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# Effects of free ammonia on volatile fatty acid accumulation and process performance in the anaerobic digestion of two typical bio-wastes

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## ABSTRACT

The effect of free ammonia on volatile fatty acid (VFA) accumulation and process instability was studied using a lab-scale anaerobic digester fed by two typical bio-wastes: fruit and vegetable waste (FVW) and food waste (FW) at 35°C with an organic loading rate (OLR) of 3.0 kg VS/(m<sup>3</sup>·day). The inhibitory effects of free ammonia on methanogenesis were observed due to the low C/N ratio of each substrate (15.6 and 17.2, respectively). A high concentration of free ammonia inhibited methanogenesis resulting in the accumulation of VFAs and a low methane yield. In the inhibited state, acetate accumulated more quickly than propionate and was the main type of accumulated VFA. The co-accumulation of ammonia and VFAs led to an “inhibited steady state” and the ammonia was the main inhibitory substance that triggered the process perturbation. By statistical significance test and VFA fluctuation ratio analysis, the free ammonia inhibition threshold was identified as 45 mg/L. Moreover, propionate, iso-butyrate and valerate were determined to be the three most sensitive VFA parameters that were subject to ammonia inhibition.

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## Introduction

The rapid increasing disposal of municipal solid wastes (MSWs) has resulted in severe environmental problems in China. Two types of typical bio-wastes, including fruit and vegetable waste (FVW) and food waste (FW), contributed to high organic and water content in MSW (Zhang et al., 2014). As compared with conventional treatment technologies, anaerobic digestion has emerged as one of the most promising alternative technologies for the treatment of high organic content waste as well as recovery of renewable energy-biogas (De Clercq et al., 2016). Despite this finding, various operational problems still prevent the anaerobic process from being widely applied. The substrates’

compositions, usually referred as C/N ratio and ammonia concentration, are considered to be the key parameters affecting process stability and performance (Mata-Alvarez et al., 2014; Yuan and Zhu, 2016).

An optimum C/N ratio in the range of 20 to 30 is essential for anaerobic digestion which can help to keep an appropriate nutrient balance for the microbial growth and to maintain a stable environment (Li et al., 2015; Mata-Alvarez et al., 2014). Therefore, the anaerobic digestion performance of low C/N ratio substrate, such as FW and FVW, is usually not very effective and stable. Low C/N ratio substrates contain a relatively higher percentage of nitrogenous organic matters. Ammonia produced by the biological degradation of nitrogenous organic matters

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was in excess for microorganism utilizing. The excess ammonia usually accumulates during the process and leads to an increase of pH, inhibitory effects, and eventually, process deterioration (Ariunbaatar et al., 2015; Sheng et al., 2013). Ammonium ion ( $\text{NH}_4^+$ ) and free ammonia (FA) ( $\text{NH}_3$ ) are the two principal forms of total ammonia nitrogen (TAN) of which FA has been suggested to be the main cause of inhibition. Hydrophobic free ammonia molecules may diffuse passively through the membrane and into the cell, resulting in proton imbalance and/or potassium deficiency (Belmonte et al., 2011; Chen et al., 2008). A high concentration of ammonia can inhibit methanogenesis resulting in the accumulation of volatile fatty acids (VFAs) and, as a result, low methane yield. In the literature, the inhibitory concentrations of TAN and free ammonia were in the range of 1500–7000 mg/L and 53–1450 mg/L, respectively (Rajagopal et al., 2013). This wide range is mainly due to the different substrates, inocula, environmental conditions (affecting pH and temperature, for instance) and acclimation (Chen et al., 2008; Rajagopal et al., 2013; Yenigün and Demirel, 2013).

Previous research on ammonia inhibition in anaerobic digestion has mainly been focused on inhibition concentration thresholds, inhibition mechanisms, and microbial community shift, etc. (Gao et al., 2015; Poirier et al., 2016; Rajagopal et al., 2013). Furthermore, feasible and sensitive indicators of the anaerobic digestion (AD) process subject to ammonia inhibition are equally important to monitor system health and to prevent the system from collapse. Parameters of biogas production rate, methane yield, pH, VFAs, etc., have been recommended as the process indicators; however, these parameters are either not sensitive enough to reflect the process instability or not feasible for *in situ* measurement (Nielsen et al., 2007). Biogas production rate and methane yield are the most commonly used monitoring indicators, but these indicators respond slowly and as a result, cannot indicate process instability timely (Boe et al., 2010; Nielsen et al., 2007). The pH measurement is easy to obtain, but not reliable when used in highly buffered systems. Under such conditions, even rapid increases in VFAs or ammonia cannot lead to significant pH fluctuation. Many researchers have suggested that VFAs could be good indicators of the process. VFAs are the most predominant intermediates during the AD process and their accumulation indicates the imbalance between sequential steps of AD process (Boe et al., 2010; Madsen et al., 2011). Ahiring et al. (1995) suggested that butyrate and iso-butyrate concentration might be reliable for indicating process instability because they are sensitive to different types of perturbation imbalances. Nielsen et al. (2007) suggested that propionate might be the best indicator during a process disturbance caused by overloading because it has proven significant and long-lasting. Nakakubo et al. (2008) studied thermophilic digestion of pig manure with intermittent  $\text{NH}_4\text{Cl}$  pulsing, and found that iso-butyrate, butyrate, and iso-valerate, rather than propionate, were useful indicators for acute ammonia induced perturbation. Two of the most abundantly produced VFAs (*i.e.*, acetate and propionate) did not accumulate with increased ammonia concentration.

In addition to being intermediates and indicators, VFAs are also essential buffering agents in the AD system. Moreover, high concentrations of VFAs show an inhibitory effect to methanogenesis (Yuan and Zhu, 2016). VFAs accumulate at a

high organic loading rate or during perturbations when methanogens cannot utilize hydrogen and VFAs as quickly as they are produced by acidogens and acetogens. High concentrations of free VFAs are thought to freely permeate the cellular membrane and damage the macromolecules in low-pH environments, especially for the gram-positive bacteria (Wang et al., 2009; Yuan and Zhu, 2016). Accumulation of VFAs leads to rapid pH decrease, and eventually, process deterioration. Wang et al. (2009) reported that a propionate concentration of 900 mg/L resulted in the significant inhibition of methanogens. Xu et al. (2014) found that acetic acid was the main VFA inhibitor in methanogenesis when treating kitchen wastes. The initial inhibitory concentration of acetic acid was between 1.5 and 2.5 g/L and the methanogenesis activities were inhibited completely at the VFA concentration of 5.8–6.9 g/L.

Ammonia and VFAs are both inhibitory to methanogenesis and can lead to pH fluctuation. High concentrations of both ammonia and VFAs usually lead the system to fall in an “inhibited steady state”, in which the digester runs stably within a neutral pH range, but where methane production rate and volatile solid (VS) reduction rates are quite low (Angelidaki and Ahiring, 1993; Chen et al., 2008). Although the individual effects of VFAs or ammonia on methanogenesis have been widely reported, comprehensive analysis of ammonia-VFA interaction has seldom been demonstrated. Whether ammonia or VFAs are the main inhibitory substances that trigger the process perturbation is undefined. Finding useful indicators for potential process perturbation is also unclear. Thus, in this study, the anaerobic digestion of two kinds of typical bio-wastes was conducted to investigate the ammonia inhibition effects on VFA accumulation and process performance. The interaction of ammonia-VFAs, which led the digester into an “inhibited steady state” was also studied. In addition, this study aimed to identify an ammonia inhibition threshold and to determine sensitive VFA parameters as indicator subject to ammonia inhibition. This study provided useful insight into preventing ammonia inhibition from causing low efficient biogas production and process deterioration.

## 1. Materials and methods

### 1.1. Substrates and inocula

Raw FVWs were collected from a fruit and vegetable market in Beijing in during July to January of the next year. The FVW mainly contained residues of Chinese cabbage, carrot, lettuce, apple, banana, and watermelon. Raw FW, which mainly consists of leftovers from cooked foods, was collected from students' restaurants in Tsinghua University, Beijing, China. The FWV and FW were pre-treated and homogenized using a food grinder after manually sorting out bones, paper and plastics etc. The samples were stored at 4°C before use. Inocula were anaerobic granular sludge taken from a full-scale upflow anaerobic sludge bed (UASB) reactor treating starch processing wastewater at 35°C. The granular sludge was ground into slurry before the experiment. The characteristics of substrates and inocula are summarized in Table 1 (Lin et al., 2011).

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