



# A comparative study of single- and two-phase anaerobic digestion of food waste under uncontrolled pH conditions

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## ABSTRACT

This study compared single- versus two-phase systems for semi-continuous anaerobic digestion of food waste without pH control at varying organic loading rates (OLRs). The methanogenic reactors of both systems required trace element supplementation for stable operation at 3.0 g VS (volatile solids)/L-d or higher OLRs. Under trace-element supplemented conditions, both systems achieved stable and efficient performance at OLRs up to 4.0 g VS/L-d. The two-phase system outperformed the single-phase system at 1.0–4.0 g VS/L-d OLRs, but it failed at an OLR of 5.0 g VS/L-d. Meanwhile, the single-phase system maintained the stable performance and reached its maximum methane production at this OLR. These results suggest that a single-phase configuration is more advantageous for robust treatment of food waste without pH control at high organic and hydraulic loads. Hydrogenotrophic methanogens dominated the methanogen community throughout the experiment in both systems. Microbial community structure shifts correlated with reactor operation and performance characteristics.

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## 1. Introduction

Food waste (FW) is increasing worldwide with population and economic growth and has become a major sustainability issue in most countries. Annual production of FW in Korea is approximately 5 million tons, and this accounts for over 22% of the total municipal solid waste production (Nguyen et al., 2017). Direct landfilling and ocean dumping of FW and other organic wastes have recently been banned in Korea. Therefore, alternative methods for proper management of FW on land are highly needed. Anaerobic digestion is considered a viable means to treat FW because it converts organic waste to methane-rich biogas. FW, characterized by high organic content, is a good feedstock for methane production through the AD. However, the AD of FW has practical limitations related to the characteristics of FW such as low pH, low alkalinity, and low trace element content (Cho et al., 1995). Readily biodegradable FW is rapidly fermented to organic acids under anaerobic conditions, leading to reactor souring and process deterioration (Shen et al., 2013). Low pH and alkalinity of FW can lower the buffering capacity and thus the process stability of a digester, particularly under high organic loading conditions. Deficiency of trace elements, which function as cofactors in essen-

tial enzymes involved in methanogenic pathways, is also reported as a common cause of process upset in anaerobic FW digestion processes (Zhang et al., 2015).

Conventional AD plants treating FW have typically employed a single-phase continuously stirred tank reactor (CSTR) configuration. In a single-phase system, all reaction steps of the AD pathway, from hydrolysis to methanogenesis, occur in one reactor operated under optimal conditions for methanogens, i.e., neutral pH and long hydraulic retention time (usually more than 20–30 days). This means that acidogens, which have different physiological and growth characteristics from methanogens, are under suboptimal conditions in such a reactor (Ahiring, 2003). A two-phase AD system, consisting of two reactors in series operating under different conditions, was proposed in an attempt to resolve this limitation (Pohland and Ghosh, 1971). The first reactor (i.e., acidogenic phase) is often operated under moderately acidic conditions (pH 5–6) at a short hydraulic retention time (HRT; <5 days) to form a favorable environment for the growth of acidogens and prevent methanogenic activity, while the second reactor (i.e., methanogenic phase) is run under optimal conditions for methanogens as for the single-phase system. This two-phase configuration is anticipated to enhance the overall performance as far as an ideal biphasic ecosystem is maintained. Owing to such advantages, it has been proposed to be advantageous over single-phase configuration and widely applied in the pilot- and field-scale processes treating various organic wastes including FW, sewage sludge, animal manure, and

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their mixtures (Demirel and Yenigün, 2002; Kim et al., 2014b; Ratanatamskul et al., 2015). However, the significance of its effect is debatable because AD involves complex syntrophic relationships between acidogens and methanogens (Cooney et al., 2007).

Reactor pH is a major factor in determining the fermentation type (Chen et al., 2015). FW is readily fermented and prone to acidification; thus, it is often required to control the pH of the acidogenic phase to avoid highly acidic conditions that may disrupt both acidogenic and methanogenic activities. The pH control is generally achieved by adding alkaline compounds such as NaOH, NaHCO<sub>3</sub>, and KOH into the reactor. However, the use of a buffer solution results in additional operating costs, and salts from alkaline compounds, such as Na<sup>+</sup> and K<sup>+</sup>, that can negatively affect microbial activity (Chen et al., 2008). These limitations should be considered when operating AD processes, particularly those with two-phase configuration. Minimizing or avoiding the use of a buffer solution could be a direct way to mitigate such limitations. However, if pH is not controlled, the acidogenic pH can quickly drop to <4, which is well below the optimal range for acidogenesis, when treating readily biodegradable substances such as FW (Lim et al., 2013; Shin et al., 2010). This means that acidogens will be under suboptimal pH conditions, which is inconsistent with the original intention of the two-phase configuration.

This study aimed to compare the process performance and microbial community dynamics between a single- and a two-phase AD system treating FW in continuous mode without pH control. The experimental systems were operated at increasing organic loading rates (OLRs) by reducing HRT to examine their responses to increasing hydraulic and organic loads for an 18-month period. Trace element supplementation was also employed to stabilize the process performance and assess its effect on methanogenesis. The acidogenic phase of the two-phase system remained highly acidic (pH 3.3–3.4), which may exert inhibitory effects, throughout the experiment, and particular attention was paid to its influence on acidogenesis as well as methanogenesis. To gain a more comprehensive understanding of the systems, the microbial communities in the reactors were analyzed and compared using a combination of molecular and statistical tools. Characterization of microbial community structure in AD processes has been attracting growing interest because understanding the underlying microbial ecology is critical for improving the performance at the fundamental level. Fermentative *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* bacteria, involved in hydrolysis and acidogenesis, commonly occur in abundance, and most archaea are methanogens of the phylum *Euryarchaeota* in AD systems. However, microbial ecology of AD systems is still largely unknown due to its highly diverse and complex mixed-culture nature, particularly in waste treatment processes. Insights into the process performance and the underlying ecology obtained from this study would help better understand and operate a continuous AD process treating FW.

## 2. Materials & methods

### 2.1. Substrate and inoculum

FW was collected from a cafeteria at Ulsan National Institute of Science and Technology and consisted of mainly cooked rice and smaller amounts of flour products, soup, vegetables and meat. The collected FW was ground into a slurry using a household blender and passed through a 3 mm mesh. The prepared FW slurry was adjusted to a volatile solid (VS) content of 10% (w/v) with tap water and stored at 4 °C until use. FW was collected on four occasions during the course of the experiment (i.e., four different batches of FW), and the reactors were fed with the same batch for fair comparison. The average physicochemical characteristics of the FW

substrate are summarized in Table 1. Anaerobic sludge from a full-scale AD plant co-digesting sewage sludge and FW were used to inoculate the reactors. The sludge was passed through an 860 µm mesh to remove coarse particles before inoculation.

### 2.2. Reactor operation

A single-phase and a two-phase semi-continuous AD system treating FW (10% VS, w/v) were operated in parallel at stepwise increasing OLRs from 0.5 g VS/L·d, a sufficiently low OLR for stable start-up, by reducing the HRT until system failure (Table 2). A CSTR with a working volume of 2 L was used as the single-phase AD system (Rs). The two-phase system consisted of an acidogenic (Ra) and a methanogenic (Rm) CSTR with a working volume of 0.5 and 2 L, respectively. Rs and Rm were initially filled up with anaerobic sludge while Ra was with 0.4 L of anaerobic sludge (80%, v/v) and 0.1 L of food waste. Both single- and two-phase systems were started up at a low OLR of 0.5 g VS/L·d. In the two-phase system, Ra was operated at a fixed HRT of 4 days throughout the experiment while Rm was fed with a portion of the effluent from Ra to adjust the overall OLR. The reactors were manually fed using a syringe on a daily (Rs and Rm at OLRs below 4.0 g VS/L·d) or semi-daily (Rs and Rm at 4.0 g VS/L·d or higher OLRs and Ra) basis. All reactors were operated without pH control and maintained at 35 ± 1 °C. Fe, Co, and Ni (100 mg Fe/L as FeCl<sub>3</sub>·6H<sub>2</sub>O, 2 mg Co/L as CoCl<sub>2</sub>·6H<sub>2</sub>O, and 1 mg Ni/L as NiCl<sub>2</sub>·6H<sub>2</sub>O in the substrate) were added to Rs and Rm every other day from days 227 and 101, respectively, and onwards as trace elements for the growth of methanogens (Zhang et al., 2015). Biogas production was continuously measured by a MilliGascounter (Ritter) connected to the reactor headspace. The pH in each reactor was continuously monitored using a pH electrode installed in the reactor.

### 2.3. DNA extraction and next-generation sequencing

The 16S rRNA gene libraries for next-generation sequencing (NGS) were prepared from the purified DNA samples by poly-

**Table 1**  
Physicochemical characteristics of food waste.

Parameter	Unit	Value <sup>a</sup>
pH		4.2 (1.9)
Total COD	g/L	133.3 (14.9)
Soluble COD	g/L	58.7 (8.5)
Total solids	g/L	103.4 (5.9)
Volatile solids	g/L	97.0 (5.9)
Total nitrogen	g/L	2.6 (0.5)
Total phosphorus	g/L	0.5 (0.1)
Total carbohydrates	g/L	61.4 (11.4)
Carbon	% dw <sup>b</sup>	48.1 (2.8)
Hydrogen	% dw	7.1 (0.3)
Oxygen	% dw	37.3 (2.0)
Nitrogen	% dw	3.7 (1.2)
Sulfur	% dw	0.1 (0.1)
C/N ratio		13.9 (3.3)
Alkalinity	mg/L as CaCO <sub>3</sub>	31.0 (27.0)
Fe	mg/L	1.987 (2.088)
Ni	mg/L	0.158 (0.248))
Co	mg/L	0.002 (0.002)
Al	mg/L	0.817 (0.189)
Cr	mg/L	0.013 (0.009)
Cu	mg/L	0.141 (0.112)
Mn	mg/L	1.081 (0.599)
Zn	mg/L	1.163 (0.956)
Mo	mg/L	0.398 (0.652)
W	mg/L	0.021 (0.011)

<sup>a</sup> Determined from four different batches of food waste prepared at different points during the course of the experiment. Standard deviations are in parentheses.

<sup>b</sup> dw, dry weight.

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