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# Hydrolysis–acidogenesis of food waste in solid–liquid-separating continuous stirred tank reactor (SLS-CSTR) for volatile organic acid production

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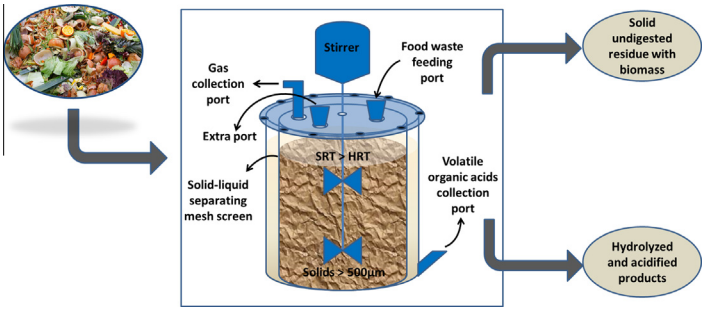
## HIGHLIGHTS

- Novel CSTR design incorporated a solid–liquid separator for SRT–HRT decoupling.
- The design effectively retained the active biomass and un-hydrolyzed solids.
- The organic acid leaching rate and enzyme activities were high with SLS-CSTR.
- The design could be potentially used for many bio-refinery projects.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The use of conventional continuous stirred tank reactor (CSTR) can affect the methane ($CH_4$) recovery in a two-stage anaerobic digestion of food waste (FW) due to carbon short circuiting in the hydrolysis–acidogenesis (*Hy–Aci*) stage. In this research, we have designed and tested a solid–liquid-separating CSTR (SLS-CSTR) for effective *Hy–Aci* of FW. The working conditions were pH 6 and 9 (SLS-CSTR-1 and -2, respectively); temperature-37 °C; agitation-300 rpm; and organic loading rate (OLR)-2g VS $L^{-1}$ $day^{-1}$. The volatile fatty acids (VFA), enzyme activities and bacterial population (by qPCR) were determined as test parameters. Results showed that the *Hy–Aci* of FW at pH 9 produced ~35% excess VFA as compared to that at pH 6, with acetic and butyric acids as major precursors, which correlated with the high enzyme activities and low lactic acid bacteria. The design provided efficient solid–liquid separation there by improved the organic acid yields from FW.



## 1. Introduction

Food waste (FW) is generally characterized with high organic contents (20–45% carbon; 80–90% volatile solids), lipids (10–40%) and protein (5–10%). They are readily available feed stock in urban centers for production of platform molecules and biofuels that can offset fossil-fuel consumption. In Hong Kong, the FW generation is ~3648 tons per day (~38% of municipal solid waste; HKEPD, 2015), which can be efficiently handled to produce 800,000–850,000 kWh energy assuming 250 kWh/ton FW (USEPA, 2008) by anaerobic digestion (AD) processes. Anaerobic digestion is a reliable and mature technology to convert organic carbon into methane ($CH_4$) gas that is mainly governed by groups of bacteria/archaea (Rani et al., 2012; Karthikeyan and Visvanathan, 2013; Kavitha et al., 2014). Bacteria playing a major role in hydrolysis (*Hy*), acidogenesis (*Aci*) and acetogenesis (*Ace*) processes converting organic

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substrates into volatile fatty acids (VFA), $CO_2$ and $H_2$, while archaea convert these accumulated acids, $CO_2$ and $H_2$ into $CH_4$ via methanogenesis (*Metho*) processes, if steady-state-operating conditions were maintained (Fantozzi and Buratti, 2009; Karthikeyan and Visvanathan, 2013; Zhang et al., 2014). While in common, even under very low organic loading rates, the single-phase FW–AD systems are turned out to be sore digesters due to quick *Hy–Aci* and slow *Metho* rates (Zhang et al., 2014, 2015).

Therefore, physical separation of *Hy–Aci* from *Metho* process are researched for FW treatment, thereby the optimum conditions for two different groups of microbes can be maintained to maximize the biogas yield (Kim et al., 2008; Selvam et al., 2009; Aslanzadeh et al., 2013; Xu et al., 2014). The greatest advantages of two-phase AD systems are (i) higher organic loading rate in the first stage (*Hy–Aci* system); (ii) consistent feed for the second stage (*Metho* system); (iii) easy to manipulate the reactor conditions to facilitate the reactions faster; and (iv) much smaller reactor foot print area (Bouallagui et al., 2004; Wang et al., 2003; Dogan and Demirer, 2009). On contrary, a unique challenge is to maintain high ratio of active biomass for *Hy–Aci* of FW to produce continuous and constant feed for $CH_4$ production. Since the *Hy–Aci* is the heart of two-phase AD technology, developing a bioreactor for efficient *Hy–Aci* of FW is vital.

In a continuous stirred tank reactor (CSTR), which is most commonly used bioreactor design for AD, the FW that are partially hydrolyzed is constantly removed with active attached microbial biomass. This leads to carbon loss via short circuiting since solids and hydraulic retention times are equal (SRT = HRT). Undigested larger particles will be removed from the system; otherwise it requires longer SRT–HRT for complete digestion even at low organic loading rates (Boe and Angelidaki, 2009). Therefore, decoupling active biomass and FW solids from hydrolyzed products (i.e., SRT > HRT) are necessary to improve the process efficiency. Although leach bed reactors (LBRs) provide better solid–liquid separation (Selvam et al., 2009; Xu et al., 2011) for effective hydrolysis of FW, only limited /no studies have been demonstrated for the continuous mode of LBR operations for FW treatment (Wang et al., 2003). Also, addition of bulking agent is a pre-requisite to facilitate leachate movement in LBR for FW treatment (Xu et al., 2011). Besides, bulking agents will occupy the effective reactor volume making it more difficult for continues operations. In another study, the novel rotating drum mesh (membrane pore size of 100 μm) bioreactor was developed and SRT–HRT decoupling effects was evaluated (Walker et al., 2009). But, the organic leaching/acidification rates were found to be very poor (see Table 1), while the scale-up of reactor for field application was found impractical.

Considering this predicament, a bench scale solid–liquid separating CSTR (SLS-CSTR) is designed for *Hy–Aci* of FW to produce consistent feed stock (i.e., organic acids) for subsequent *Metho* processes (and any other bio-refinery projects). The proposed design provides high SRT (with active biomass) and low HRT thereby the yield of VFA per gram of FW is expected to be improved for subsequent *Metho* processes. As a preliminary study, two identical SLS-CSTRs are tested under semi-continuous operational mode under two different pH conditions and the *Hy–Aci* rates as the measure of VFA yield are compared. In addition, the enzyme activities and bacterial populations are quantified by standard protocols and compared between the test conditions.

## 2. Methods

### 2.1. Feed stock and inoculum preparation

Around 20 kg of simulated FW consisted of bread, boiled rice, cabbage and cooked meat at 35%, 25%, 25% and 15% on wet weight basis, respectively were prepared to provide homogeneous feed stock for subsequent experimental use (Selvam et al., 2009; Xu et al., 2011). The feedstock was stored at 4 °C and brought to room temperature before feeding the reactors. The properties of FW were: total and volatile solids (TS and VS) contents of ~39.5% and ~97.1%, respectively. Anaerobic digested (AD) sludge with ~7% TS and ~98% VS of TS were collected from a laboratory scale upflow anaerobic sludge blanket reactor, operated for more than one year with FW leachate as substrate and was used as the seed culture for the startup of the *Hy–Aci* reactor.

### 2.2. Reactor design and operational sequences

Two identical CSTRs designed with the total and working volume of 6.0 L and 3.7 L, respectively (~60%) were used. To provide the solid–liquid separation in the SLS-CSTR, a non-corrosive cylindrical screen of 0.5 mm mesh size with the dimension of 28 cm height and 6.5 cm in diameter, was placed inside the reactor. The reactors were initially loaded with 2 kg of FW pre-mixed with 500 mL AD sludge and 1 L of water. The content of each reactor was continuously mixed with a stirrer at 600 rpm (during start-up and stabilization phase) and pH was monitored daily using a pH probe (Orion, Model 920). After 10 days start-up phase, semi-continuous feeding for the stabilization phase was initiated with pH adjustment continuously to acidic (pH = 6) and alkaline (pH = 9) range in SLS-CSTR-1 and -2, respectively using 2 M NaOH. After 30 days of stabilization, the agitation speed was set to 300 rpm (from 600 rpm) in SLS-CSTR-1 and -2 and the acidification rate was monitored at 37 °C with pH maintained continuously with 2 M NaOH in the experiment phase. The HRT and OLRs were calculated using below equations,

$$\mathrm{HRT} = \frac{\mathrm{LV_r}}{\mathrm{LW_{eff}}}\left(\frac{\mathrm{m^3}}{\mathrm{m^3\ d^{-1}}}\right) \tag{1}$$

$$\mathrm{OLR} = \frac{\mathrm{FW_{fed}} \times \mathrm{OC_{FW}}}{\mathrm{WV_r}}\left(\frac{\mathrm{m^3\ d^{-1}} \times \mathrm{kg\ m^{-3}}}{\mathrm{m^3}}\right) \tag{2}$$

where,

$LV_r$ – liquid volume of the reactor;

$LW_{eff}$ – acidified liquid withdrawn as effluent from the reactors per day;

$FW_{fed}$ – Food waste loading rate into CSTRs;

$OC_{FW}$ – organic carbon content of FW (as VS);

$WV_r$ – working volume of the reactor.

Every alternate day, ~40 g of FW was loaded into the SLS-CSTRs, while 100 mL of *Hy–Aci* leachate was withdrawn daily and replaced with 100 mL of DI water to compensate the loss and/or to maintain the TS contents in the reactors (~8–10%). Based on the calculations, the OLR in SLS-CSTR was ~2.0 kgVS $m^{-3}$ $day^{-1}$ and HRT was 15 days during the experimental phase of operation, while SRT is greater than 15 days. The leachate samples were collected for physiochemical and biological analyses i.e., electrical conductivity (EC), chemical oxygen demand (COD), total organic carbon, VFAs, total bacteria and Lactobacilli populations by qPCR, once in every week from SLS-CSTRs, except pH that was measured daily and buffered to 6 or 9 using NaOH. Both the systems were naturally buffered during experimental phase and the addition of NaOH was largely reduced (from 12 g to <1 g in SLS-CSTR-1 and 16 g to <3 g in SLS-CSTR-2) as compared to that of initial start-up or stabilization phase. The acidification rate was calculated from the sCOD and VFA concentrations within the SLS-CSTRs.