



# Effects of organic loading rate and effluent recirculation on the performance of two-stage anaerobic digestion of vegetable waste

Zhuang Zuo<sup>a</sup>, Shubiao Wu<sup>b,\*</sup>, Wanqin Zhang<sup>a</sup>, Renjie Dong<sup>b</sup>

<sup>a</sup> College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, PR China

<sup>b</sup> Key Laboratory of Clean Utilization Technology for Renewable Energy in Ministry of Agriculture, College of Engineering, China Agricultural University, Beijing 100083, PR China

## HIGHLIGHTS

- The performance of VW anaerobic digestion under various OLRs was demonstrated.
- Dynamics of hydrolysis and methanogenesis in AD of VW were investigated.
- Hydrolysis process was inhibited in the acidogenic reactor under high OLRs.
- Recirculation alleviated VFA inhibition due to effects of dilution and pH adjustment.
- Recirculation improved acidogenesis and biogas production in acidogenic reactor.

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## ABSTRACT

The effects of organic loading rates (OLR) and effluent recirculation on dynamics of acidogenic and methanogenic processes in two-stage anaerobic digestion of vegetable waste were investigated. Two systems were performed at OLRs of 1.3, 1.7, 2.1 and 2.6 g VS/L/d. One system recirculated the effluent from the methanogenic reactor to acidogenic reactor. With increasing OLRs, total volatile fatty acid (VFA) concentration increased to approximately 8500 mg/L in acidogenic digester, where pH decreased from 6.4 to 5.2. Daily biogas production and methane content in methanogenic reactor increased from 1.2 to 4.4 L/d and from 27.4% to 60.5%, respectively. However, inhibition of hydrolysis in acidogenic reactor was demonstrated under the OLR of 2.6 g VS/L/d without recirculation, thus indicating system overloading. Effluent recirculation shown a considerable positive effect on alleviating VFA inhibition and improving biogas production in acidogenic reactor because of the effect of dilution and pH adjustment, particularly at high OLRs.

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## 1. Introduction

China is a large vegetable producing country. However, more than 30% of produced vegetable are lost as waste throughout the vegetable supply chain, which includes collection, storage and transportation (Singh et al., 2012). Vegetable waste (VW) is often a source of nuisance as leachate when it is disposed of in landfills because of its high moisture (>80%) and organic contents (Arvanitoyannis and Varzakas, 2008). Anaerobic digestion has shown to be an alternative with multi-environmental benefits such as waste disposal and renewable energy production (Bouallagui et al., 2004; Arvanitoyannis and Varzakas, 2008). However, the digester performance is highly sensitive to organic loading rate (OLR), and waste composition (Sharma et al., 1999). It is known that carbohydrate-rich substrates, such as VW, can rapidly produce volatile fatty acid (VFA). During anaerobic treatment of VW, inhibition of

methanogenesis often occurs because of rapid hydrolysis rate and VFA accumulation (Mata-Alvarez et al., 2000; Bouallagui et al., 2005). Preliminary investigations also found that anaerobic VW treatment is unstable, particularly at high OLRs. Thus, investigating the performance of anaerobic digestion of VW in terms of its characteristics is critical.

A stable anaerobic digestion of VW can be achieved by reducing the OLR of the feedstock and by controlling pH by using chemical agents (Mata-Alvarez et al., 2000; Romano and Zhang, 2011). However, a low organic feeding rate will increase the requirements for larger reactor investment and operational cost. The chemicals used for pH control are also costly (Jiang et al., 2012). Thus, developing a process with high efficiency and low cost is highly important. Two-stage anaerobic digestion systems have been operated using a wide variety of substrates, such as municipal solid waste (Held et al., 2002), wastewater, and activated sludge (Demirel and Yenigün, 2002). Compared with the single-stage systems, phase separation is helpful in anaerobic digestion because of the buffering effect of organic loadings in the first stage, and a more constant

\* Corresponding author. Tel.: +86 10 62737852; fax: +86 10 62737885.

E-mail address: [wushubiao@gmail.com](mailto:wushubiao@gmail.com) (S. Wu).

environment for the methanogenic process in the second stage. These features accordingly promote the efficiency in terms of organic degradation and biogas production (Bouallagui et al., 2004, 2005). Considering the fast hydrolysis of VW and the negative effect of VFA accumulation on microorganism/enzyme activity, phase separation is a potential approach for controlling pH. Based on phase separation, effluent recirculation from the methanogenic stage to the acidic stage can help buffer the rapidly produced VFAs from VW hydrolysis and maintain a suitable pH. Effluent recirculation can also decrease the total operational cost of the treatment because of considerable savings in alkali addition (Cavinato et al., 2011). However, these ideas have not been well evaluated sufficiently in anaerobic digestion for treating VW.

The effluent from methanogenic reactors often contains an established microbial population. Its recirculation in acidogenic reactors makes easily microbial cells attachment on vegetable and flower wastes surface which enhances the hydrolysis (Zhang et al., 2007). It is also reported that recirculation could accelerate the rate of municipal waste degradation in anaerobic landfill (Bilgili et al., 2007). In addition, Lee et al. (2009) investigated the thermophilic two-stage anaerobic digestion of high-solid food waste for  $H_2$  and  $CH_4$  production at three different OLRs and the results implicated the function of recirculation on pH adjustment and dilution. Considering the dependence of recirculation effect on a variety of factors such as characteristics of feedstock and OLR, however, the effects of recirculation on the performance of anaerobic digestion of VW under continuously increasing OLRs are still unclear.

In this study, VW treatment was investigated in two laboratory-scale two-stage anaerobic reactors. The response of the performance to increasing OLR was evaluated in terms of biogas production, chemical oxygen demand (COD) degradation and the dynamics of pH and VFAs. Moreover, the extracellular enzyme activities were also examined to evaluate hydrolysis of insoluble organic materials. The performance of the two-stage system on VW treatment was further investigated by considering the potential limitations of hydrolysis under high OLRs. Effluent recirculation was conducted in one system. The effect of recirculation at different OLRs was identified.

## 2. Methods

### 2.1. Experimental setup

The experiments were carried out in two identical two-stage anaerobic digestion systems (A1–M1 and A2–M2). The acidogenic stages were run in completely stirred tank reactors (A1 and A2) with a volume of 5 L (diameter was 16 cm and height was 25 cm) and an effective volume of 3 L. The methanogenic stages were run in anaerobic fixed-bed biofilm reactors (M1 and M2) with a volume of 6 L (diameter was 16 cm and height was 30 cm) and an effective volume of 4 L. The fixed-bed reactor was packed with active carbon fiber (Yongtong Environmental Science and Technology Company, Jiangsu, China). Four cylindrical active carbon fiber

textiles (inner diameter was 6 cm, height was 20 cm and thickness was 2 mm) were bundled together by stainless steel wire and placed in the reactor as biofilm carriers. Gasbags were used in reactors for biogas collection. Data were recorded every day.

### 2.2. Substrate

The VW used in this study was collected from Xiyuan vegetable market in Northwest Beijing, China. Leafy waste materials (e.g., cabbage and Chinese cabbage) were selected. The raw materials were shredded to obtain a particle size less than 2 cm and stored in a refrigerator at  $-20\text{ }^{\circ}\text{C}$  before feeding. Analysis of raw shredded waste was carried out thrice, and the average composition was described as average  $\pm$  standard deviation. The results are shown in Table 1.

### 2.3. Experimental procedure

The laboratory-scale semi-continuous experiments were conducted under the controlled temperature of  $37 \pm 2\text{ }^{\circ}\text{C}$ . The acidogenic reactors were fed with different VW dilutions to vary the OLRs. The output of the acidogenic reactors was fed directly to the methanogenic reactors. The overall hydraulic retention time (HRT) of the systems was 14 d. To investigate effects of OLRs on two-stage anaerobic digestion, the systems were operated at four different OLRs of 1.3 g VS/L/d (phase A), 1.7 g VS/L/d (phase B), 2.1 g VS/L/d (phase C) and 2.6 g VS/L/d (phase D). During phase A and phase B, the HRTs of the acidogenic reactors and methanogenic reactors were fixed at 6 and 8 d, respectively, without recirculation. Considering the inhibition caused by organic overloading, part of the effluent from the methanogenic reactor M2 was recycled into the acidogenic reactor A2, during phase C and phase D. The effluent recycle ratio ( $R$ ) was defined as the ratio of the returned flow rate ( $Q_r$ ) to that of the base inlet flow rate ( $Q_i$ ) (Saritpongteeraka and Chaiprapat, 2008; Lee et al., 2009). The whole experimental characteristics are summarized in Table 2.

### 2.4. Analytical methods

Total solids (TS) and volatile solids (VS) were measured according to the standard method (APHA, 1998). The pH was measured using a portable Orion 3-Star pH meter with a pH electrode (ORION, 9172BNWP). Alkalinity was measured by the Nordmann-titration method using 0.25 N  $H_2SO_4$  to endpoints of pH 5.0 (Kafle and Kim, 2011). Biogas volume was measured from gasbags by a calibrated wet-type gas flow meter (LML-1, China), and  $CH_4$  content was determined using biogas analyzer EHEIM Visit 03 (Messtechnik Eheim, Germany). The COD for the effluent was determined by spectrophotometry at 620 nm according to the HACH method (DRB-200, USA). VFA concentrations were quantified in a gas chromatograph (Shimadzu, GC-2010 Plus, Kyoto, Japan) using aflame ionization detector and a rtx-wax capillary column ( $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ ) with high purity nitrogen as the carrier gas at a flow rate of 40 mL/min and a split ratio of 30. The column had an initial temperature of  $60\text{ }^{\circ}\text{C}$  (2 min holding time), which increased at  $10\text{ }^{\circ}\text{C}/\text{min}$  to  $140\text{ }^{\circ}\text{C}$ , then increased further at  $20\text{ }^{\circ}\text{C}/\text{min}$  to  $230\text{ }^{\circ}\text{C}$  (5 min holding time). The temperatures of the injector and detector were 230 and  $250\text{ }^{\circ}\text{C}$ , respectively. Samples were separated by high-speed centrifugation and then acidified using formic acid. Concentrations were determined using a standard curve obtained by injecting standard solutions of ethanol, acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid, valeric acid, caproic acids. The concentrations were summed as total VFA (TVFA). The cell-free amylase enzyme activities were measured according to the description by Zhang et al. (2007). The measurements of cellulose, hemicellulose and

**Table 1**  
Characteristics of the feed solids.

Item	Unit	Average values
Total solids (TS)	%	$5.4 \pm 0.6$
Volatile solids (VS)	%	$4.5 \pm 0.6$
Total COD	mg/g (humid weight)	$87 \pm 10.5$
Cellulose	% TS	$18.5 \pm 0.2$
Hemicellulose	% TS	$7.9 \pm 0.3$
Lignin	% TS	$3.8 \pm 0.2$
Total Kjeldhal nitrogen	% TS	$3.5 \pm 0.5$

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