Research progress on the effect of antibiotics on anaerobic fermentation gas production and microorganisms of animal manure

Xin Zhang$^{1,2,3,4,*}$

$^1$Shaanxi Provincial Land Engineering Construction Group Co. Ltd, Xi'an 710075, China;  
$^2$Institute of Land Engineering and Technology, Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Xi'an 710075, China  
$^3$Key Laboratory of Degarded and Unused Land Consolidation Engineering, the Ministry of Natural Resources of China, Xi'an 710075, China  
$^4$Shaanxi Provincial Land Consolidation Engineering Technology Research Center, Xi'an 710075, China 

*Corresponding author: xinzhang@shanxidichan.com

Abstract. Antibiotics are widely used in animal husbandry as veterinary drugs and feed additives, resulting in a large amount of antibiotic residues in animal feces. In order to achieve harmless and resourceful utilization of livestock manure, anaerobic fermentation is the most extensive treatment method in the world. It briefly introduces the residues of antibiotics such as oxytetracycline, chlortetracycline, and tetracycline, which are currently used the amount. The effects of antibiotics on anaerobic fermentation gas production performance and research progress are discussed in recent years. A large number of results indicate that the influence of antibiotics on the fermentation system mainly occurs in the initial stage of fermentation. There are mainly H$_2$ / CH$_4$ reduction pathways, acetic acid fermentation pathways and methyl conversion pathways. Methanogenic and acidogenic microorganisms are closely related to these pathways in the fermentation system. And the effects of antibiotics on the methanogenic microorganisms in the fermentation system are described. It is suggested that the future research direction should further study the effect of antibiotics on antibiotic resistance genes in livestock and poultry manure.

1. Antibiotic content in livestock manure

Since the 1870s, the three scientists Fleming, Chain and Florey used the antagonistic effect of microorganisms to make penicillin an anti-bacterial infection drug for the first time after mass production and use, Save countless lives. At the same time, the massive use of antibiotics has brought new distress to humans. More and more microorganisms have become resistant to antibiotics, and the "magic effect" of antibiotics has continued to weaken or even disappear [1]. In addition, antibiotics can prevent livestock and poultry diseases, increase the growth rate of livestock and poultry, and are favored by the majority of farmers. The farm has also become a severe disaster area for antibiotic abuse [2]. From 2010 to 2030, it is estimated that the proportion of antibiotics used in the production of global food animals has inc
reased by 67%. By then, most of the approximately 105,000 tons will be used in China, the United States, Brazil, India and Mexico. In 2012, the number of antibiotics used in poultry production in China was estimated to be about 38,500 tons, of which 23,176 tons can be attributed to sulfonamides, tetracyclines and penicillins. In 2013, the consumption of veterinary antibiotics in China was nearly 90,000 tons, while in 2016 it reached 98,000 tons [3]. Because the animal's own absorption capacity is limited, about 83% of antibiotics and primary metabolites of antibiotics remain in the feces [4]. China produces about 4 billion tons of livestock and poultry manure every year, and the problem of environmental pollution caused by antibiotics should not be underestimated, and should be highly regarded [5]. Table 1 shows some commonly used antibiotic types.

2. Effect of antibiotics on anaerobic fermentation methane production
Antibiotic as a toxic substance will have a certain effect on the formation of anaerobic fermentation biogas. Its effect is relative. When the concentration is low, it will be used as an influencing substance to promote biogas generation. The concentration to achieve inhibition is different. Some scholars have found that oxytetracycline can reduce the biogas production of dairy cow manure by 50-60% [6]. Alvarez (2010) [7] found that compound OTC and CTC pollution reduced methane production by 62%. Arkan et al. (2006) [8] by oral administration of OTC to calves, the concentration of OTC detected in cow manure was 3.1 mg/L, and the biogas production after fermentation was reduced by 27%. Tylosin between 130 and 913 mg/L reduces biogas production by 10–38%. Florfenicol reduced biogas by 5%, 40% and 75% at 6.4, 36 and 210 mg/L, respectively. Gartiser et al. (2007) [9] found that another macrolide antibiotic, erythromycin, can inhibit biogas production by 6–24% at 6–100 mg/L without dose dependence. Lallai et al. (2002) [10] found that amoxicillin at 60 and 120 mg/L reduced methane production by 25% and 32%, respectively, after about 10 days.

The degradation degree of antibiotics in anaerobic fermentation of livestock and poultry manure is also the reason for the difference in antibiotics. The removal rate of tetracycline after 20 days of anaerobic fermentation of cow manure is less than 20%[11]; sulfadiazine, sulfamethazine, sulfamethoxazole, sulfamethoxazole, trimethoprim, Sulfamethoxazine can be almost completely removed by half-month a naerobic digestion, while sulfathiazole, sulfamethazine, and sulfachloropyridazine are almost indegradable; tylosin undergoes 4 days of anaerobic fermentation in cow dung After detection, it is almost impossible to detect, but it is difficult to degrade in pig manure. Inc et al. (2013)[6] research shows that when the concentration of OTC is highest on the first day of fermentation, the inhibition of biogas is the most serious. By the fifth day of fermentation, the inhibition level drops, and the inhibition effect disappears after the 15th day. There is no obvious difference. Stone et al. (2009) [12] found that the presence of CTC inhibited biogas production by about 28% compared with the control fertilizer. Lallai et al. (2002) [10] showed that when the concentration of oxytetracycline reached 250 mg/L has no inhibitory effect. Therefore, further research is needed on the effect of antibiotics on anaerobic fermentation performance.

3. Effect of antibiotics on methanogenic microorganisms
Anaerobic fermentation technology is a process that uses the symbiotic metabolism of microorganisms under anaerobic conditions to convert complex organic substances such as straw, livestock and poultry manure, kitchen waste, sludge, etc. into CH4 and CO2. MP Btyaut proposed in 1979 that anaerobic fermentation can be divided into three stages. The main fermentation process is methanogenic bacteria and acidogenic bacteria. In the same year, JG Zeikus and others proposed the four-stage theory of anaerobic fermentation at the first international anaerobic fermentation conference, including Hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis. In anaerobic fermentation systems, methanogens are more sensitive to the presence of antibiotics than acidogenic bacteria. Due to the different types of microorganisms and living conditions, different responses to antibiotics are shown. With the contact time with antibiotics, some microorganisms will gradually disappear, while some will gradually adapt and the abundance will increase.
Hydrogen-producing acetogens: Only when hydrogen-producing methanogens and hydrogen-producing acetogens coexist, long-chain organic acids (propionic acid, butyric acid) can be degraded when the hydrogen concentration is very low [13]. This physiological metabolism between hydrogen-producing microorganisms and hydrogen-consuming microorganisms is called a joint venture. The hydrogen transfer between the joint ventures is the biological force that promotes the stable fermentation [14]. The more abundant carbohydrates and substrates in the anaerobic reactor, the more conducive to the survival and proliferation of hydrogen and acetogenic bacteria [15]. The methanogens in the anaerobic fermentation process include hydrogen vegetative methanogens and acetic acid vegetative methanogens, which convert acetic acid and H2/CO2 to CH4/CO2 in the absence of external hydrogen acceptors [17]. Hydrotrophic methanogens include all Methanococci and Methanobacteria, and part of Methanobacteria [18]. The acetic acid vegetative methanogens are only Methanosarcina and Methanothrix or Methanothrix [19].

Ma Yi et al. (2013) [20] through Biolog research showed that ciprofloxacin reduced the utilization of carbon sources of carbohydrates, carboxylic acids, amino acids, polymers, phenols and amines by soil microorganisms, ciprofloxacin concentration $\geq 0.1 \mu g / g$ significantly affected the carbon source metabolic intensity and metabolic diversity of soil microbial communities. Ma et al. (2010) [21] showed that when the content of enrofloxacin was $0.1−100 \mu g / g$, the diversity of metabolic functions of soil microbial communities was significantly reduced ($P <0.05$). Zhang et al. (2011) [22] found that ciprofloxacin has a high ecological risk to aquatic organisms including Vibrio fischeri, Microcystis aeruginosa and Synechococcus leopoliensis, and extremely low levels are also toxic to these organisms. Aydin et al. (2016) [23] found that the combined addition of erythromycin and tetracycline will reduce Acinetobacter in the fermentation system. The combined pollution of tetracycline, erythromycin and sulfamethoxazole greatly reduced the abundance of Methanosarcinales in the acetic acid nutrition pathway in the fermentation system, which affected the gas production performance of the system [24].

4. Conclusion and outlook
Due to the rapid and high-level development of animal husbandry in China, antibiotics are used more and more frequently in the prevention and treatment of animal diseases. This has caused antibiotics to b
e excreted with animal feces and urine. Many scholars have studied the role of antibiotics in anaerobic fermentation. And the main focus is on the research of fermentation biogas production, methanogenic microorganisms and acidogenic microorganisms. However, the types of livestock and poultry manure, fermentation temperature, solid content and pretreatment technology are the key factors that affect the fermentation system during the fermentation process. It is worthwhile to study the metabolism and functional microbial structure of antibiotics through changes in the fermentation process.

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