Anaerobic Digestion of Cassava Pulp with Sewage Sludge Inocula

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The effectiveness of sludge inocula from a municipal carrousel oxidation ditch wastewater treatment plant was studied for both batch and semi-continuous anaerobic digestion (AD) of cassava pulp. In 1.25-L batch experiments, the following sludge inocula were used: I1 sludge from the hydrolysis acidification tank pre-acclimated with cassava pulp; I2, predigested sludge from the hydrolysis acidification tank; and I3, predigested sludge from the gravity thickening tank. In 1.0-L semi-continuous tests, mesophilic AD of cassava pulp inoculated with I3 was carried out at organic matter loading rates (OLRs) of 1.5 to 12.5 kg volatile solids (VS)/(m^3 d), whereas 1.5 to 18.0 kgVS/(m^3 d) was used for thermophilic AD. For batch operations, all sludge types could be used as the inoculum for the mesophilic AD of cassava pulp, whereas only I1 was used for thermophilic AD. The maximum specific methane yields were 0.333 and 0.395 $m^3/kgVS_{added}$, respectively, for mesophilic and thermophilic batch digestion with I1. During semi-continuous AD of cassava pulp inoculated with I3, high specific methane yields of 0.334 to 0.336 $m^3/kgVS_{added}$ were obtained under both mesophilic and thermophilic conditions. For thermophilic AD of cassava pulp with sludge inocula, the range of optimum OLR was 2.5 to 15.0 kgVS/(m^3 d), and that was 2.5 to 8.7 kgVS/(m^3 d) for mesophilic AD.

Keywords: Anaerobic digestion; Cassava pulp; Sludge inocula; Biogas production

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INTRODUCTION

Cassava is one of the three primary food sources from tuber crops worldwide and is mostly used to produce edible starch and methanol. However, a large amount of cassava pulp, also known as cassava dregs, is generated during processing. In South China, fresh cassava is an economically important crop with an annual production of approximately 9.11 million tons (Jansson *et al.* 2009), resulting in the production of 0.95 million tons of cassava pulp (Yang *et al.* 2011). The disposal of cassava pulp has become a major environmental problem because of the associated emission of foul odors and the pollution of soil and groundwater. Because cassava pulp contains high levels of carbohydrates (starch, cellulose, and hemicellulose), it has great potential as a raw substrate for biogas production (Pu and Liu 2009). Consequently, anaerobic digestion (AD) is considered a valuable method for treating cassava pulp and for helping to meet China’s growing energy requirements while minimizing adverse environmental effects (Ren *et al.* 2014).

AD comprises four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The hydrolysis of particulates is generally the main rate-limiting step in the AD of solid wastes (Batstone *et al.* 2002). However, Bouallagui *et al.* (2009) showed that the AD of fruit and vegetable wastes (FVW) was more inhibited by a slower rate of methanogenesis than a slower rate of hydrolysis. Similarly to FVW, cassava pulp, which contains a high starch content, may rapidly become acidified. Such acidification can result in the severe inhibition of methanogenesis because of the accumulation of volatile fatty acids (VFA) (Panichnumsin *et al.* 2010).

There are two ways to overcome rapid acidification: co-digestion and the addition of inocula. Co-digestion has the following advantages: it dilutes toxic pollutants, balances nutrients, improves microorganism diversity, increases the organic matter loading rate (OLR), and enhances the specific methane yield (Alvarez and Liden 2008; Estevez *et al.* 2014; Serrano *et al.* 2014).

Sewage sludge and manure are two commonly used co-substrates. Ratanatamskul *et al.* (2014) investigated the pilot co-digestion of food wastes and sewage sludge and found that the addition of sewage sludge accelerated the start-up procedure and increased methane production. Panichnumsin *et al.* (2010) also achieved high methane yields and the reduction of volatile solids (VS) in the AD of cassava pulp after the addition of manure.

Inocula can provide nitrogen to improve the C/N ratio of substrates rich in carbohydrates, just as co-substrates can. Moreover, inocula can introduce a more diverse microbial community and a higher initial bacterial population (Walkins *et al.* 2015). Consequently, a suitable inoculum can shorten the start-up time of AD, accelerate the biodegradation reaction, and enhance the biogas yield (Quintero *et al.* 2012). Digested manure and digested sludge are two common inocula.

Li *et al.* (2010) reported that the inoculation of corn stover pretreated with NaOH with digested swine manure obtained higher biogas and methane yields during AD than did inoculation with digested dairy manure, digested corn stover, or digested municipal sludge. Gu *et al.* (2014) also found that of the inocula tested, the AD of rice straw with digested dairy manure achieved the highest biogas production. Borowski (2015) reported that the addition of sewage sludge improved biogas production from the hydromechanically sorted organic fraction of municipal solid wastes by balancing the C/N ratio of the mixture.

The benefits of co-digesting pig manure and cassava pulp have been reported by Panichnumsin *et al.* (2010) and Ren *et al.* (2014). Compared with cassava pulp alone, the specific methane yield increased for cassava pulp/pig manure ratios in the range of 2:3 to 4:1 (Ren *et al.* 2014) or 1:4 to 4:1 (Panichnumsin *et al.* 2010). Digested sludge is more readily available than pig manure because of its ubiquitous production in urban regions. Pu and Liu (2009) found that inoculation with anaerobic granule sludge is effective for the AD of cassava pulp.

However, it is not clear whether sewage sludge is the most effective inoculum for the AD of cassava pulp. Consequently, the aim of this study was to carry out a series of batch and semi-continuous tests to evaluate sewage sludge as an inoculum and to determine the optimum OLR for both the mesophilic and thermophilic AD of cassava pulp.

EXPERIMENTAL

Substrates and Inocula

Cassava pulp was collected from a cassava starch processing plant in Guangxi province, China. The collected substrate was dried at 60 °C for 8 h and then stored at 4 °C. Sludge inocula samples were collected from a municipal carrousel oxidation ditch wastewater treatment plant. Three types of sludge inocula were used: (1) I1, sludge from the hydrolysis acidification tank pre-acclimated with cassava pulp; (2) I2, predigested anaerobic sludge from the hydrolysis acidification tank; and (3) I3, predigested excess sludge from the gravity thickening tank. The sludge was kept under anaerobic conditions at 25 °C for 30 days to remove its potential for producing biogas. During this predigestion process, I1 was acclimated by adding 7 g/L cassava pulp, whereas I2 and I3 were not acclimated. The characteristics of the cassava pulp and inocula are shown in Table 1.

Table 1. Characteristics of Cassava Pulp and Inocula

	Total Solid (TS) (%)	Volatile Solid (VS) (%TS)	C/N	Starch (%TS)	Protein (%TS)	Lipid (%TS)	Cellulose (%TS)	Hemi-cellulose (%TS)	Lignin (%TS)
Cassava Pulp	88.85	91.59	38.02	39.91	4.98	3.52	25.83	16.79	8.15
I1	8.15	66.41	11.10	1.17	16.87	1.72	22.63	10.59	5.38
I2	9.27	63.03	6.04	0.83	28.12	0.83	17.15	3.36	1.74
I3	6.46	67.58	6.08	0.46	28.06	1.04	18.37	1.02	1.60

Batch Experimental Design

Batch experiments were conducted in 1.25-L Erlenmeyer flasks. The six types of batch experiments are defined in Table 2. In each digester flask, 27.4 g cassava pulp and 58.3 g total solids (TS) of inocula were loaded, giving a substrate/inocula ratio of 3:7 (TS/TS). Deionized water was then added to give 1 L of mixed liquor. After being fed, the digesters were purged with pure nitrogen to remove the dissolved oxygen, then sealed tightly with rubber stoppers. Three digesters were operated under mesophilic conditions (35±2 °C), and the other three digesters were operated under thermophilic conditions (55±2 °C). The digesters were constantly agitated at 120 rpm. Each test lasted for 50 days.

Table 2. Experimental Conditions for the Six Batch ADs of Cassava Pulp

	S1	S2	S3	S4	S5	S6
Inoculum	I1	I2	I3	I1	I2	I3
$m_{cassava}$ (wt., g)	27.4	27.4	27.4	27.4	27.4	27.4
m_{inoculum} (wt., g)	715	629	902	715	629	902
T (°C)	35	35	35	55	55	55

Semi-continuous AD Experiments

Because excess sludge was the main solid waste from a municipal wastewater treatment, I3 was used as the inoculum for the semi-continuous AD of cassava pulp. The semi-continuous AD of cassava pulp was carried out in 1.0-L stirred glass digesters with working volumes of 0.5 L. Initially, 0.62 g of cassava pulp, 260 g of I3 (wt.), and 250 mL of deionized water were added to the digesters. TS of the initial feedstock was 3.4%. Cassava pulp was feed as sole substrate, and equal withdrawal/feeding was carried out once a day. The digesters were mechanically stirred in shakers at 120 rpm. The digesters were operated with stepwise increases in OLR from 1.0 to 18.0 kgVS/(m^3 d). The initial reaction was carried out for 12 days, and subsequent reactions with increasing OLRs were carried out for 15 days (low OLRs) or 18 days (high OLRs) (Table 3, Fig. 3(a)). The daily amounts of substrate fed to achieve the required OLRs are listed in Table 3. When the biogas production decreased quickly at the highest OLR in each bioreactor, feeding was stop for the next 10 or 8 days and then each bioreactor was run for 10 days at the second highest OLR. Digesters were run under mesophilic conditions (35±2 °C) or thermophilic conditions (55±2 °C) (Table 3). The mesophilic bioreactors lasted for 224 days, and the thermophilic bioreactors lasted for 247 days. Duplicate digesters were run at each condition.

Table 3. Daily Amounts of Substrate Fed in the Semi-continuous AD Experiments

OLR (kgVS/(m^3 d))		1.0	1.5	2.0	2.5	3.0	3.5	4.2	5.0	6.0	7.2	8.7	10.4	12.5	15.0	18.0	0
Daily Feed Amount (g)		0.62	0.92	1.23	1.54	1.85	2.16	2.59	3.11	3.72	4.47	5.36	6.44	7.72	9.27	11.13	0
Time (d)	Mesophilic	12	15	15	15	15	15	18	18	18	18	18	18, 10	18	-	-	10
	Thermophilic	12	15	15	15	15	15	18	18	18	18	18	18	15	15, 10	4	8

Analysis

Daily biogas production for each test was recorded using the acidified (pH = 3) saturated saline solution displacement method. A 0.5-L measuring cylinder was used to collect the water displaced from the gas collector. The corresponding cumulative biogas volume was also calculated. Biogas in the headspace of the digesters was pumped out to analyze the methane content, which was determined by the alkaline absorption method (KOH, saturated solution) (Borja *et al.* 1995). TS, ammonia nitrogen (NH_4^+-N), Kjeldahl nitrogen (TKN), alkalinity, VS levels were analyzed by oven drying method, trimetric method, semi-micro-Kjeldahl method, titration method, muffle furnace method (APHA 2005). The pH values of samples were measured with a pH meter (Sartorius UB-7, Gottingen, Germany). The total carbon and total nitrogen levels were determined by an element analyzer (PerkinElmer EA 2400 II, Waltham, Massachusetts) to calculate the C/N ratio. Starch and lipid levels were measured by the glucoamylase method (AACC 1990) and Soxhlet extraction method (AOAC 1995), respectively. Protein was obtained as TKN multiplied by 6.25. The levels of cellulose, hemicellulose, and lignin were determined using the Van Soest method (Van Soest and Wine 1968).

To determine the alkalinity, NH_4^+-N, VFA, 1-g digestate sample was first collected and diluted with DI water (1:5, w/w). Then, the mixed liquor was centrifuged at 12000 rpm for 10 min, and the supernatant was separated into three aliquots for the analysis of alkalinity, NH_4^+-N and VFA. One aliquot was acidified to pH 3 with 3% formic acid (v/v). The solution was filtered with a 0.45 μm membrane filter. VFA including acetate, propionate, butyrate, and pentanoic acid in the filtrate were measured using a GC (6890N, Santa Clara, California) equipped with a DB-WAX column (30 m×0.53 mm×1.0 μm) and a flame ionization detector (FID). The temperature for the column and FID were 240 and 250 °C, respectively. Nitrogen gas was used as the mobile phase at a flow rate of 2 mL/min.

RESULTS AND DISCUSSION

Feedstock Characteristics

The characteristics of cassava pulp and inocula are shown in Table 1. The primary components of cassava pulp were starch and lignocelluloses, with lesser amounts of lipid and protein. Compared with cassava pulp, inocula had more protein and less starch. Starch and cellulose were the main carbon sources for anaerobic microorganisms. The C/N ratio of cassava pulp was 38.02, which is higher than that required by anaerobic microorganisms (Panichnumsin *et al.* 2010). The C/N ratio decreased markedly after inoculation with sludge with C/N ratios of 6.0. Compared with cassava pulp alone, the inoculated mixture had more suitable C/N ratios in the range of 15.7 to 19.5, which are within the optimum range of 15 to 30 for AD (Zhang *et al.* 2014).

Effects of Inocula on the AD of Cassava Pulp

pH and VFA

The pH is important for estimating the progress of AD, which is determined jointly by VFA and alkalinity. The pH of the mixed liquor in experiments S1 and S2 (Fig. 1(a)), which was initially acidic, recovered to a pH above 7.0 during the first 5 to 8 d. Compared with S1 and S2, S3 took longer to achieve a pH greater than 7, which indicated a weaker pH buffering capacity in S3. Among the three thermophilic digesters, only in S4 did the pH recover to a value above 7 during the first 14 days; in S5 and S6 the pH did not recover throughout the 50-day run (Fig. 1(b)).

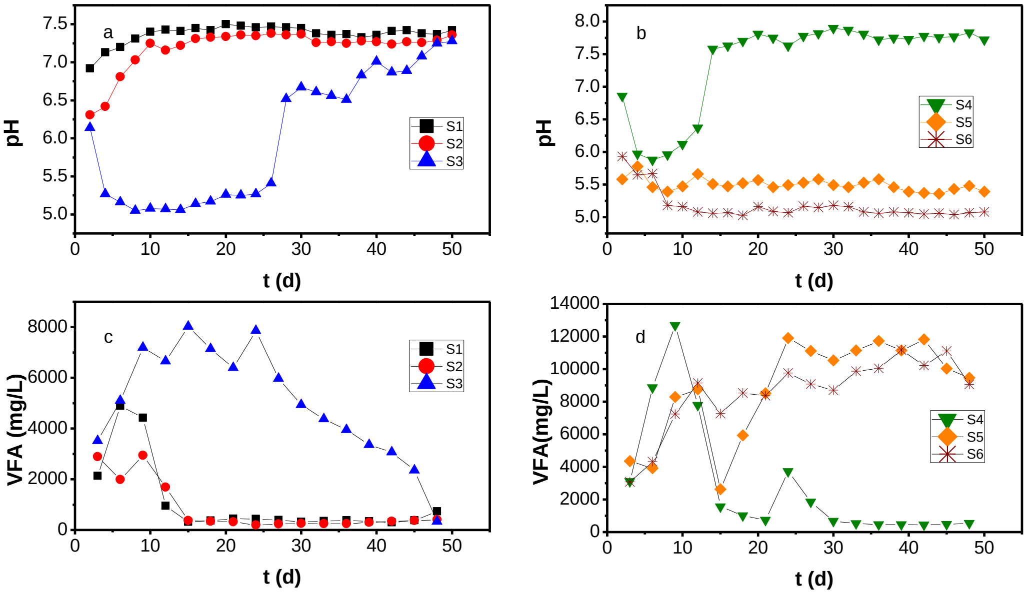

Fig. 1. pH and VFA levels during the AD of cassava pulp with sludge inocula: (a) pH, mesophilic; (b) pH, thermophilic; (c) VFAs, mesophilic; (d) VFAs, thermophilic

Mesophilic digesters exhibited a more rapid pH recovery rate than did thermophilic digesters, a fact that could be attributed to slower rates of acidogenesis. Compared with inocula I2 and I3, I1 provided a higher pH buffering capacity for both mesophilic and thermophilic AD. This improved buffering of I1 likely resulted from the higher activity and larger population of methanogens resulting from the acclimation.

Because the accumulation of VFAs reduces the pH of the digester, VFA concentration was used to monitor the stability of AD (Fonoll *et al.* 2015). A VFA concentration of 4000 mg/L was considered the lowest inhibitory concentration by Siegert and Banks (2005). In the current experiments, the VFA concentration sharply decreased between days 9 and 12 in both S1 and S2 (Fig. 1(c)), whereas the VFA concentrations in S3 were greater than 4000 mg/L between days 6 and 33. In S4, the VFA concentration decreased to below 4000 mg/L after 12 days, whereas severe accumulation of VFAs occurred in both S4 and S5 around day 9 (Fig. 1(d)). Compared with the three thermophilic digesters, the mesophilic digesters spent less time with VFA levels above the 4000 mg/L threshold.

Biogas and methane production

The levels of daily biogas production from the AD of cassava pulp with inocula are shown in Figs. 2(a) and (b). The maximum rate of biogas production in most digesters was achieved during the first two days. During this period, the daily biogas yield of each digester was within the range of 1200 to 1900 mL/d. After these high initial yields, the biogas production rates of S1 and S2 gradually declined, even though the pH value was above 7.0. In S3, the daily biogas production was below 100 mL/d between days 8 and 20, but then secondary biogas production occurred. In S3, there were two main spikes of daily biogas production and methane content at 28 and 38 days (Figs. 2(a) and (c)), which was corresponding to the curve of pH in S3 (Fig. 1(a)). The phenomenon of secondary biogas production was attributed to a long adaptation time of I3 which was resulted of less methanogens in I3 than that of I1 and I2. S4 produced less biogas than S1 or S2 during the first 12 days, but S4 produced more biogas than S1 or S2 during the final 20 days. Biogas yields remained low for S5 and S6 after day 10. In S4, there were four main spikes of daily biogas production at the 14, 16, 26 and 29 days (Fig. 2(b)), which was corresponding to neutrality of pH in S4 (Fig. 1(a)).

The phenomenon of secondary biogas production was attributed to the quick adaptation of anaerobic microbial community in S4 which was resulted of more acclimated methanogens in I4 than that of I5 and I6. Cumulative biogas production was 13.95 and 12.34 L for S1 and S2, respectively, which was 26% and 12% higher than that for S3. The cumulative biogas production by thermophilic digesters was 15.04 and 5.85 L for S4 and S5, respectively, which was 272% and 45% higher than that of S6. The AD of cassava pulp with inoculum I1 had the highest biogas production under thermophilic conditions, whereas under mesophilic conditions, the AD of cassava pulp with both I1 and I2 obtained high levels of biogas production. This result was in accordance with other recent reports.

The investigation from Toreci *et al.* (2011) showed that acclimated inoculum enhanced the rate and extent of anaerobic digestion of thickened waste activated sludge. Wilkins *et al.* (2015) also found that sludge inoculum source influenced bacterial communities and specific methane yield of cellulose.

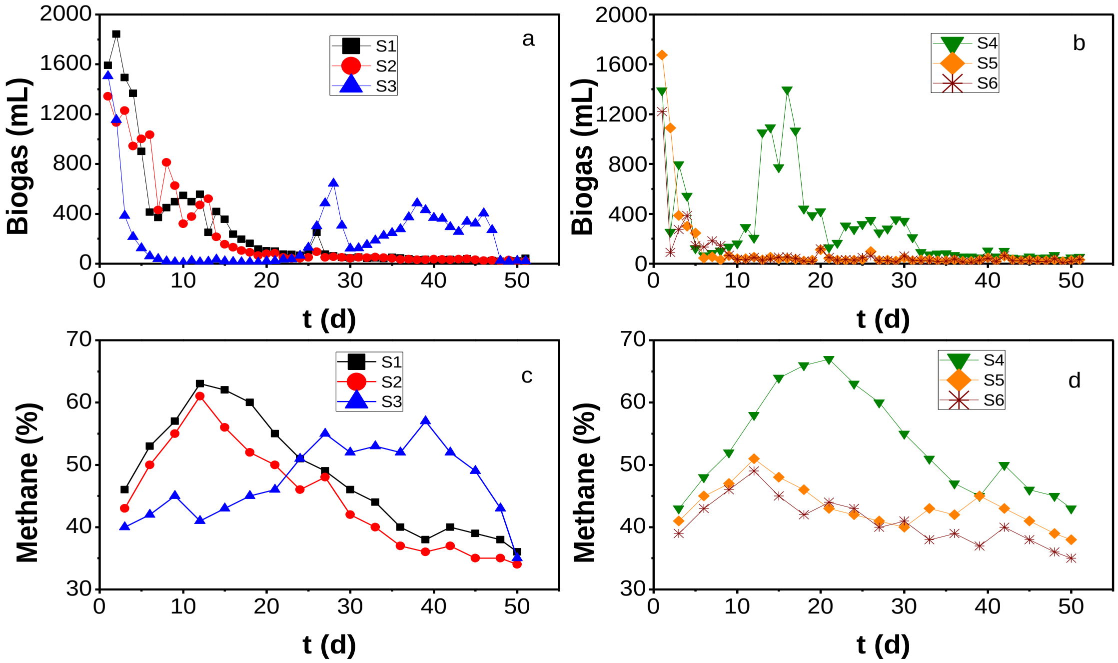

Fig. 2. Biogas production levels and methane contents during the AD of cassava pulp with sludge inocula: (a) biogas, mesophilic; (b) biogas, thermophilic; (c) methane content, mesophilic; (d) methane content, thermophilic

Figures 2(c) and (d) give the methane contents for the six digesters during the AD of cassava pulp. In mesophilic digesters, methane contents were in the range of 36% to 63%, 34% to 61%, and 35% to 57% for S1, S2, and S3, respectively. In thermophilic digesters, methane contents were in the range of 43% to 67%, 38% to 51%, and 35% to 49% for S4, S5, and S6, respectively. The maximum methane contents occurred on the 12th, 12th, 39th, 21st, 12th, and 12th day for S1 through S6, respectively, approximately three days after the corresponding time of maximum VFA concentration. The AD of cassava pulp with inoculum I1 produced higher-quality biogas than did AD with inocula I2 or I3.

Under mesophilic conditions, the total methane production volumes were 7.28 and 6.08 L for S1 and S2, respectively, which were 1.26 and 1.12 times higher than that of S3. Under thermophilic conditions, the total methane production volumes were 8.64 and 6.51 L for S4 and S5, respectively, which were 5.19 and 1.51 times higher than that of S6. Of all the digesters, S4 achieved the highest total methane production. Compared with inocula I2 and I3, I1 could improve the specific methane yields during the AD of cassava pulp under both mesophilic and thermophilic conditions.

Based on the VS contents of added cassava pulp, the average specific biogas yields over 50 days of AD were 0.638, 0.565, 0.504, 0.688, 0.268, and 0.185 $m^3/(kgVS_{added})$ for S1 through S6, respectively. The corresponding specific methane yields were 0.333, 0.278, 0.242, 0.395, 0.111, and 0.076 $m^3/(kgVS_{added})$. These results were similar to those of Panichnumsin *et al.* (2010), who reported that the ultimate methane potential for the mesophilic AD of cassava pulp was 0.344 $m^3/(kgVS_{added})$. The specific methane yield depends on the substrate used. Agro-wastes, such as corn stover, generally have lower methane yields because of their C/N ratios and high lignin contents (Ward *et al.* 2008). Compared with the AD of other substrates (Goberna *et al.* 2010; Fang *et al.* 2011; Dai *et al.* 2013; Vanegas and Bartlett 2013; Serrano *et al.* 2014), the AD of cassava pulp with pig manure generated high methane yields of 0.281 to 0.352 $m^3/kgVS_{added}$ under mesophilic conditions (Panichnumsin *et al.* 2010; Ren *et al.* 2014)(Table 4). In the current study, high specific methane yields for S1 through S4 were observed even though no co-substrates were added. This process may become important in regions without adequate supplies of manure to use as a co-substrate. All three types of inocula from sewage sludge facilitated high methanogenesis efficiency during the AD of cassava pulp in mesophilic digesters. However, under thermophilic conditions, only inoculation with pre-acclimated anaerobic sludge (I1) facilitated high levels of methane production during the AD of cassava pulp. The low specific methane yields in S5 and S6 was attributed to the imbalance between acidogenesis and methanogenesis which resulted from the faster acidification rate (Figs. 1(c) and (d)) and then higher accumulation concentration of VFA under the thermophilic condition.

Table 4. Specific Methane Yields from the AD of Different Feedstocks

Feedstock	T (°C)	Specific Methane Yield ($m^3/(kgVS_{added})$)	References
Cassava pulp	35	0.242 to 0.333	This study
Cassava pulp	55	0.076 to 0.395	This study
Cassava pulp, pig manure	25 (SBR) 37 (CSTR)	0.281 to 0.352	Ren *et al*. (2014)
Cassava pulp, pig manure	37	0.227 to 0.306 (semi-continuous)	Panichnumsin *et al.* (2010)
Cassava pulp	37	0.342 (batch)	Panichnumsin *et al.* (2010)
Cattle excreta, olive mill wastes	35	0.18 to 0.21	Goberna *et al.* (2010)
By-products from sugar production, cow manure	35	0.24	Fang *et al.* (2011)
Sewage sludge, orange peel waste	35	0.165	Serrano *et al.* (2014)
Sewage sludge, food waste	35	0.33	Dai *et al.* (2013)
Seaweed	35	0.191 to 0.335	Vanegas and Bartlett (2013)
Straw, pig manure	37	0.178-0.268	Li *et al.* (2015)
Horse manure	35	0.105	Boske *et al.* (2014)
Maize stalk, swine manure	55	0.146	Zhang *et al.* (2015)
Horse manure	55	0.118-0.155	Boske *et al.* (2015)

Semi-continuous AD of Cassava Pulp Inoculated with Digested Excess Sludge (I3)

pH, VFA, alkalinity, and NH_4^+-N

The pH levels in semi-continuous anaerobic digesters are shown in Fig. 3(b). During the initial phase, the pH in the digesters decreased more slowly than in the batch tests (Fig. 3(b)). This can be attributed to the high buffering capacity of the high amount of sewage sludge inocula. When the OLR was increased from 1.5 to 8.7 kgVS/(m^3 d) (Fig. 3(a)), the pH gradually became stable in the ranges of 6.8 to 7.2 and 7.2 to 7.4 for mesophilic digesters and thermophilic digesters, respectively. In mesophilic digesters, when the OLR was 12.5 kgVS/(m^3 d), the pH rapidly fell to below 6.6. In thermophilic digesters, when the OLR was in the range of 12.5 to 18.0 kgVS/(m^3 d), the pH decreased gradually to below 6.6. The pH recovered to levels above 6.9 after the digesters were not fed for several days and then continued with a lower OLR.

The VFA levels in four semi-continuous anaerobic digesters are shown in Fig. 3(c). High concentrations of VFA severely inhibit biogas production (Siegert and Banks 2005). Consequently, the VFA was used to monitor the stability of the digesters. Severe fluctuations were observed during the initial few days of operation, indicating unstable processes. After about 50 days of operation, the VFA was relatively stable in an operating range of 175.5 to 419.3 mg/L and 146.3 to 596.9 mg/L for mesophilic digesters and thermophilic digesters, respectively. These VFA levels indicate that no accumulation of VFA was taking place. When the OLR was increased to 12.5 and 18.0 kgVS/(m^3 d) for mesophilic digesters and thermophilic digesters, respectively, the concentration of VFA rapidly increased, with peak values of 2126.8 mg/L (mesophilic) and 2605.6 mg/L (thermophilic). These spikes could be explained by the fact that the concentrations of VFA were more than the maximum degradation capacity of methanogens in those bioreactors. These maximum concentrations were below the glucose inhibition concentration of VFA of 4000 mg/L suggested by Siegert and Banks (2005). However, the accumulation of VFA indicates that methanogenic activities were close to their limits at OLRs of 10.4 kgVS/(m^3 d) (mesophilic) and 15.0 kgVS/(m^3 d) (thermophilic). After recovery procedures were implemented, the concentrations of VFA in the digesters decreased to below 600 mg/L, which showed that the AD in this study had a high capacity for resisting short-term shock loading of organic matter.

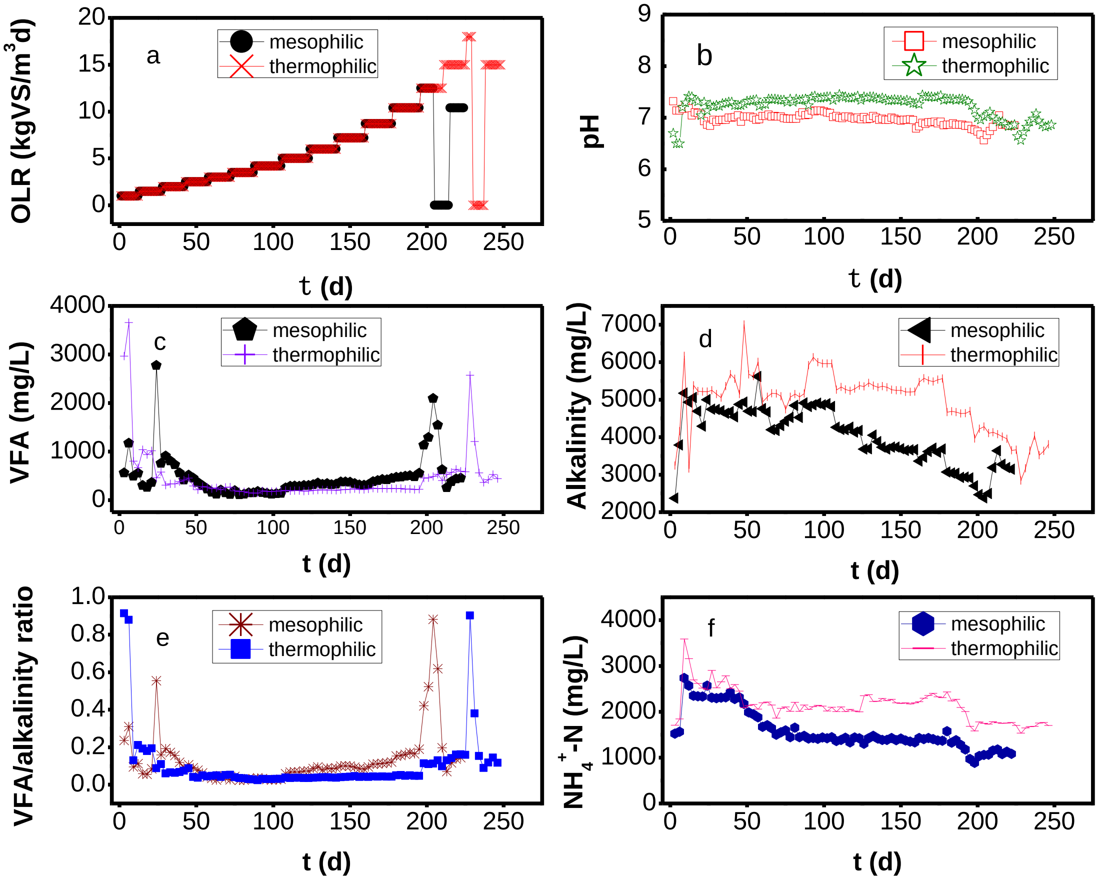

Fig. 3. (a) OLRs; (b) pH levels; (c) VFA concentrations; (d) alkalinity levels; (e) VFA/alkalinity ratios; and (f) ammonia nitrogen concentrations during the semi-continuous anaerobic digestion of cassava pulp

The alkalinity levels in four semi-continuous anaerobic digesters are shown in Fig. 3(d). These spikes in Fig. 3(d) were attributed to the reduction of VFA (Fig. 3(c)) and the increase of specific methane yield at the relative OLRs. The maximum levels of alkalinity occurred during the first 60 days of operation. After this period, the alkalinity of the mesophilic digester decreased gradually, whereas the alkalinity of the mesophilic digester remained above 5000 mg/L until the OLR was increased to 10.4 kgVS/(m^3 d). Thermophilic digesters exhibited higher alkalinity levels and better buffering capacity compared with those of mesophilic digesters.

However, the ratio of the VFA level to the alkalinity level (VFA/ALK) is reportedly a more effective parameter for assessing the stability of AD (Callaghan *et al.* 2002). When the VFA/ALK is less than 0.4, the system is considered stable, whereas when the VFA/ALK is greater than 0.8, the system becomes unstable. In all digesters, the spikes of VFA/ALK (Fig. 3(e)) were observed for the reason of peak values of VFA (Fig. 3c). Peak values of VFA/ALK greater than 0.4 were observed in all digesters during the first 30 days of operation, and then values declined to below 0.1, indicating that the AD process had become stable (Fig. 3(e)). When the OLRs were increased to 12.5 kgVS/(m^3 d) (mesophilic) and 18.0 kgVS/(m^3 d) (thermophilic), VFA/ALK values in the digesters rapidly increased, and the peak values were above 0.8. The thermophilic digestion of cassava pulp, therefore, had a better buffering capacity than that of mesophilic digestion. This finding was consistent with the results for pH, VFA, and alkalinity.

Ammonia nitrogen produced from the degradation of organic nitrogen during AD may enhance the buffering capacity of digesters. However, high ammonia nitrogen concentrations can inhibit methanogenic activity. During the first 40 days, one spike of ammonia nitrogen content occurred in each digester, which could be interpreted as the active microbial deamination of protein from sewage sludge (Fig. 3(f)). After severe fluctuations during the first 40 days of operation, the concentration of ammonia nitrogen gradually decreased (Fig. 3(f)). For OLRs in the range of 4.2 to 8.7 kgVS/(m^3 d), ammonia nitrogen levels remained at approximately 1500 mg/L and 2100 mg/L for mesophilic digesters and thermophilic digesters, respectively. These levels were below the reported inhibition concentration of ammonia nitrogen of 3860 mg/L (Benabdallah EI Hadj *et al.* 2009). When the OLR was increased to 10.4 kgVS/(m^3 d), the concentration of ammonia nitrogen in both the mesophilic and thermophilic digesters rapidly fell, indicating the inhibition of ammonifying bacteria.

Biogas and methane production

Because of the degradation of partial residues from the sludge inoculum, abnormally high values of biogas production and specific biogas yields were observed in the first 12 to 20 days (Figs. 4(a) and (b)). When the OLR was increased from 1.5 to 8.7 kgVS/(m^3 d), biogas production rates in both types of digesters increased gradually and were almost identical. During this phase of the operation, the specific biogas yields in both types of digesters were approximately 0.540 m^3/kgVS$_{\text{added}}$. When the OLR was increased to 10.4 to 12.5 kgVS/(m^3 d), the specific biogas yield of the mesophilic digester decreased to 0.453 m^3/kgVS$_{\text{added}}$, which implied that the OLR levels had exceeded the maximum degradation capacity of the mesophilic digester. On account of the acidification of digestate (Fig. 3(b)) and subsequent intermitting feeding (Fig. 3(a)), daily biogas production yield rapidly decreased during 196 to 214 days for the mesophilic digester and 226 to 237 days for the thermophilic digester (Fig, 3(a)), as did specific biogas yield and specific methane yield (Figs. 3(b) and (d)). Therefore, 8.7 kgVS/(m^3 d) was the highest acceptable value of OLR for the mesophilic production of biogas from cassava pulp using a sludge inoculum in semi-continuous AD. However, for the thermophilic digester, when the OLR was in the range of 10.4 to 15.0 kgVS/(m^3 d), biogas yields as high as 0.589 to 0.620 m^3/kgVS$_{added}$ were obtained. This biogas yield was higher than that of the mesophilic digesters. Therefore, the current AD of cassava pulp achieved higher specific biogas yields than did the AD of a mixture of rice straw and pig manure, which produced a specific biogas yield of 0.413 m^3/kgVS$_{added}$ (Li *et al.* 2015). Consequently, cassava pulp is a more efficient raw feedstock than rice straw and pig manure for the commercial production of biogas.

The methane contents of the four semi-continuous anaerobic digesters are shown in Fig. 4(c). In the first 20 days of operation, the methane content in both types of digesters rose above 50%, which implied the rapid proliferation of methanogenic bacteria. When the OLR was in the range of 2.0 to 10.4 kgVS/(m^3 d) (mesophilic) or 2.0 to 15.0 kgVS/(m^3 d) (thermophilic), the methane content was in the range of 55% to 60%. However, when the OLR was above these ranges, the methane content decreased rapidly as a result of the inhibition of methanogenesis.

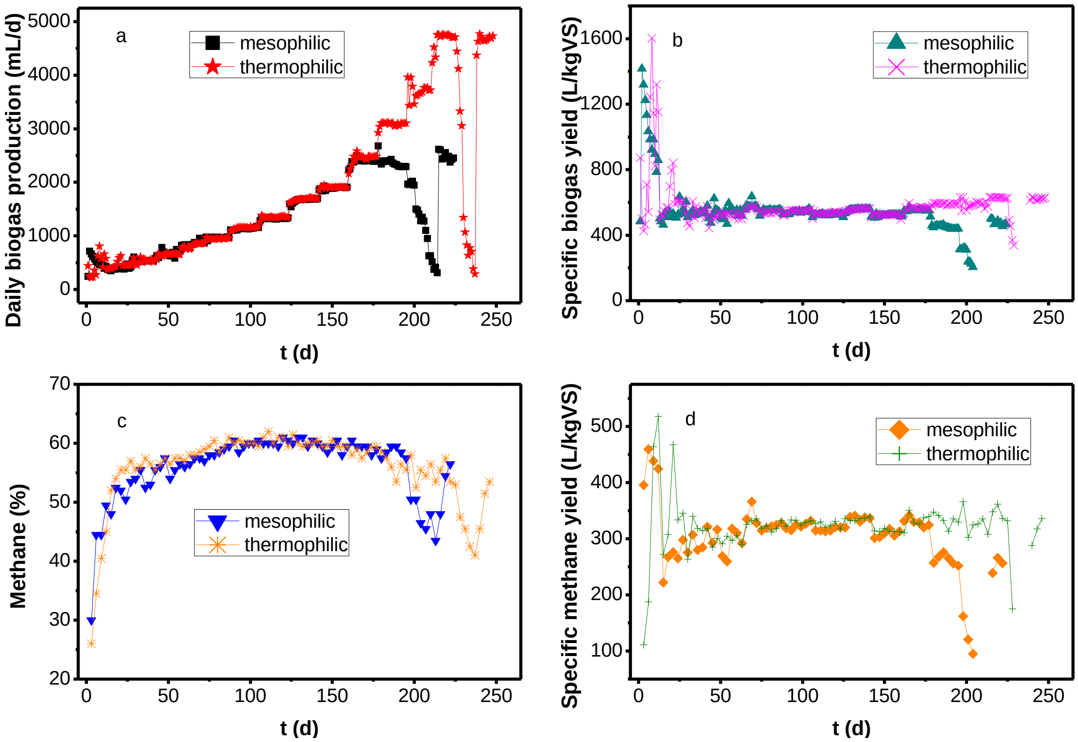

Fig. 4. (a) Daily biogas production levels; (b) biogas yields; (c) methane contents; and (d) methane yields during the semi-continuous anaerobic digestion of cassava pulp

During the first 20 days or so, specific methane yields oscillated in a similar way to that of specific biogas yields (Figs. 4(b) and (d)). When the OLR was increased from 2.5 to 8.7 kgVS/(m^3 d), the specific methane yields for both types of digesters were similar and were in the range of 0.308 to 0.334 m^3/kgVS$_{added}$ and 0.314 to 0.336 m^3/kgVS$_{added}$ for the mesophilic and the thermophilic digesters, respectively. In the above range of OLRs, cassava pulp at mesophilic conditions produced the almost same methane yield as that at thermophilic conditions, and it was different from several other investigations in which the methane production and methane yields at thermophilic conditions were 52.2 to 66.5% (Boske *et al.* 2015) or 36.4% (Pohl *et al.* 2102) higher than that at mesophilic conditions. The contradiction was due to chemical component of the substrate. In this study, cassava pulp contained more easily degradable organic matter such as starch than horse manure (Boske *et al.* 2015) and wheat straw (Pohl *et al.* 2102). In the mesophilic digester, when the OLR was 10.4 kgVS/(m^3 d), the specific methane yield decreased to 0.262 m^3/kgVS$_{added}$. However, under thermophilic conditions, the specific methane yield of cassava pulp decreased rapidly only when the OLR exceeded 15.0 kgVS/(m^3 d). It was concluded that the AD of cassava pulp under thermophilic conditions could operate at a higher OLR, and therefore produce more methane, than it could under mesophilic conditions. The higher OLR tolerance in the thermophilic digesters was be explained that thermophilic methanogens could consume VFA more quickly than mesophilic methanogen.

The specific methane yields for cassava pulp were between those of food wastes and cellulose-lignin agricultural waste, such as straw (Table 4). The highest stable specific methane yield of cassava pulp in semi-continuous AD in this study was close to the value of 0.344 m^3/kgVS$_{added}$ reported by Panichnumsin *et al.* (2010) for the mesophilic AD of cassava pulp. To avoid the rapid acidification of cassava pulp, researchers commonly have used pig manure as a co-substrate to achieve successful AD. Panichnumsin *et al.* (2010) and Ren *et al.* (2014) reported maximum specific methane yields of 0.306 and 0.352 m^3/kgVS$_{added}$ for the AD of cassava pulp and pig manure mixed in a ratio of 3:2 (dw/dw). Sludge inocula had high organic nitrogen contents that provided a high pH buffering capacity similar to that of pig manure. Furthermore, highly efficient methane production was maintained in the current study at OLRs 2 to 4 times higher than those for cassava pulp and pig manure. The final TS value was 13.4% in the current mesophilic digester and 16.1% in the current thermophilic digester, both of which were greater than the values of 3% to 5% reported by Panichnumsin *et al*. (2010) and Ren *et al*. (2014). Therefore, because operation is possible at higher OLRs and higher TS levels, the AD of cassava pulp inoculated with excess sludge could more effectively produce methane than the co-digestion of pig manure and cassava pulp under both mesophilic and thermophilic conditions.

CONCLUSIONS

1. During the mesophilic batch AD of cassava pulp, sludge inocula obtained from a municipal wastewater treatment hydrolysis acidification tank had a more effective buffering capacity against acidification than sludge inocula from the gravity thickening tank. Based on the specific methane yields, all three types of sewage sludge tested could be used as inocula for the mesophilic AD of cassava pulp, whereas sludge pre-acclimated with cassava pulp was clearly optimal as an inoculum for the thermophilic AD of cassava pulp.
2. For the batch operations, the highest levels of methane production were observed during the AD of cassava pulp inoculated with acclimated sludge from the hydrolysis acidification tank. The specific biogas yields and specific methane yields were, respectively, 0.638 and 0.333 m^3/kgVS$_{added}$ for S1 (mesophilic) and 0.688 and 0.395 m^3/kgVS$_{added}$ for S4 (thermophilic).

3. The semi-continuous AD of cassava pulp inoculated with digested excess sludge achieved high specific methane yields in the range of 0.304 to 0.334 $m^3/kgVS_{added}$, 0.314 to 0.336 $m^3/kgVS_{added}$ at the optimal OLR under mesophilic and thermophilic conditions, respectively.
4. The thermophilic semi-continuous AD of cassava pulp with sludge inocula maintained high levels of biogas production with OLRs in the range of 2.5 to 15.0 kgVS/(m^3 d), which was broader than the range of 2.5 to 8.7 kgVS/(m^3 d) for mesophilic AD.

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REFERENCES CITED

AACC (1990). "Approved methods of the AACC," American Association of Cereal Chemists, Minnesota.

Alvarez, R., and Lidén, G. (2008). "Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste," *Renew. Energ.* 33(4), 726-734. DOI: 10.1016/j.renene.2007.05.001

AOAC (1995). "Official method of analysis," Association of Official Agricultural Chemists, Virginia

APHA (2005). *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., Sanders, W. T. M., Siegrist, H., and Vavilin, V. A. (2002). "The IWA anaerobic digestion model no. 1 (ADM 1)," *Water Sci. Technol.* 45(10), 65-73.

Benabdallah El Hadj, T., Astals, S., Gali, A., Mace, S., and Mata-Alvarez, J. (2009). "Ammonia influence in anaerobic digestion of OFMSW," *Water Sci. Technol.* 59(6), 1153-1158. DOI: 10.2166/wst.2009.100

Borja, R., Martin, A., Banks, C. J., Alonso, V., and Chica, A. (1995). "A kinetic study of anaerobic digestion of olive mill wastewater at mesophilic and thermophilic temperatures," *Environ. Pollut.* 88(1), 13-18. DOI: 10.1016/0269-7491(95)91043-K

Borowski, S. (2015). "Co-digestion of the hydromechanically separated organic fraction of municipal solid waste with sewage sludge," *J. Environ. Manage.* 147, 87-94. DOI: 10.1016/j.jenvman.2014.09.013

Boske, J., Wirth, B., Garlipp, F., Mumme, J., and Weghe, H., V., D. (2014). "Anaerobic digestion of horse dung mixed with different bedding materials in an upflow solid-state (UASS) reactor at mesophilic conditions," *Bioresour. Technol.* 158, 111-118. DOI: 10.1016/j.biortech.2014.02.034

Boske, J., Wirth, B., Garlipp, F., Mumme, J., and Weghe, H., V., D. (2015). "Upflow anaerobic solid-state (UASS) digestion of horse manure: Thermophilic vs. mesophilic performance," *Bioresour. Technol.* 175, 8-16. DOI: 10.1016/j.biortech.2014.10.041

Bouallagui, H., Lahdheb, H., Romdan, E.B., Rachdi, B., and Hamdi, M. (2009). "Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition," *J. Environ. Manage.* 90(5), 1844-1849. DOI: 10.1016/j.jenvman.2008.12.002

Callaghan, F. J., Wase, D. A. J., Thayanithy, K., and Forster, C. F. (2002). "Continuous codigestion of cattle slurry with fruit and vegetable wastes and chicken manure," *Biomass Bioenerg.* 22(1), 71-77. DOI: 10.1016/S0961-9534(01)00057-5

Dai, X., Duan, N., Dong, B., and Dai, L. (2013). "High-solids anaerobic co-digestion of sewage sludge and food waste in comparison with mono digestions: Stability and performance," *Waste Manage.* 33(2), 308-316. DOI: 10.1016/j.wasman.2012.10.018

Estevez, M. M., Sapci, Z., Linjordet, R., and Morken, J. (2014). "Incorporation of fish by-product into the semi-continuous anaerobic co-digestion of pre-treated lignocellulose and cow manure, with recovery of digestate's nutrients," *Renew. Energ.* 66, 550-558. DOI: 10.1016/j.renene.2014.01.001

Fang, C., Boe, K., and Angelidaki, I. (2011). "Anaerobic co-digestion of by-products from sugar production with cow manure," *Water Res.* 45(11), 3473-3480. DOI: 10.1016/j.watres. 2011.04.008

Fonoll, X., Astals, S., Dosta, J., and Mata-Alvarez, J. (2015). "Anaerobic co-digestion of sewage sludge and fruit wastes: Evaluation of the transitory states when the co-substrate is changed," *Chem. Eng. J.* 262, 1268-1274. DOI: 10.1016/j.cej.2014.10.045

Goberna, M., Schoen, M. A., Sperl, D., Wett, B., and Insam, H. (2010). "Mesophilic and thermophilic co-fermentation of cattle excreta and olive mill wastes in pilot anaerobic digesters," *Biomass Bioenerg.* 34(3), 340-346. DOI: 10.1016/j.biombioe.2009.11.005

Gu, Y., Chen, X., Liu, Z., Zhou, X., and Zhang, Y. (2014). "Effect of inoculum sources on the anaerobic digestion of rice straw," *Bioresour. Technol.* 158, 149-155. DOI: 10.1016/j.biortech. 2014.02.011

Jansson, C., Westerbergh, A., Zhang, J., Hu, X., and Sun, C. (2009). "Cassava, a potential biofuel crop in (the) People's Republic of China," *Appl. Energ.* 86(S1), 95-99. DOI: 10.1016/ j.apenergy.2009.05.011

Li, L., Yang, X., Li, X., Zheng, M., Chen, J., and Zhang, Z. (2010). "The influence of inoculum sources on anaerobic biogasification of NaOH-treated corn stover," *Energ. Source Part A* 33(2), 138-144. DOI: 10.1080/15567030902937192

Li, D., Liu, S., Mi, L., Li, Z., Yuan, Y., Yan, Z., and Liu, X. (2015). "Effects of feedstock ratio and organic loading rate on the anaerobic mesophilic co-digestion of rice straw and pig manure," *Bioresour. Technol.* 187, 120-127. DOI: 10.1016/j.biortech.2015.03.040

Panichnumsin, P., Nopharatana, A., Ahring, B., and Chaiprasert, P. (2010). "Production of methane by co-digestion of cassava pulp with various concentrations of pig manure," *Biomass Bioenerg.* 34(8), 1117-1124. DOI: 10.1016/j.biombioe.2010.02.018

Pohl, M., Nordberg, J., Heeg, K., and Nettmann, E. (2012). "Thermo- and mesophilic anaerobic digestion of wheat straw by the upflow anaerobic solid-state (UASS) process," *Bioresour. Technol.* 124, 321-327. DOI: 10.1016/j.biortech.2012.08.063.

Pu, Y., and Liu, J. (2009). "Study on biogas production of cassava dregs by anaerobic fermentation," *J. Anhui Agric. Sci.* 37(29), 14308-14310.

Quintero, M., Castro, L., Ortiz, C., Guzmán, C., and Escalante, H. (2012). "Enhancement of starting up anaerobic digestion of lignocellulosic substrate: Fique's bagasse as an example," *Bioresour. Technol.* 108, 8-13. DOI: 10.1016/j.biortech.2011.12.052

Ratanatamskul, C., Onnum, G., and Yamamoto, K. (2014). "A prototype single-stage anaerobic digester for co-digestion of food waste and sewage sludge from high-rise building for on-site biogas production," *Int. Biodeter. Biodegr.* 95, 176-180. DOI: 10.1016/j.ibiod.2014.06.010

Ren, J., Yuan, X., Li, J., Ma, X., Zhao, Y., Zhu, W., Wang, X., and Cui, Z. (2014). "Performance and microbial community dynamics in a two-phase anaerobic co-digestion system using cassava dregs and pig manure," *Bioresour. Technol.* 155, 342-351. DOI: 10.1016/j.biortech.2013.12. 120

Serrano, A., Ángel Siles López, J., Chica, A. F., Martin, M., Karouach, F., Mesfioui, A., and El Bari, H. (2014). "Mesophilic anaerobic co-digestion of sewage sludge and orange peel waste," *Environ. Technol.* 35(7), 898-906. DOI: 10.1080/09593330.2013.855822

Siegert, I., and Banks, C. (2005). "The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors," *Process Biochem.* 40(11), 3412-3418. DOI: 10.1016/j.procbio.2005.01.025

Toreci, I., Droste, R. L., and Kenndy, K. J. (2011). "Mesophilic anaerobic digestion with high-temperature microwave pretreatment and importance of inoculum acclimation," *Water Environ. Res*. 83(6), 549-559. DOI: 10.2175/106143010X12780288628651

Van Soest, P. J., and Wine, R. H. (1968). "Determination of lignin and cellulose in acid-detergent fiber with permanganate," *J. Assoc. Off. Anal. Chem.* 51, 780-785.

Vanegas, C. H., and Bartlett, J. (2013). "Green energy from marine algae: Biogas production and composition from the anaerobic digestion of Irish seaweed species," *Environ. Technol.* 34(15), 2277-2283. DOI: 10.1080/09593330.2013.765922

Walkins, D., Rao, S., Lu, X., and Lee, P. K. H. (2015). "Effect of sludge inoculum and organic feedstock on active microbial communities and methane yield during anaerobic digestion," *Front. Microbiol.* 6, 1114. DOI: 10.3389/fmicb.2015.01114

Ward, A. J., Hobbs, P. J., Holliman, P. J., and Jones, D. L. (2008). "Optimisation of the anaerobic digestion of agricultural resources," *Bioresour. Technol.* 99(17), 7928-7940. DOI: 10.1016/j.biortech.2008.02.044

Yang, J., Tan, H., Yang, R., Sun, X., Zhai, H., and Li, K. (2011). "Astaxanthin production by *Phaffia rhodozyma* fermentation of cassava residues substrate," *Agric. Eng. Int.: CIGR J.* 13(2), 1-9.

Zhang, C., Su, H., Baeyens, J., and Tan, T. (2014). "Reviewing the anaerobic digestion of food waste for biogas production," *Renew. Sust. Energ. Rev.* 38, 383-392. DOI: 10.1016/j.rser.2014.05.038

Zhang, T., Mao, C., Zhai, N., Wang, X., and Yang, G. (2015). "Influence of initial pH on thermophilic anaerobic co-digestion of swine manure and maize stalk," *Waste Manage*. 35: 119-226. DOI: 10.1016/j.wasman.2014.09.004

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