

# Microorganisms and functional genes that play a key role in anaerobic fermentation and nitrification

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## Abstract

With the rapid development of the aquaculture industry, livestock manure has become the main source of agricultural pollution, and its reasonable disposal is an urgent problem to be solved. Anaerobic digestion (AD) is a microbially-mediated biotechnology employed for renewable energy production and livestock manure management. The digestate can be applied as organic fertilizer to the farmland to realize resource utilization of manure. Nitrogen is an important nutrient element necessary for crops. The anaerobic digestion process of manure can convert nitrogen into nitrogen-containing gas through denitrification. This may have an effect on anaerobic digestion process and reducing the fertilizer efficiency of digestate. Therefore, it is important to know the mechanism of nitrogen conversion and its influencing factors in anaerobic digestion for the anaerobic resource utilization of livestock manure. As antibiotics and heavy metals are widely used in livestock and poultry breeding to prevent diseases and promote growth. In the meantime, it causes a large amount of residue in the livestock manure which brings new challenges to the treatment and resource utilization of livestock manure.

## Keywords

Anaerobic digestion, Microbial community, antibiotics.

## 1. Anaerobic fermentation principle and main influencing parameters

In recent years, with the rapid development of large-scale and intensive livestock and poultry breeding, livestock and poultry breeding pollution has become the main environmental pollution, and its reasonable disposal has become an urgent problem to be solved. As of 2016, the waste livestock and poultry manure generated by my country's aquaculture industry has reached 3.8 billion tons (Wang Yahui et al. 2017). When the farm does not implement the treatment of manure, it will endanger the water, atmosphere, soil and other environmental systems. At present, compared with the rapidly developing animal husbandry industry, my country's pollution prevention and control work is relatively backward, and it is necessary to strengthen the demonstration and promotion of pollution prevention and control technology. The use of anaerobic digestion technology to treat livestock and poultry waste not only completes the centralized treatment of waste, but also realizes the secondary utilization of resources. It is a treatment process that integrates energy, environmental protection and economic benefits. It is listed as one of the most environmentally friendly and efficient biomass energy production methods (Zhou Dandan et al. 2014). Anaerobic fermentation of aquaculture waste can not only produce clean energy, but the fermented products can be used as fertilizers, which can be used as organic fertilizers and applied to farmland, realizing the utilization of waste resources (Alvarez and Lidén 2008).

Temperature is an important factor affecting anaerobic fermentation (Suryawanshi et al. 2010), which has a direct impact on microbial community composition, ability to degrade pollutants, and transformation of organic nutrients during fermentation (Lee et al. 2012; Scaglia et al. 2014). The common anaerobic fermentation of livestock and poultry manure is carried out at medium temperature (30 C – 40 C) or high temperature (50 – 60 C). The practical fermentation temperature of high-temperature fermentation is 52 C – 55 C, and the substrate degradation rate and methane production during the fermentation process are high. Although higher temperature can increase the rate of microbial metabolism and reduce the abundance of pathogenic bacteria, it has higher operating costs (Suryawanshi et al. 2010; Mladenovska and Ahring 2000). Lee et al. (2012) showed that high-temperature anaerobic fermentation has good advantages in processing organic solid wastes rich in protein and fat, but fermentation intermediates (ammonia nitrogen, volatile fatty acids, etc.) or toxic substances Accumulation can negatively affect anaerobic systems (Ahn and Forster 2002). In contrast, mesophilic fermentation requires less energy, and a suitable temperature provides a good living environment for more diverse microorganisms and ensures better fermentation stability (Shi et al. 2013; Weiland 2010). However, with the consumption of substrates in the fermentation system, nutrient deficiency and imbalance are prone to occur, which in turn reduces the gas production efficiency of late fermentation (Bowen et al. 2014).

## 2. Microbial-dominated nitrogen cycle reaction pathways

The material conversion process of nitrogen-containing compounds and nitrogen elements in nature is collectively referred to as the nitrogen cycle, and the nitrogen metabolism mediated by microorganisms is the main driving force of the nitrogen cycle (Li Dan et al. 2015). Traditional pure culture distribution has been unable to meet modern needs, most functional microorganisms cannot be isolated and pure culture, and there is a complex mutualistic relationship between microbial communities, it is difficult to simulate the natural environment of microbial growth in a laboratory environment. The development of modern molecular biology, and its application in the field of environmental microbes, provides new strategies and approaches for a deeper understanding of microbial function and metabolism. Nitrogen cycle includes organic nitrogen synthesis, ammonification, nitrogen fixation, nitrification, dissimilatory nitrate reduction, anaerobic ammonia oxidation and denitrification, etc. Most of the current research focuses on nitrogen fixation, nitrification, denitrification and ammonification.

Nitrogen fixation refers to the process by which Nitrogen fixation bacteria convert nitrogen ( $N_2$ ) in the atmosphere and the environment into ammonia ( $NH_3/NH_4^+$ ), which is catalyzed by Nitrogenase (Dos Santos and Dean 2011). Ferritin in nitrogenase is an electron transporter, which is powered by adenosine triphosphate (ATP) to transfer electrons to molybdenum ferritin, which then catalyzes the reduction of the substrate (Howard et al. 1996). Biological nitrogen fixation can be divided into Associative nitrogen fixation, Symbiotic nitrogen fixation and Free-living nitrogen fixation. Of course, the nitrogen-fixing principle of different species of microorganisms with nitrogen-fixing function is the same, and it is the main way of nitrogen-fixing in nature. It is generally believed that nitrogen-fixing microorganisms belong to prokaryotes and exist in more than 100 genera of bacteria and archaea, most of which belong to Proteobacterial and Cyanobacterial. Studies have shown that  $\alpha$ -Proteobacteria,  $\beta$ -Proteobacteria,  $\gamma$  The four classes -Proteobacteria and  $\delta$ -Proteobacteria are nitrogen-fixing microorganisms that mainly carry the *nifH* gene (Gaby and Buckley 2011). In recent years, studies have found that some sulfate-reducing bacteria, methanotrophs and methanogens in the ecosystem have nitrogen-fixing functions. Bertics et al. (2010) studied the nitrogen fixation process in marine sediments and found that most of the nitrogen-fixing bacteria detected in the

sediments were derived from *Desulfovibrio* and *Desulfobacter*, indicating that sulfate Reducing bacteria (SRB) play an important role in nitrogen fixation in the deep sea. With further in-depth research, it was found that the nitrogen-fixing activity and the activity of sulfate-reducing bacteria in sediments changed with seasons, showing a consistent response trend (Bertics et al. 2013).

### 3. Research methods of denitrification functional genes

The analysis of functional gene diversity helps to understand the microecology and biogeochemical cycles of different environments (Guo Liyun et al. 2011). The number and distribution of species is the basis for the interaction between organisms and the environment (Fang Fang and Chen Shaohua 2010). The species, distribution and abundance of species in the environment are very important for the study of microbial ecology. The development and promotion of molecular ecology has greatly promoted the dynamic study of environmental microbial community diversity, which can directly reflect the distribution and existence of microorganisms. Denaturing gradient gel electrophoresis (DGGE), functional gene high-throughput sequencing, metagenomics, terminal restriction fragment length polymorphism (T-RFLP), reverse transcription PCR (RT-PCR) and quantitative PCR (qPCR) Modern molecular biology methods such as these have been used in the study of environmental denitrifying microorganisms. This paper summarizes the recent progress in the investigation and research on the abundance and community structure of denitrifying bacteria in the environment, as shown in Table 1.

Table 1 Summary of studies on denitrification in the environment

No.	Aims and conclusions	Methods	Functional genes
1	Different concentrations of Cr affect specific denitrification genes in high nitrate wastewater process	qPCR	napA, nirS, nosZ, nirK,
2	Under autotrophic conditions, denitrification electrochemistry and pathway gene expression revealed that nosZ gene expression is a key step in N <sub>2</sub> O accumulation	RT-PCR	narG, napA, nirK, nirS, nosZ, norB
3	Succession and functional gene changes of forest soil microbial communities	GeoChip 4.0	nir、 nar、 gdh
4	Effects of BM-S-1 bacteria on denitrification in wastewater process	Metagenomics	nrfA, narG, napA, nirK, nirS, nosZ
5	Soil pH affects N <sub>2</sub> O and N <sub>2</sub> release and denitrification activity	15N, qPCR	narG, napA, nirS, nirK, nosZ
6	Denitrification kinetics in North Sea seafloor waters and sediments	RT-PCR, qPCR	nirS
7	Effects of long-term sewage irrigation on soil denitrifying bacterial communities and genes, pH and NO <sub>2</sub> <sup>-</sup> are the main driving factors, in fact, secondary pollutants	T-RFLP, qPCR	nirS

### 4. Conclusion

At present, the influence of heavy metals and antibiotics on denitrifying microorganisms is mainly concentrated in the soil and water environment, and there are a large number of heavy metals and antibiotics residues in livestock and poultry manure, which are bound to have certain effects on denitrifying microorganisms and nitrogen transformation during anaerobic fermentation. impact deserves further study.

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