

Enhanced Biomethanation in Co-Digestion of Cassava Pulp and Pig Manure Using A Two-Phase Anaerobic System

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Abstract: In this study, the co-digestion of cassava pulp (CP) and pig manure (PM) in a two-phase continuously stirred tank reactor (CSTR) was examined to find the optimum CP-to-PM mixture ratio (w/w volatile solids; VS) and to evaluate the effect of phase separation on the efficiencies of solid destruction and methane production. The results showed that the highest VS removal (29%) and the greatest production of intermediates, such as volatile fatty acids and ethanol (24%) in the hydrolysis/acidification reactor were obtained using a 50:50 CP:PM ratio. Using feedstock that had a higher CP ratio, the activity of the hydrolytic/acidogenic bacteria was inhibited at approximately pH 4. In addition, the distribution of the VFA produced was shifted at approximately pH 4.5. The removal of solids and the production of methane increased in the two-phase CSTR with increasing cassava pulp ratio, up to 60% of the feedstock. The highest reduction in solids (68%) and the greatest methane yield (370 mL g⁻¹VS_{added}) were obtained using a 60:40 CP:PM ratio. Co-digestion in a two-phase CSTR reduced the solids by 14% and increased the production of methane by 36% over those obtained using a single-phase CSTR.

Keywords: Biomethanation; Cassava pulp; Mixture ratio; Pig manure; Two-phase CSTR.

1. Introduction

Renewable energy production from animal and agro-industrial wastes should be adopted to compensate for fossil fuel consumption in order to reduce green house gas emissions and consequently to prevent global warming. Anaerobic digestion is considered to be one of the most economic ways of producing bio-energy from biomass and has been used efficiently to treat various household, industrial and agricultural organic waste [1].

Approximately 5.2 Mt of fresh cassava pulp is generated annually from the production of cassava starch in Thailand [2]. At present, a part of cassava pulp is used as a low cost animal feed and some part become a major solid waste problem [2]. In the rainy season especially, improper management of cassava pulp leads to odor problems and leachate contamination of the soil and ground water. Cassava pulp containing 65% starch (dry basis) is regarded as a great potential substrate for biogas production. However, biogas production from cassava pulp might be limited due to its very high carbon to nitrogen ratio (C:N ratio), the high level of readily degradable compounds (65% starch of total solid), and the high level of recalcitrant organic matter (30% ligno-cellulose of total solid) [3]. It has been reported that methanogenesis is the rate limiting step of digestion of high content of easily degradable organic materials to methane while hydrolysis is the rate limiting step in degradation of particulate organic matter [4-5].

Thailand has an extensive pig industry with an average of 8 million heads per year which generate a large amount of manure; approximately 2 Mt dry matter per year [2]. Anaerobic digestion of pig manure typically produces a low methane yield and it has been operated at long hydraulic retention time (HRT) due to a low C:N ratio and a high amount of remaining recalcitrant compounds. Furthermore, its excessive ammonia content might cause an inhibitory effect on methanogenesis [6]. However, its high buffering capacity and richness of macro and micro nutrients is beneficial for biogas production process [7].

Thus, improving the efficiency of biogas production through waste co-digestion would be a rational way to deal with individual inferior characteristics of cassava pulp and pig manure.

Anaerobic digestion uses a series of metabolic interactions among various groups of microorganisms. It is considered a complex biochemical process that comprises 4 steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Anaerobic digestion is commonly used in the conventional single-phase process because of the simplicity of its configuration and operation [8]. However, in terms of the effluent quality, the single-phase process has often been found to be less efficient in treating waste that contains highly recalcitrant organic materials than a phased reactor system [9]. The acidogenic phase and the methanogenic phase are very different in their nutritional and pH requirements, their growth kinetics, and in their sensitivity to environmental stresses. To optimize the environmental conditions for each phase the two-phase system has separate units for acidogenesis and methanogenesis [10]. The two-phase system has demonstrated several advantages over the conventional single-phase system including selection and enrichment of different bacteria in each reactor, a higher organic degradation rate, a higher rate of methane production, the stability of the process, and a significant reduction in the risk of digester overloading [4,11]. It has been suggested that the two-phase system be used for waste that contains a high amount of readily-degradable organic compounds to achieve a balanced process at high organic loading rates [10]. In addition, studies have indicated that the two-phase system could achieve higher degradation rates of particulate organic compounds. The hydrolysis rate of ligno-cellulose was enhanced using a slightly acidic pH, as this improved the working conditions for the hydrolytic/acidogenic bacteria [12-14], and the volatile fatty acids (VFA) produced can improve the accessibility of hydrolytic enzymes [15-16].

In order to achieve higher destruction rates of insoluble particulate organic matter and an increase in production rates of

methane and to obtain greater process stability for the co-digestion of cassava pulp and pig manure, a two-phase system was used in this study. The objectives of this study were to evaluate the effect of phase separation on the performance of a two-phase anaerobic co-digestion process and to determine the optimum substrate mixture ratio that provides good working conditions for the hydrolytic/acidogenic bacteria, without controlling the pH of the system.

2. Experimental

2.1 Feed materials and inoculum

Fresh cassava pulp (CP) was collected from a cassava starch factory in Rayong Province, Thailand. Pig manure (PM) was taken directly from excretion of pig fatteners at a medium scale farm in Nakornprathom Province, Thailand. The CP and PM were stored at -20°C in small plastic bags before use. CP had a higher VS:TS ratio than PM, and most of its COD was in suspended forms (very low $\text{COD}_{\text{soluble}}:\text{COD}_{\text{total}}$ ratio). CP contained on average 30% TS with 98% VS. The TS content in PM was 25% with 72% VS. The C:N ratios were about 210 in CP and 14 in PM. The feedstocks were prepared every 2-3 days by diluting the cassava pulp and pig manure with tap water, such that their volatile solid content was brought to 5.25%, and stored at 4°C . The mixtures of cassava pulp (CP) and pig manure (PM) at CP:PM ratios of 0:100, 20:80, 40:60, 50:50, 60:40, 80:20 and 100:0 were prepared by mixing 5.25% of each feedstock together based on the volatile solids content (w/w). The volatile solids of the substrates were fixed at 5.25%. The characteristics of the feedstocks are shown in Table 1. Partially digested pig manure taken from an industrial anaerobic plug flow reactor was used as the starting inoculum.

2.2 Reactor setup and operation

The two-phase system was composed of two continuously-stirred tank reactors (CSTR) of different volumes (Fig. 1). The reactors were double-walled glass cylinders that had been fitted with stainless steel plates on the top and bottom. The reactors were operated in semi-continuous mode using the withdraw/feed method once a day, and they were mechanically stirred at 100 rpm using an electric motor for 15 minutes each half hour. The reactors were maintained at 37°C by circulating water through a water jacket from a temperature-controlled water bath. The daily production of biogas from each reactor

was measured using a liquid displacement system that was connected to the reactor, and the gas production was recorded automatically.

The hydrolysis/acidification phase was performed in a reactor that had an active volume of 0.5 L. The reactor was operated at a constant organic loading rate (OLR) of $24.47 \text{ kg VS m}^{-3} \text{ d}^{-1}$ and a hydraulic retention time (HRT) of 2 days. It was initially fed with pig manure alone, and then fed stepwise with a mixture of cassava pulp (CP) and pig manure (PM) at CP:PM ratios of 20:80, 40:60, 50:50, 60:40, 80:20 and 100:0 (w/w, based on the volatile solids contents). The reactor was kept fed with each mixture for at least thirteen HRTs. The methanogenic phase was carried out in a 5 L reactor that had an active volume of 3 L. The reactor was fed with the effluent from the hydrolysis/acidification reactor at an OLR in the range of $3.27 \pm 0.32 \text{ kg VS m}^{-3} \text{ d}^{-1}$ and a HRT of 13 days.

2.3 Analysis and calculation

The technical composition of the influent and effluent were determined three times a week, with the exception of pH which was measured daily. The effluent from each digester was analyzed for its total solid (TS), volatile solid (VS), total chemical oxygen demand (COD-T), pH, total nitrogen (TKN) and ammonium-nitrogen ($\text{NH}_4\text{-N}$) levels, according to standard methods [17]. Total alkalinity was measured by titration to pH 4.0, using $0.1 \text{ M H}_2\text{SO}_4$. The production of biogas was measured using the water displacement method [17]. The percentages of methane and carbon dioxide in the biogas were analyzed using gas chromatography (Shimadzu, Class-GC 14B, Japan), using a Porapak-N column equipped with a thermal conductivity detector (TCD). The oven, injector and detector temperatures were 70 , 120 and 120°C , respectively. Helium was used as the carrier gas at a flow rate of 30 mL min^{-1} . Effluents taken from the reactors were centrifuged at $10,000 \text{ rpm}$ for 10 min , and the supernatant was passed through a $0.45\text{-}\mu\text{m}$ membrane for the analysis of the volatile fatty acids (VFA) and soluble COD (COD-S) levels. The individual VFA concentrations (acetic acid, propionic acid, butyric/isobutyric acids and valeric/isovaleric acids) were analyzed using the same gas chromatograph equipped with a flame ionization detector (FID) and Carbowax B-DA column. The oven, injector and detector temperatures were 170 , 200 and 200°C , respectively. Helium was used as the carrier gas at a flow rate of 50 mL min^{-1} , and nitrogen was used as a makeup gas at a flow rate of 50 mL min^{-1} .

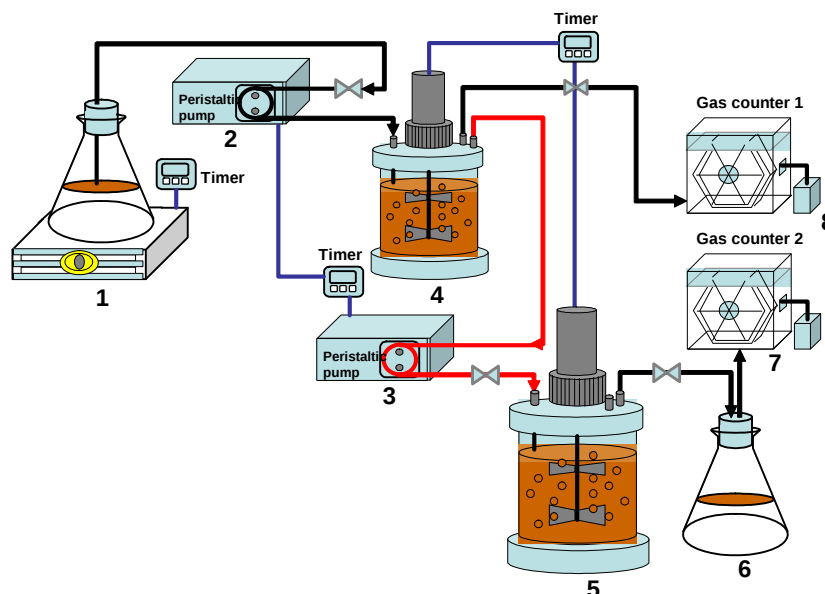


Figure 1. Schematic diagram of a two-phase CSTR set up. 1: Influent vessel. 2-3: Influent pump. 4: Hydrolysis/acidification reactor. 5: Methanogenic reactor. 6: Effluent vessel. 7-8: Gas counter.

The percentages of soluble COD and organic acid produced in the hydrolysis/acidification reactor were calculated using the following equations:

$$\text{Soluble COD (COD-S) production (\%)} = [(\text{COD-S}_{\text{eff}} - \text{COD-S}_{\text{inf}}) / \text{COD-T}_{\text{inf}}] \times 100 \quad (1)$$

$$\text{VFA and ethanol production (\%)} = [((\text{VFA+E})_{\text{eff}} + (\text{VFA+E})_{\text{CH}_4}) - (\text{VFA+E})_{\text{inf}}] / \text{COD-T}_{\text{inf}} \times 100 \quad (2)$$

Where, $\text{COD-S}_{\text{inf}}$ and $\text{COD-S}_{\text{eff}}$ are the influent and effluent concentrations of soluble COD (g COD L^{-1}), respectively. $(\text{VFA+E})_{\text{inf}}$ and $(\text{VFA+E})_{\text{eff}}$ are the influent and effluent concentrations of organic acids and ethanol (g COD L^{-1}), respectively, $(\text{VFA+E})_{\text{CH}_4}$ is the concentration of organic acids and ethanol (g COD L^{-1}) converted to methane, and $\text{COD-T}_{\text{inf}}$ is the influent concentration of total COD (g L^{-1}).

3. Results and Discussion

3.1 Hydrolysis/acidification reactor performance

The performance efficiency of the hydrolysis/acidification reactor was evaluated from the level of solid destruction and the production of soluble COD and VFA/ethanol. Fig. 2 shows the efficiency of VS reduction and the production of soluble COD and VFA/ethanol. The results show that the removal of VS and the production of VFA/ethanol increased with increasing CP proportions compared to PM alone. The greatest reduction of VS (29%) was achieved using a CP:PM ratio of 50:50, and this was 93% greater than when PM was used alone. The production of soluble COD and VFA/ethanol corresponded to the efficiency of the reduction of the VS. The highest production levels of soluble COD and VFA/ethanol were 26% and 25%, respectively, and were obtained when 50% CP was used in the feedstock. The high efficiency of solid reduction obtained

during the co-digestion process was due to the increase of the readily degradable fraction (i.e., the starch-rich carbohydrate in CP) and an improved C:N ratio in the feedstock (from 14 to 27, Table 1) that enhanced the growth of the hydrolytic/acidogenic bacteria. When higher CP ratios were used in the feedstock, the reduction of the VS declined remarkably. The reduction of the VS decreased to 10% when we used a CP:PM ratio of 80:20. Correspondingly, the production of soluble COD and VFA/ethanol decreased to 17% and 11%, respectively.

As seen in Table 2, the biogas productivity and the percentage of CO_2 in the biogas increased as the CP proportion of the feedstock increased, up to 50%. The biogas productivity increased from $540 \text{ mL L}^{-1} \text{ d}^{-1}$ to $940 \text{ mL L}^{-1} \text{ d}^{-1}$ when the feedstock was changed from PM alone to a 50:50 CP:PM mixture. Correspondingly, the percentage of CO_2 in the biogas increased from 52% to 78%. At higher CP:PM ratios, the productivity of biogas and the CO_2 content decreased, indicating that the removal of organic matter was limited. The productivity of biogas and the CO_2 content decreased from $940 \text{ mL L}^{-1} \text{ d}^{-1}$ and 78% at a CP:PM ratio of 50:50 to $160 \text{ mL L}^{-1} \text{ d}^{-1}$ and 61% at a CP:PM ratio of 80:20. When the CP ratio of the feedstock was increased to 80%, the production of methane ceased (Table 2). This might be due to the lowering of the pH to a level that is inhibitory to the growth of methanogenic bacteria (Fig. 2 and Table 2). In addition, PM is different from other protein wastes. It contained lots of anaerobic bacteria from pig intestines. High VFA (Table 1) resulted from natural acidification prior to the experiment. High methane content in biogas (Table 2, CP:PM ratio 0:100) showed that methanogenesis occurred under short SRT (and HRT). The 2-day HRT was not sufficient for cell growth unless some bacteria were added along with input, (see Table 2-CP:PM ratio 80:20).

Table 1. Operational duration and characteristics of feedstocks in CSTR treating PM and five mixtures (values are the mean $\pm$ S.D. of 5 determinations).

	CP:PM ratios						
	0:100	20:80	40:60	50:50	60:40	80:20	100:0
Duration							
Days	38 (1-38)	26 (39-64)	26 (65-90)	26 (91-118)	26 (119-144)	15 (145-160)	
Feedstock							
TS (%)	7.2 ± 0.2	6.8 ± 0.1	6.5 ± 0.2	6.2 ± 0.3	6.1 ± 0.2	5.7 ± 0.2	5.3 ± 0.1
VS (%)	5.2 ± 0.2	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
COD-T (%)	8.6 ± 0.3	8.1 ± 0.2	7.5 ± 0.2	7.2 ± 0.2	7.0 ± 0.2	6.4 ± 0.3	5.89 ± 0.17
COD-S (%)	0.91 ± 0.03	0.75 ± 0.03	0.59 ± 0.02	0.52 ± 0.02	0.44 ± 0.02	0.28 ± 0.01	0.12 ± 0.01
pH	7.42 ± 0.3	7.32 ± 0.2	7.25 ± 0.2	7.22 ± 0.2	7.16 ± 0.1	6.88 ± 0.3	4.49 ± 0.3
VFA (g COD L^{-1})	3.26 ± 0.1	2.63 ± 0.1	1.99 ± 0.05	1.68 ± 0.05	1.37 ± 0.05	0.74 ± 0.05	0.11 ± 0.05
Ammonium N (g L^{-1})	0.18 ± 0.04	0.15 ± 0.03	0.11 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	N.D. ¹
TKN (g L^{-1})	2.30 ± 0.08	1.92 ± 0.07	1.48 ± 0.06	1.18 ± 0.05	0.95 ± 0.05	0.54 ± 0.07	0.10 ± 0.04
Total Alkalinity ($\text{g CaCO}_3 \text{ L}^{-1}$)	3.3 ± 0.3	2.7 ± 0.2	2.0 ± 0.2	1.7 ± 0.2	1.3 ± 0.2	0.7 ± 0.1	0.2 ± 0.1
C:N ratio	14	17	23	27	33	59	210

¹N.D. = not detectable

Table 2. Steady-state performance data of hydrolysis/acidification reactor.

	CP:PM ratios					
	0:100	20:80	40:60	50:50	60:40	80:20
VS (%)	4.5 ± 0.2	4.2 ± 0.2	3.9 ± 0.1	3.7 ± 0.2	4.2 ± 0.1	4.8 ± 0.3
Total COD (%)	7.7 ± 0.6	6.9 ± 0.7	6.2 ± 0.4	5.9 ± 0.6	5.9 ± 0.5	5.8 ± 0.8
Soluble COD (%)	1.68 ± 0.13	1.85 ± 0.16	2.18 ± 0.05	2.42 ± 0.08	1.89 ± 0.04	1.36 ± 0.33
Total VFA (g COD L^{-1})	11.25 ± 1.01	14.79 ± 1.58	16.30 ± 2.15	18.07 ± 1.93	12.00 ± 2.09	6.90 ± 1.90
Ammonium-N (g L^{-1})	1.15 ± 0.19	0.78 ± 0.06	0.37 ± 0.09	0.26 ± 0.03	0.20 ± 0.02	0.08 ± 0.05
COD-T reduction (%)	11 ± 5	14 ± 3	17 ± 3	19 ± 5	15 ± 4	9 ± 4
Volumetric biogas productivity ($\text{mL L}^{-1} \text{ d}^{-1}$)	540 ± 120	840 ± 260	840 ± 200	940 ± 260	240 ± 200	160 ± 100
CH_4 productivity ($\text{mL L}^{-1} \text{ d}^{-1}$)	220 ± 40	260 ± 120	160 ± 60	120 ± 40	40 ± 10	0 ± 0
CH_4 content (%)	39 ± 1	30 ± 5	19 ± 5	12 ± 2	4 ± 3	0 ± 0
CO_2 content (%)	52 ± 1	60 ± 4	76 ± 4	78 ± 4	71 ± 5	61 ± 7

When we used a high CP ratio in the feedstock, the lower ammonium-nitrogen (with a consequent decrease in the buffering capacity) and the rapid production of organic acids led to the reduction of the pH. The pH was 6.3 when the reactor was fed with PM alone, and it decreased sequentially to 3.9 as the CP ratio in feedstock was sequentially increased (Fig. 2). When we used a CP ratio of 60-80% in the feedstock, the reduction in solids decreased at ~pH 4, even though the feedstock contained higher levels of easily-degradable organic matter. The possible inhibitory effects of pH, VFA and non-ionized VFA at the hydrolysis/acidification stage are controversial [5]. Previous studies have demonstrated that the pH affects the hydrolysis/acidification phase significantly. There are conflicting results regarding the optimum pH for the hydrolysis/acidification phase. The optimum pH for hydrolysis lies in the range from 5 to 6 [18-21]. Contrarily, acidic pH had a significantly inhibitory effect on the hydrolysis of particulate organic matter. The rate constants for the hydrolysis of carbohydrate and protein in the fermentation of kitchen waste were very low at pHs 5.5 and 5, and these

increased at pHs 6 and 7 [22]. It is generally considered that the inhibitory effect of acids increase as the pH decreases, as non-ionized acid is strongly toxic. At a pH of 4.5, the majority of the VFA will be in their toxic, unionized forms. In this study, the maximum destruction efficiency of solids was obtained, even though the pH in the reactor was around 4.5. It has been reported that non-ionized acetate does not noticeably inhibit the hydrolysis of carbohydrate. However, an acidic pH (5 and 6) greatly suppressed the hydrolysis of carbohydrate and proteins in potatoes [23].

The total concentration of VFA in the hydrolysis/acidification reactor, when fed with PM alone, was 11.25 g COD L⁻¹. The total VFA concentration increased by 61%, to 18.07 g COD L⁻¹, as the CP ratio in the feedstock was increased to 50% (Table 2). At a CP:PM ratio of 50:50, the VFA produced was composed of 10.21 g COD L⁻¹ acetic acid, 3.25 g COD L⁻¹ propionic acid, 1.94 g COD L⁻¹ n-butyric acid, 1.54 g COD L⁻¹ n-valeric acid, 1.55 g COD L⁻¹ iso-valeric acid and 0.9 g COD L⁻¹ ethanol (Fig. 3). The results show that the highest concentrations of the total VFA and the individual VFAs did not depress the

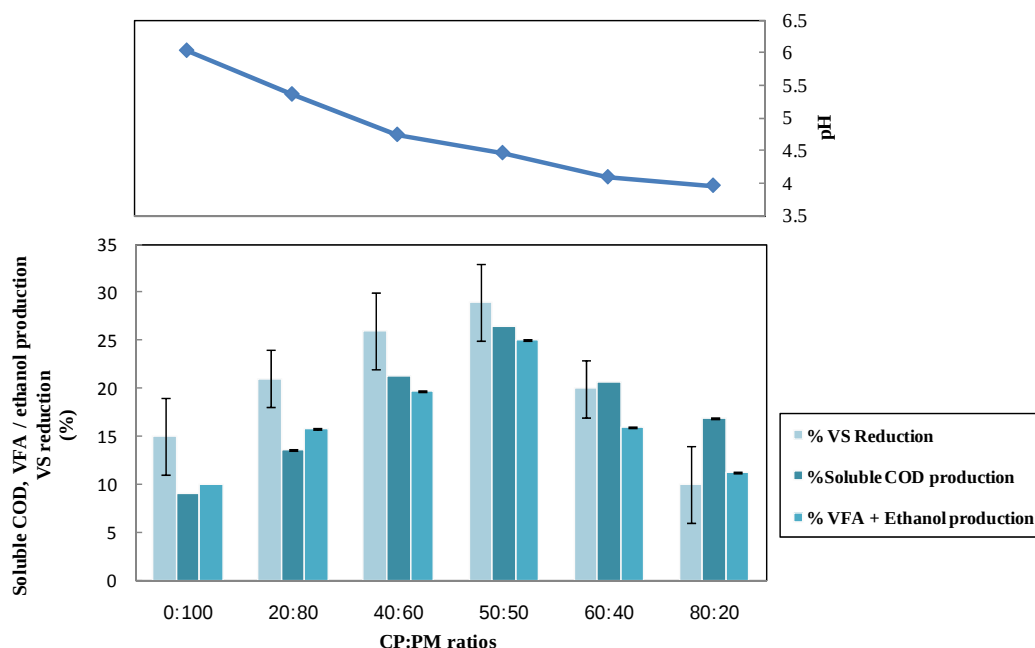


Figure 2. Average pH value, Vs reduction and the production of soluble COD and volatile fatty acids/ethanol in hydrolysis/acidification reactor fed with various CP:PM ratios.

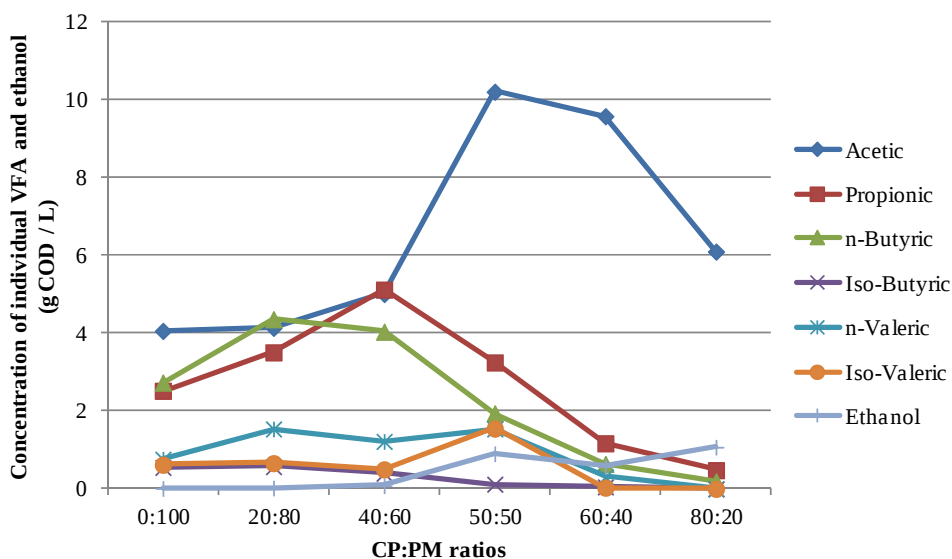


Figure 3. Concentration of volatile fatty acids and ethanol in the effluent of hydrolysis/acidification reactor fed with various CP:PM ratios. (HVA: n-valeric, i-HVA: iso-valeric, HBU: n-butyric, i-HBU: iso-butyric, HPr: propionic, HAC: acetic and ETOH: ethanol).

hydrolysis of solid organic matter throughout the experimental run when the feedstock CP:PM ratio was 50:50. At CP levels up to 40%, acetic acid, propionic acid and n-butyric acid were the major VFAs produced, and small amounts of n-valeric acid and iso-valeric acid were also detected. At CP proportions of 50%-80%, acetic acid was the main VFA produced. The ethanol concentration increased, and the productions of propionate and butyrate decreased drastically by 91% and 95%, respectively, compared with those of 40% CP. The results indicate that the critical pH, at which the distribution of the individual VFAs was altered, was 4.5. It has been reported that the distribution of individual VFAs is pH-dependent [24-27]. Acetate was the dominant product of acid fermentation of the primary sludge, in both batch assays and in semi-continuous reactors [28]. However, it was observed that the acetate fraction increased with increasing pH, whereas the butyrate fraction decreased with increasing pH, during the acidogenesis of cattails using rumen cultures [29].

3.2 Methanogenic reactor performance and stability

The steady-state performance of the methanogenic reactor, fed with various ratios of CP and PM, is given in Table 3. The removal of organic matter and the production of methane increased with increasing CP ratios up to 60%. The maximum reduction efficiencies of VS (62%) and of total COD (65%) were achieved using feedstock containing 60% CP. Despite the inhibition of hydrolytic/acidogenic bacteria at this ratio, the VS portion was degraded continuously in the methanogenic reactor. Co-digestion removed solids more effectively (35%) than digestion of PM alone. Likewise, the yield and productivity of methane from co-digestion were 32% and 23% higher, respectively, than those obtained from the digestion of PM alone. The highest specific methane yield (505 mL g⁻¹ VS), obtained using a 50:50 CP:PM ratio, corresponded to the highest removal

of VS in the hydrolysis/acidification reactor that we obtained. However, the volumetric methane productivity (1,490 mL L⁻¹ d⁻¹) was maximal when a 60:40 CP:PM ratio was used. The methane content in the biogas varied slightly (in the range 62-66%) when the feedstock contained up to 60% CP, and it dropped to 54% when using 80% CP.

As shown in Table 4, a well-balanced digestion process was achieved using substrate mixtures containing up to 60% CP. The pH ranged between 7.18-7.45, and the VFA levels were low (200-240 mg COD L⁻¹). Since the VFA levels were very low, and the total alkalinity ranged between 2,000-5,000 mg CaCO₃ L⁻¹, the VFA/alkalinity ratios ranged from 0.04 to 0.12. When the reactor was fed with feedstock that contained 80:20 CP:PM, the digestion of this mixture was limited, as indicated by the lowest levels of solid removal and methane production, and by the instability of the process. In this case the VFA increased greatly to 6,210 mg COD L⁻¹, and the major VFAs produced were 2,800 mg COD L⁻¹ acetic acid, 1,940 mg COD L⁻¹ propionic acid and 1,140 mg COD L⁻¹ n-butyric acid (data not shown). An increase in the proportion of CP in the feedstock caused a decline in the level of ammoniacal nitrogen in the digestate, resulting in the decrease of the total and partial alkalinity. As the total alkalinity decreased to 1,800 mg CaCO₃ L⁻¹, the VFA/alkalinity ratio rose to approximately 1, and the pH dropped to less than 6 (indicating improper environmental conditions for methanogenesis). Previous authors have reported that 6,000 mg L⁻¹ of total VFA inhibited the production of biogas [30] and that 900 mg L⁻¹ of propionic acid inhibited mesophilic methanogens significantly [31].

The results indicate that the production of methane from CP requires high buffering capacity to neutralize the pH when VFA is produced rapidly. In co-digestion, the degradation of proteins in PM produces ammoniacal nitrogen, providing an important source of alkalinity. However, the stability of the reactor depends on the ratio of the wastes in the feedstock,

Table 3. Steady-state performance data of methanogenic reactor.

	CP:PM ratios					
	0:100	20:80	40:60	50:50	60:40	80:20
VS (%)	2.4 ± 0.1	2.3 ± 0.2	2.1 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	2.4 ± 0.3
COD-T (%)	3.7 ± 0.5	3.3 ± 0.4	2.7 ± 0.5	2.4 ± 0.4	2.2 ± 0.4	2.9 ± 0.4
COD-S (%)	0.27 ± 0.08	0.21 ± 0.07	0.16 ± 0.03	0.14 ± 0.03	0.15 ± 0.07	0.47 ± 0.29
pH	7.45 ± 0.11	7.40 ± 0.10	7.27 ± 0.08	7.22 ± 0.08	7.18 ± 0.07	6.78 ± 0.65
VFA and Ethanol (g COD L ⁻¹)	0.22 ± 0.05	0.21 ± 0.05	0.22 ± 0.03	0.20 ± 0.04	0.24 ± 0.02	2.94 ± 2.23
Ammonium N (g L ⁻¹)	1.00 ± 0.12	0.98 ± 0.20	0.65 ± 0.11	0.50 ± 0.05	0.37 ± 0.03	0.28 ± 0.06
Total Alkalinity (g CaCO ₃ L ⁻¹)	5.00 ± 0.30	4.44 ± 0.28	3.81 ± 0.15	3.42 ± 0.18	2.00 ± 0.25	1.81 ± 0.21
VFA / alkalinity ratio	0.04 ± 0.003	0.05 ± 0.003	0.06 ± 0.002	0.06 ± 0.001	0.12 ± 0.005	1.08 ± 0.775
VS reduction (%)	46 ± 3	45 ± 1	46 ± 2	52 ± 4	62 ± 3	49 ± 3
Total-COD reduction (%)	52 ± 3	52 ± 4	57 ± 3	60 ± 4	65 ± 3	50 ± 6
Volumetric CH ₄ production (mL L ⁻¹ d ⁻¹)	1210 ± 160	1200 ± 120	1310 ± 160	1430 ± 170	1490 ± 170	1100 ± 430
Specific CH ₄ yield (mL g ⁻¹ VS _{added})	352 ± 48	372 ± 40	436 ± 54	505 ± 60	463 ± 50	299 ± 130
CH ₄ content (%)	64 ± 2	64 ± 3	66 ± 1	66 ± 1	62 ± 2	54 ± 5
CO ₂ content (%)	32 ± 2	34 ± 3	31 ± 1	31 ± 1	34 ± 2	43 ± 5

Table 4 Comparison of the efficiencies of single-phase (SP) and two-phase (TP) systems.

CP:PM ratios	VS reduction (%)		COD reduction (%)		Specific CH ₄ yield (mL g ⁻¹ VS _{added})		Volumetric CH ₄ productivity (mL L ⁻¹ d ⁻¹)		CH ₄ in biogas (%)	
	SP	TP	SP	TP	SP	TP	SP	TP	SP	TP
0:100	46 ± 2	54 ± 3	50 ± 3	57 ± 3	217 ± 16	310 ± 40	740 ± 60	1080 ± 150	64 ± 1	64 ± 2
20:80	49 ± 2	56 ± 2	51 ± 2	59 ± 4	227 ± 10	308 ± 37	790 ± 30	1070 ± 120	63 ± 1	64 ± 3
40:60	54 ± 1	60 ± 2	52 ± 2	64 ± 3	266 ± 10	331 ± 40	930 ± 20	1160 ± 140	61 ± 1	66 ± 1
50:50	59 ± 1	66 ± 4	57 ± 1	67 ± 2	290 ± 16	361 ± 38	1010 ± 40	1260 ± 150	59 ± 1	66 ± 1
60:40	61 ± 1	68 ± 2	57 ± 1	68 ± 3	306 ± 13	370 ± 42	1060 ± 30	1290 ± 150	57 ± 0	62 ± 2
80:20	60 ± 4	54 ± 6	57 ± 4	55 ± 7	263 ± 50	273 ± 110	920 ± 180	950 ± 370	55 ± 1	54 ± 5

correlating to the C:N ratio. The methanogenic reactor can be highly stable when PM is co-digested at the optimum C:N ratio (C:N = 33). In this study, the enhancement of solid reduction and the efficiency of methane production by co-digestion were apparently correlated to an overall increase in the biodegradability of the co-substrate. This resulted from an increase in the readily digestible fraction, and an improved C:N ratio that could meet the requirements of microbial growth and supply sufficient buffering capacity for the process to be stable.

3.3 The overall performance efficiency of two-phase systems, as compared to conventional single-phase systems

Table 4 summarizes the performance efficiency of two-phase and single-phase CSTRs. Our study on the continuous co-digestion of CP and PM in a single-phase CSTR [32], operating at a constant OLR of $3.5 \text{ kg VS m}^{-3} \text{ d}^{-1}$ and a HRT of 15 days, demonstrated that co-digestion could improve the VS reduction by 33% and increase the methane yield by 41%, over the use of PM alone. The highest reduction of VS (61%) and methane yield ($306 \text{ mL g}^{-1} \text{ VS}_{\text{added}}$) were obtained using a 60:40 CP:PM ratio. Using 80% CP in the feedstock, the reactor performance deteriorated, as indicated by the decreased production of methane and rate of solid reduction, and by the imbalance in the stability of the system. In the two-phase system, the greatest removal of VS (68%) and the greatest methane yield ($370 \text{ mL g}^{-1} \text{ VS}_{\text{added}}$) were achieved using a CP:PM ratio of 60:40. The results indicate that two-phase CSTR performed better, in terms of methane production and solid reduction, than the single-phase CSTR, under conditions where the methanogenic reactions were not inhibited. The two-phase system could improve the effluent quality, as indicated by the higher efficiencies of solid destruction and total COD reduction than in the single-phase system. When the systems were fed with PM alone, the VS was 35% greater, and the total COD removal was 18% greater, using the two-phase CSTR rather than a single-phase CSTR. When using 60:40 CP:PM, the two-phase CSTR achieved 21% greater reduction of VS and 23% greater total COD removal.

To compare the efficiencies of methane production, we expressed the methane productivities in terms of the VS load ($\text{mL g}^{-1} \text{ VS}$) and the volume of active volume ($\text{mL L}^{-1} \text{ d}^{-1}$). Based on this study, we concluded that the two-phase CSTR produced energy more efficiently than the single-phase CSTR. At the optimum CP:PM ratio (60:40) for both systems, the methane productivity using a two-phase CSTR was approximately 20% higher than that using a single-phase CSTR. The two-phase CSTR yielded 43-46% higher methane productivity than the single-phase CSTR, when the reactor was fed with PM alone. In addition, the two-phase CSTR improved the methane content of the biogas. For example, the percentage of methane in the biogas increased from 59% using the single-phase CSTR to 66% when using the two-phase CSTR, when the system was fed using 50:50 CP:PM in the feedstock.

As both systems were operated using the same total retention time, the methanogenic reactor of the two-phase system was run using a shorter retention time than in the single-phase reactor. Even though the retention time decreased from 15 days to 13 days, the methanogenic reactor yielded better gas production and solid reduction efficiencies, and the process had good stability, when the reactor was fed with feedstock containing up to 60% CP. As mentioned above, acidogens and methanogens prefer broadly different environments for their growth. Therefore, the separation of the phases in the two-phase system enhanced the destruction of solids, resulting in a greater efficiency of biogas production. Shorter HRT in the methanogenic tank may be compensated by lower COD and VS concentrations in the influent, better characteristics- more VFA,

as well as lower organic loading rates when compared with the single-phase CSTR.

As the methanogenic reactor was run under a higher hydraulic loading than the single-phase reactor, it was more sensitive to the organic load. When the systems were fed with an 80% CP feedstock with a C:N ratio of 59, their performance deteriorated. The performance of both systems was similar, but the two-phase system was more inhibited, especially in terms of VS reduction and methane productivity. For the anaerobic co-digestion process, the two-phase system is generally suggested for treating highly biodegradable wastes, because this system allows for a higher OLR and is more resistant to shock loading [33]. The acidogenic phase of the two-phase system is also sensitive to the high organic loading of easily degradable matter, and it did not show significant advantages over the single-phase system for the anaerobic digestion of fruit and vegetable wastes [34].

Anaerobic co-digestion of CP with PM has been proven to be an efficient way to achieve a high performance and stable digester. Co-digestion of both waste types could be applied in north-eastern, eastern and western parts of Thailand, where many cassava starch factories and pig farms are found. However, in order to evaluate the benefits of co-digestion of waste from different sources, the cost of transportation of waste should be taken into account.

4. Conclusions

The anaerobic digestion of CP and PM mixtures, at a CP ratio up to 60% of the feedstock, in a two-phase CSTR was able to improve the methane yield and solids removal compared to the digestion of pig manure alone. This result was achieved through an increase in the amount of readily-degradable compounds and an improvement of the C:N ratio of the feedstock. Comparing the performance efficiency of the two-phase to the single-phase CSTR, the results reveal that phase separation in the two-phase system enhanced the rate of solids reduction, resulting in 20-46% greater methane productivity than when using the single-phase CSTR.

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