Effects of Anaerobic Digestion on Antibiotic Resistance Genes in Sludge

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Abstract. Sludge treatment and reuse in municipal sewage treatment plants is beneficial to improve economic effect and reduce environmental pollution. Sludge is an important source of contamination of antibiotic resistance genes. Anaerobic fermentation is an important way to treat activated sludge. Effective treatment of municipal sludge can avoid the release of pathogenic microorganisms and resistance genes (ARGs) into the environment and cause secondary pollution. The research results will be of certain guiding significance to the resource utilization of sludge.

Keywords: Sludge, anaerobic digestion, ARGs.

1. Introduction
In recent years, domestic water and industrial water consumption are increasing, and sewage treatment capacity is also rising. Sludge, as the main by-product of sewage treatment plants, has reached 43.28 million tons (water content 80%) in 2017, and is expected to reach 89.09 million tons in 2022 [1]. The sludge is rich in nutrient elements such as nitrogen and phosphorus, which is considered to be the most potential disposal method as a good organic fertilizer for land consolidation and improvement. However, the premise of land use is that not only the nutrient content of sludge should be high, but also the toxic substances contained in the sludge should not exceed the environmental capacity of the application site. Therefore, it is very necessary to seek reasonable methods to realize the harmless treatment and resource utilization of sludge. Anaerobic fermentation has obvious advantages in recovering clean energy, reducing the volume of sludge, killing pathogenic microorganisms and improving the performance of sludge due to its low energy consumption. Directly on the sludge anaerobic digestion process, however, there are 40% ~ 50% of organic matter into methane, and the process is slow, degradation of performance is poor, the difficulty or barrier is to Extracellular polymers in the sludge (Extracellular polymeric substances, EPS) the microbial cells aggregation mesh, EPS and microorganism, respectively, in the form of physical and chemical barrier layer limits the
hydrolysis rate of sludge [2]. Only by breaking the cell wall (membrane) to release these organic matters can microorganisms utilize them for anaerobic fermentation [3].

2. Effect of sludge pretreatment on anaerobic digestion performance

Sludge pretreatment can promote the degradation of EPS and effectively improve the anaerobic fermentation performance of sludge. At present, the commonly used pretreatment methods include chemical pretreatment, mechanical and physical method, biological method and different pretreatment combination treatment. Microwave pretreatment is a new method of sludge cracking in recent years. This method can break the cell wall (membrane) of sludge bacteria through two ways: thermal effect and non-thermal effect, and improve the hydrolysis rate in the process of sludge anaerobic fermentation. The thermal effect of microwave means that microwave can cause the oscillation of water molecules in the sludge, increase the temperature of sludge, produce thermal effect, and then change the secondary and tertiary structure of microbial protein. Non-thermal effect refers to the hydrogen bond breaking of macromolecules on the cell wall (membrane) of sludge bacteria in the alternating electric field formed by microwave, which destroys the structure of cell wall (membrane), releases intracellular substances to the outside of the cell, and improves the hydrolysis rate. In addition, ozone is a strong oxidizing gas [4], which has obvious advantages in sterilization, disinfection, decolorization, deodorization, oxidation of some refractory organic matter and other aspects. The ozone-ultraviolet combined process combines ultraviolet irradiation with ozone, and utilizes the strong oxidizing oxidants generated by the decomposition of ozone under ultraviolet irradiation to oxidize organic matter [5]. It is also a difficult task for environmental microbiologists to explore the technology of sludge pretreatment.

3. Antibiotic resistance genes in sludge

Sludge generally contains a large amount of organic matter, heavy metals and pathogens. If it is not properly treated, it will be enriched in the process of crop growth and can also harm human health through the food chain. Sources of antibiotics in sewage treatment plants include People's Daily use, animal husbandry, aquaculture, and drug residues and discharges from medical and pharmaceutical processes. The presence of antibiotics can induce antibiotic resistance genes (ARGs), which makes sewage treatment plants, especially activated sludge, become a huge gene pool for resistance. The spread of ARGs to pathogenic microorganisms through horizontal gene transfer can threaten human health [6]. If ARGs persist, spread and spread in the environment, the harm is greater than the antibiotic itself.

With the use of a large number of antibiotics, the sensitivity of more and more microorganisms in the environment to antibiotics is decreasing or even disappearing, leading to the decrease of efficacy or even failure of antibiotics. More than 25,000 deaths are caused by drug-resistant bacterial infections in the European Union each year, resulting in an annual healthcare expenditure of more than $1.5 billion [7]. Pruden et al. (2006) proposed ARGs as new environmental pollutants for the first time, which attracted extensive attention in the academic community [8]. ARGs carried by bacterial-containing wastes and those located on removable genetic elements were defined as contaminants in the world since 2007. ARGs are non-degradable, but self-replicating pollutants. With the utilization of sludge, resistance genes can be spread to indigenous microorganisms through horizontal gene transfer. When soil, surface water and groundwater in the indigenous microbial resistance genes, a large number of breeding and through the vertical gene transfer, the resistance genes passed on generation after generation, and the pollution of farmland soil biological systems, breeding resistance genes in animal food products also is likely to be hazardous to health through the food chain into the human body. At present, the way and mechanism of the antagonistic genes entering human body through the food chain of plant products are still under further study.

Antibiotics and heavy metals can be directly induced animal resistant microorganisms, and with the animal waste directly into the environment, the environmental microorganisms constitute a selective pressure, induced resistance microbes in the environment, and to the spread of pathogenic
microorganisms ARGs can cause multiple drug-resistant pathogens, a threat to human health [7]. The persistent residue, transmission and diffusion of ARGs in the environment are more harmful than antibiotics themselves. Cheng et al. (2013) investigated resistance genes in feces and wastewater of different types and scales of livestock farms in eastern China and found that 12 target genes (TETA, TEB, TETC, TEG, TEL, TEM, TETO, THEQ, TEW, TEX, SUL1, SUL2) and class I integron integrase gene (INTI1) were detected in high abundance in all samples [9]. Ju et al. (2015) detected the changes of 323 ARGs in sludge during medium-temperature anaerobic digestion, indicating that most of the ARGs could not be effectively removed [10]. The research results of Zhang et al. (2015) showed that only 8 species of 35 Args could be greatly removed by medium temperature anaerobic digestion [11], while 13 species could be removed by high temperature anaerobic digestion. However, the abundance and diversity of total Args were not effectively removed. Yang et al. (2014) used high-throughput sequencing based metagenomic method to analyze chemical genes in sewage treatment plants, and detected a total of 271 resistance genes, which belonged to 18 types of resistance genes [12].

4. Research progress in anaerobic digestion of sludge

Anaerobic fermentation can remove ARGs to some extent. At present, there are relatively many literatures on the change of ARGs in sludge anaerobic digestion. Ma et al. (2011) studied the reduction of nine resistance genes in sludge by medium-temperature [13] and high-temperature anaerobic digestion under laboratory conditions, and the results showed that medium-temperature anaerobic digestion could significantly reduce Suli, Sulii, TETC, TETG and TETX, and the reduction effect was more obvious with the increase of hydraulic residence time, but TETW, ERMB and ERMF increased. High temperature anaerobic digestion at 47℃, 52℃ and 59℃ could reduce TETW, ERMB, TETO, TETX, ERMB and ERMF more effectively, but the reduction effect on other resistance genes and INTL1 was poor, and TETC and TETG increased. It is generally believed that sludge anaerobic fermentation has a better reduction of ARGs and can achieve certain risk control.

Domestic and foreign scholars have carried out in-depth research on sludge anaerobic fermentation treatment. In Europe, anaerobic fermentation has become the main method of sludge treatment. Anaerobic digestion accounts for 67%, 64%, 97% and 65% of all sludge treatment methods, respectively. In most sewage treatment plants in Japan, anaerobic fermentation is also used to treat sludge, and in recent years, the sludge fermentation process has been further improved, through mechanical concentration and dehydration, anaerobic digestion, and stirring and thermal efficiency improvement. In order to reduce digestion time and tank volume, the number of high-temperature anaerobic fermentation plants is also increasing. Dai Qianjin et al. (2006) showed that the biogas yield of fresh sludge (moisture content 96%-97%) was 0.2L/g, which only accounted for 34.5% of theoretical sludge gas production (theoretical sludge gas production was about 0.58L/g) [14]. Battimelli et al. (2003) also showed that the COD removal rate of sludge anaerobic digestion was only 38%. Sludge pretreatment can break the cell wall (membrane), release organic matter to be decomposed and utilized by microorganisms, increase the hydrolysis rate of sludge and improve the fermentation performance [15]. Park et al. (2004) showed that the ratio of SCOD to TCOD increased by 8.5 times through 600kJ/L microwave treatment of sludge [16]. Eskicioglu et al. (2007) adopted microwave pretreatment process to simulate the traditional water bath heating method, and the results showed that when the microwave pretreatment temperature reached 50, 75 and 96℃, respectively, the SCOD/TCOD increased from 9.1% without treatment to 12.1%, 21.0% and 24.1%, respectively, and the microwave pretreatment temperature showed a certain linear correlation with the degree of sludge hydrolysis wall breaking [17].

In conclusion, anaerobic fermentation is undoubtedly one of the important ways for sludge resource utilization because it can reduce ARGs carried by microorganisms to a certain extent. In the process of anaerobic digestion, although it has been preliminarily proved that nutrients, pH, temperature and microbial community structure will affect digestion efficiency to varying degrees, nutrients and microbial community structure play an important role in improving digestion efficiency [4].
5. Conclusion and outlook
A comprehensive review of existing domestic and foreign research literature shows that there are also few studies on the change process and mechanism of targeted functional microorganisms and ARGs in the process of anaerobic fermentation under the pretreatment method. The existing studies mainly focus on the study of large categories of microorganisms and the change of the abundance of some types of ARGs. There is a lack of research on the types, abundances and influencing mechanism of ARGs in the whole system, and the distribution and trend of ARGs in the process of soil utilization are rarely explored.

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References
[1] Hang Shijun, Fu Tao, Dai Xiaohu, et al. How to break the dilemma of unsolved technical route, unintegrated industry and lack of policy through sludge outlet? [J]. Journal of Environmental Economics, 2019, 242 (02): 34-39.
[2] TYAGI V K, LO S L. Application of physico-chemical pretreatment methods to enhance the sludge disintegration and subsequent anaerobic digestion: an up to date review [J]. Reviews in Environmental Science and Bio/Technology, 2011, 10 (3): 215-242.
[3] Zhang Wan-qin, Qi Dan-dan, Wu Shu-biao, Dong Ren-jie, Zhao Wan-sheng. Effects of different pretreatment methods on anaerobic fermentation of sludge. Transactions of the Chinese Society for Agricultural Machinery, 2014, 45 (9), 187-198.
[4] Qiao Wangyan, Wang Huisheng, Yang Peng, et al. Application of ozone in advanced treatment of vegetable waste fermentation broth [J]. Environmental Engineering, 2012, 30 (1): 35-38, 46.
[5] Li Hua, Wang Wendong, Wang Xiaochang, et al. Effect of ozone-UV pretreatment on the coagulation effect of high organic matter raw water [J]. Environmental Science, 2010, 31 (8): 1807-1812.
[6] TYAGI V K, LO S L. Application of physico-chemical pretreatment methods to enhance the sludge disintegration and subsequent anaerobic digestion: an up to date review [J]. Reviews in Environmental Science and Bio/Technology, 2011, 10 (3): 215-242.
[7] An, X. L., Su, J. Q., Li, B., Ouyang, W. Y., Zhao, Y., Chen, Q. L. Tracking antibiotic resistome during wastewater treatment using high throughput quantitative PCR [J]. Environment International, 2018, 117: 146-153.
[8] Pruden, A., Pei, R., Storteboom, H., Carlson, K. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado [J]. Environmental Science & Technology, 2006, 40 (23): 7445.
[9] Cheng W, Chen H, Su C, et al. Abundance and persistence of antibiotic resistance genes in livestock farms: A comprehensive investigation in eastern China [J]. Environment International, 2013, 61: 1-7.
[10] Ju F, Li B, Ma L, et al. Antibiotic Resistance Genes and Human Bacterial Pathogens: Co-occurrence, Removal, and Enrichment in Municipal Sewage Sludge Digesters [J]. Water Research, 2015, 91: 1-10.
[11] Zhang T, Yang Y, Pruden A. Effect of temperature on removal of antibiotic resistance genes by anaerobic digestion of activated sludge revealed by metagenomic approach [J]. Applied Microbiology and Biotechnology, 2015, 99 (18): 7771-7779.
[12] Yang Y, Li B, Zou S, et al. Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach [J]. Water Research, 2014, 62 (Complete): 97-106.
[13] MA Y, WILSON C a., NOVAK J T et al. Effect of various sludge digestion conditions on
sulfonamide, macrolide, and tetracycline resistance genes and class I integrons [J]. Environmental science & technology, 2011, 45 (18): 7855–7861.

[14] Dai Qianjin, Li Yi, Fang Xianjin. Experimental study on anaerobic digestion of residual sludge in municipal sewage treatment plant [J]. China Water & Wastewater, 2006, 22 (23): 95-98.

[15] Battimelli A, Millet C, Delgenès J P, et al. Anaerobic digestion of waste activated sludge combined with ozone post-treatment and recycling [J]. Water Science & Technology, 2003, 48 (4): 61-68.

[16] Park, B., Ahn, J. H., Kim, J., Hwang, S. Use of microwave pretreatment for enhanced anaerobiosis of secondary sludge. Water Science and Technology, 2004, 50 (9), 17-23.

[17] Eskicioglu, C., Terzian, N., Kennedy, K. J., Droste, R. L., Hamoda, M. Athermal microwave effects for enhancing digestibility of waste activated sludge [J]. Water Research, 2007, 41 (11), p.2457-2466.