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2 Ammonia determines transcriptional profile of 3 microorganisms in anaerobic digestion

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ABSTRACT

Anaerobic digestion is important for the management of livestock manure with high ammonia level. Although ammonia effects on anaerobic digestion have been comprehensively studied, the molecular mechanism underlying ammonia inhibition still remains elusive. In this study, based on metatranscriptomic analysis, the transcriptional profile of microbial community in anaerobic digestion under low (1500 mg L^{-1}) and high NH_4^+ (5000 mg L^{-1}) concentrations, respectively, were revealed. The results showed that high NH_4^+ concentrations significantly inhibited methane production but facilitated the accumulations of volatile fatty acids. The expression of methanogenic pathway was significantly inhibited by high NH_4^+ concentration but most of the other pathways were not significantly affected. Furthermore, the expressions of methanogenic genes which encode acetyl-CoA decarbonylase and methyl-coenzyme M reductase were significantly inhibited by high NH_4^+ concentration. The inhibition of the co-expressions of the genes which encode acetyl-CoA decarbonylase was observed. Some genes involved in the pathways of aminoacyl-tRNA biosynthesis and ribosome were highly expressed under high NH_4^+ concentration. Consequently, the ammonia inhibition on anaerobic digestion mainly focused on methanogenic process by suppressing the expressions of genes which encode acetyl-CoA decarbonylase and methyl-coenzyme M reductase. This study improved the accuracy and depth of understanding ammonia inhibition on anaerobic digestion.

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Introduction

22 Anaerobic digestion (AD) is a promising technology in the field
23 of waste treatment and renewable energy production. Live-
24 stock manure has been processed increasingly by AD to reduce
25 pathogens and to generate bioenergy such as methane.[1,2]
26 Thus, the improvement of stability and efficiency of AD is
27 crucial for the comprehensive application of this technology.
28 Ammonia concentration is one of crucial factors regulating
29 AD stability.[3] Optimal ammonia concentrations provide suffi-
30 cient buffer capacity and nutrient for microbial growth, which improves the AD stability and efficiency. However, low or

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excessive ammonia concentrations usually result in the failure of AD. It is reported that low ammonia concentration (<500 mg L^{-1}) decreases methane production, biomass and the aceticlastic methanogenic activity.[4] The high ammonia (>4000 mg L^{-1}) results in the inhibition of microbial activity and accumulation of volatile fatty acids, which finally causes decreased stability and even failure of AD.[3–6] Due to high protein content in manure, there are usually high ammonia concentrations in the AD of manure.[3] Thus, AD of livestock manure has to confront the inhibition from high ammonia. It is necessary to reveal the mechanisms underlying ammonia inhibition on the AD process.

Temperature is considered as a prominent factor regulating ammonia toxicity on AD process.[3,7,8] The thermophilic temperatures compared to mesophilic temperatures usually cause higher ammonia toxicity.[7,8] The thermophilic temperatures enhance metabolic activities of microorganisms, which strengthens hydrolysis of substrates including proteins. This process increases ammonia concentration in the slurry, so that ammonia toxicity is undoubtedly strengthened. Thus, under mesophilic temperatures, the ammonia toxicity more depended on the initial ammonia concentration, which facilitates the understanding of the relationship between ammonia concentration and ammonia toxicity. Different methanogens are distinguishably sensitive to ammonia concentrations, which partly determines ammonia toxicity on AD process. Due to spherical *Methanosarcina* with higher ratio of volume/surface than that of rod-shaped *Methanosaeta*, the diffusion of ammonia is less into the *Methanosarcina* than *Methanosaeta*,[9] which results in their different sensitivities to ammonia concentrations. Thus, high ammonia more probably causes failure of AD where *Methanosaeta* are dominated in the methanogens than that where *Methanosarcina* are dominated. The recovery of AD from failure depends on a reconstruction of methanogenic species,[10] so that *Methanosarcina* as dominated methanogens replace *Methanosaeta*. This process probably causes the decrease of acetoclastic methanogenesis.[11] These studies of ammonia toxicity on AD process mainly focus on methanogens and methanogenic process.[3,5,6,12,13]

Although the ammonia toxicity on AD process has been comprehensively revealed mainly based on methanogenic microflora,[3,5,12–14] the transcriptional profiles of specific pathways and genes in response to ammonia have rarely been discussed. The AD process consists of AD food web (hydrolysis, acidogenesis, acetogenesis and methanogenesis), so besides methanogenesis, the other three steps of the food web also play important roles in final methane production. Thus, the transcriptional profiles of methanogenesis-related processes are necessary to be revealed based on metatranscriptomic analysis, which can provide a new sight underlying ammonia toxicity on AD process.

In this study, based on metatranscriptomic analysis, the transcriptional profiles of microbial community in response to low and high NH_4^+ concentrations, respectively, were revealed in AD. Specifically, we focused on the expressions of pathways and genes responsible for methane production under different NH_4^+ concentrations to further reveal the mechanism underlying ammonia toxicity on AD process.

Materials and methods

Setup of AD system

The AD experiment of swine manure was conducted with working volume of 2.5 L digestion sludge containing 0.75 L initial inoculum, and the total solid content was 7% (Supplement Table S1). The high ammonia treatment with NH_4^+ (5000 mg L^{-1}) (HN) and low ammonia treatment with NH_4^+ (1500 mg L^{-1}) (LN) were determined by adding NH_4Cl at the beginning of AD, which was mainly based on previous report[6] and pre-experiments. All the treatments were conducted in triplicate at 37 °C. Seed slurry was prepared by anaerobic digestion of swine manure (obtained from a pig farm in Neijiang, Sichuan Province, China) at 37 °C, for one hydraulic retention time (HRT). After methane production reached the first peak (6th day) in the reactor, we performed a semi-continuous feeding mode that 500 mL of digestate was exchanged every three days with HRT of 15 days and organic loading rate of 4.5 g VS (volatile solid) L^{-1} day^{-1}. The anaerobic digestion was performed for two HRT. The feeding slurry was adjusted to the corresponding NH_4^+ concentration using NH_4Cl. Details about parameters at the start of fermentation were shown in Supplement Table S1.

Sampling and analysis

At each feeding, methane content in biogas, volatile fatty acids (VFA), NH_4^+ and pH were measured to monitor AD dynamic. The methane content in biogas was measured using an Agilent 6890 gas chromatography system (Agilent Technologies, USA), equipped with a thermal conductivity detector and carrier gas of argon. The injection port, column oven, and detector were operated at 100, 70, and 150 °C, respectively. The daily volume of biogas was detected by water replacement method. The VFA in the slurry was detected using Agilent 1260 Infinity liquid chromatography (Agilent Technologies, USA), equipped with a differential refraction detector (RID) and mobile phase of H_2SO_4 (0.005 M). Total solid and volatile solid were measured based on previous report.[15] NH_4^+ concentration was quantified with Nessler's reagent colorimetric method.[16] At the end of the second HRT, the digestate were sampled in triplicate for total RNA extraction with the RNeasy PowerMicrobiome Kit (Cat. No. 26000-50; MO BIO, USA). The quality of RNA was checked with a NanoDrop 2000 spectrophotometer (Thermo, USA). Ribosomal RNA was removed from the total RNA with the RiboMinusTM kit (Lot. No. 1539791; Invitrogen, USA). The metatranscriptomic (mRNA) sequencing was performed using an Illumina Hiseq 2000 (Illumina Inc., USA). The sequencing raw data from total 6 samples were uploaded to MG-RAST with assigned MG-RAST ID (mgs589946, mgs589949, mgs589952, mgs589955, mgs589958 and mgs589961) for further analysis.[17] Prior to the analysis, the quality control pipeline in MG-RAST[17] was performed to remove poor quality sequences. The annotation of functional profiles was based on the KEGG Orthologs database including 4 levels. The functional categories was presented as level 2. Level 3 reflected the KEGG pathways, and level 4 (gene expression level) showed expressions of specific

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