High-Solids Anaerobic Digestion of Cassava Pulp in Semi-continuous Bioreactors

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The effects of two total solids contents (TS) and two inocula were studied for the semi-continuous high-solids anaerobic digestion (HS-AD) of cassava pulp under mesophilic conditions (35 ± 2 °C). In the 1.0-L bioreactors, two TS of 15% and 20% with digestate as a sole inoculum were chosen to run the HS-AD, and two inocula (the digestate from the AD of cassava pulp and sewage sludge) were used separately under TS 20%. All treatments were carried out at the organic matter loading rates (OLRs) of from 3.0 to 10.0 kg volatile solids (VS)/(m^3·d), with each phase of 6 days followed by two 3-days phases of no feeding and then low OLR of 6.5 kg VS/(m^3·d). Compared with TS 15%, the bioreactors of TS 20% with the digestate had a higher buffering capability to alleviate the rapid acidification and a higher level of the specific methane yields (SMYs) of from 0.212 to 0.233 m^3/($kgVS_{added}$) at the OLRs of 4.0 to 6.5 kgVS/(m^3·d), while TS 15% obtained the highest SMY of from 0.152 to 0.182 m^3/($kgVS_{added}$) at the OLR of 4.0, 6.5, and 8.0 kgVS/(m^3·d). In contrast, sewage sludge did not restrain the rapid acidification and only yielded quite small SMYs under TS 20%.

Keywords: Cassava pulp; High-solids anaerobic digestion; Biogas production; Mesophilic; Inoculum

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INTRODUCTION

As one of the major crops, cassava is grown ubiquitously in tropical areas (Trakulvichean *et al.* 2019) to produce edible starch and ethanol. Processing fresh cassava creates enormous by-product cassava pulp, which is also known as cassava dregs (Sriroth *et al.* 2010). In South China, about 0.95 million tons of cassava pulp is produced annually (Yang *et al.* 2011), which is derived from more than 9 million tons of fresh cassava in the local provinces. If cassava pulp is discarded in the environment without being properly treated, it generally emits odors and leachate owing to much easily-biodegraded carbohydrate in cassava pulp. In contrast, a high-level content of carbohydrate means a high biogas production potential of cassava pulp (Sanchez *et al.* 2017). Allowing for urgent requirement for clean energy in the recent decade, anaerobic digestion (AD) is considered as a promising method for the treatment of cassava pulp, which not only minimizes the solid waste but also produces a large amount of biogas (Ren *et al.* 2014).

Total solids content (TS) is one key technical parameter when developing the technology of cassava-pulp AD. Based on TS, AD is divided into two types: low-solids AD (LS-AD) (TS < 15%) and high-solids AD (HS-AD) (TS ≥ 15%) (Xu *et al.* 2021).

Compared with LS-AD, HS-AD generally has some advantages, such as more endurance at a high OLR (organic loading rate), less addition of water, less biogas slurry, and a lower cost (Nkemka and Hao 2018). A recent full-scale study showed that HS-AD under TS 21.9% demonstrated similar biogas yields as that from LS-AD for co-digestion of cow manure and agricultural products (Chiumenti *et al.* 2018). However, an excessive accumulation of volatile fatty acids (VFAs) could happen in HS-AD of cassava pulp because cassava pulp has much easily biodegradable organic matter, which in effect could be enhanced by the strong heterogeneity derived from a weak mixing of matrix during HS-AD. The overload VFA accumulation will deteriorate the acidification of organic matter and lower microorganism activity in AD. Thus, it is necessary to assess the effect of TS on biogas production during HS-AD of cassava pulp for optimizing the range of TS.

Besides TS, the addition of inoculum is another key factor when HS-AD of cassava pulp is carried out. The advantages of adding inoculum include: accelerating the start-up of AD (Walkins *et al.* 2015; De la Rubia *et al.* 2018), moderating the rapid acidification (Wang *et al.* 2014), and also promoting the C/N ratio. There are three necessary groups of enzymes for AD of organic solid waste, which include hydrolytic enzymes, acidogenic enzymes, and methanogenic enzymes (Amin *et al.* 2021; Xing *et al.* 2020). These enzymes can be enriched in raw material by adding inoculum to accelerate the start-up (Xing *et al.* 2020). Rapid acidification is a common problem for AD of cassava pulp and other similar substrates, such as fruit and vegetable wastes (Bouallagui *et al.* 2009), which severely reduces microbial activity of methanogenesis and inhibits the biogas production (Panichnumsin *et al.* 2010). To overcome the rapid acidification in AD of cassava pulp, co-digestion and adding inoculum were developed by some researchers (Panichnumsin *et al.* 2012; Zhang *et al.* 2016). With some co-substrate of low C/N ratio, such as manure, the C/N ratio of the mixed feedstocks was adjusted to be at the range of from 20:1 to 30:1, which was considered an optimal value for biodegradation of organic substrate (Zeshan *et al.* 2012). However, the co-substrate of a low C/N ratio is not sustainably sufficient in some rural areas for the continuous feeding. Adding inoculum has the similar benefits and an extra advantage of a once-through operation that is beneficial for the area lacking co-substrates. Thus, the addition of an optimal inoculum is a reasonable alternative under that condition. Sewage sludge and digestate are two inocula used commonly in AD (Cordoba *et al.* 2018; Alrefai *et al.* 2020). Sewage sludge was used successfully as an inoculum to improve the biogas production of the organic fraction of municipal solid wastes (Borowski 2015) and food wastes (Ratanatamskul *et al.* 2014). The digested manure was found to increase the biogas and methane yields of corn stover (Li *et al.* 2010) and rice straw (Gu *et al.* 2014). Nevertheless, only few reports discussed the effects of inoculum on HS-AD of cassava pulp until now, so that it is not clear whether sewage sludge or digestate is an effective inoculum for HS-AD of cassava pulp.

In our previous study (Zhang *et al.* 2016), the authors found that sewage sludge can be used as an inoculum of LS-AD of cassava pulp, and its effects on AD process vary with temperature, OLR, and operation mode. Considering that cassava pulp usually has been dewatered before it is fed into a digestor, HS-AD is actually a more advantageous method for industrial setting of cassava pulp. However, it is unknown about whether HS-AD is applicable for cassava pulp. Therefore, the aim of this study is to assess the feasibility of HS-AD for cassava pulp by investigating the effects of two TS (15%, 20%) and inocula (digestate, sewage sludge) on the HS-AD of cassava pulp in a series of bench-scale semi-continuous bioreactors.

EXPERIMENTAL

Substrates and Inocula

The wet cassava pulp was derived from a factory processing cassava-starch nearby Guilin in Guangxi province, China. After collection, the substrate was oven-dried for 24 h at 60 °C, and then it was stored at 4 °C after passing through the 0.25-mm sieve. Two inocula were used in this study, in which the digestate was from the dry batch bioreactors of cassava-pulp lasting 42 days and sewage sludge was obtained after being dewatered mechanically from a municipal wastewater treatment plant in Guilin. The biogas-production potential of the digestate was considered negligible in this study because it was predigested for approximately 7 days until yielding no biogas. The basic physicochemical characteristics of the cassava pulp and two inocula are listed in Table 1.

Table 1. Characteristics of Cassava Pulp and Sewage Sludge (n = 3)

	TS (%)	Volatile Solids (%)	Total Carbon (%)	Total Nitrogen (%)	C/N
Cassava Pulp	88.21 ± 0.33	88.42 ± 0.33	37.70 ± 0.18	0.44 ± 0.01	85.68
Digestate	15.25 ± 0.18	61.39 ± 0.24	30.25 ± 0.26	3.52 ± 0.03	8.59
Sewage Sludge	14.23 ± 0.21	57.74 ± 0.41	27.86 ± 0.22	4.48 ± 0.04	6.22

Semi-continuous AD Experiments

HS-AD of cassava pulp was run in a series of 1.0-L glass serum bottles as bioreactors in a water bath equipment. In each bioreactor, the effective volume was around 0.9 L for matrix, and the left aerospace was used to collect biogas. The cumulated biogas entered into another 1.0-L serum bottle by replacing salt solution in this bottle, which was automatically controlled by the difference of air pressure between the air space and the atmosphere. Two groups of HS-AD experiments were performed were operated in semi-continuous mode with daily feeding (once a day draws-off and feeding) and at mesophilic temperature (35 ± 2 °C). The first group included two TS treatments of 15% (A1) and 20% (A2) with the digestate as an inoculum. The second group composed of two treatments under the same TS (20%) with the different inoculum of digestate (B1) and sewage sludge (B2). In each treatment, approximately 800-g of inoculum was added initially into the bioreactor, and then the wetted cassava pulp of 14.25 to 63.33 g was fed daily after draws-off of equal-mass matrix (Table 2). Then, the matrix was only mixed manually with a glass rod after every feeding, and pure nitrogen gas was injected into the bioreactor for 1 min to totally remove oxygen from the bioreactor. After the mixing and nitrogen-gas injection, the bioreactor was sealed for HS-AD running and biogas collection.

The OLR of digesters increased step-wisely from 3.0 to 10.0 kgVS/(m^3·d) and each step lasted 6 days (Table 2, Fig. 1). When the deterioration of digestors happened at the highest OLR, a special operation was run to recover the digestion that included 3-days of no feeding and another 3-days OLR of 6.5 kgVS/(m^3·d). The OLRs procedure and the feedstock amounts are listed in Table 2. All treatments were operated for 42 days except B2, which was stopped at the 21st day due to the VFA accumulation. All the treatments were performed in duplicate.

Table 2. Experimental Conditions for the Four Treatments of AD of Cassava Pulp

OLR (kgVS/ (m^3·d))	A1 (Digestate, TS=15%)		A2 (Digestate, TS=20%)		B1 (Digestate, TS=20%)		B2 (Sewage Sludge, TS=20%)	
	Cassava Pulp (g)	Water (g)	Cassava Pulp (g)	Water (g)	Cassava Pulp (g)	Water (g)	Cassava Pulp (g)	Water (g)
3.0	3.23	15.77	3.23	11.02	3.23	11.02	3.23	11.02
4.0	4.31	21.02	4.31	14.69	4.31	14.69	4.31	14.69
5.0	5.38	26.28	5.38	18.37	5.38	18.37	5.38	18.37
6.5	7.00	34.16	7.00	23.87	7.00	23.87	7.00	23.87
8.0	8.61	42.05	8.61	29.39	8.61	29.39	-	-
10.0	10.77	52.56	10.77	36.72	10.77	36.72	-	-

Analysis

TS of cassava pulp was measured by the oven-drying method, and then volatile solids (VS) was obtained by the muffle-furnace method in which the oven-dried sample was aerobically incinerated at 600 °C for 6 h (Rice *et al.* 2017), while the total carbon (TC), total nitrogen levels (TN), and C/N ratio were obtained *via* an element analyzer (EA 2400 II; PerkinElmer, Waltham, MA, USA). The starch level was measured *via* the glucoamylase method (AACC 1990), while the cellulose, hemicellulose and lignin levels were analyzed by the Van Soest method (Van Soest *et al.* 1968). The pH values of samples were analyzed with a pH meter (CT-6023; Kedida, Shenzhen, China). To determine the VFAs, a 1-g digestate sample was diluted with deionized water (1:5, w/w), and then the mixture was centrifuged at 12000 rpm for 10 min to collect the supernatant. A 1-mL aliquot of the supernatant was filtered with a 0.45-μm membrane to obtain the filtrate, and then adjusted to pH 3 with 3% formic acid (v/v) for determining VFAs. Four VFAs including acetate, propionate, butyrate, and pentanoate were measured by a gas chromatograph (GC) (6890N; Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-WAX column (30 m × 0.53 mm × 1.0 μm) and a flame ionization detector (FID). The temperature were set at 240 °C and 250 °C for the column and FID, respectively.

Daily biogas production was measured *via* the solution displacement method with the saturated salt solution of pH 3.0. The methane content of produced biogas was determined by GC (102A; Jingke, Shanghai, China) with a stainless steel column (3 m × 3 mm) and a thermal conductivity detector as the detector. Hydrogen was applied as the carrier gas, and the temperature program was set as inlet port 70 °C, column chamber 35 °C, and detector 120 °C.

RESULTS AND DISCUSSION

Feedstock Characteristics

Cassava pulp contained a high mass content of easily bioavailable carbohydrate (starch, 31.95%), which implied that cassava pulp had a considerable potential of methane production compared with other lignocellulose agricultural wastes such as wheat straw. It also had high contents of cellulose (17.08%), hemicellulose (24.40%), and a small amount of lignin (2.19%). Both of the two inocula had a low C/N ratio in the range of 6 to 10, which was beneficial to sustain AD of cassava pulp by promoting the initial C/N ratio of the raw material from 38.2 to the optimal range of 15 to 30 for the requirement of anaerobic microorganisms (Panichnumsin *et al.* 2010; Zhang *et al.* 2016).

Effects of Solids Content on HS-AD of Cassava Pulp

pH, VFAs

The pH is a key index for assessing the stability of AD, which is affected by both VFAs and alkalinity (Zhang *et al.* 2015). Figure 1(a) shows the bioreactor under TS 20% had a stronger buffering capacity for the acidification than that under TS 15%. During the first 9 days, the pH decreased rapidly from 7.78, 7.68 to 5.40, 5.76 for A1, A2, respectively (Fig. 1(a)). That was attributed to a high acidification rate of the starch in cassava pulp, which could not be neutralized completely by only alkalinity provided from the inoculum. During the 9th through 21st days, the pH in A1 and A2 rose gradually even though the OLR increased from 4.0 to 6.5 kgVS/(m^3·d), which indicated that methanogenic microbes had finished the acclimation for the new feedstock and could utilize VFAs more quickly (Toreci *et al.* 2011). Then, the increase of OLR resulted in the secondary acidification, which was weaker than the first one in each treatment. The pH in A2 fell slowly to approximately 6.0 when the OLR was 8.0 kgVS/(m^3·d), while the pH in A1 decreased to below 5.5.

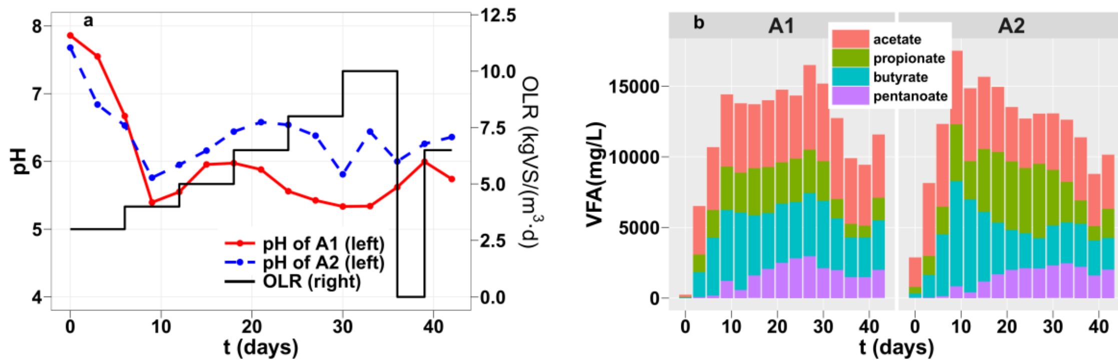

Fig. 1. pH and VFA levels during the HS-AD of cassava pulp inoculated with digestate under different TS (A1, TS = 15%; A2, TS = 20%): (a) pH; (b) VFAs

The total VFA is also considered an important index to monitor the stability of AD, of which the accumulation generally causes a rapid acidification of matrix in AD (Fonoll *et al.* 2015). Figure 1(b) shows that the two TS resulted a similar trend of the temporal variation of VFA levels in A1 and A2. The VFA levels in A1 and A2 firstly increased sharply during the initial 9 or 12 days, and then kept a high level until the feeding of feedstock was stopped. The high concentrations of total VFA were observed in both A1 and A2. A high concentration of the total VFA generally was the main reason of rapid acidification, which normally suppresses the biogas production during AD (Siegert and Banks 2005). Compared with A2, more of the total VFA was obtained in A1, which was due to a high activity of acidogenic bacteria in the higher moisture under TS 15%. In both A1 and A2, the pH showed a relatively low level only when the total VFA was more than 15,000 mg/L, which was much higher than the threshold value of 4,000 mg/L in LS-AD (Siegert and Banks 2005). Otherwise, the VFA accumulation was mitigated by the recovery procedure in this study, which meant that HS-AD of cassava pulp under TS of 15% to 20% could endure a short-term shock of a high OLR. Compared with A2, there were higher concentrations of acetate and lower concentrations of propionate in A1, but A1 produced less biogas and had a lower pH. That cannot be interpreted by the previous selection theory of methanogen during the mesophilic phase (Horiuchi *et al.* 2002; Franke-Whittle *et al.* 2014), in which a high concentration of acetate and butyrate were considered beneficial to LS-AD but propionate gave an adverse effect. The inconsistent result implies that the microbial community under HS-AD of cassava pulp has a different selection effect of VFAs from that under LS-AD. As for the loss of VFAs by evaporation, each of these magnitudes was estimated by multiply the Henry's constant (0.76 to 3.4 × 10^{-5}) at 20 to 25 °C (Hudson and Ayoko 2008) with the volume ratio of total overlying gas (including biogas and injected nitrogen gas) to liquid in each bioreactor, and the results showed the losses of VFAs were in the range of 0.15 to 0.68 % and were negligible.

Biogas and methane production

Figure 2(a) shows the daily biogas production from the HS-AD of cassava pulp under the two TS. With the increase of OLR, the biogas-production rate ascended gradually in both treatments and achieved the maximum values at the 31st and 22nd day at the OLR of 10.0, 6.5 kgVS/(m^3·d) for TS 15%, TS 20%, respectively. A sharp decrease occurred from the relatively higher biogas production at the phase of OLR 10.0 kgVS/(m^3·d). The biogas production increased again at the 37th day after stopping feeding into the bioreactors at the 36th day, which showed a fast recovery capacity for both treatments. Compared with A1, the higher TS in A2 promoted more biogas yield during the moderate OLR of 5.0 to 6.5 kgVS/(m^3·d), while less biogas at the initial phase of OLR 3.0 kgVS/(m^3·d) and high OLR phase of 8.0 to 10.0 kgVS/(m^3·d).

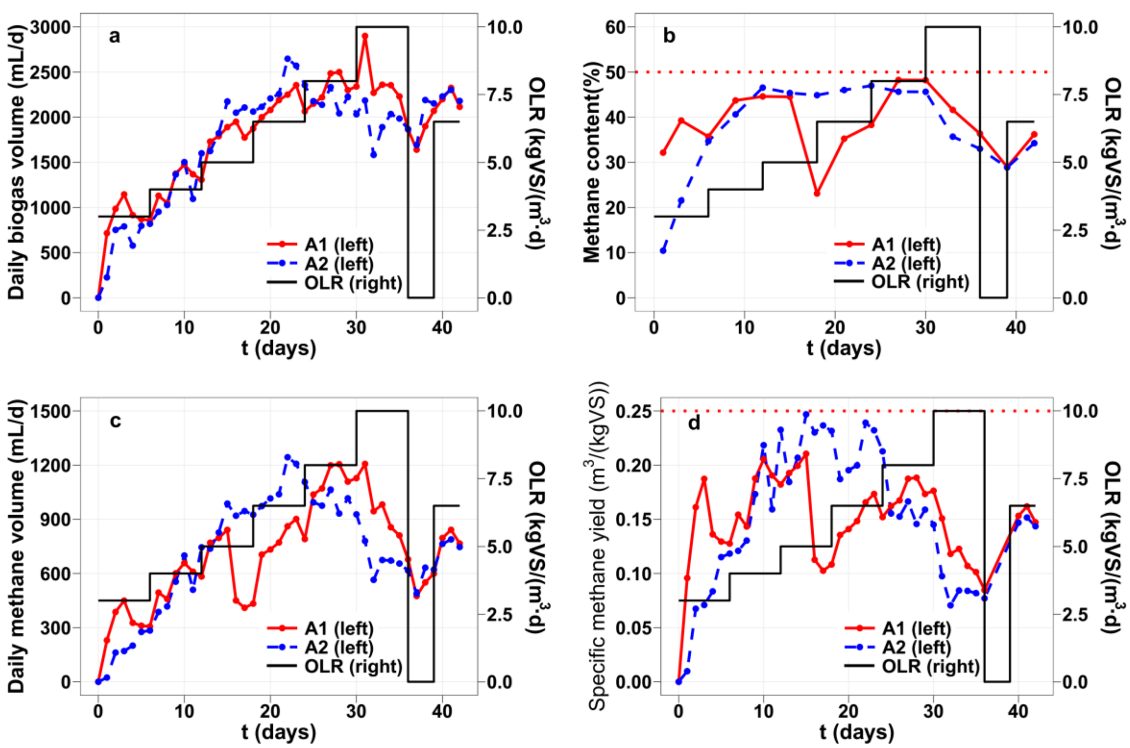

Fig. 2. Biogas and methane production during the HS-AD of cassava pulp inoculated with digestate under different TS (A1, TS = 15%; A2, TS = 20%): (a) daily biogas production; (b) methane content; (c) daily methane production; (d) specific methane yield

Compared with A2, the higher moisture in A1 was beneficial to microorganisms from the inoculum and then resulted in higher biogas production in A1 during the initial phase of AD. Then, the higher biogas production at the moderate OLR in A2 was attributed to the more appropriate pH (Fig. 2(a)), while the low pH lightly inhibited the methanogenesis in A1. At the OLR of 8.0 kgVS/(m^3·d), the less biogas production in both treatments was due to the insufficient methanogenesis caused by rapid acidification. However, both treatments stably produced biogas at the OLR range of 5.0 to 8.0 kgVS/(m^3·d), which was generally considered too high an OLR for LS-AD of cassava pulp (Panichnumsin *et al.* 2010).

Figure 2(b) shows the temporal variation of the methane contents (MC) during the HS-AD of cassava pulp in A1 and A2. The MC were in the range of from 23.1% to 48.2% and from 10.4% to 47.0% for A1 and A2, respectively, while the maximum values occurred at the 27th and 24th days for these trials. Both peak values were less than 50%, which was probably due to a high CO_2 concentration produced in the acidic environment (Fig. 1(a)). Compared with A1, A2 obtained a higher methane content at the OLR of 5.0, 6.5 kgVS/(m^3·d), while slightly lower at almost all of the other OLRs except for 3.0 kgVS/(m^3·d).

The temporal variations of the daily methane volume were similar to the trends of the daily biogas production in both treatments at almost of all OLR phases except the moderate OLR (Fig. 2(a) and 2(c)). The peak values at each OLR phase were in the range of 450 to 1, 205 mL/d, and from 284 to 1, 244 mL/d in A1, A2, respectively (Fig. 2(c)), which indicated that the daily methane volumes were of the appropriate range in two trails. Whereas the trends were different between two treatments. Compared with A1, the higher methane was produced in A2 at the phase of the moderate OLR, while lower at the other phases (Fig. 2(c)).

During the 42-day operation, SMY based on VS of the added cassava pulp were in the range of from 0.091 to 0.210 m^3/(kgVS$_{added}$), from 0.010 to 0.247 m^3/(kgVS$_{added}$) for TS 15%, TS 20%, respectively (Fig. 2d). Considering the microorganisms had to adapt in the first several days, the value at the 6th day in each phase of OLR was used as the typical SMY. The typical SMYs under TS 15% were 0.128, 0.182, 0.108, 0.152, 0.176, and 0.084 m^3/(kgVS$_{added}$) with the increase of OLR, while the corresponding typical SMYs were 0.118, 0.233, 0.232, 0.212, 0.145, and 0.077 m^3/(kgVS$_{added}$) for TS 20%. The relatively high values of SMY were obtained at the OLR of 4.0 to 6.5 kgVS/(m^3·d) for TS 20%, while the high values at the OLRs of 4.0, 6.5, and 8.0 kgVS/(m^3·d) for TS 15%. Compared with TS 15%, cassava pulp at TS 20% produced more methane at the optimal OLR range, which was approximately 30% higher than the value from TS 15%. These results were less than the values of 0.242 to 0.344 m^3/(kgVS$_{added}$) from the previous studies using the batch mesophilic experiments (Panichnumsin *et al.* 2010; Zhang *et al.* 2016), which was attributed to an insufficient retention time for the complete biodegradation of the substrate. In regards to the semi-continuous AD, the typical SMY in this study were slightly less than the values from 0.227 to 0.352 m^3/(kgVS$_{added}$) from the other LS-AD of cassava pulp (Panichnumsin *et al.* 2010; Ren *et al.* 2014; Zhang *et al.* 2016), which was attributed to the less microbial activity in this study than that in LS-AD with a low TS of less than 5% in those studies. Though the value of SMY at TS 20% was lower than that from LS-AD of cassava pulp, it was higher than the values from many other agricultural wastes (Serrano *et al.* 2014; Li *et al.* 2015; Mustafa *et al.* 2017). The abundant starch was the main cause of the high SMY in LS-AD of cassava pulp, which had much higher methane-production potential than the lignin-cellulose agricultural wastes. Therefore, it was acceptable to choose TS 20% rather than TS 15% as the optimal parameter to operate the HS-AD of cassava pulp.

Table 3. Specific Methane Yields from the AD of Different Feedstocks

Feedstock	T (°C)	TS	OLR (kgVS/(m^3·d))	Specific Methane Yield (m^3/(kgVS$_{added}$))	References
Cassava pulp, digestate	35 (Semi-continuous[a])	15%	3.0 to 10.0	0.077 to 0.233	This study
Cassava pulp, digestate	35 (Semi-continuous[a])	20%	3.0 to 30.0	0.108 to 0.182	This study
Cassava pulp, pig manure	37 (CSTR[b])	3.5% to 4.8%	4.0	0.281 to 0.352	Ren *et al.* 2014
Cassava pulp, pig manure	37 (CSTR[b])	3.5%	3.5	0.227 to 0.306	Panichnumsin *et al.* 2010
Cassava pulp, sewage sludge	35 (CSTR[b])	3.4%	2.5 to 8.7	0.308 to 0.334	Zhang *et al.* 2016
	55 (CSTR[b])	3.4%	2.5 to 8.7	0.314 to 0.336	
Straw, pig manure	37 (CSTR[b])	4.3%	3.0 to 12.0	0.178 to 0.268	Serrano *et al.* 2014
Rice straw	37 (Batch)	20%	0.33	0.064	Li *et al.* 2015
Sewage sludge, orange peel waste	35 (CSTR[b])	6.1%	0.4 to 1.6	0.165	Mustafa *et al.* 2017

[a] Semi-continuous mode was applied with daily feeding (once a day draws-off and feeding).
[b] CSTR meant continuously stirred tank reactor.

Effects of Inocula on AD of Cassava Pulp

pH and VFAs

The pH values decreased first in B1 and B2, and then they rose slightly during the following 9 to 12 days before a secondary acidification (Fig. 3(a)). The acidic environment with pH less than 5.0 sharply depressed the biogas production (Fig. 4(a)), so that the treatment with sewage sludge had to be turned off after the weak digestion of 21 days. Compared with the sewage sludge, pH in B1 with the digestate increased above 6.0 after the first acidification, which was acceptable for AD. The pH in B2 increased again rapidly to above 6.0 after stopping or reducing the feeding of cassava pulp, which indicated that the treatment with the digestate had a fast recovery rate. The higher buffering capacity in B1 for acidification was attributed to the larger and more diverse population of methanogens carried with the digestate than sewage sludge (Ma *et al.* 2013; Shi *et al.* 2014). The acidification-buffering phenomenon was accordance with that in A1 and A2 with the digestate as the inoculum (Fig. 1(a)).

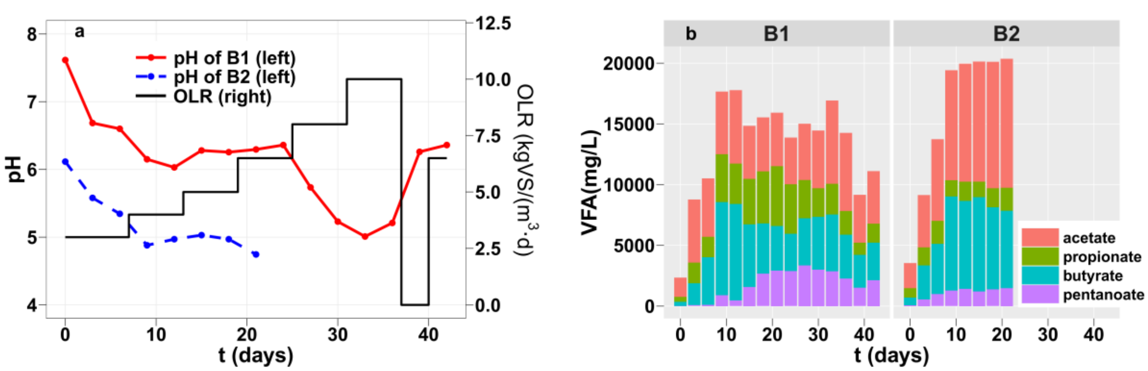

Fig. 3. pH and VFA levels during the HS-AD of cassava pulp with two inocula (B1, digestate; B2, sewage sludge): (a) pH; (b) VFAs

In both B1 and B2, the total VFAs sharply increased during the first 9 to 12 days, which was in accordance with the acidification in these two bioreactors (Fig. 3(b)). Then, the VFAs in B2 remained constant at a high level while the VFAs in B1 gradually decreased except at the 33rd day when the highest OLR was loaded. Compared with the sewage sludge, the digestate resulted in much lower total VFAs, which demonstrated that the digestate provided more methanogens than sewage sludge to utilize the VFAs. Additionally, B1 showed a much higher threshold of the total VFAs of about 15,000 mg/L than the value of 9,000 mg/L in B2. Nevertheless, both of those thresholds were obviously much higher than the values from LS-AD (Siegert and Banks 2005), which could be attributed to a lower chemical activity of VFAs in HS-AD. Among the four VFAs, more acetate and less propionate were observed, which cannot be explained by the selection mechanism of metabolic pathways for methanogen in LS-AD (Franke-Whittle *et al.* 2014). Thus, methanogen in HS-AD probably has a different priority to select the VFA substrates from that in LS-AD.

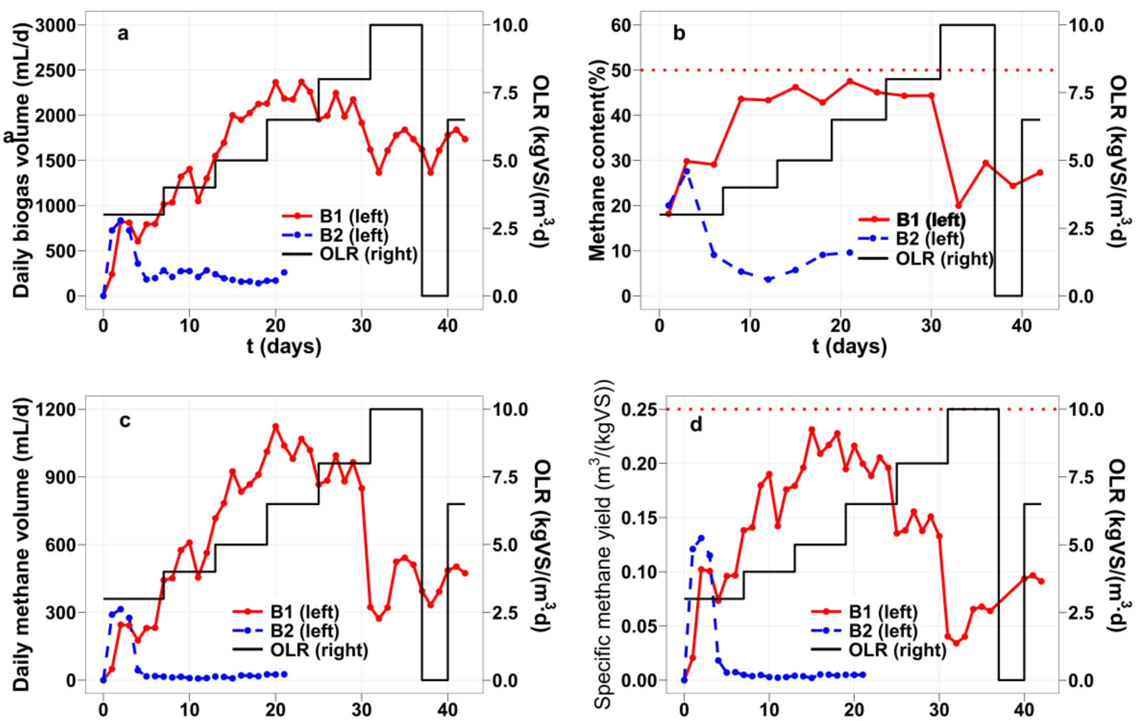

Fig. 4. Biogas and methane production during the HS-AD of cassava pulp with two inocula (B1, digestate; B2, sewage sludge): (a) daily biogas production; (b) methane content; (c) daily methane production; (d) specific methane yield

Biogas and methane production

Figure 4(a) shows the daily biogas production in the HS-AD with the two inocula. With the increase of the OLR, the biogas-production rate with the digestate rose rapidly at the initial phase and achieved the maximum value at the 23rd day when the OLR was 6.5 kgVS/(m^3·d). A sharp descend of the biogas production occurred at the OLR of 10.0 kgVS/(m^3·d), which followed the peak at the OLR of 8.0 kgVS/(m^3·d). The biogas production increased again when the loading was stopped or lowered during the 36th to 42nd days, which showed that the bioreactor ran well again after the temporary malfunction.

Compared with the sewage sludge, the digestate was a better inoculum for HS-AD of cassava pulp based on the biogas production (Fig. 4(a)). This was due to a favorable bacterial community included in the acclimated inoculum such as the digestate (Walkins *et al.* 2015).

The MC are shown in Fig. 4(b) to assess the methane production in two treatments with the two different inocula under TS 20%. The MC were in the ranges of 3.6% to 28.6%, and 18.2% to 47.5% for sewage sludge and the digestate, respectively, and the corresponding maximum values arrived at the 3rd and 21st days. The MC in the two treatments were no more than 50%, which was caused by more CO_2 production occurring at the low pH. The bioreactors with sewage sludge only got low values of MC, of which most were less 10%. In contrast, the HS-AD with the digestate made cassava pulp yield high values of MC in the OLR range of from 3.0 to 8.0 kgVS/(m^3·d), and the maximum value of MC was close to that reported in the previous AD under TS 20% (Fig. 2(b)).

Under TS 20%, the daily methane volumes in each OLR phase were from 50 to 1,124 mL/d, and from 7 to 315 mL/d for the digestate and sewage sludge, respectively, (Fig. 3(c)), which indicated that more methane was obtained in the treatment with the digestate at most OLR phases except the low OLR of 3.0 kgVS/(m^3·d). The digestate broadened the OLR range and achieved a higher and more stable methane production than sewage sludge, which was attributed to the fact that the bacteria community in the digestate had been acclimated to use the specific organic compounds in cassava pulp.

The SMPs were in the range of from 0.021 to 0.231 m^3/($kgVS_{added}$), and from 0.002 to 0.131 m^3/($kgVS_{added}$) for the treatments with the digestate and sewage sludge, respectively (Fig. 4(d)). The typical SMYs with the digestate were 0.097, 0.176, 0.228, 0.196, 0.133, and 0.091 m^3/($kgVS_{added}$), and the relatively high values were achieved at the OLR range from 4.0 to 6.5 kgVS/(m^3·d). The low yields at the OLR of 3.0 kgVS/(m^3·d) were due to the unfinished acclimation of microorganisms caused by the rapid acidification during the first 12 days (Fig. 3(a) and 3(b)). Due to the high OLR and low water activity at the HS-AD, the typical SMYs in B2 were slightly less than the values from LS-AD of cassava pulp in previous studies (Panichnumsin *et al.* 2010; Ren *et al.* 2014; Zhang *et al.* 2016). These results were close to results from A2 (Fig. 2(d)), which also showed the HS-AD was stable under the TS 20%. Compared with the digestate, sewage sludge was not an applicable inoculum in HS-AD of cassava pulp, on account of the quite small typical SMYs in B2 even at the lowest OLR of 3.0 kgVS/(m^3·d).

CONCLUSIONS

1. Compared with a total solids (TS) of 15%, TS 20% was a better condition for mesophilic semi-continuous anaerobic digestion (AD) with the digestate as an inoculum, which resulted in a higher buffering capability for the rapid acidification and more methane yields at the moderate OLR range. Cassava pulp with TS 20% had the typical values of SMY from 0.212 to 0.233 m^3/($kgVS_{added}$) at the OLRs of 4.0 to 6.5 kgVS/(m^3·d), while TS 15% produced typical SMYs from 0.152 to 0.182 m^3/($kgVS_{added}$) at the OLR of 4.0, 6.5, and 8.0 kgVS/(m^3·d).
2. During the mesophilic semi-continuous AD of cassava pulp under TS 20%, the digestate as an inoculum was more effective than sewage sludge to protect the bioreactor from the rapid VFA accumulation and then yield more methane.

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