



## Technical Note

# Effects of temperature and organic loading rate on the performance and microbial community of anaerobic co-digestion of waste activated sludge and food waste



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## HIGHLIGHTS

- Co-digestion of WAS and FW was investigated at different temperatures and OLRs.
- The CH<sub>4</sub> yield and VS removal efficiency were decreased as OLR gradually increased.
- The thermophilic system had the highest endurable OLR of 7 g VS L<sup>-1</sup> d<sup>-1</sup>.
- The mesophilic system showed the best stability at low OLR (< 5 g VS L<sup>-1</sup> d<sup>-1</sup>).
- The microbial community was more affected by temperature than the OLR.

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## ABSTRACT

Anaerobic co-digestion of waste activated sludge and food waste was investigated semi-continuously using continuously stirred tank reactors. Results showed that the performance of co-digestion system was distinctly influenced by temperature and organic loading rate (OLR) in terms of gas production rate (GPR), methane yield, volatile solids (VS) removal efficiency and the system stability. The highest GPR at 55 °C was 1.6 and 1.3 times higher than that at 35 and 45 °C with the OLR of 1 g VS L<sup>-1</sup> d<sup>-1</sup>, and the corresponding average CH<sub>4</sub> yields were 0.40, 0.26 and 0.30 L CH<sub>4</sub> g<sup>-1</sup> VS<sub>added</sub>, respectively. The thermophilic system exhibited the best load bearing capacity at extremely high OLR of 7 g VS L<sup>-1</sup> d<sup>-1</sup>, while the mesophilic system showed the best process stability at low OLRs (< 5 g VS L<sup>-1</sup> d<sup>-1</sup>). Temperature had a more remarkable effect on the richness and diversity of microbial populations than the OLR.

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## 1. Introduction

Anaerobic digestion (AD) is widely applied for sludge stabilization to reduce the sludge volume, generate methane gas, and yield a nutrient-rich final product (Appels et al., 2011). However, its efficiency is largely limited due to the relatively slow hydrolysis process, as waste activated sludge (WAS) is mainly composed of microbial cells within extracellular polymeric substances, and cell walls are physical barriers that do not permit intracellular organics to be easily biodegraded through digestion (Toreci et al., 2011). In previous studies, pretreatments such as mechanical (Nah et al., 2000), microwave (Toreci et al., 2011), alkaline (Li et al., 2012)

and ultrasonic (Xu et al., 2011) were reported to improve the efficiency of AD by disrupting sludge membranes to release the intracellular nutrients, but extra energy or chemicals were greatly consumed simultaneously.

Co-digestion of sludge with other organic-rich residues seems to be an attractive method which has been used to overcome its low digestibility in several studies (Habiba et al., 2009; Silvestre et al., 2011). Meanwhile, proper co-digestion could dilute potential hazardous compounds, promote synergistic effects of microorganisms and enhance biogas yields (Wan et al., 2011). Food waste (FW), with its high organic contents and excellent biodegradability, was regarded as an appropriate substrate that can be treated by AD (Zhang et al., 2007). Furthermore, plenty of attention has been paid due to its huge production from daily life in China. Nevertheless, some literatures pointed out that the digestion of FW alone may lead to the accumulation of abundant volatile fatty acids (VFA)

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especially at high organic loading rate (OLR), which could inhibit the methanogenesis and even destabilize the anaerobic process (El-Mashad et al., 2008; Nagao et al., 2012). These findings led to the investigation of its co-digestion with WAS as an alternative. Moreover, co-digestion of WAS and FW could be a strategic and cross-sectorial solution to deliver beneficial synergies for the water industry and FW management authorities (Iacovidou et al., 2012).

Efficiency and process stability are proved to be the criteria for the performance of AD (Lv et al., 2010). Most recently, impacts of mixing ratios and hydraulic retention time (HRT) on the performance of co-digestion of WAS and FW have been discussed (Heo et al., 2004; Kim et al., 2007; Lee et al., 2009). In fact, the equilibrium and productivity of the fermentation process can also be greatly disturbed by the OLR (Luste and Luostarinen, 2010). The major problem is that with an extremely high OLR, the rate of hydrolysis/acidogenesis could be higher than methanogenesis, and the high concentration of VFA accumulated from hydrolysis/acidogenesis can eventually lead to an irreversible acidification (Nagao et al., 2012). Nevertheless, previous studies mainly focused on the methane production and volatile solids (VS) removal efficiency of co-digestion system in a tolerable range of OLR (Heo et al., 2004; Kim et al., 2004, 2006), the information on maximum feasible loading rate is still lacking. Therefore, it is interesting to investigate the critical value by increasing the OLR stepwise through long-term experience.

Temperature, another important environmental factor, directly affects the dynamic situation of microorganisms. Earlier studies mainly concentrated on improving the efficiency of co-digestion process at a certain operating temperature, such as mesophilic (Dai et al., 2013), thermophilic (Kim et al., 2011), and even under hyperthermophilic condition (Lee et al., 2009). It seems that more data were demanded to compare the co-digestion performances at different temperatures applying the similar OLR, especially using continuously stirred tank reactors (CSTRs) in which all of the reactions (hydrolysis, acidogenesis, and methanogenesis) happened simultaneously. In addition, although the microbial community has been analyzed in the acidogenic fermenter for anaerobic co-digestion of kitchen garbage and sewage sludge (Lee et al., 2009), the information concerning the effects of the gradient temperature and OLR on microbial community structures in CSTRs is currently insufficient.

Consequently, the objective of this study was to evaluate the efficiency and stability of anaerobic co-digestion of WAS and FW under a wide range of OLRs at different temperatures in CSTRs. Meanwhile, the microbial community involved in anaerobic co-digestion process was also investigated by means of 16S rDNA polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE).

## 2. Methods

### 2.1. Preparation of feed stocks and inoculums

WAS used in this study was obtained from the secondary clarifier of the second municipal wastewater treatment plant in Changsha, China. Fresh sludge was concentrated by sedimentation for 6 h before being used. FW was collected continuously over 5 consecutive working days from a refectory in Hunan University, Changsha, China. To facilitate the digestion effectively, the FW was crushed to a mean size of 3–5 mm by an electrical food grinder. Samples were stored at 4 °C for no longer than one week.

WAS and FW were mixed with a total solids (TS) ratio of 2:1, which was demonstrated to have the best stability and efficiency in our preliminary experiments, and the value was approximately equal to the optimal mixture suggested by Kim et al. (2007). The

**Table 1**  
Characteristics of feed stocks and inoculums.

Characteristics	Unit	Inoculums	WAS	FW	Co-substrate
TS	g L <sup>-1</sup>	20	25	150	45
VS	% of TS	58	63	90	72
pH	–	7.8	7.2	5.6	6.8
SCOD	mg L <sup>-1</sup>	2324	2165	7260	14838
C/N	–	5.8	7.2	34	13
VFA	mg L <sup>-1</sup>	860	467	842	748
Alkalinity	mg L <sup>-1</sup> as CaCO <sub>3</sub>	212	143	453	3662
Ammonium	mg L <sup>-1</sup>	221	163	113	160

detailed characteristics of these substrates are summarized in Table 1.

### 2.2. Semi-continuous anaerobic co-digestion systems

Three series of lab-scale CSTRs were installed with a working volume of 2.0 L. The temperatures were controlled at 35 ± 2, 45 ± 2 and 55 ± 2 °C by three water baths, and were referred to as R1, R2 and R3, respectively. Mixing was performed intermittently by magnetic stirrers at uniform speed of 200 rpm before and after the new substrate was added. About 60% of digester working volume was filled with inoculums, and the co-substrates were introduced into the reactors as the starting material. The systems were flushed with N<sub>2</sub> for 3 min to create an anaerobic environment before sealing. Then the feeding and withdrawing were conducted once a day according to the needed OLR. Starting-up OLR was 1 g VS L<sup>-1</sup> d<sup>-1</sup> and the corresponding HRT was 33 d. The OLR was then increased to next step when the system reached a steady state. Corresponding operation process is shown in Table 2.

### 2.3. Analytical methods

Biogas volume was daily measured with water displacement, and methane content was analyzed by a gas chromatograph (GC 2010 Shimadzu) equipped with a thermal conductivity detector and a 2 m × 3 mm stainless-steel column packed with Porapak Q (80/100 mesh). Samples from the reactors were immediately centrifuged at 5000 rpm for further analyses. For the analysis of soluble chemical oxygen demand (SCOD) and ammonium, the supernatant was filtrated through a 0.45-μm membrane filter (Whatmann, USA). TS, VS, total VFA (TVFA), alkalinity, total nitrogen, ammonium, and SCOD were determined according to the Standard Methods (APHA, 2005).

### 2.4. Microbial community analysis

Samples were taken at steady state of each temperature under various OLRs for microbial community analysis. Total genomic DNA was extracted from 0.2 g digestion samples (wet weight) according to the method described by Yang et al. (2007). DNA was dissolved in 200 μL of 50 × TAE (2 M Tris, 1 M Acetate, 0.1 M Na<sub>2</sub>EDTA·2H<sub>2</sub>O) buffer and 4 μL of DNA was used for agarose gel electrophoresis.

The PCR amplification was performed on an iCycler IQ5 Thermocycler (Bio-Rad, USA). The primer set GC341F and 534R were used for amplification. DGGE was carried out by using the Dcode Universal Detection System in accordance with the manufacturer's instructions (Bio-Rad, USA). The detailed running procedures for PCR and DGGE were operated as described by Zhang et al. (2011).

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