LaPara, T.M. (2010) Effects of temperature on the fate of genes encoding tetracycline resistance and the integrase of class 1 integrons within anaerobic and aerobic digesters treating municipal wastewater solids. *Environ Sci Technol*, 44: 9128-9133.
- [18] Ma, Y., Wilson, C.A., Novak, J.T., et al. (2011) Effect of various sludge digestion conditions on sulfonamide, macrolide and tetracycline resistance genes and class I integrons. *Environmental Science & Technology*, 45: 7855-7861.
- [19] Zhang, T., Yang, Y., Pruden, A. (2015) Effect of temperature on removal of antibiotic resistance genes by anaerobic digestion of activated sludge revealed by metagenomic approach. *Applied microbiology and biotechnology*, 99: 7771-7779.
- [20] Sun, W. (2017) Study on the variation mechanism of antibiotic resistance genes in the anaerobic fermentation process of livestock and poultry feces. *Northwest agriculture and forestry university*, 04: 20-23.
- [21] Miller, J.H., Novak, J.T., Knoke, W.R., et al. (2016) Survival of antibiotic resistant bacteria and horizontal gene transfer control antibiotic resistance gene content in anaerobic digesters. *Frontiers in Microbiology*, 7: 263.
- [22] Liu, W.T., Chan, O.C., Fang, H.H. (2002) Microbial community dynamics during start-up of acidogenic anaerobic reactors. *Water Research*, 36: 3203-3210.
- [23] Sui, L.X., Hu, Q., Gao, D.W. (2015) Study on microbial community structure and membrane pollution in normal temperature anaerobic MBR. *Chinese environmental science*, 35: 110-115.
- [24] Davison, J. (1999) Genetic exchange between bacteria in the environment. *Plasmid*, 42: 73-91.
- [25] Baker-Austin, C., Wright, M.S., Stepanauskas, R., et al. (2006) Co-selection of antibiotic and metal resistance. *Trends in Microbiology Cell Press*, 14: 176-182.
- [26] Graham, D.W., Olivares-Rieumont, S., Knapp, C.W., et al. (2011) Antibiotic resistance gene abundances associated with waste discharges to the Almendares River near Havana, Cuba. *Environmental Science & Technology*, 45: 418-424.
- [27] Zhang, J.Y., Sui, Q.W., Tong, J., et al. (2016) Sludge bio-drying: Effective to reduce both antibiotic resistance genes and mobile genetic elements. *Water Research*, 106: 62-70.
- [28] Berg, J., Tom-Petersen, A., Nybroe, O. (2014) Copper amendment of agricultural soil selects for bacterial antibiotic resistance in the field. *Letters in Applied Microbiology*, 40: 146-151.