Ammonia Inhibition

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Anaerobic digestion is one of the most widely used treatment methods for animal manure. Chicken manure has high methane production potential and is thus a suitable substrate for biogas plants.

Keywords: anaerobic digestion ; chicken manure ; ammonia inhibition

1. Introduction

Anaerobic digestion technology, the primary treatment strategy for animal manure, can efficiently treat organic matter to generate clean energy. In China, the annual output of animal manure reaches 3800 million tons [1]. This potential bioenergy from anaerobic treatment represents an ideal alternative to fossil energy sources and can reduce greenhouse gas emissions. Chicken manure is a bioenergy source that is very special as compared to pig and cow manure. Firstly, the total solids (TS) content of chicken manure is greater than 20%. It can be treated by high solids anaerobic digestion technology. However, a large amount of water is required to dilute chicken manure to about TS 10% before anaerobic treatment in the actual project [2]. This increases the production of digestate and operating costs. In addition, chicken manure contains considerable protein, uric acid, and other nitrogen-containing organic matter, with the nitrogen content usually being higher than 4% [3]. Anaerobic digestion technology, however, recommends a suitable carbon/nitrogen (C/N) ratio of 20–30 [4], while the C/N ratio of chicken manure is as low as 5–10 [5].

High ammonia level (total ammonia nitrogen, TAN > 3 g/L) is the primary bottleneck and is one of the main factors affecting microbial community structure and the methanogenic pathway in the anaerobic digestion of chicken manure. Previous studies demonstrated that hydrogenotrophic methanogens (converting H_2 and CO_2 into methane) were more tolerant of ammonia nitrogen than acetoclastic methanogens (cracking acetate to produce methane), which were dominant in high ammonia conditions [6]. However, recent studies found that acetate generated methane through syntrophic acetate oxidation combined with the hydrogenotrophic methanogenesis pathway under high ammonia stress [6]. Syntrophic oxidation is the primary metabolism of acetate at high ammonia levels [6]. Therefore, comprehensive knowledge of the links among ammonia levels, microbial community structure, and methanogenic pathways is critical to improving biogas production performance through improved operating strategies.

2. Ammonia Inhibition

2.1. Anaerobic Decomposition of Protein and Uric Acid in Chicken Manure

During the anaerobic treatment of chicken manure, process instability may occur due to high levels of ammonia nitrogen produced as a byproduct of protein and uric acid degradation. About 40%–70% of organic nitrogen in chicken manure comes from uric acid and 30%–60% from protein [7]. Protein was converted into amino acids by hydrolytic bacteria and then further converted into organic acids and ammonia by the action of acidogenic bacteria (**Table 1**). The protein degradation efficiency in chicken manure is less than 50% [8]. Notably, the degradation of proteins is complex and more sensitive to ammonia inhibition in the hydrolysis stage. For example, the peptone degradation efficiency was 50% under TAN levels of 2.0 g/L and rapidly decreased to 30% when the TAN increased to 5.0 g/L. However, peptone degradation almost ceased at a TAN of 6.5 g/L, and high ammonia levels mainly inhibit the deamination of peptone [9]. Therefore, due to the low degradability of proteins, uric acid degradation is one of the leading causes of high ammonia levels in the anaerobic digestion of chicken manure.

Table 1. Anaerobic degradation of protein and uric acid.

The Methanation of Substrate	Equations
Protein	

The Methanation of Substrate	Equations
$C_{16}H_{24}O_5N_4 + 14.5\ H_2O = 8.25\ CH_4 + 3.75\ CO_2 + 4\ NH_4HCO_3$	(1)
Uric acid by *Clostridium purinolyticum*:	
$C_5H_4N_4O_3 = 0.58\ CH_3COOH + 0.25\ HCOOH + 0.06\ C_2H_5NO_2 + 3.47\ CO_2$	(2)
Uric acid by *Streptococcus* sp.:	
$C_5H_4N_4O_3 + HCOOH = CH_3COOH + 4\ CO_2 + 4\ NH_3$	(3)
Uric acid by *Bacteroides ternitidis*:	
$C_5H_4N_4O_3 = 0.75\ CH_3COOH + 3.5\ CO_2 + 4\ NH_3$	(4)

The degradation of uric acid is complicated. Uric acid can be anaerobically degraded by *Clostridium* sp., *Enterobacter* sp., *Streptococcus* sp., *Bacteroides* sp., and so on (**Table 1**) [10]. These microorganisms were ubiquitous in anaerobic digestion systems, especially *Clostridium* and *Bacteroides* sp. [11]. *Clostridium purinolyticum*, for example, degrades uric acid into acetate, formate, glycine, and CO_2 (Equation (2)) [12]. In addition, the uric acid degradation by *Streptococcus* sp. requires the presence of formate, which acts as a reducing agent (Equation (3)) [13]. The degradation of uric acid by *Bacteroides* sp. does not require the participation of formate (Equation (4)) [13]. Therefore, the anaerobic degradation of uric acid involves a variety of microbial metabolic reactions. The degradation of uric acid should be given adequate attention during anaerobic digestion. Current research lacks knowledge of nitrogen balance for the anaerobic treatment of chicken manure.

2.2. Ammonia Inhibition Threshold for Anaerobic Consortia

Anaerobic digestion is performed by various microorganisms, with the degradation of substrate divided into hydrolysis, acidogenesis, acetogenesis, and methanogenesis (**Figure 1**) [14]. These steps need to be performed in balance in order to obtain stable operation. Ammonia nitrogen exists mainly as ammonium bicarbonate (NH_4HCO_3) under anaerobic alkaline conditions [15]. Ammonium bicarbonate can provide alkalinity, buffer the pH value of the digestate and enhance the buffering capacity of the system (**Figure 1**). In addition, minor TAN levels (0.05–0.2 g/L) benefit microorganisms [16]. High ammonia nitrogen levels (TAN > 3 g/L), however, can inhibit the activity of anaerobic consortia, especially acetoclastic methanogens. The anaerobic digestion process produces undesirably higher TAN concentrations, even more than 10 g/L for chicken manure [17]. TAN is composed of free ammonia nitrogen (FAN, NH_3) and ammonium ions (NH_4^+). Temperature and pH modulate the balance between NH_4^+ and NH_3, and the latter has been reported to be the leading cause of microbial inhibition [16].

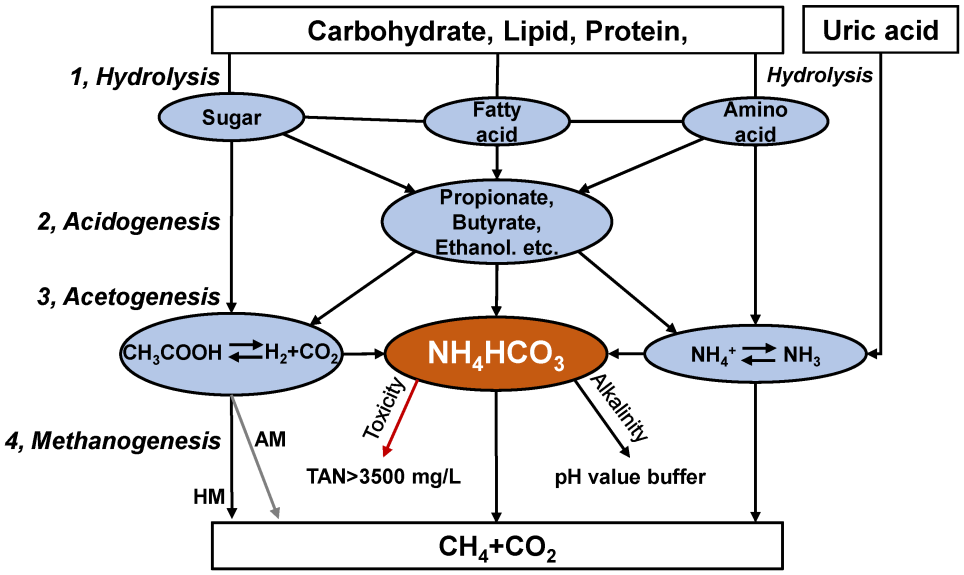

Figure 1. Biological metabolism of organic matter in chicken manure during anaerobic digestion.

Hydrolysis and acidogenesis bacteria have higher ammonia tolerance than methanogens, which is the main reason for organic acid accumulation at high ammonia levels [18]. The IC_{50} (50% inhibition) of hydrolysis and acidogenesis efficiency was reached at TAN of 5.7 and 5.3 g/L in thermophilic conditions [18]. The hydrolysis efficiency of chicken manure was 75–77% (FAN of 1.1 g/L) under mesophilic conditions and only about 66% under thermophilic conditions (FAN of 2.2 g/L) with a similar TAN concentration (5.6–5.7 g/L) [19]. The IC_{50} of the hydrolysis and acidogenesis processes was 2.3 and 2.2 g/L

of FAN [18]. This also illustrates that the hydrolysis and acidogenesis processes were less susceptible to ammonia inhibition.

The concentration of ammonia nitrogen can influence acetogenesis efficiency. The efficiency of acetogenesis increased by 52% when the concentration of TAN was decreased from 5.6 g/L to 3.8 g/L [20]. Because the acetogenic step is not considered the bottleneck, few studies have examined the effect of ammonia levels on acetogenic bacteria. However, it has been shown that an increased ammonia concentration (TAN 0.8 to 6.9 g/L) dramatically influenced the putative acetogenic population structure and caused two distinct changes in the most abundant microbial members [21]. In addition to converting organic matter to acetate, hydrogen can be converted to acetate through homoacetogens, the process being thermodynamically positive ($\Delta G0' = -104.6$ kJ/mol). Homoacetogens are suited to survival under low temperatures and acidic or alkaline conditions and are more competitive for hydrogen than hydrogenophilic methanogens [22]. The FAN concentration increased from 0.1 to 0.4 g/L, and the hydrogen consumption efficiency of the homoacetogenic process decreased from 68.5% to 4.5% [23]. It can be seen that homoacetogens have a lower ammonia inhibition threshold, being more sensitive to ammonia inhibition than hydrolysis, acidogenesis, and acetogenesis processes.

Ammonia levels have been found to inhibit methane production performance, with the ammonia threshold for inhibition ranging widely from 1.5 g/L to 7.0 g/L [16][17]. Inhibition phenomena were observed at 1.5–2.5 g/L of TAN during the anaerobic digestion of chicken manure [16][17]. The acetoclastic *Methanosaeta* sp. tolerated TAN concentrations of up to 3.0 g/L. In comparison, the facultative hydrogenotrophic *Methanosarcina* sp. could grow in environments containing as much as 7.0 g/L, whereas growth of hydrogenotrophic *Methanobacterium* sp. was observed up to 9.0 g/L [24]. Hydrogenophilic methanogens can tolerate higher ammonia levels and often were most dominant in high ammonia-level reactors.

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