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Responses of microbial community and acidogenic intermediates to different water regimes in a hybrid solid anaerobic digestion system treating food waste

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HIGHLIGHTS

- Effects of different water regimes on LBR performance were studied.
- Methanogenic effluent recirculation accelerated the protein hydrolysis in LBR.
- Different water regimes significantly affected the bacterial community in LBR.
- *Lactobacillus* predominated in LBR when water replacement was applied.
- *Clostridium* and hetero-fermentation LAB dominated with effluent recirculation.

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ABSTRACT

This study investigated the effects of different water regimes in an acidogenic leach bed reactor (LBR) during 16-day batch mode food waste digestion. LBRs were operated under five water replacement ratios (WRRs) (100%, 75%, 50%, 25% and 5% in LBRs R1, R2, R3, R4 and R5, respectively) and methanogenic effluent (ME) addition with two leachate recirculation frequencies (once in 24 h and 12 h in LBRs R6 and R7, respectively). Results showed that 50–100% WRRs accelerated the hydrolysis and acidogenesis with butyrate as the dominant product (~35% of COD); whereas 5–25% WRRs promoted propionate production. The ME recirculation enhanced protein decomposition and reduced ethanol production. *Lactobacillus* dominated in LBRs with water addition (R1–R5), while *Clostridium* and hetero-fermenting lactic acid bacteria dominated in LBR with ME addition (R7). The highest volatile solid degradation (82.9%) and methane yield (0.29 L-CH_4/g VS) were obtained with ME addition at 0.7 d hydraulic retention time.

1. Introduction

Leach bed reactor (LBR) is a technically simple and flexible system that can be used as either single-stage or two-stage anaerobic digester. In a hybrid solid anaerobic digestion (HSAD) system, leachate collected from first-stage LBR containing various hydrolysates and acidogenic intermediates is fed into the second-stage methanogenic reactor for methane generation (Xu et al., 2011). The advantage of LBR is its ability to handle solid wastes with varied solids content; however, it suffers from a slow and limited solubilization rate. Due to the channeling problems and absence of agitation in LBR, the intimate contact between microorganisms and substrate is retarded and the hydrolysis rate decreases consequently. Until now, the most popular methods to hasten the particulates hydrolysis are water flushing and leachate or methanogenic effluent recycling within the LBR (Cirne et al., 2007; Lü et al., 2008; Selvam et al., 2010). Their positive effects include increased moisture content, enhanced mass transportation, redistribution of the enzymes and microbes; and minimization of local shortages of nutrients in reactor (Cirne et al., 2007; Cysneiros et al., 2008).

However, only limited design data and operation guidance have been reported and very little is known about the microbial interactions of two-phase anaerobic digesters with various water regimes. Charles et al. (2009) compared the effect of adding sterile (filtered) anaerobic liquid and the non-sterile anaerobic liquid (buffer + microbes) during the startup of an anaerobic digester with municipal solid waste; and the results showed that the essential role of anaerobic liquid was its buffering capacity. Meanwhile, nitrogen,

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phosphorous and other micro-nutrients in the methanogenic effluent were expected to be favorable for the microbial growth in the acidogenic reactor.

In fact, the appropriate microbial community exists in the recycling methanogenic effluent would play an essential role in the rapid start-up of methanogenesis in LBR. Up to 20–40% of the theoretical methane potential was found to be generated in the acidogenic phase (Cysneiros et al., 2008). Usually the biogas generated in LBR is not collected due to economic reasons, thus the introduction of methanogenic bacteria into the first stage should be avoided in order to realize phase-separation. Indeed, aeration and micro-aeration have been proposed to inhibit the methanogenic activity in the first stage (Xu et al., 2014). Furthermore, hydraulic retention time (HRT) should be a critical factor to regulate the microbial community. With a HRT of 4 d, 14 g/L of volatile fatty acids (VFA) accumulated in a continuous stirred tank reactor (CSTR) with acetate as the main fatty acid; but when the HRT was increased to >4 d, evolution of methane was observed (Ueno et al., 2007). However, HRT of LBR is decoupled from solid retention time (SRT) by separating solid/biomass from liquid phase, therefore the response of microbial community and metabolites distribution should be different from CSTR.

To expand the knowledge on the link between microbial community and regulation of water regimes, this study was performed in a LBR connected with an upflow anaerobic sludge blanket (UASB) system using synthetic food waste as substrate. Tap water and methanogenic effluent were supplemented into the acidogenic LBR; and the reactor performance was monitored and correlated with enzyme activities and microbial population dynamics of the hydrolytic/acidogenic LBRs.

2. Methods

2.1. Experimental setup

The synthetic food waste (FW) with a total solids content (TS) of 38.5% and volatile solids content (VS) of 37.3% was used in the study, and the composition and preparation of the FW have been described previously (Selvam et al., 2010). Anaerobically digested sludge (ADS) collected from the Shek Wu Hui wastewater treatment plant, Hong Kong, with TS and VS/TS of 4.7% and 83.1%, respectively, was used as the inoculum. A two-phase system combining a LBR connected to an UASB with the working volumes of 4.6 L and 10.0 L, respectively, was used in this study. One kg FW was mixed with 200 mL ADS and fed into the LBRs and then 1.0 L of leaching solution (tap water/methanogenic effluent) was added from the top of the LBRs on Day 0. Leaching occurred naturally and the leachate was collected in the lower chamber of the LBRs. Different water regimes (Table 1) were applied as described below and the reactors were operated for 16 d under mesophilic condition (35 °C).

In Run-1, five different water replacement ratios (WRRs) of 100% (R1), 75% (R2), 50% (R3), 25% (R4) and 5% (R5) were investigated. Following the addition of FW, 1.0 L of tap water was percolated through the LBRs on Day 0 to initiate natural leaching, and about 1 L of acidogenic leachate (AL) was expected to be collected. The volume of collected AL was measured; and depending on the WRR applied, ~1.0 L to 0.05 L (corresponding to the LBRs R1–R5, respectively) was replaced with tap water and made up to 1 L with pH adjusted to 6.0 using $NaHCO_3$ and was recycled back to the respective LBRs. The replaced AL was fed to the UASB for methanization. This practice was continued for the rest of the 16 d experimental period.

In Run-2, methanogenic effluent (ME) was added instead of tap water on Day 0. The ME was collected from the UASB, stored in 20-L tanks and aerated for 12 h before adding into the LBRs. Since the WRR 75% was identified as the optimum ratio from Run-1 experiment, it was adopted in Run-2. Therefore a ratio of 75% ME and 25% AL was recycled back to the LBR daily. Since the ME could carry alkalinity, pH adjustment was not practiced in Run-2. Two different recycling frequencies of ME/AL (0.75/0.25) were applied in this Run: once in 24 h and 12 h (LBRs R6 and R7, respectively).

2.2. Characterization of leachate and digestate

The characteristics of FW-ADS mixture, including TS and VS contents, total organic carbon (TOC_{solid}) and total Kjeldahl nitrogen (TKN_{solid}) were analyzed before and after digestion to calculate the organic removal efficiency. The TS and VS were determined through oven drying at 105 °C for 24 h and igniting at 550 °C for 16 h in a muffle furnace, respectively, while the TOC and TKN were analyzed according to the Standard Methods (APHA, 2005). Acidogenic leachate (AL) from LBR and ME from the UASB reactor were collected daily and analyzed for pH, chemical oxygen demand (COD), NH_4^+-N, TKN and VFA. The concentrations of COD, NH_4^+-N and TKN in leachate samples were determined according to the Standard Methods (APHA, 2005). VFA and ethanol (EtOH) concentrations were determined using a HP 6890 Series gas chromatograph (Hewlett Packard) with flame ionization detector as reported previously (Xu et al., 2011). The sum of acetate (HAc), propionate (HPr), *n*-butyrate and *iso*-butyrate (HBu), and *n*-valerate and *iso*-valerate (HVa) are reported as total VFA (tVFA). Biogas produced in the UASB was measured using a wet gas meter (BSD-0.5, Shanghai) and further qualitatively analyzed using a gas chromatograph (GC-HP7890, 0.53 mm × 30 m PLOT-Q column) equipped with a thermal conductivity detector.

2.3. Analyses of enzyme activities

Enzyme activities were determined using API ZYM™ strips (BioMerieux, France), which is a commercial semi-quantitative micro-method designed for systematic and rapid analyses of 19 enzymatic reactions (Tiquia, 2002). Leachate samples collected from Day 1, 9 and 16 were allowed to settle for 30 min and then 65 μL of supernatant was used for the analysis of extracellular enzyme activities using API ZYM™ strips. After incubation at 37 °C for 4 h, ZYM-A and ZYM-B reagents were added into each well and illuminated under light for color development. The results

Table 1
Experimental design.

	Reactors	Water regimes[a]	pH regulation to 6.0	Exchange frequency	HRT (d)
Run-1	R1	Water/AL = 1.0/0, WRR 100%	Yes	1.0 L/d to LBR	1
	R2	Water/AL = 0.75/0.25, WRR 75%	Yes	1.0 L/d to LBR	1.33
	R3	Water/AL = 0.50/0.50, WRR 50%	Yes	1.0 L/d to LBR	2
	R4	Water/AL = 0.25/0.75, WRR 25%	Yes	1.0 L/d to LBR	4
	R5	Water/AL = 0.05/0.95, WRR 5%	Yes	1.0 L/d to LBR	17
Run-2	R6	ME/AL = 0.75/0.25	No	1.0 L/d to LBR	1.33
	R7	ME/AL = 0.75/0.25	No	1.0 L/12 h to LBR	0.67

[a] Acidogenic leachate, AL; methanogenic effluent, ME.