

## **CHAPTER 3**

### **ANAEROBIC FOOD WASTE DIGESTION**

#### **3.1 Introduction**

The fermentation of food waste is affected by various factors such as the biodegradability of the substrate, the degrading capacity of microorganisms and the environmental conditions. While much attention has been paid to the methane production phase, as it is the energy phase, the first phase of hydrolysis and acidogenesis have been considered as rate limiting steps in the process of anaerobic digestion of particulate organic substrates like food waste and needs to be improved to enable faster digestion of solid organics. The experimental procedure has been developed in two stages:

a) Selection of the suitable operating condition for anaerobic acidogenesis of food waste. Lab-scale batch experiments were carried out to improve the first step of hydrolysis and acidogenesis of food waste (from a university campus restaurant) at 10% TS using two different techniques, pH adjustment and dilution of leachate. The performance of the acidogenic digester is known to be a function of the pH and studies with varying pH (5, 6, and 7) were carried out to investigate its effect on overall hydrolysis-acidogenesis activities. Liquid phase parameters, i.e. pH, alkalinity, SCOD, and TVA (in terms of yield and rate) were investigated to define the optimum pH environment for the acidogenic-phase reactor treating food waste. The influence of the pH adjustment and the leachate dilution techniques on VFA production in an acid-phase digester was determined, together with their roles on inhibitory product control, mainly focusing on VFA and their undissociated form. It was hypothesized that undissociated acid (UA) was the major factor contributing for inhibition of acidogenesis. To increase the VFA production, the most efficient way to decrease the concentration of undissociated inhibitors had to be found.

b) Validation of operational parameters on acid phase treating 10%TS food waste treatment for elevated solid content of food waste at 15%TS (semi-dry) with the attention on maximizing precursor VFA supply for an effort to increase biogas production and waste degradation as well.

## 3.2 Methodology

### 3.2.1 Feedstock and Seed Sludge Preparation

Unsorted food waste was obtained from the cafeteria of King Mongkut's University of Technology Thonburi in Bangkok, Thailand. It was fed into the reactor after separating out non-biodegradable matter (e.g. rubber band, plastic, drinking straws, bone, and food picks). Food waste was manually mixed to promote the homogeneity and then diluted to 10% and 15% on a TS basis specified in each experiment by tap water. The pH, moisture content, VS/TS, and C/N ratio of the food waste were 3.96-4.59, 71.5-75.9%, 0.92-0.94, and 11.0-11.2, respectively, indicating the abundance of organic matter in the food waste. Feedstock food waste contained 54.7-61.0% total carbohydrate, 16.2-19.5% lipid, and 14.5-29.0% protein (on dry weight basis).

The sludge was taken from up flow anaerobic sludge blanke (UASB) tanks of an anaerobic wastewater treatment plant of a pig farm, located in Ratchaburi. The pH, TS, VS, and VS/TS of the seed sludge were 6.66-7.13, 30.9-33.1 g/l, 20.2-25.8 g/l, and 0.61-0.83, respectively.

**Table 3.1** Food waste characteristics

Parameters	Raw FW	FW-10%TS	FW-15%TS
Moisture content (% by ww)	73.4±1.8	88.0±1.4	85.8
Total solid (% by ww)	26.6±1.8	12.0±1.4	14.2
Volatile solid (% by dw)	91.9±2.3	93.2±0.9	91.9
Ash (% by dw)	8.1±2.3	6.8±0.9	8.1
Carbon content (% by dw)	51.1±1.3	52.2±1.3	51.0
Hydrogen content (% by dw)	5.5±1.5	6.2±0.1	6.1
pH	4.28±0.35	4.25±0.20	3.75

### 3.2.2 Experimental Set-Up and Operation

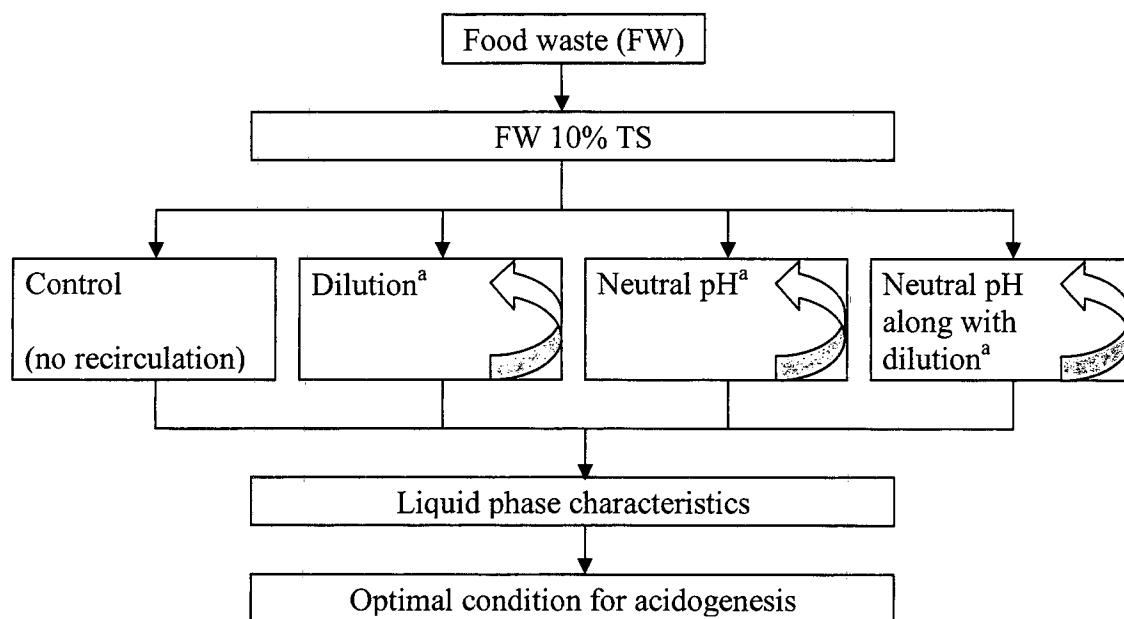
#### 3.2.2.1 Study of the pH Effects on Acidogenesis in AD

Batch experiments were programmed to select the optimum pH for anaerobic acidogenesis of food waste. The 2-l volumetric flasks as acidogenic digesters, with 2/3 of the flask filled with food waste and anaerobic seed (70/30, v/v), were frequently adjusted to pH sets 5, 6, and 7 with sodium bicarbonate (NaHCO<sub>3</sub>) as

buffer. To achieve the anaerobic condition, immediately before sealing the flasks with rubber stoppers they were all flushed with nitrogen gas (N<sub>2</sub>) for approximately 5 minutes. Liquid samples were collected from the flask at regular interval after manual shaking using a measuring pipette to analyze COD as total COD (TCOD), VFA, alkalinity, and pH.

### **3.2.2.2 Acidogenesis Improvement with Leachate Dilution and pH Adjustment**

The aim of this experimental part was to find out the suitable operating conditions for the anaerobic acidogenesis of food waste. Four lab-scale reactors were operated under different regimes: (a) leachate dilution, (b) neutral pH, and (c) neutral pH control along with a dilution application (see Figure 3.1, Table 3.2) on food waste degradation. The remaining reactor without pH control and leachate recirculation was set-up to obtain the baseline data (control). A closed acrylic pipe of 5-l (3.8-l working volume) with a 14-cm internal diameter and a 33-cm height was set as an experimental acidogenic digester (Figure 3.2). A 5-cm thickness of gravel, covered with nylon screens, was placed at the bottom part of the reactor to facilitate the filtering of the leachate. Mixed food waste (10%TS) and seed sludge (without any acclimation), having ratio of 70:30, v/v, were added up to 3/4 of the reactor's height and then flushed with N<sub>2</sub> gas before sealing. Three separate ports were used for different functions: pH control/leachate recirculation-dilution, biogas composition and biogas production were installed at the cover of each reactor. Liquid products were recirculated from the bottom through the upper part of the reactor in the upward direction to enhance the contact between the waste feedstock and the seed sludge. In the dilution reactor, leachate was withdrawn with 20-25% volume of liquid waste produced and replaced by an equivalent amount of distilled water. Acidogenic reactors with neutral pH control were conformed by 2 reactors. Effects of leachate dilution with neutral pH control were investigated after reaching the steady-state condition based on the measurement of the neutral pH.

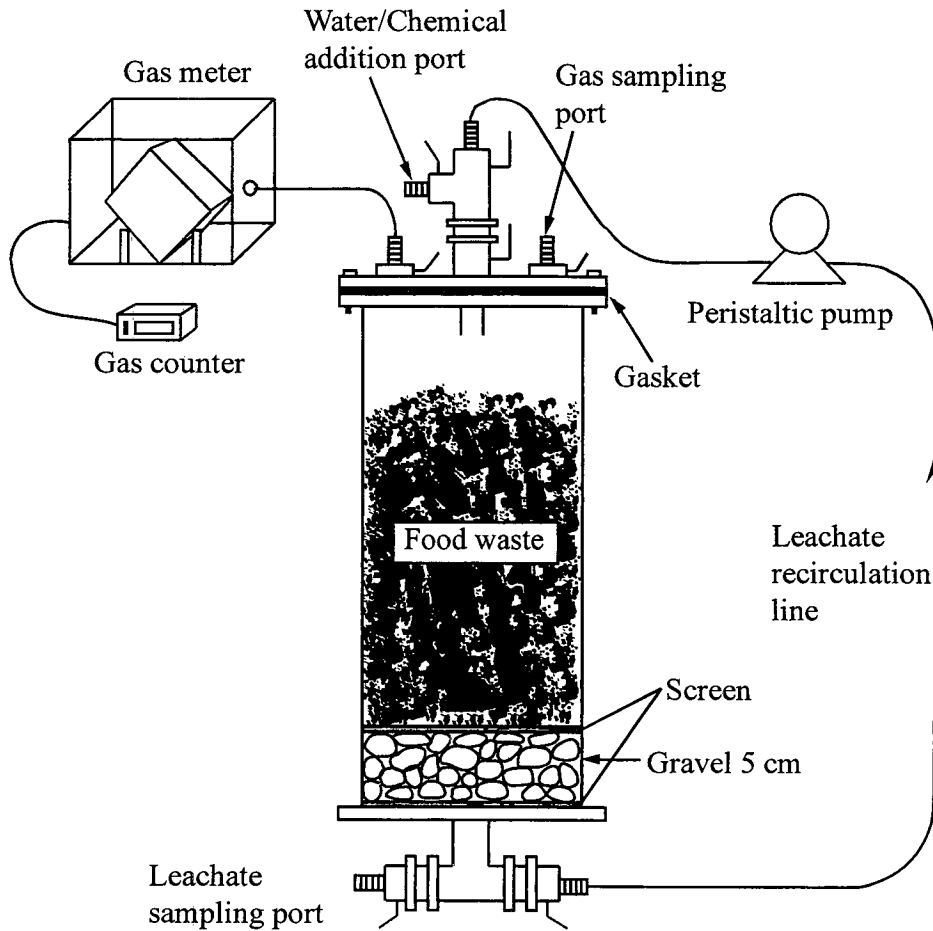


**Figure 3.1** Acidogenesis improvement study flowchart

<sup>a</sup> with leachated recirculation

**Table 3.2** Experimental procedure of acid-phase anaerobic digestion treating food waste

Operation period	Reactor operating conditions			
	Dilution	Neutral pH	Neutral pH along with dilution	Control
Period I		-Add buffer (d4-d13)	-Add buffer (d3-d13)	-Without leachate recirculation and pH adjustment
Period II	-Leachate dilution 3 times (d14, d23, d33)		-Leachate dilution 3 times (d14, d23, d33)	
	Routine leachate recirculation			



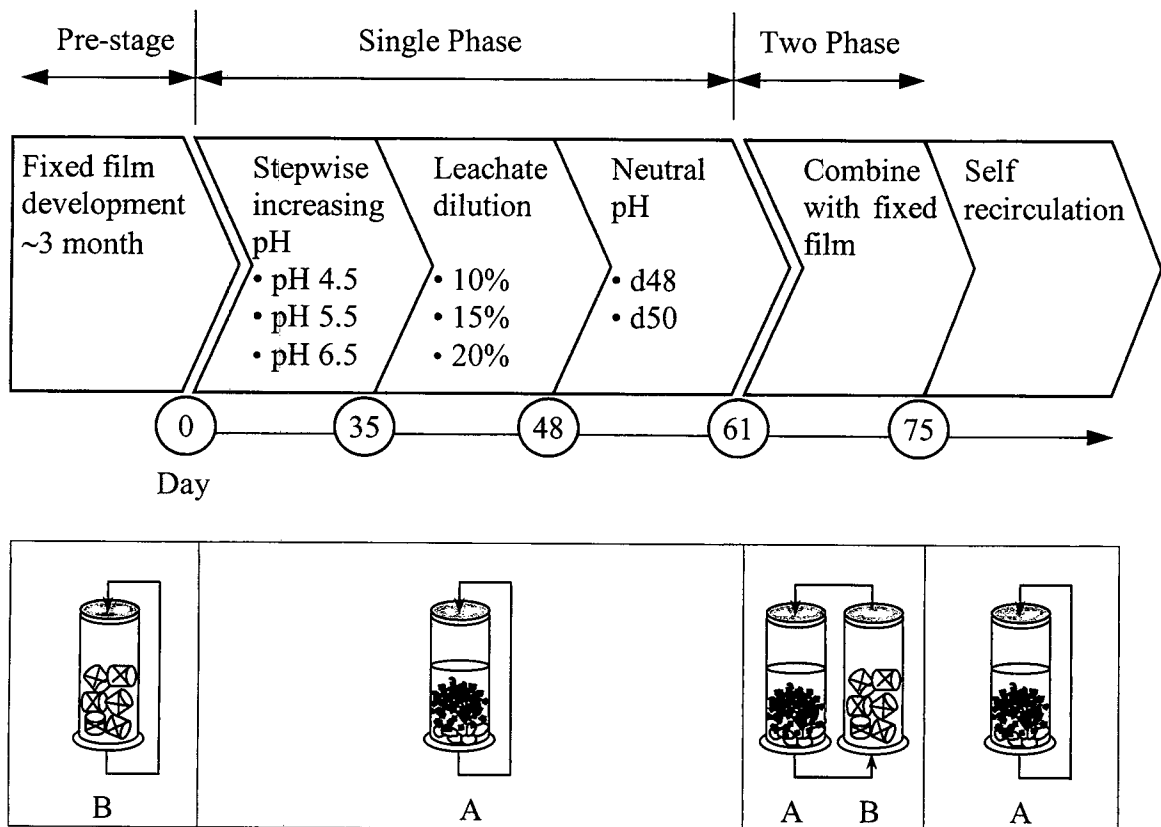
**Figure 3.2** Schematic diagram of experimental laboratory reactor set-up

### 3.2.2.3 Products (VFA and Undissociated VFA) Inhibition Control and Phase Separation for High Solid Food Waste Treatment

Food waste with a TS content of 15% was selected as a model feedstock for high solid food waste anaerobic digestion. Three consecutive operating conditions of stepwise increasing pH 4.5 to 6.5, leachate dilution application, and neutral pH adjustment were designed to determine their influences on anaerobic high-solid food waste digestion. The 15% TS food waste and seed sludge with 70:30 (v/v) were added into a 5 l closed acrylic pipe reactor. Seed sludge was acclimated for 3 months with ground fruit and vegetable on polypropylene (PP) packing media with characteristics of 40 mm diameter, 128 kg/m<sup>3</sup> density, 240 m<sup>2</sup>/m<sup>3</sup> surface area, and void fraction of 90%.


The pH, alkalinity, VS, and VS/TS of anaerobic seed were 7.07, 5.42 g CaCO<sub>3</sub>/l, 37.80 g/l, and 0.66, respectively. The methanogenic reactor was randomly

filled with packed media bed as mentioned above before 2.26 l of inoculum (1.6 l of acclimatized seed sludge and 0.66 l of supernatant) were added. The methanogenic effluent was circulated back to the acid digester through the port installed at the upper part of reactor sidewall. Leachate from the bottom of the acidogenic reactor was fed to the fixed film reactor with the initial OLR of 1 g COD/l d, in intervals of once per day, in the up flow mode. Subsequent OLR increased to 1.6, 2, and 3 g COD/l d by reducing HRT to accelerate the VFA conversion to biogas. The combination of acidogenic and methanogenic reactors ended when the undissociated acid concentration of the acidogenic reactor reached the threshold level for methanogenesis of 30-60 mg/l (Anderson *et al.*, 1982; Kroeker *et al.*, 1979).



**Figure 3.3** Representation of high solid food waste (15%TS) digestion: (A) acidogenic reactor; (B) methanogenic fixed film reactor

**Table 3.3** Packing media (Pallring 40×40 mm) specification

Surface area	240 m <sup>2</sup> /m <sup>3</sup>	
Void ratio	90 %	
Dimension	40 x 40 mm. (Dia. H.)	
Bulk density	128 kg/m <sup>3</sup>	

### 3.2.3 Analytical Method

Chemical properties of food waste were determined in accordance with AOAC and Standard Methods (APHA, 2005). The market waste feedstock in terms of the total lipids was determined using the Soxhlet method. The protein content was determined based on the total nitrogen content (Micro Kjeldahl method) with a conversion factor of 6.25 (Hattingh *et al.*, 1967). The carbohydrate content was analyzed using the phenol-sulfate method. Liquid samples were taken from the bottom of the digester and analyzed for pH, soluble chemical oxygen demand (SCOD), and total chemical oxygen demand (TCOD) daily in the initially experimental period and periodically measured thereafter. The pH values were measured with a Hach sension 378 pH meter. The total VFA and alkalinity were measured by a titration technique that accounts for approximately 70–80% of the total VFA concentration (DiLallo and Albertson, 1961). The SCOD was analyzed in the supernatant (centrifuge at 10000 rpm for 15 minute, in duplicate) by HACH DR/4000U, USA. The individual VFAs were analyzed using gas chromatography (Agilent 6890) equipped with a flame ionization detector (FID) and a capillary column type DB-FFAP (30 m × 0.25 mm, 0.25 μm). The flame ionizing detector temperature was 300 °C, and the oven temperature was programmed from 100 to 200 °C at 25 °C/min. Helium at 2 ml/min was used as the carrier gas. Liquid samples were first centrifuged at 10,000 revolutions per minute (rpm) for 10 minutes and then acidified to pH < 2.0 using 0.02 N oxalic acid to ensure that the VFAs were in their acidic forms. The supernatants of the acidified samples were filtered using a 0.45-μm membrane filter and analyzed for their C2-C5 volatile fatty acid content using a standard curve obtained by injecting a standard mixture of five VFAs of known concentrations. The daily gas production was measured using a water substitution gas collector connected to a counter (Omron H7EC, Thailand). Biogas samples were withdrawn from the reactor headspace using a

1-ml gas tight syringe (VICI, Precision Sampling Inc, USA) and subsequently injected into the gas chromatography. The gas composition (CO<sub>2</sub> and CH<sub>4</sub>) was analyzed using a gas chromatograph (Shimadzu 14B) equipped with a thermal conductivity detector (TCD) and Porapak Q column (2 m × 3 mm, 80/100 mesh). The injector, column, and detector were operated at 100, 70, and 120 °C, respectively. Helium was used as the carrier gas at a flow rate of 30 ml/min.

### 3.2.4 Calculations

The undissociated acid was determined from the equation:

$$[HA]=C_T[H^+]/K_A+[H^+] \quad [3.1]$$

where  $[HA]$  is the undissociated acid concentration (mg/l),  $C_T$  is the total acid concentration (mg/l),  $[H^+]$  is the hydrogen ion concentration (mole/l);  $K_A$  is the ionization constant of each acid (acetic acid  $1.75 \times 10^{-5}$ , propionic acid  $1.40 \times 10^{-5}$ , butyric acid  $1.48 \times 10^{-5}$ , and valeric acid  $1.60 \times 10^{-5}$ ).

The mass carbon distribution was calculated following the product form in each of the anaerobic biochemical degradation stages. The total feed carbon was calculated from the sum of carbon content in food waste and seed sludge added into the reactor in the first day. Daily released carbon biogas (CO<sub>2</sub>+CH<sub>4</sub>) was monitored. Measured biogas volume was corrected to standard condition (0 °C and 1 atmosphere) before the calculation of mass biogas was done by dividing with the molecular weights of CH<sub>4</sub> and CO<sub>2</sub> (16 and 44 g/mole, respectively) as follows (Rao and Singh, 2004):

$$G_m = V_d \times [16 \times CH_4/100 + (44 \times CO_2/100)]/22.413 \quad [3.2]$$

where  $G_m$  is the biogas mass (g);  $V_d$ , the dry biogas volume at STP (l);  $CH_4$ , the methane content (%vol);  $CO_2$ , the carbon dioxide content (%vol).

The carbon content in the feedstock feed and remaining in solid-liquid in the last day was estimated based on the VS contents by assuming 55 percent since the carbon content for most biological material is between 45-60% of the VS fractions; Carbon (%) = (%VS)/1.8 (Adams *et al.*, 1951). Soluble carbon in the leachate was

determined from COD using a theoretical conversion for COD/TOC of 2.66 (Ramsay and Pullammanappallil, 2005). With regard to the quality of biogas, it was expressed in terms of methane yield, which reflects the amount of gas produced in reference to the quantity of convertible material added; our principal reference is the content of VS.

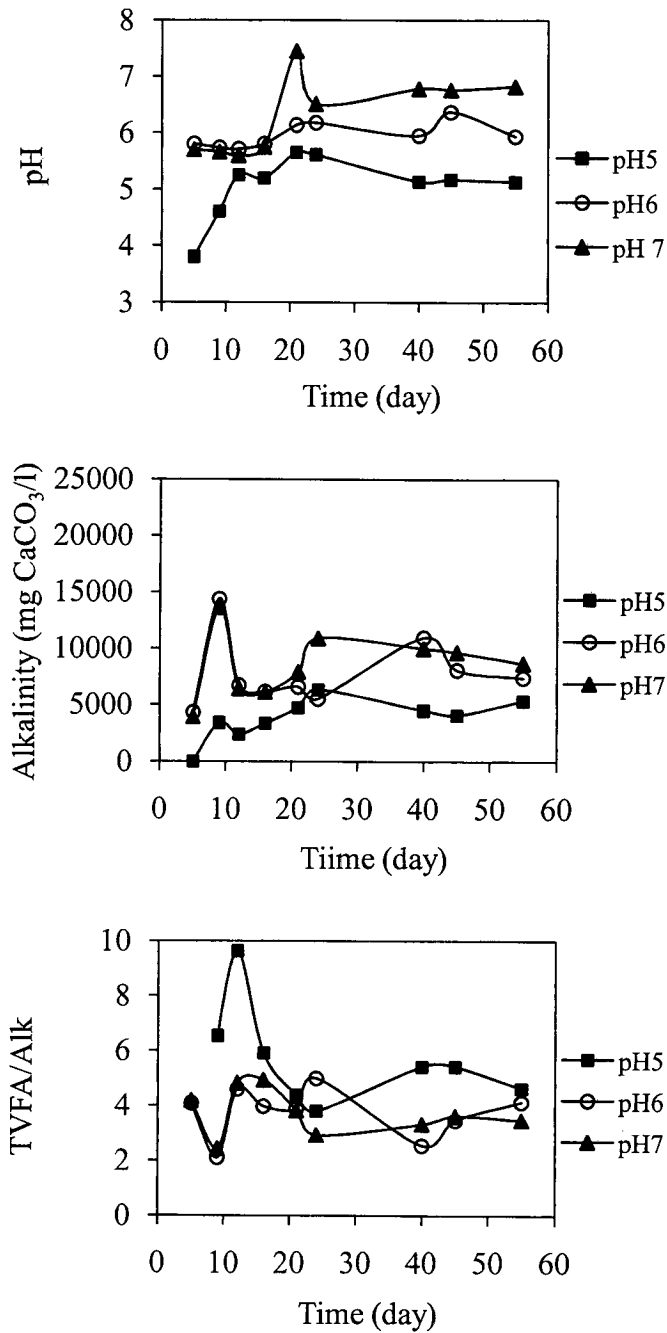
### **3.3 Result and Discussion**

#### **3.3.1 Effect of pH on Acid-Phase Digestion of Food Waste**

##### **3.3.1.1 Leachate pH and Alkalinity**

Figure 3.4 shows the pH and alkalinity variations in acid digesters at operating pH of 5, 6, and 7. An attempt was made to adjust the desired pH set points of 5, 6, and 7 by introducing 0.08, 0.13, and 0.24 g NaHCO<sub>3</sub>/g DM, respectively. However, the desirable pH adjustment of pH 7 could not be achieved due to unstable condition with excessive loss of alkalinity from rapid acid formation of food waste coupled with the generation of carbon dioxide. Thus, it should be noted that the results reported here understated the true values due to incomplete pH 7 adjustment (pH 5.60-5.75 or 5.68 on average). As a result, a close similarity in the plots of the operating pH 6 and pH 7 during the first 2 weeks was observed.

Alkalinity fluctuation was greatest in the initial operation period of the operating pH 7 reactor from an effort to raise the pH to the desired value under the sharp rise in VFA production. Operating pH 7 reactor showed the lowest TVA/Alk (alkalinity) ratio of 2.9-3.8 when compared to the other reactors, 3.8-9.6 (pH 5) and 2.1-5.0 (pH 6). The TVA/Alk of all reactors was out of the optimum range for the anaerobic process through the end of the experiment, finally leading to failure of the methanogenic stage. For the anaerobic treatment, the TVA/Alk ratio of 0.1-0.25 is considered favorable without the risk of acidification, whereas at a ratios of 0.3-0.4 (indicating digester upset) and 0.8 (inhibition of mathanogenesis) may result in digester failure (Khanal, 2008).



**Figure 3.4** Changes in pH, alkalinity, and TVA/Alk at operating pH of 5, 6, and 7

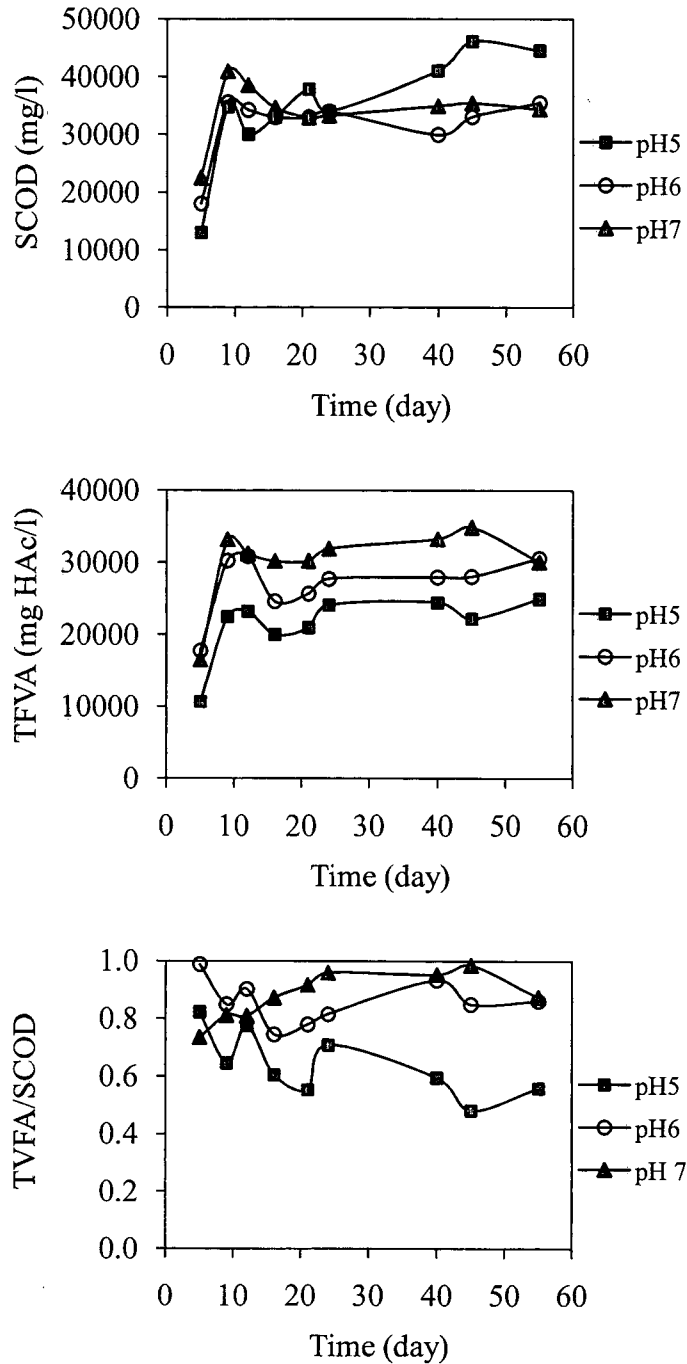
*Remark:* Incomplete pH 7 adjustment during day 0-16

### 3.3.1.2 COD Solubilization and VFA Production

The levels of hydrolyzed and acidified VFA products (yield and rate) increased in a stepwise manner with increased pH. The liquid SCOD portions of pH of 5, 6, and 7 at the beginning were 13, 18, and 22.5 g/l, respectively. It seems the hydrolytic phase occurred in the first two weeks and was characterized by a 9-day period to reach the maximal SCOD concentrations of pH 6 (35.6 g/l) and pH 7 (35.4 g/l). A typical food waste decomposition pattern with a fast start up phase beginning within the first 5-days from the start-up has been reported by Forster-Carneiro *et al.* (2008). This result was in line with a previous study (Jash and Ghosh, 1996). The authors found at higher concentration of soluble substrate amenable to acidification, lowering of pH is observed which slows down solubilization process.

The VFA production has followed a similar pattern as soluble COD. At pH 7, VFA production was the highest compared to the other pH tested. The highest VFA generated at pH 5, 6, and 7 were 24.5, 30.9, and 34.9 g HAc/l, respectively. The acid yield obtained at pH 7 (320.4 g HAc/kg DM), increased by 54% and 11% compared to pH 5 (207.6 g HAc/kg DM) and pH 6 (289 g HAc/kg DM), respectively. It should be noted that the yields and rates of operating pH 7 reported here are shown as apparent values due to a failure to maintain a consistent pH 7 and a relatively low frequency of liquid sampling (rate of VFA production calculated from the slope of data on day 5 and day 9).

After 9 days of operation, the VFA production at pH 5 and 6 almost ceased, while the VFA of pH 7 was slightly increased until day 45 of the operation. Food waste is carbohydrate-rich (Chu *et al.*, 2008). Around 75% (on dry weight basis) in this food waste was composed of carbohydrate. An abrupt increase in VFA level could be occurred at the initial digestion period from a relatively high hydrolysis rate constant of carbohydrate ( $K=0.5-2 \text{ d}^{-1}$ ), leading to a drop in pH and reactor instability if the buffer capacity is insufficient (Alvarez, 2003). The VFA concentration of pH 7 showed a consistent increase, up to 34.9 g HAc/l with a high level of alkalinity by the addition of  $\text{NaHCO}_3$ . This result showed the extent of which acidogenesis can be promoted at a controlled pH 7.



**Figure 3.5** Changes in SCOD, TVA, and TVA/SCOD at operating pH of 5, 6, and 7

*Remark:* Incomplete pH 7 adjustment during day 0-16

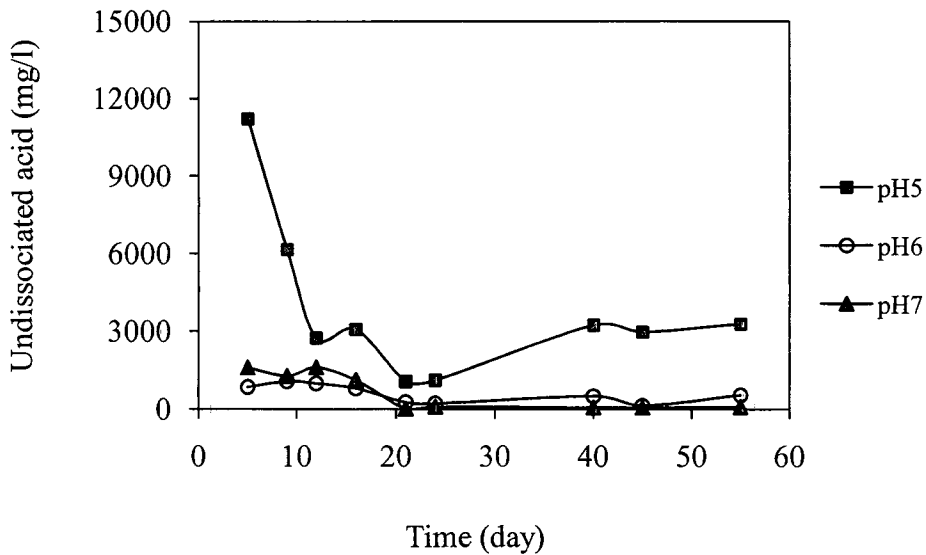
The positive benefit of buffer addition and neutral pH on acidogenic step also could be seen in the TVA/SCOD ratio, which represents the proportion of soluble compounds that were further converted to VFA. Operating pH 6 and 7 showed a relatively high TVA/SCOD (around 0.9, on average), while lowering the

TVA/SCOD ratio to 0.6 by operating pH 5 implied a high non-VFA form in soluble SCOD. This result matched with previous study of pH effects on hydrolytic and acidogenic step by Cysneiros *et al.* (2012). Low pH inhibited not only hydrolysis but also acidogenesis. They found 75-97% of the SCOD produced in the buffered reactor (pH ~ 6.5), while in the unbuffered one they only accounted for 31-75%.

Anaerobic digestion of food waste over a period of 55 days showed the same acid distribution patterns by acid type at all pH treatments. Butyric and acetic acid dominated with the 34-37% and 29-31% of all individual VFAs detected, followed by propionic, valeric, and iso-butyric acids, which amounted to 17-20, 10-12, and 3-5%, respectively.

### **3.3.1.3 Undissociated VFAs Concentration**

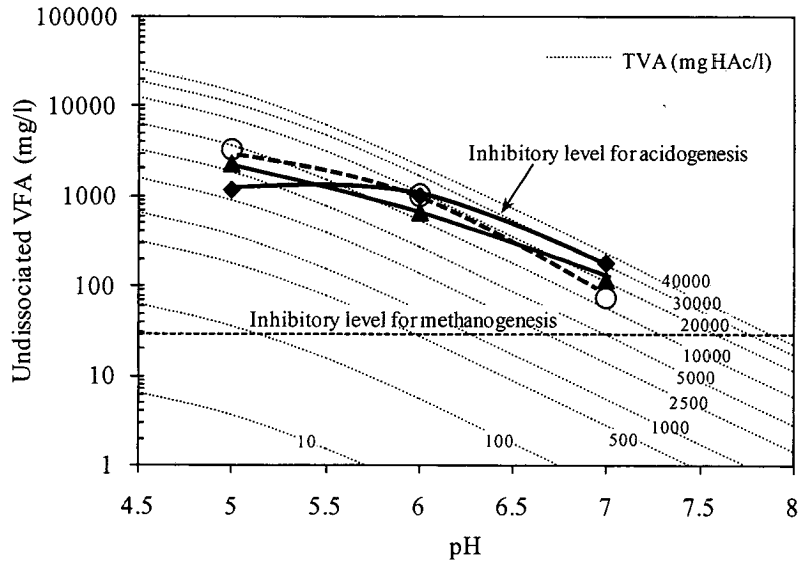
Operating at pH of 7 has showed better performance in the acid phase through its remarkable suppression of the undissociated form of VFAs, an inhibitory product for acidogenesis-methanogenesis, than at other pHs. Microbial inhibition is likely related to the concentration of membrane-permeable undissociated acid molecules, which lead to a decrease in the pH of the bacterial cell, and eventually become toxic to the bacteria (Russell and Diez-Gonzalez, 1998). A more than an 8-fold reduction was found in the average undissociated acid content of pH 7 (14-95 mg/l) compared with pH 6 (146-1,080 mg/l). Low TVA production at pH 5 was probably caused by a high concentration of undissociated acid throughout the end of the experiment, ranged 1,065-11,222 mg/l (Table A.1 to Table A.3).



**Figure 3.6** Undissociated VFAs content at different operating pH tested

*Remark:* Incomplete pH 7 adjustment during day 0-16

The maximum TVA formations at of pH of 5, 6, and 7 were 24.5, 30.9, and 34.9 g HAc/l, respectively. Corresponding undissociated VFAs, as calculated using equation 3.1, were responsible for the inhibition. From the result of this study, inhibition undissociated VFAs levels for acidogenesis of food waste were 3230, 994, and 73 mg/l at pH 5, 6, and 7, respectively. Inhibitory level for acidogenesis in terms of undissociated VFA from different type of food waste (mainly pineapple peel (Babel *et al.*, 2004), kitchen waste (Li *et al.*, 2010) and from this experiment) were compared in Figure 3.7. The inhibitory undissociated acid levels for acidogenesis for these three different substrates showed a similar curve pattern. The notable differences in inhibitory values (2300, 650, and 120 mg/l, Babel *et al.*, 2004, and 1185, 979, and 178 mg/l, Li *et al.*, 2010 at pH 5, 6, and 7) for these feedstocks may be probably related to the variations in the amount and type of substrate used. With respect to the heterogeneous component of kitchen waste and food waste, it is interesting to see the similarity of kitchen waste and food waste, particularly taking into account that the pineapple peel was more as a straight line.



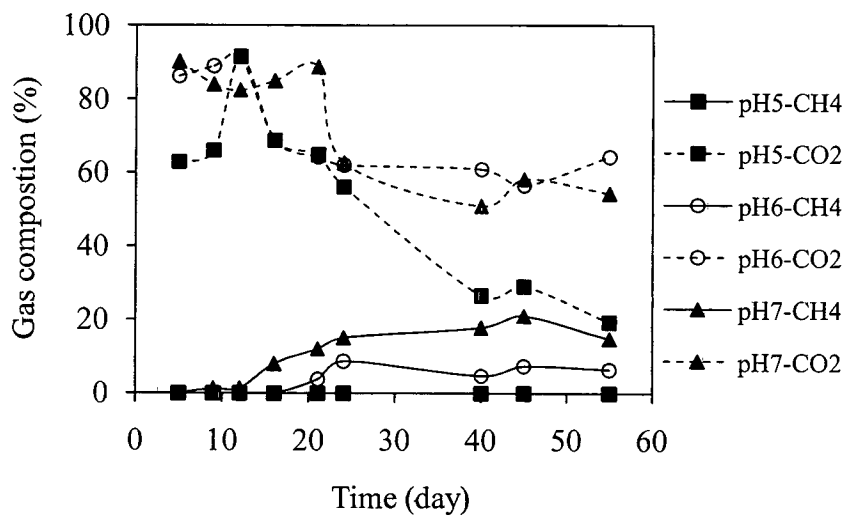
**Figure 3.7** Inhibitory undissociated VFAs level for acidogenesis of food waste: (▲) peel pineapple, Babel; *et al.*; (◆) kitchen waste, Dong Li, *et al.*; (○) food waste, this study. Dotted lines in the studies by Babel and Dong Li denote the VFA concentration (mg/l as acetic acid) and total VFAs (mg/l), respectively

#### 3.3.1.4 Biogas Composition

The gas composition results are presented in Figure 3.8. Carbon dioxide was the majority of gas composition in all pHs tested over the digestion period of 55 days. Methane composition slightly increased and reached the maximum value of 8.6% (pH 6) and 20.9% (pH 7) on day 24 and day 45, respectively. Increasing methane concentration in operating pH 7 indicated more proper condition for methanogenesis, which was developed by buffer addition into the system. High-level  $\text{NaHCO}_3$  supplementation favored methane production as the alkalinity was sufficient to buffer against pH drops and enough to prevent toxic levels of undissociated VFA fraction even during the VFA accumulation period (Florencio, 1995).

Many researchers have been reported on the toxic effects of undissociated form of VFA on methanogen. Anderson *et al.* (1982) recommended the suitable inhibitory level for methanogenesis of 30 mg/l. The corresponding inhibitory undissociated VFAs on methanogenesis reported by Kroeker (1979) ranged 30-60 mg/l. Thus, a 30-60 mg/l of undissociated acid was selected as the inhibitory level for methanogenesis guideline for this study. For the reactor operating at pH 7, the

undissociated VFAs level during day 21-55 (14 to 95 mg/l) was closed to the threshold level for methane production, and the methane content was higher than that found in other pHs tested. Unfavorable conditions of low-pH (pH 5 and 6) created undissociated VFA accumulation, thus inhibiting methanogens. When a methanogen was exposed to high undissociated acid for a prolonged period, it was slowly wiped out, consequently inducing reactor upset (Florencio *et al.*, 1995). The biogas production results were not shown in this experiment due to a technical problem with the equipment. Therefore, the performance in terms of biogas production could not be determined.



**Figure 3.8** Gas compositions at different operating pHs of 5, 6, and 7

*Remark:* Incomplete pH 7 adjustment during day 0-16

Table 3.4 shows the hydrolysis-acidogenesis performances by pH adjustment. It was clearly seen that pH played an important factor affecting the solubilization and acidification of food waste. The neutral pH adjustment can efficiently control the inhibitory undissociated fractions which further promote the acid production. The acidified products with a lower concentration of undissociated VFA were more favorable to the subsequent methanogenesis. The acid yield was 320 g HAc/kg DM (not including VFA conversion toward the gas phase), which was 1.1-1.5 times higher than the yield with lower pH (5 and 6). This result is similar to that

observed by Xu *et al.* (2011) in previous work; the two-stage anaerobic digestion of food waste could be significantly improved by the daily leachate recirculation practice in acidogenic reactors with pH control. The authors also observed that among the different condition tests, neutral pH control was identified as potential method for hydrolysis/acidification in terms of organic leaching. At pH 7, most of the protein was degraded into ammonia nitrogen ( $\text{NH}_4\text{-N}$ ), resulting in additional buffer of acidified liquid. However, the author recommended the amount of buffer introduced into the first stage reactor needs to be further optimized to reduce the stressing condition of the hydrolysis-acidification and methane yield. Miron *et al.* (2000) also reported that protein hydrolysis was rate limiting under acidic conditions. Therefore, controlling the system pH also effectively hydrolyzed the protein content. Regarding high VFA productions (rate and yield) and efficiency in reduction of undissociated acid concentration, the pH of 7 was chosen as the appropriate pH value for acidogenesis of food waste for further studies. It is in good agreement with the study of Li *et al.* (2010) who investigated the effects of pH adjustment on fermentative product distribution of kitchen waste. They found an acidogenesis pathway that was VFAs-type without alcohol production when the operating pH was 6.0-7.0, and also reported that the optimal pH for VFAs production was 7.0.

**Table 3.4** Performances of acid phase food waste at various pH values

Parameter	Unit	Operating pH		
		5	6	7 <sup>c</sup>
Hydrolysis yield <sup>a</sup>	g SCOD/kg DM	321.2	340.4	394.9
Acid yield <sup>a</sup>	g TVA/kg DM	207.6	289.0	320.4
Acid generation rate <sup>b</sup>	g HAc/l d	2.95	3.12	4.16
Undissociated acid	mg/l	1,065-11,222	146-1,080	14-95

<sup>a</sup> maximum value

<sup>b</sup> the slope of VFA was calculated by using only two concentration values (day 5 and 9)

<sup>c</sup> incomplete pH 7 adjustment during days 0-16

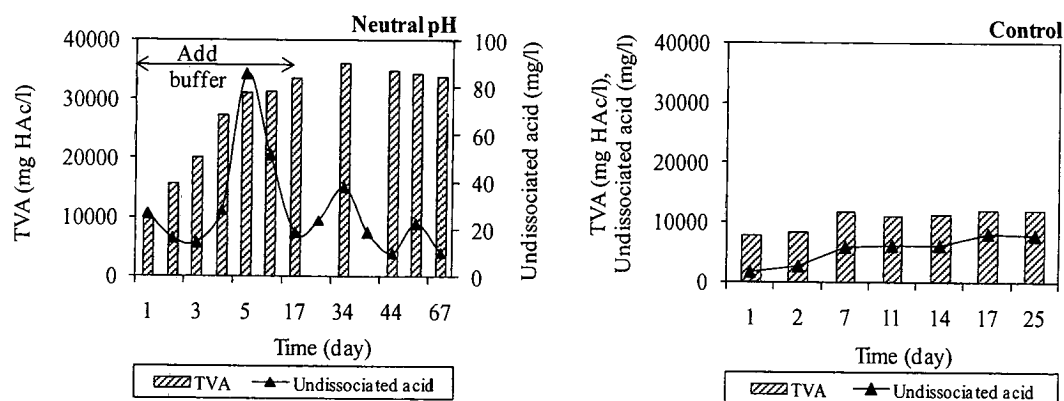
### 3.3.2 The pH Adjustment and Dilution Techniques for Acid Phase AD

Improving anaerobic acidogenesis of food waste was evaluated by two different techniques selected (neutral pH control and leachate dilution) during a period of 67 days. The experiment was conformed by four laboratory scale reactors; namely, control 'FC', dilution 'F1', neutral pH 'F2', and neutral pH along with dilution 'F3', at single-phase condition under batch operation.

#### 3.3.2.1 Hydrolyzed-Acidified Products

##### 1) Application of Neutral pH Adjustment

The pH adjustment effects on acidogenic phase performance were mainly focused on the first week of the operation due to the methanogenesis onset with a consistent increase in methane concentration from 10.8% to 60.2% for the first 6 days of the operation. In the first week, neutral pH conditions took 5 days for acid production to reach above 30 g HAc/l, a 2.8-fold on average higher than in the unbuffered reactor, with the maximum acid generation rate of 5.6 g HAc/l d (Table A.7). The TVA concentration under neutral pH condition increased until reaching a peak of 36.8 g HAc/l on day 31. Similar result attaining by Zhang *et al.* (2005) reported the rates of solubilization and the VFA production with controlled pH at 7 increased to a peak on day 4. The relative high stable of SCOD and TVA concentration with the increasing methane content, signifying that the high VFA production. Undissociated acid concentration dropped closed to the inhibition level for acidogenesis (73 mg/l) previously obtained (section 3.3.1.3) after start-up (Figure 3.9). High levels of  $\text{NaHCO}_3$  supplementation improved the acid production since the alkalinity was strong enough to prevent toxic levels of undissociated acid, which inhibited the acidogenesis (Florencio *et al.*, 1995).

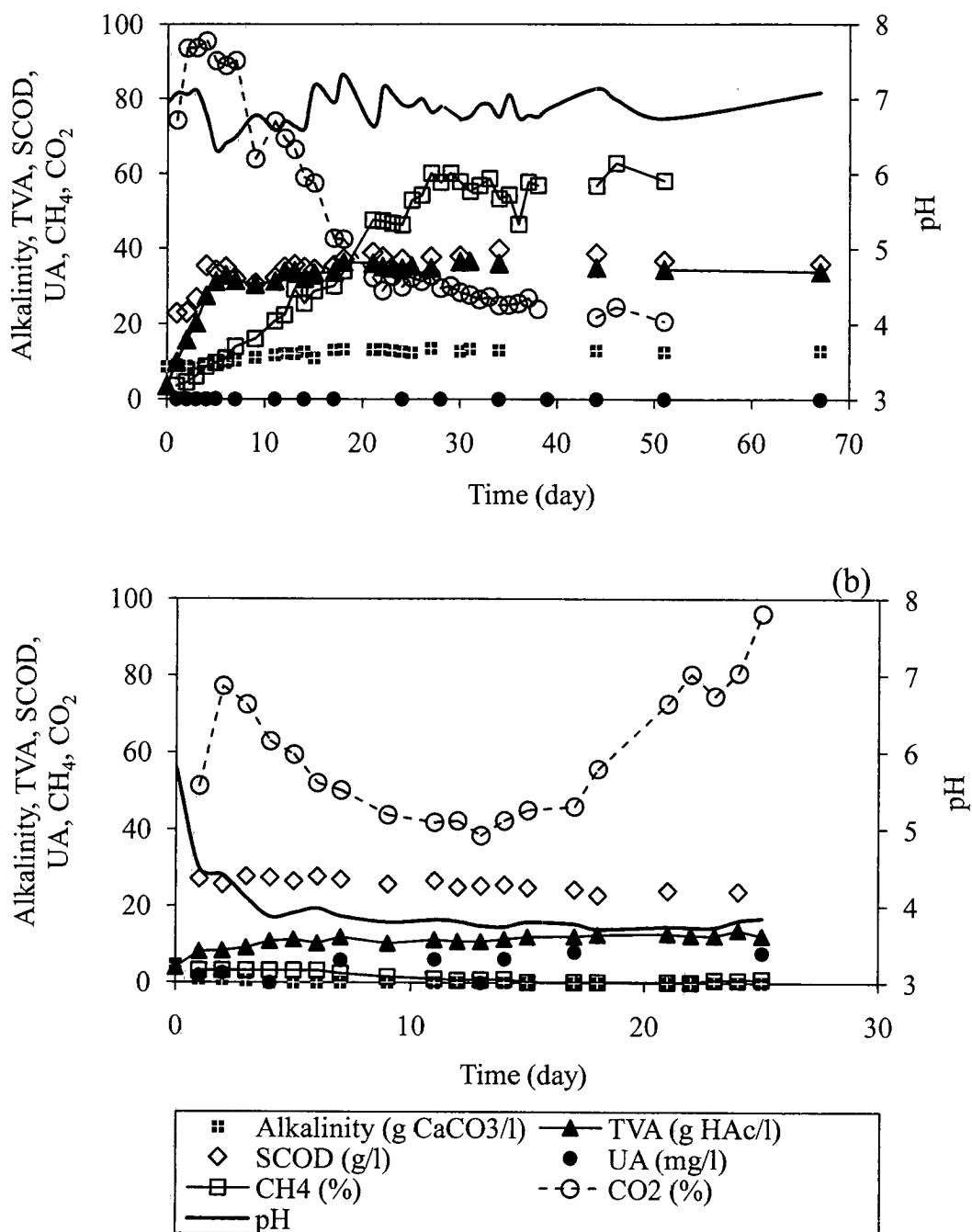


**Figure 3.9** Change in TVA and undissociated VFAs with and without pH adjustment

The hydrolysis degree is expressed in terms of soluble COD (SCOD) to total COD (TCOD) ratio (SCOD/TCOD, %) (Park *et al.*, 2009). TCOD indicates the total organic material contained in solids and liquids and SCOD indicates the organic material in the liquid form. An increase in the ratio of SCOD to initial TCOD (SCOD/TCOD<sub>0</sub>) displays released organic matters from solid state to liquid state. The SCOD/TCOD<sub>0</sub> at neutral pH condition almost reached 75% within 4 days and thereafter increased slightly to 76% on average (85% was the maximum). Similarly, Cysneiros *et al.* (2012) reported the solubilisation of substrate improved by stabilization of pH around 6.5 even when VFAs accumulated in control reactor. The poor performance of uncontrolled pH reactor (FC) with the low SCOD/TCOD<sub>0</sub> of 43% (on average) implied their most COD remained in the insoluble form, whereas obtaining organic matter of mostly VFA-soluble form in liquid fraction was presented by neutral pH reactor. Lehtomäki *et al.* (2008) also reported COD solubilization values of 80% for grass-silage in one stage with pH adjusted continuously to 7 (without leachate replacement). The study on effects of pH and bulking agents on hydrolysis/acidogenesis of food waste by Xu *et al.* (2011) proved that the daily leachate recirculation with regulated pH control (6.0) accelerated the hydrolysis rate (59% higher VFA) and methane production (more than 88%) compared to that of control without pH adjustment.

The performance of the control reactor is presented in Figure 3.10. During start-up a rapid drop in the pH in the uncontrolled pH reactor was observed. This indicated that readily biodegradable matter like carbohydrates was converted to VFA, resulting in a pH drop due to the insufficient alkalinity. Due to the fast VFA production, the control reactor was upset with low pH and high carbon dioxide content. After the startup day, the pH in all unbuffered reactors including dilution and control reactors (F1 and FC) drastically dropped from the initial pH of 5.80-5.82 to below 5 and it subsequently decreased further to a pH value below 4 on day 4-6. Thereafter, pH remained between 3.70-3.97 in the control reactor due to accumulated VFA; thus, the alkalinity was zero under these conditions. The VFA production curve indicates an inhibition of acidogenesis early in the batch runs in the unbuffered reactors. Failure in the control reactor with a rapid decline in pH levels, from 5.8 to less than 4 after day 3, and a high TVA/Alk ratio, rising above 18 on day 4, implied a supplemental buffer requirement. Previously, 33% higher COD leaching

potential from food waste under controlled pH conditions compared to that of control reactor was reported by Stabnikova *et al.* (2008). Although acidogens' growth have a broadened optimum pH range (between 5.2 and 6.5), VFA production is reduced if pH is not controlled and drops to 4.0 (Solera *et al.*, 2002). In fact, food waste has quite low pH buffering capacity as indicated by alkalinity level of  $\sim 56$  mg  $\text{CaCO}_3/\text{l}$ . Although, theoretically, protein degradation releases ammonia-N by each mole of organic nitrogen generates one equivalent of alkalinity, various carbohydrate-rich wastes could not contribute alkalinity sufficiently due to a lack of organic nitrogen (Khanal, 2008). A similar result was reported by Zhu *et al.* (2008) who studied the influence of pH buffering on hydrogen production and cited that the food waste had a low pH buffering capacity indicated by a low total acidity and alkalinity (340 and 40 mg/l as  $\text{CaCO}_3$ ). The poor performance of the unbuffered reactors on hydrolysis could be explained by inhibition of some hydrolytic microorganisms due to low pH (Cysneiros *et al.*, 2012). This is clear as the pH in the reactors was in the range considered inhibitory for cellulolytic microorganisms, which are usually inactive below pH 6.0 (Russell and Wilson, 1996), while strains of amylolytic bacteria have been shown to be inhibited at pH values below 5.4 (Therion *et al.*, 1982). Veeken *et al.* (2000) also confirmed that the concentrations of hydrolysates mainly depended on the system pH. Another reason may have been the low pH of the control reactor which shifted the VFA towards the most toxic form which is undissociated acid. In our case undissociated acid was in the range 1.75-7.98 g/l.



**Figure 3.10** Overall performances of (a) neutral pH reactor and (b) control reactor

## 2) Application of the 20-25% Leachate Dilution

During the first 14-days of the operation (before applying the dilution), the acid production ceased after the fourth day of operation in the presence of increasing undissociated VFA concentrations, from 1925 to 6868 mg/l (Table A.6). The digester pH dropped down from 5.82 (start-up) to 3.91 (day 6) due to ongoing

depletion of alkalinity with a high TVA/Alk ratio around 20. After 5 days of operation, the pH moved below 4, and the SCOD and VFA productions seemed to be inhibited indicated by the stable concentrations. Typically, food and garden waste have with high contents of readily biodegradable matters. Acidogenesis in these substrates can readily create inhibitory concentrations of VFA, which inhibit further breakdown. The acidification can be very rapid, as food waste is rich in small molecules that can be directly utilized by acidogen without extracellular hydrolysis (Martin, 1999). A similar observation was reported during AD of ligno-cellulosic feedstocks (Jagadabhi *et al.*, 2010). The rapid VFA production and fluctuating pH values found during the initial 4 days of operation in dilution reactor was attributed to the acidifying nature of the feedstock and/or buffering capacity of the system.

To investigate the product inhibition effect, the production of acids at a lower TVA concentration was monitored by dilution of the leachate in the acidogenic reactor. The dilutions were performed 3 times: 1<sup>st</sup> dilution on day 14, 2<sup>nd</sup> dilution on day 23, and the 3<sup>rd</sup> dilution on day 33. The first dilution was performed at 25% (v/v); about 0.9 l of liquid in digester was withdrawn and substituted with the same amount of distilled water. The results of leachate dilution on VFA production are shown in Table 3.5. The rate of TVA generated by the first dilution (1.71 g HAc/l d) was almost 2 times higher than that obtained before applying the leachate dilution (0.94 g HAc/l d). After 1 h of operation, distilled water was recirculated in place of the acidogenic leachate, and increase in the leachate strength was noticed, indicating the possibility of further extraction of the organic fraction of food wastes. The first dilution gave an increase of 53% in the total amount of TVA, from 26.6 g HAc (before dilution) to 40.7 gHAc. As low VFA concentrations had tendency to produce more acid than a high VFA concentrations, the second dilution was then made with the same 25% dilution degree. This led to a decrease in the rate of TVA production, 0.87 g HAc/l d, whereas the TVA production showed a slightly increase (62%), from 26.6 g HAc (before dilution) to 42.9 g HAc. The third dilution was conducted on day 33. The leachate TVA was diluted from 8.96 to 7.19 g HAc/l, that is, a 20% dilution. Given that TVA production then increased by 73%, but with a TVA generation rate of 0.45 g HAc/l d, which was lower than that of before dilution, the leachate dilution was ended. The application of the leachate dilution had a positive effect on reactor performance, as solubilization and acidification of substrate performed better on acidification than

before dilution. These results are in agreement with Bhattacharya *et al.* (2008) showing that leachate replacement with water may have diluted the inhibitory products of hydrolysis/acidogenesis and thus enabled further hydrolysis. A similar result was obtained by Hao *et al.* (2008). Leachate replacement with tap water showed COD solubilization and methane production improvement through dilution of the hydrolysis/acidogenesis products in one-stage. In simulated landfill experiment, Sanphoti *et al.* (2006) also reported the supplemental water addition and recirculation in the early acid phase of the reactor (day 203) helped to dilute inhibitory substances and negated the need for buffer addition to overcome acid phase and resulted in higher COD removal (85%) than without supplemental water addition and recirculation (81%). It is similar to that observed by Jagadabhi *et al.* (2008) in previous work with the leachate replacement with tap water. They showed that the solubilization effect, amount of SCOD leached from the substrate, of leachate replacement without pH adjustment resulted in the highest COD solubilization of 0.51 SCOD/g VS<sub>added</sub> compared with control and leachate replacement with initial pH adjustment. The leachate replacement can dilute inhibitory products and lead to improve COD solubilization (Hao *et al.*, 2008).

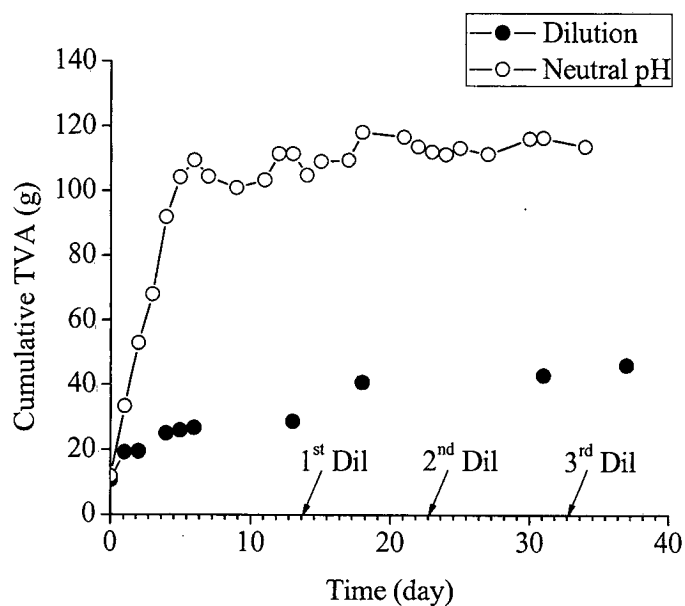
Reduction of undissociated acid was large by the first dilution, from 6868 (day 14) to 2622 mg/l, which corresponds to a 62% reduction (Table 3.5), but the subsequent dilution showed the decline in the efficient control of undissociated VFA. The introduction of leachate dilution led to a noticeable drop in undissociated VFA levels on the following day, but a few days later the levels increased. Undissociated acid was reduced by around 75% on average of the undissociated acid present, resulting in an increase of the TVA concentration (73%) and the rate of production (1.8 fold) compared to before the dilution. Although the proportion of undissociated VFA and total VFA (22%) were lower than that obtained before the dilution (76%), large fractions of the undissociated form still remained. Digesters failed at the start-up of control reactor as a result of the build-up of VFA and reduction in pH. Using leachate replacement with fresh water as dilution water proved to be an effective means of restoring stable operating conditions for hydrolysis/acidogenesis, as illustrated by the continuing raise of TVA production from 27 to 46 gHAc. Thus, the leachate dilution should be used as a supportive technique for acid formation improvement since the undissociated acid efficient control was quite lower than with

the pH adjustment (neutral pH). Another point should to be considered is that, the water requirement by the dilution technique resulted in more effluent from the AD system that needs further treatment, causing a higher operation cost.

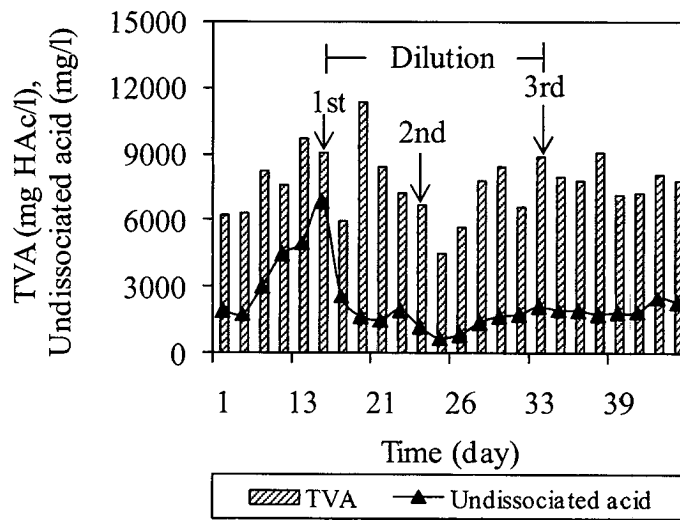
**Table 3.5** Concentration of VFA and undissociated acid by the leachate dilution

No	Day	Mass TVA (g HAc)		Undissociated acid (mg/l)		Rate of TVA generation (g HAc/l d)
		Before dilution	After dilution (%Inc)	Before dilution	After dilution <sup>a</sup>	
1	14	26.56	40.69 (53.2%)	6868.3	2,621.9	1.71
2	23		42.94 (61.7%)	1,224.5	701.8	0.87
3	33		46.04 (73.4%)	2,135.0	1,988.8	0.45

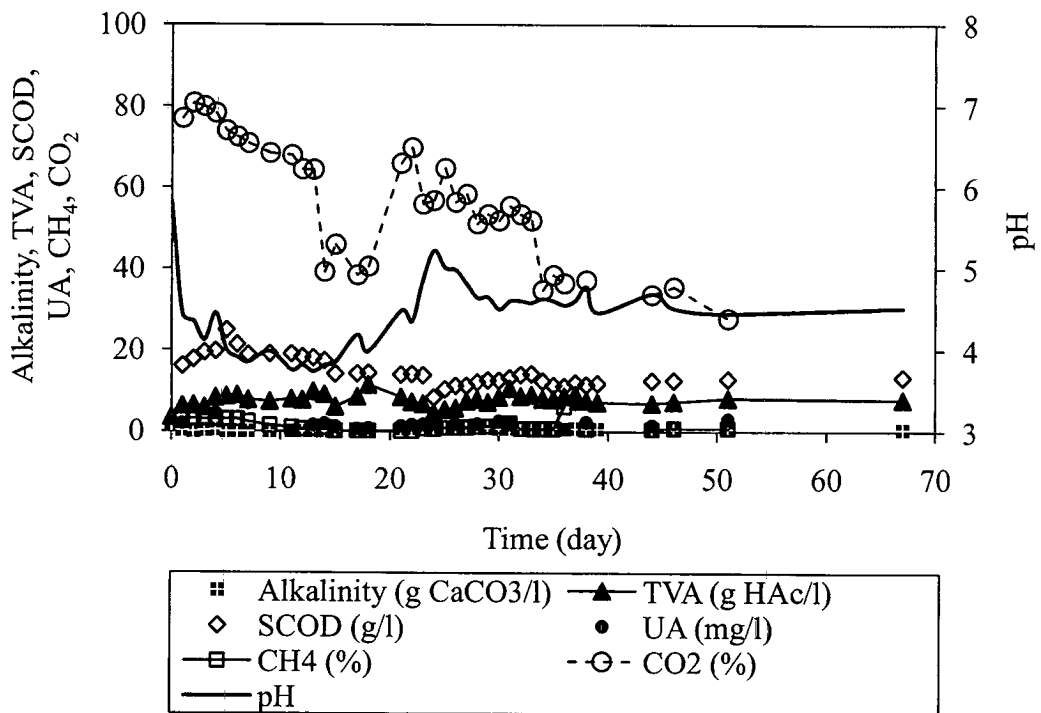
<sup>a</sup> analyzed on the following day after dilution application



**Figure 3.11** Cumulative TVA of neutral pH and dilution reactors



**Figure 3.12** Profile of total VFA and undissociated VFA fraction by dilution method

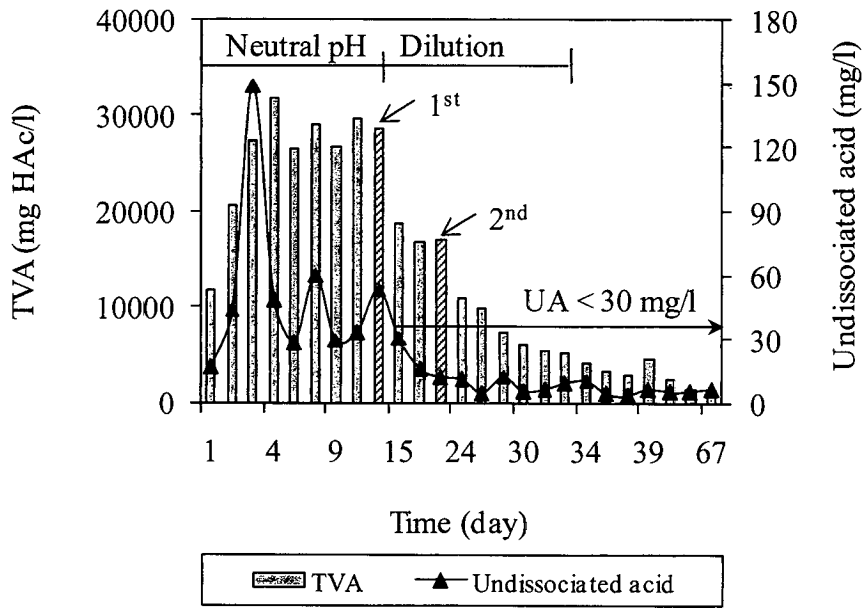


**Figure 3.13** Overall performance of dilution reactor

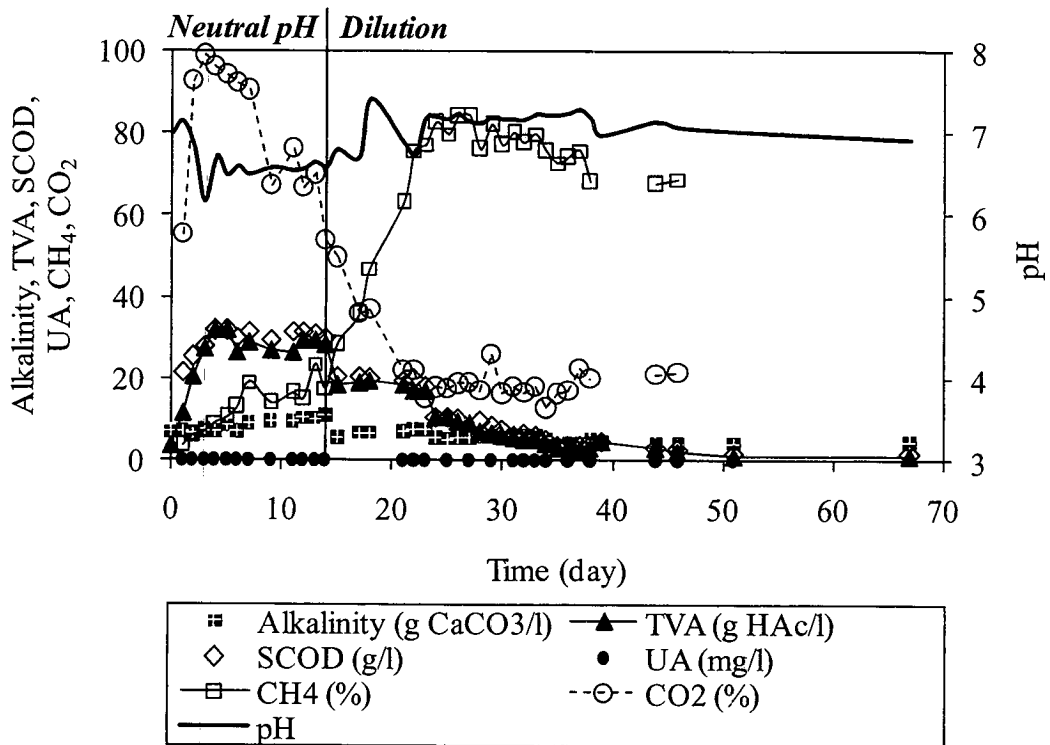
### 3) Application of Dilution after Neutral pH Control

In order to determine the effect on anaerobic food waste digestion of the neutral pH control combined with the dilution step, two duplicate neutral pH reactors were set-up. One reactor focused only on the effect of neutral pH on acid formation while another buffer supplied reactor was evaluated for the dilution effect after the result of neutral pH control was in a steady state.

As expected, the neutral pH control improved the COD and VFA leaching. Rapid degradation of readily available organic contents in the food waste resulted with more SCOD leaching at the start up phase, which was similar to the result obtained by Stabnikova *et al.* (2008). During the pH control process (day 0-13), leachate variations in terms of pH, alkalinity, SCOD, and TVA from the reactor F3 followed pattern similar to that observed in the neutral pH reactor (F2). The alkalinity increased from an initial 6.96 g CaCO<sub>3</sub>/l noticed on day 1 to 11.1 g CaCO<sub>3</sub>/l by day 14. The concentrations of TVA and SCOD sharply increased within the first week of operation and slightly decreased thereafter, together with a steadily increasing methane concentration. The SCOD/TCOD reached a peak of 0.68 on day 5, while the TVA/SCOD ratio went above 0.9 within the first 3 days of the operation. The hydrolytic-acidification of food waste digestion can be improved by neutral pH control, resulting in an increase in the TVASCOD. In F3, no further VFA was produced when the addition of external buffer ended (by day 13). Introducing dilution following neutral pH control did not have any apparent affect on acidogenesis, but improved methanogenesis characterized by a gradual increase of methane content from 23.8% to 63.2% (max. 84.3%CH<sub>4</sub>) within 7 days after introducing dilution compared with the maximum of 62.9% obtained by the neutral control reactor (F2). The results also showed that the buffer supplied reactors with dilution of leachate (F3), performed, on average, 28% better than the dilution experimental reactors (F1) in the VS degradation.



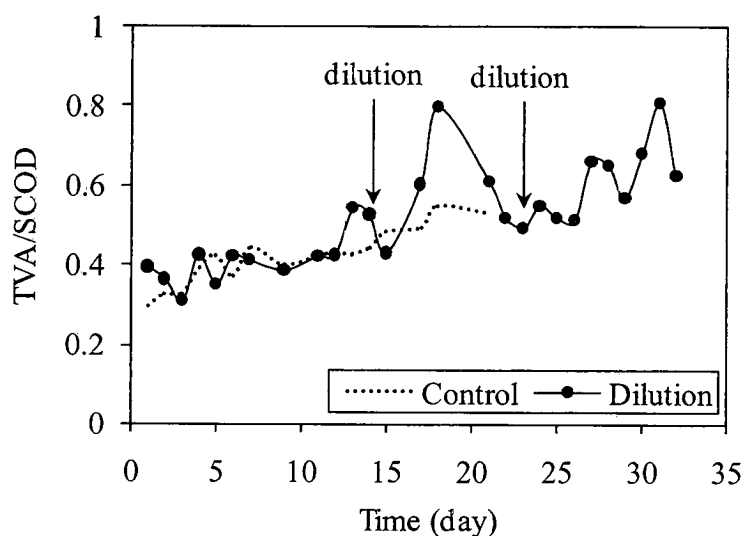
**Figure 3.14** Changes in TVA and undissociated acid concentrations by dilution after neutral pH



**Figure 3.15** Overall performance of dilution with initial pH adjustment

### 3.3.2.2 Acid Phase Transfer (TVA/SCOD) Comparison

The influence of leachate dilution on the acid phase transfer was explored and expressed in terms of the soluble COD conversion to VFA. The total VFA to SCOD (TVA/SCOD) ratio displays the amount of soluble matter that has been converted to VFAs. A TVA/SCOD value is a measure of the degree of success of acidogenesis (Maharaj and Elefsiniotis, 2001). The leachate dilution induced an increase in TVA/SCOD from 0.4 to 0.8 while a low stable ratio of 0.4, on average, appeared in the control reactor by the end of the experiment (Figure 3.16). The increase in TVA levels but not in leachate SCOD imply that leachate dilution enhanced SCOD transfer to VFA but could not alleviate the inhibition of hydrolysis by VFA.

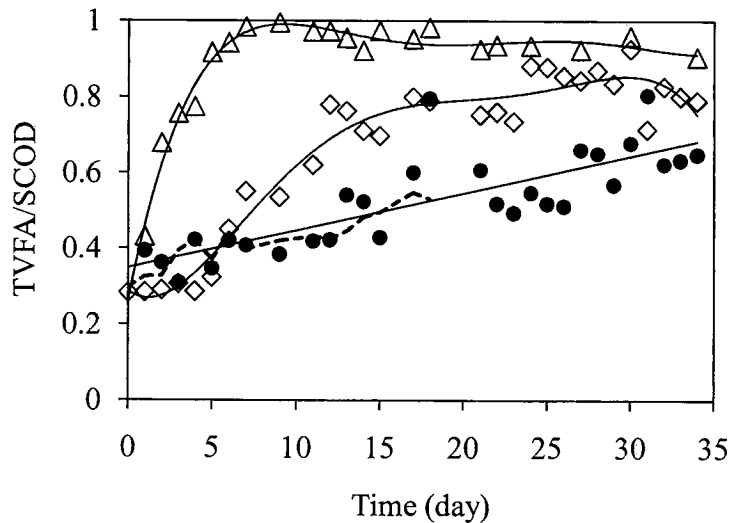


**Figure 3.16** The TVA/SCOD ratio in the unbuffered reactors

In order to validate the effect of the pH control technique, an additional batch was run with stepwise increasing pH (to about 6.5) to further confirm its effect on VFA production. The TVA/SCOD of F2 was near completion (0.98) within 7 days, while a pH adjustment closed to neutral retarded the COD conversion, reaching 0.93 only by day 35. In the neutral pH reactor, the VFA contributed to almost whole of COD since by day 5 of the operation the SCOD level had changed from 22.9 to 34.1 g/l and the TVA level from 3.5 to 31.2 g HAc/l, indicating that the higher pH reduced the lag phase and improved the VFA production (Cysneiros *et al.*, 2012). In contrast,

in the dilution reactor the TVA was just 60%, on average, of the soluble COD. It was clearly seen that the control pH regimes - both neutral pH and stepwise increasing pH - led to positive effects on the acidification step with increasing the VFA/SCOD ratio.

In fact, the VFAs are the most important products or nearly the only compounds in the soluble COD fraction under suitable working conditions. System pH plays an important factor in VFA formation; low pH in unbuffered reactors favors the undissociated acid, leading to the inhibition of the fermentation process. The acidogenic step, which transforms the soluble organic compounds produced in the hydrolysis phase into short chain volatile fatty acids, was only partially developed in such low pH conditions, such as in the dilution reactor. This finding means that the neutral pH adjustment is better than the dilution technique in helping to phase transform the soluble organic content in volatile acid was.

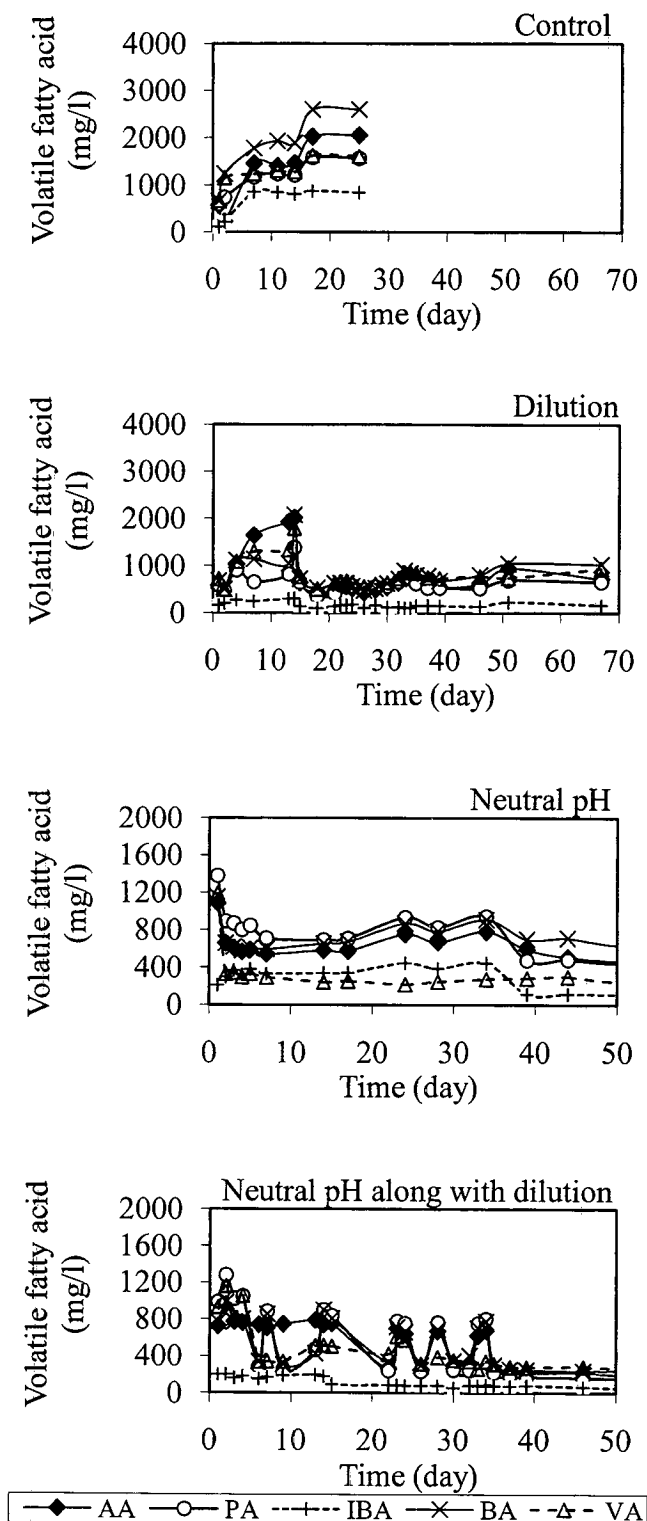


**Figure 3.17** The variation of TVA/SCOD ratio under different treatments :

(Δ) neutral pH, (◇) stepwise pH 5 to 6.5, (●) dilution, (---) control

### 3.3.2.3 Individual Volatile Fatty Acid

Short chain fatty acids are a key intermediate in the process of anaerobic digestion and are also an indicator of process stabilization. Figure 3.18 shows the composition of iVFAs with and without pH control. The order of average iVFA in the four treatments followed the same sequence of butyric acid > acetic acid > propionic acid > valeric acid > iso-butyric acid. Butyric acid under uncontrolled-pH presented in higher concentrations than other fatty acids, in the range of 0.66-2.60 g/l (FC) and 0.48-2.07 g/l (F1). Serious VFA accumulation appeared in all pH tested. Among VFAs, propionic and butyric acids are more inhibitory to the methanogens (Ward *et al.*, 2008). Butyrate and iso-butyrate have been reported to indicate process stability as an increase in fatty acid can be indicative of an overload of the OLR or suboptimal balance in VFA production and consumption. Many studies about propionic acid accumulation on methanogen activity and fermentation have been carried out. In accordance with Boone and Xun (1987), propionic acid concentrations over 3,000 mg/l have been shown to cause digester failure. Wang *et al.* (2009) finding indicated that propionic acid did not have negative effects on the methanogen and acidogen growth rates when its concentration was below 300 mg/l. However, when the propionic acid concentration reached 900 mg/l, the acidogen activity became repressed, leading a drop in the rate of VFA degradation and consequent VFA accumulation. Increase in the propionic acid retrained not only acidogenic bacteria but also methanogenic bacteria growth, followed by decreases in the methane production. Demirel and Yenigun (2002) found that propionic inhibits methanogen growth when its level more than 951 mg/l. Yeole *et al.* (1996) pointed out that methane yield decreased by 22-38% when the propionic acid concentration was 5,000 mg/l and pH was 7, and found that the inhibition would be greatly strengthened when pH was decreased. In addition, the low methane concentration of unbuffered reactors (FC and F1) may be related to the inability to metabolize the acetate produced by the acetogenic organisms by insufficiently increasing the number of methanogen.

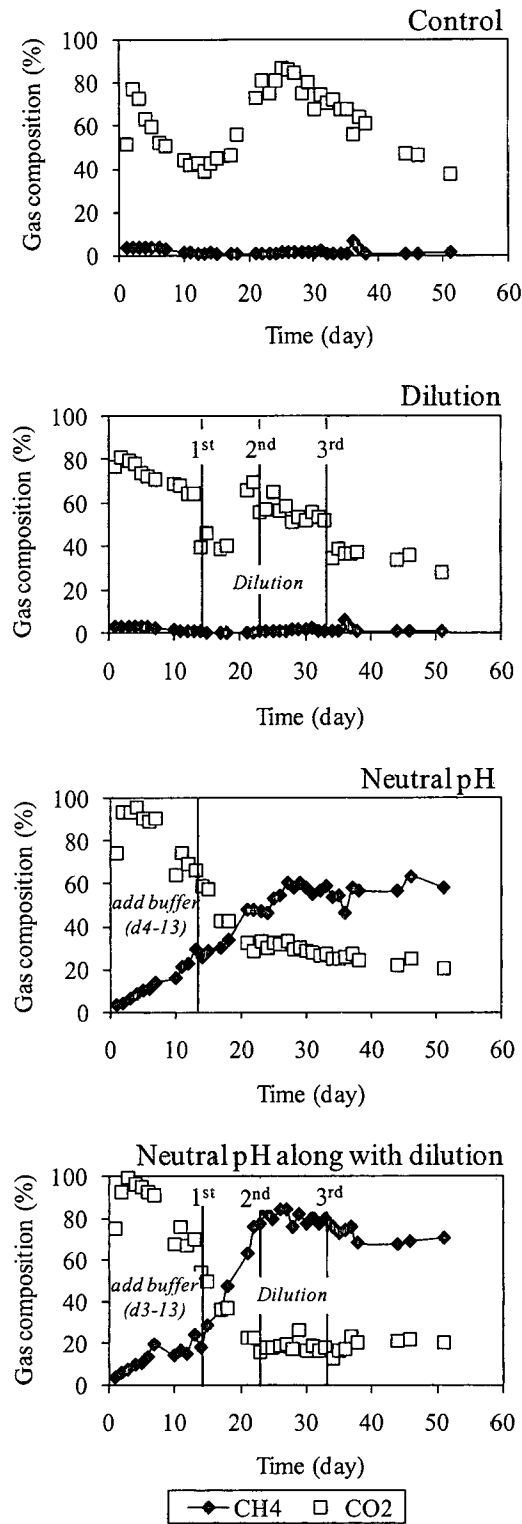


**Figure 3.18** The evolution of iVFA under various experimental conditions  
*(Note: control reactor clogged)*

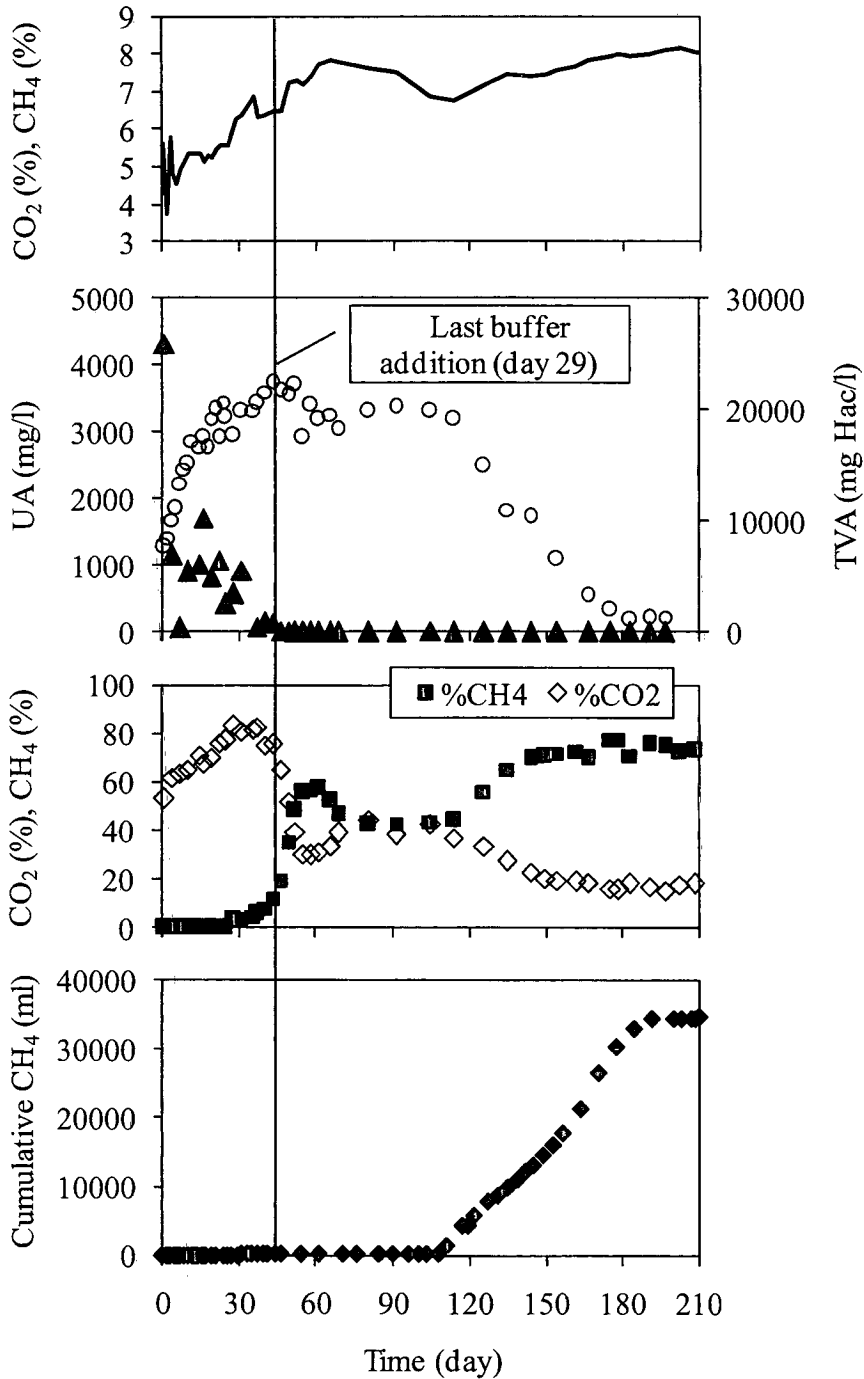
### 3.3.2.4 Biogas Production

The profile of biogas composition is shown in Figure 3.19. It can be clearly seen that CO<sub>2</sub> was the predominant gas generated in FC and F1 (represented ~60-70% of biogas content), indicating that the acidogenesis step prevailed in treating food waste under unbuffered conditions. It seemed that the anaerobic digestion in the uncontrolled-pH reactors was inhibited owing to the build-up of undissociated VFA (ranged from 702 to 7984 mg/l) as well as to the fact that methane production was severely inhibited when the pH dropped below 6.5 (Xie *et al.*, 2012). The results clearly showed the strong effect of pH on methanogenesis. The methane content represented up to 50% at pH 7 (F2), while it reached a maximum of 6% and 3% at acidic pH ~5 (F1) and pH ~4 (FC). Similarly, Hwang *et al.* (2004) reported completely inhibited methanogenesis at pH below 4.5. Fang and Liu (2002) also reported that the biogas was free of methane already at pH lower than 5.5. The neutral pH along with the dilution reactor 'F3' showed the highest methane concentration among all pH-controlled experiments tested. The dilution of leachate after neutral pH adjustment showed the rapid initiation of methanogenesis, characterized by the sharp increase in methane content, 23.8% (day 14 or the first day of introducing leachate dilution) to 63.2% (day 21), and then reaching a peak of 84.3%, higher than that of 62.9% attained by the neutral pH reactor. It should be noted that the applied dilution following neutral pH control (3-16 mg/l) was much more effective in suppressing undissociated VFAs than the neutral pH control (28-149 mg/l) and reduced an accumulated VFA stressing by leachate replacement. This condition probably caused a higher methane concentration in the neutral pH along with dilution reactor.

An approaching final gas process developed on day 108 of the operation in the stepwise increasing pH reactor 'F4'. This might have been caused by the fact that the methanogens needed a longer adaptation period. Negligible amount of biogas could only be detected during the first 108-days of the operation even though almost 60% CH<sub>4</sub> approached was already noticed by day 41. At this time, the system pH reached neutral combined with undissociated acid surpassing the critical level for methanogenesis as reported elsewhere created favorable conditions for the methane formation, signifying a prolonged inhibition under the single stage environment.



**Figure 3.19** Gas composition profile with and without pH control



**Figure 3.20** Overall performance of stepwise pH adjustment

The particularly interesting point was the fact that low pH itself is not toxic to methanogens (Maestrojuan and Boone, 1991). Some methanogenic bacteria, *Methanosarcina berkeri* strain, have been reported to grow well in a broad pH range from 4.3 to 7.8. The most important that the undissociated VFA fraction, toxic substance to methanogen, increased with the pH decreased. Undissociated VFA has

been reported to be toxic to methanogens (Duarte and Anderson, 1982). Excessive fatty acids as inhibitors toxic the methanogenic step especially in their non-ionized forms (Ward *et al.*, 2008). For acetoclastic methanogens, the toxic level of undissociated acid lies between 0.26 and 1 mM (Attal *et al.*, 1988; Kroeker *et al.*, 1979). For thermophilic methylotrophic methanogens, complete inhibition was evident at 8.87 mM undissociated acetic acid whereas partial inhibition was found at around 4 mM (Yamagushi *et al.*, 1989). When the concentration of the undissociated VFA was produced, the methanogens were slowly wiped out. The addition of bicarbonate increased the pH and consequently diminished the fraction of undissociated acid in the reactor, which might explained why methane gas was highly formed in buffered reactors.

The overall process improvement in acidogenesis in different operating regimes was compiled and compared in Table 3.6. The VS removal efficiencies of supplied buffer reactors were in the range of 53% to 75% (F2 and F3) and a slightly better value of 78% was found by stepwise increasing pH (F4), while the dilution method (F1) attained only 53%. Leachate replacement caused a possible wash out of acidogen. This wash out is likely to occur due to the fact that these microorganisms are mostly hydrophilic (Daffonchio *et al.*, 1995) and therefore mainly suspended in the liquid phase of bioreactors (Akunna and Clark, 2000). Similarly, Cysneiros *et al.*, 2012) observed numbers of cellulolytic microorganisms in buffered experimental reactor showed 10-fold higher than the unbuffered one, indicating that when the pH was close to optimum there was some growth to compensate for the loss of cellulolytic microorganisms in the leachate.

Total VFAs, pH, and alkalinity are common stress indicators for AD process imbalance (Ahring *et al.*, 1995). The acidogenesis of easily degradable organics of carbohydrate-rich food waste could reduce the pH. Negative observations in rapid increment of acids and their undissociated form were noted initially at the start-up in the unbuffered reactors, causing entire system failure. In our study, control TVA and undissociated acid levels could be distinguished in two ways; stepwise increase of pH, and the flushing of inhibitory products in the acidogenic digester with leachate replacement. Alkalinity limitation in the food waste unfavored acid formation. Without controlling the pH, the very low pH and high VFA accumulation inhibited the hydrolysis/acidification, resulting thereby in the failure of the digester.

The addition of external buffer to attain a neutral pH restored the digester performance; biogas was produced with a methane content 56.5%, on average. Based on the experimental data, the dilution of leachate, especially after neutral pH operation, could be particularly helpful in assisting the methane forming stage, reaching 80% CH<sub>4</sub> in a short period.

**Table 3.6** Reactor performance data of improving acid phase food waste

Parameter	Uncontrolled pH		Controlled pH			
	Control	Dilution	Neutral pH	Neutral pH along with dilution		Stepwise pH 5-6.5 <sup>a</sup>
				Neutral pH	Dilution	
pH	3.70-5.80	3.85-5.22	6.31-7.32	6.17-6.71	6.68-7.41	3.77-8.45
Hydrolysis yield (g SCOD/g VS)	351	296	486	546		563
Acid yield (g HAc/g VS)	165	142	455	501		478
TVA generation rate (g HAc/l d)	1.49	1.71 (1 <sup>st</sup> ) 0.87 (2 <sup>nd</sup> ) 0.45 (3 <sup>rd</sup> )	5.60	7.22	0.30 (1 <sup>st</sup> )	0.74
Lowest UA (mg/l)	1752-7984	702-2537	10-86	28	3-16	28-96
Max. CH <sub>4</sub> (%)	3	6	63	24	84	78
VS reduction (%)	33	53	76	74		78

<sup>a</sup> operation time 210 days

### 3.3.3 Anaerobic High Solid Food Waste (15%TS)

#### 3.3.3.1 Effects of pH Adjustment and Dilution Technique on Anaerobically High Solid Food Waste

From our previous results, a single-stage digestion system can be operated with 10% TS food waste. Considering future practical applications, further attempts to treat food waste with higher TS content (15% TS) were employed by integrating pH control and leachate dilution techniques with the attention on maximizing VFA precursor for energetic purposes in the form of methane. Jash and Ghosh (1996) investigated the effect of organic loading on the solubilization rate of some organic residues using mixed cultures. They found that the rate of solubilization decreased with an increase of substrate concentration due to lowering of pH from a

higher concentration of soluble substrate amenable to acidogenesis. To avoid a rapid acid build-up from the high solid fermentation encountered at the start-up phase, the stepwise increase of pH technique was proposed to solve the acid stresses problem occurring during the initial digestion of food waste. Dilution technique and neutral pH adjustment were introduced in the second and third phase, respectively.

### **Phase I: Effect of Stepwise Increase of pH 4.5 to 6.5**

Since 15%TS food waste has low pH (3.75), the daily buffer addition was necessary to maintain stable pH during the first week of the operation (start-up stage). Table 3.7 shows the reactor performance data under a stepwise pH gradient (pH 4.5-6.5). On the first week (adjusted pH 4.5), leachate alkalinity, TVA, and SCOD dramatically increased to 42.9% SCOD/TCOD<sub>0</sub> and 33.5% TVA/SCOD. Carbon dioxide was the main gas produced, indicating that hydrolysis and acidogenesis were the major stages in this period. Efforts for VFA production was done by stepwise increase in pH to 5.5, 6, and 6.5.

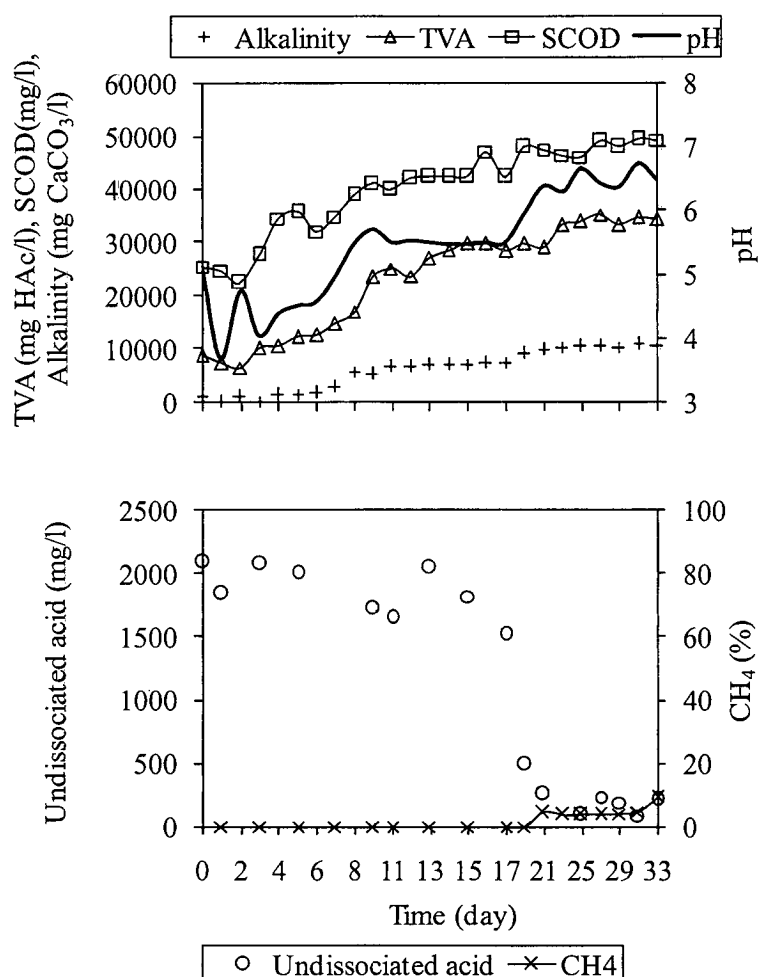
In week 2 (adjusted pH 5.5), an increase in TVA from 38 to 76 g HAc was shown to be accompanied by a decrease in undissociated VFAs from 3217 to 1518 mg/l. The SCOD/TCOD<sub>0</sub> and TVA/SCOD reached 61.3% and 59.3%, on average. An attempt to reduce the concentration of undissociated acid by the stepwise increase of pH was done in the 3<sup>rd</sup> week (day 21) but the adjusted system pH (6.38) was higher than the desired pH value (6). There were slight increases of leachate TVA and SCOD, a SCOD/TCOD<sub>0</sub> of 70% was reached, and a small percentage of methane (4% on average). From benefits of pH effects on the VFA production, a further attempt to raise the system pH to 6.5 was performed in the 4<sup>th</sup> week. As a result, the SCOD/TCOD<sub>0</sub> and TVA/SCOD reached 72.5% and 69.7%, respectively, but the undissociated acid concentration still was far beyond the commonly recommended threshold level for methanogenesis. The temporary drop in the level of undissociated acid (below 30 mg/l) was found during the buffer addition before it increased thereafter.

**Table 3.7** Acidogenesis performance under stepwise increase in pH 4.5-6.5

pH	Day	TVA (g HAc)	Rate of TVA generated (g HAc/l d)	SCOD/TCOD <sub>0</sub> (%)	TVA/SCOD (%)	Lowest UA (mg/l)
4.5	0-6	35.5	0.77	33.1-53.1 (42.9)	28.9-39.3 (33.5)	1,844
5.5	7-17	82.2	1.83	51.7-69.9 (61.4)	42.4-70.3 (59.3)	1,518
6.5	21-33	95.2	1.03	68.3-73.3 (71.1)	61.5-73.6 (69.7)	89 <sup>a</sup>

<sup>a</sup> temporary decline; it increased by the next day with no additional buffer

Note: average values show (in parentheses)

**Figure 3.21** Overall performances by stepwise pH gradient 4.5-6.5

### Phase 2: Effect of Leachate Dilution

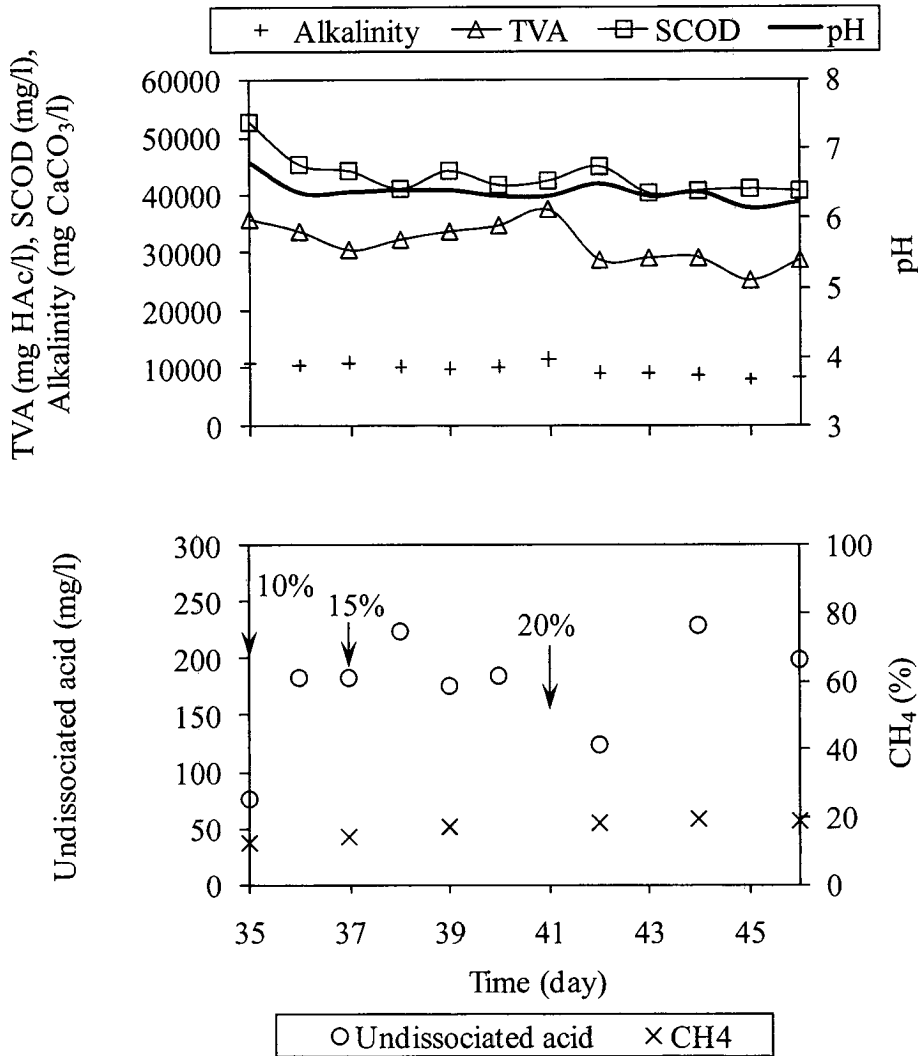
Since the dilution application led to the flushing of inhibitory products in the acidogenic reactor found in the previous 10%TS food waste treatment study (~75% of undissociated acid reduction), an introduction of dilution water into digester was selected as the second option. Before applying dilution water, a small dose of NaHCO<sub>3</sub> was added into digester on day 33, 36, and 41 to help suppress the undissociated VFAs concentration. Three different dilution degrees of 10%, 15%, and 20% were tested. The 10% dilution was performed on day 35 by a 173-ml leachate removed and replaced with the same volume of distilled water. Applying dilution followed by pH adjustment for high solid anaerobic digester did not show evidence of supportive methanogenesis as obtained in a low solid digester (10%TS food waste). Undissociated acid concentration was still higher than the threshold level for methanogenesis mentioned in the literature (30-60 mg/l). It dropped from 214 to 76 by the first 10% dilution but it reversed by the next day. Further attempts with higher dilution degree (15% and 22%) gave the same result, 225 mg/l (15% dilution) and 123.8 mg/l (20% dilution).

**Table 3.8** Change in undissociated VFAs and methane content at stepwise pH and leachate dilution

Operational process	Undissociated acid (mg/l)	Maximum CH <sub>4</sub> content (%)
Day 0-35 (Stepwise pH to 6.5)	214 <sup>a</sup>	9.0
Day 35-46 (Dilution)	76 <sup>b</sup> -230	19.5

<sup>a</sup> analyzed before introducing the leachate dilution

<sup>b</sup> a temporary drop of undissociated VFAs concentration before it increased thereafter

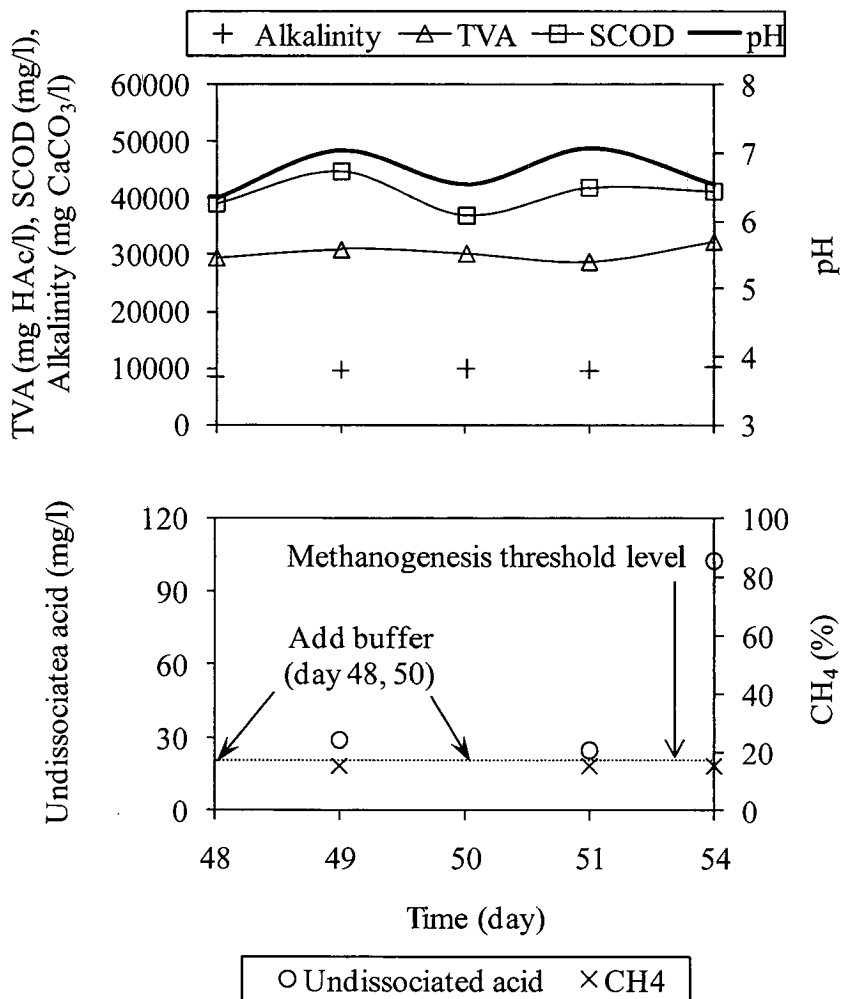


**Figure 3.22** Overall performances under dilution application

### Phase 3: Effect of Neutral pH Adjustment

An attempt was then made to treat high solid food waste in a single-stage digester by neutral pH adjustment. There was a temporary drop of undissociated VFAs after neutral pH adjustment on day 49 (29.4 mg/l) and day 51 (24.9 mg/l) but the level increased thereafter when the process of pH adjustment ended, reaching 102.2 mg/l by day 54 (Figure 3.23). These results indicated that the controlling inhibitor product techniques (pH adjustment and dilution) successfully applied on 10% TS under one phase failed for the high TS. The concentrations of TVA (28.7 to 32.1 g HAc/l) and SCOD (36.9 to 44.6 g/l) were constant with a low methane concentration of 15% (max. value), signifying the failure of entering into

methanogenic phase of high solid food waste under single-phase system. It seems that this failure was related to other factors besides the undissociated acid level. Accumulated VFA has been identified as the significant cause of inhibition in the anaerobic digestion process (Fukuzaki *et al.*, 1990). A high level of organic acid in the acid digester from the high organic matter containing in 15%TS food waste may obstruct the onset of methanogenesis. As inhibition effects of accumulated VFA could not be solved under a one-stage condition, a two-stage anaerobic reactor was then employed in the next experiment.



**Figure 3.23** Overall performances of neutral pH adjustment condition

**Table 3.9** Performances of anaerobic acidogenic process treating high solid food waste

Operation and performance	Acidogenic process operations		
	Phase I Stepwise pH (4.5-6.5)	Phase II Dilution	Phase III Neutral-pH
<i>Applied conditions</i>			
pH	3.68-6.74	6.15-6.82	6.31-7.06
Buffer amount (g/g VS)	0.29	0.01 <sup>a</sup>	0.03
Lowest level of undissociated VFAs (mg/l)	88.6	76.5	24.9
Operational period (days)	33	12	7
<i>Performances</i>			
TVA produced (g HAc/kg VS)	285	< 285 <sup>b</sup>	< 285 <sup>a</sup>
Rate of TVA generated (g HAc/l d)	1.03	1.01	0.38
Volumetric CH <sub>4</sub> production (ml/g VS)	0.15	0.34	0.15
Max. CH <sub>4</sub> content (%)	9.0	19.5	15.3

<sup>a</sup> small amounts of buffer were added before introducing dilution technique

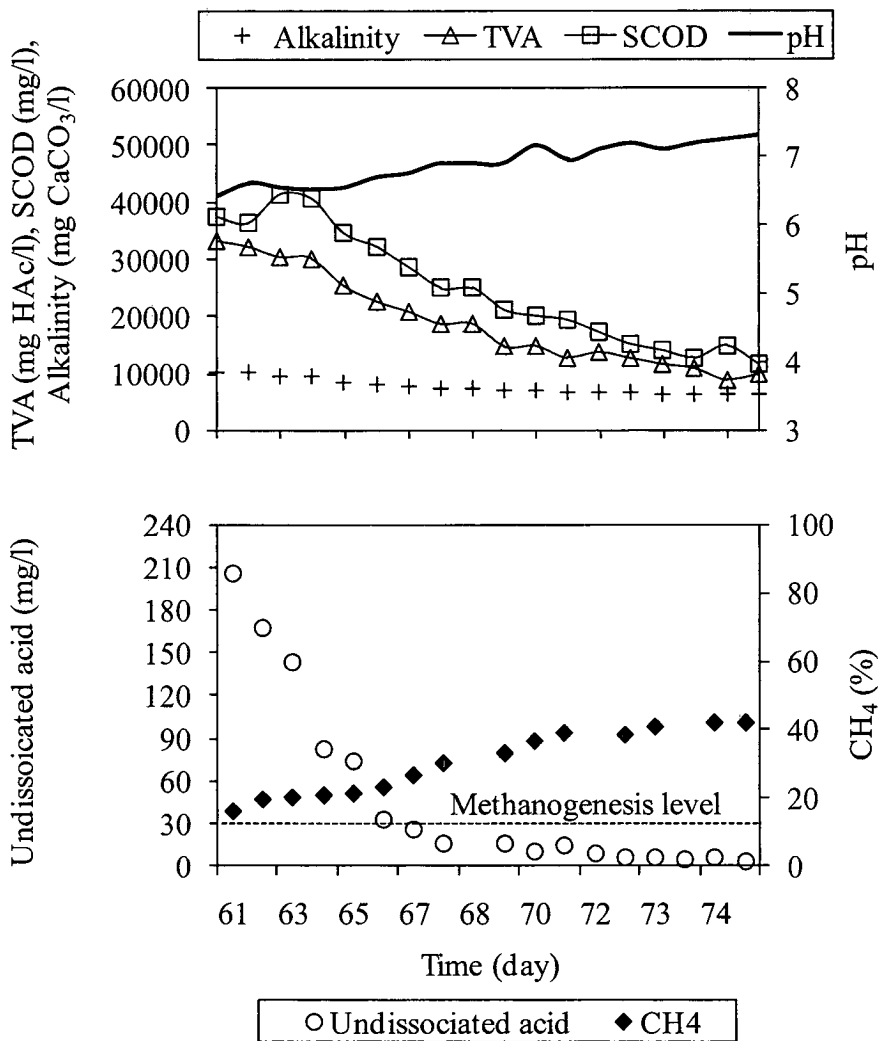
<sup>b</sup> the total VFA concentrations decreased from the effects of diluted leachate

### 3.3.3.2 Two-Phase Fixed Film Reactor

The two-phase anaerobic system treating 15%TS food waste was carried out after the failure of single-stage AD. Due to the limitation of remaining head space in the fixed film reactor, the effect of OLR was tested by methanogenic effluent withdrawal before loading the acidogenic leachate from F5 using discontinuously feeding regime. The reactor was first operated at the HRT of 24 hours, from day 61 to 68 of operational period. The HRT was then reduced in a stepwise fashion to 16 and 11 hour in order to increase the OLR. The acidogenic leachate from the bottom part of F5 was consecutively fed into FF at the organic loading of 1, 1.6, 2, and 3.2 g COD/l d with the corresponding HRT ranging from 11 to 24 hours.

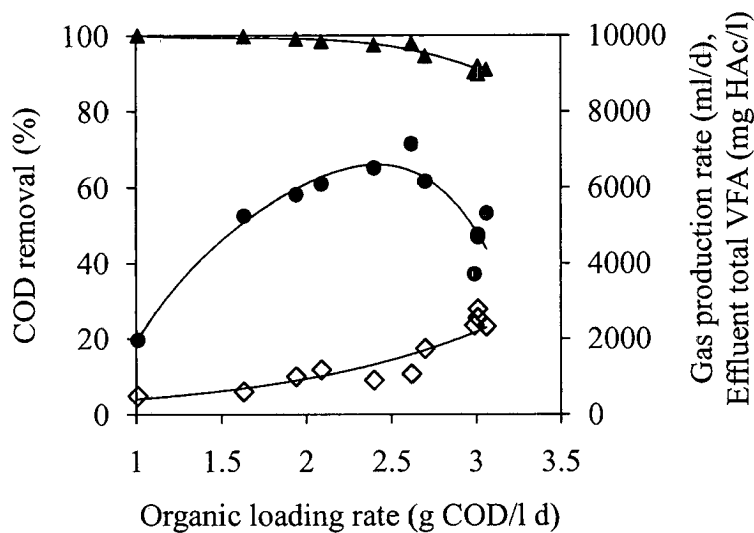
The two-phase process was first operated with HRT of 24 hours (day 61). Beneficial effects were seen, the decrease in undissociated acid concentration (from 205.2 to 167.7 mg/l) and the slight increase in system pH (from 6.43 to 6.62)

and methane content (from 16 to 20%). To accelerate the formation of VFA, an attempt was then made to speed up OLR by threefold, from 1.0 to 1.6, 2, and 3.2 g COD/l d (keeping the HRT of 24 hours). The COD results in Figure 3.24 indicated that the FF reactor eventually achieved a consistent lowering of effluent COD and maintained > 99% COD removal. This was followed by the dramatic increase in the volume of biogas from 1.96 to 9.45 l/d (or 6.7 l CH<sub>4</sub>/d) despite the fluctuation of influent concentration. The volumetric methane production reflected the COD loading rate; as the OLR increased, the methane production rate increased.



**Figure 3.24** Changes in liquid and gas phase of F5 during combination period

Due to the dramatically decline in influent concentration, which required adjusting the influent COD to the desired OLF difficult, the OLR was decreased to  $2.6 \pm 0.2$  gCOD/l d on the 6<sup>th</sup>-8<sup>th</sup> combination (day 66-68) by keeping the HRT of 24 hours. As showed in Figure 3.25, FF reactor consistently performed well in term of COD removal ( $> 97\%$ ) with TVA concentration less than 1.1 g/l, with COD removal usually higher than 90% until the end of the combination. The concentrations of SCOD and TVA in the effluent were quite low, often below 1 g/l, particularly the 24 hours HRT. An increase in effluent SCOD and TVA concentrations at this time presumably due to wash-out of sludge.



**Figure 3.25** Methanogenic reactor performances at various OLR: (▲) COD removal; (●) gas production rate; (◇) effluent total VFA for circulate back to acid digester

A change in the acidogenic influent resulted in a change in the OLR if the HRT was kept constant. The acidogenic concentration of F5 declined gradually with time that made unable to keep the OLR by increase the concentration of organic loading. Therefore, the second loading regime was accomplished by decreasing the HRT to 16 and 11 hours for six days (day 69 to 74). During the course of such operation, there were fluctuations in the methanogenic effluent of FF. In this way the concentrations of effluent SCOD and TVA increased with the decreased HRT. Keeping the OLR of 3 g COD/l d batch run was continued by reducing HRT from 24 hour to 16 hour (day 69), 15.6 hours (day 70) and 11.2 hours (day 71). The SCOD and

TVA concentrations sharply increased from 1,705 to 2,742 mg/l and from 1,728 to 2,780 mg HAc/l, respectively. F5 achieved high COD reduction (above 90%) even at the 16, 13 and 11 hour HRTs. This was followed by a decline in methane production and COD removal from 4.73 to 1.62 l CH<sub>4</sub>/day and from 98 to 91%, respectively (Figure 3.25). In addition, this experiment also shows the ability of the methanogenic reactor to endure with increasing loading rates even with a low HRT.

The large decreases in the COD and VFA concentrations in the leachate from the acidogenic reactors 'F5' resulted in a decrease of OLR applied to the methanogenic reactors during day 72 to 74, the last period of the combination of acidogenic and methanogenic reactors. The OLR decreased from 3 to 2.0, 1.5, and 1 g COD/l d on the 13<sup>th</sup> - 16<sup>th</sup> combination. On day 72 and 74, HRT was increased to 22 h as the variation of the acidogenic influent allowing the methanogenic reactor treatment efficiency showed the consistent COD removal was 90%. Once the OLR of 1.5 and 2 g COD/l was operated by reducing HRT to 12.7 hours, a decrease in COD removal efficiency was observed, becoming stable at about 88%, and consequently, the biogas and methane production decreased. Thus, the combination of an acid reactor and a methane fixed film reactor was ended on day 75 due to the poor quality of the methanogenic effluent. Deteriorating COD reduction can be explained by the fact that the methanogenic reactor (FF) was not fed with any substrate for a period longer than 1 month after decoupling the FF from the acid reactor treating market waste (M5). One additional factor may be related with the up flow operation mode, as the pore may be easily blocked by the particles, which make it less useful in real practice, particularly for long operation times. It seems that the methanogenic fixed film had consistent performances under the fluctuating organic loads as long as the organic load referred to influent concentration, not increasing load by shortening HRT.

Finally, after 5 combinations attempts, the undissociated acid concentration in F5 (32.9 mg/l) achieved the threshold level for methanogenesis onset. It should be noted that this threshold level of undissociated acid for methanogenesis was considerably lower than most of threshold reported by others. However, methane content has been found to be relatively low (23%) and this may be related with accumulation of VFA, which remained higher than 22 g HAc/l over this period.

The methane production rate proportionally increased with OLR, ranging from 1.1 to 6.7 l CH<sub>4</sub>/d at OLR 1 and 3.2 g COD/l d (HRT 24 hour),

respectively, whereas the production of methane at OLR 3 g COD/l d (under decreasing HRT) was 5-l CH<sub>4</sub>/d and was reduced to 1.6-l CH<sub>4</sub>/d after the last OLR (1 g COD/l d).

During the coupling process, it was found that the TVA and SCOD concentrations of F5 gradually decreased with methanogenic effluent recirculation, leading to the decline of product inhibition both in terms of VFA and undissociated acid. The two phase process had positive effects on biogas and methane productions. The addition of methanogenic effluent as a dilution waster has resulted in the dilution of COD and VFA and a change in the pH of the acidogenic leachate of F5, producing a pattern similar to that seen in dilution reactor 'F1'. The drops of SCOD and VFA on subsequent days can be attributed to the methanogenic effluent addition to the acid reactor. Methane concentration in the acid digester rose steadily during the following weeks, with a methane composition of about 40-50% by volume. On day 87, 12 days after decoupling, the methane content of F5 reached 62.5% with an increase of gas production, corresponding the decrease in TVA and SCOD levels with time, implying that the entering into the methanogenic stage could be achieved in the high acid digester. The subsequent slow decline was probably due to substrate depletion. Notwithstanding this decline, during the 14 days of combination, the methane production by the methanogenic fixed film reactor alone was equivalent to 2/3 for the total amount of methane produced (98% of volumetric CH<sub>4</sub> occurred in fixed film reactor). The system pH of FF remained within the functional range of methanogens (7.99±0.1). The concentrations of VFA and the alkalinity detected in the effluent were on an average 1.62 g HAc/l and 5.19 g CaCO<sub>3</sub>/l, respectively, indicating the good performances of the methanogenic reactor of this system. The coupled acid digester demonstrated promoted methanogenesis performance by retaining buffering and providing anaerobic inoculums of methanogenic bacteria allowing removal of VFA in acid digester, and the methanogenic fixed film reactor showed rapid conversion of leachate VFA into methane.

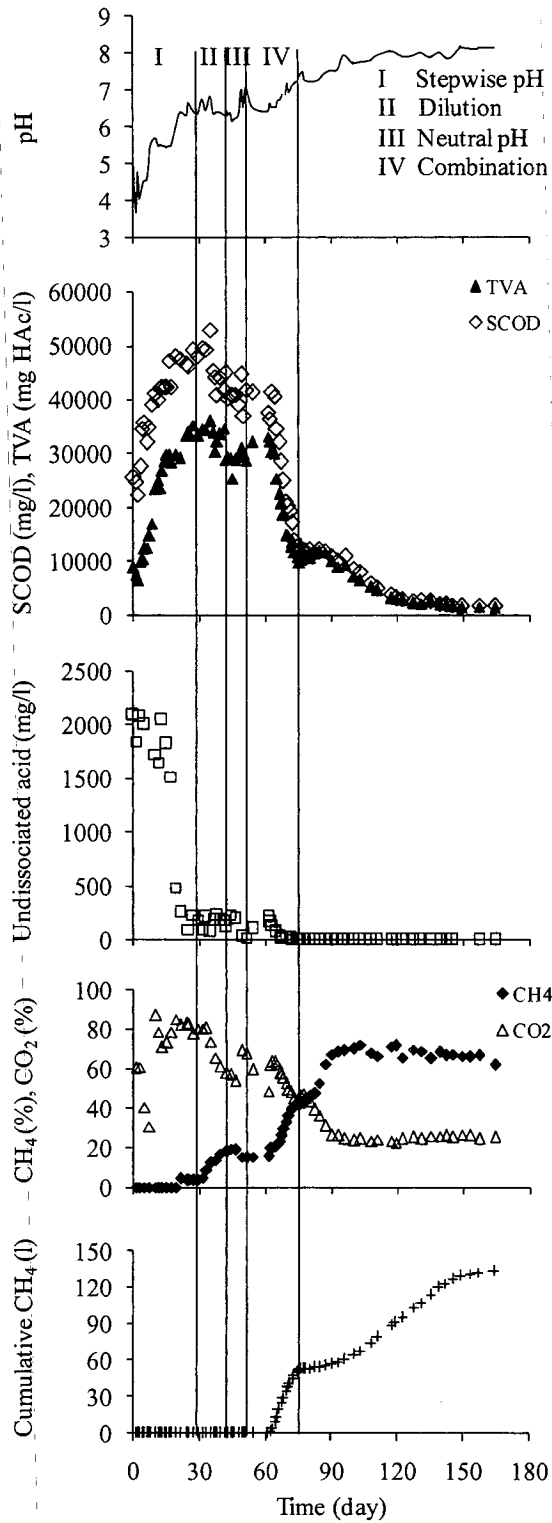
The carbon mass balance showed that high food waste (15% TS) degraded into 35.1% CH<sub>4</sub>, 16.9% CO<sub>2</sub>, and 26.2% soluble carbon, with 7.1% of remaining organic carbon (solid), using the product inhibition control technique (pH control and dilution) and phase separation (Table 3.10).

**Table 3.10** Carbon balance of high solid food waste

Mass Carbon, C (g)						% Recovery
Solid in OC+IC	Soluble	Gas	Solid out		Biomass seed	
			OC	IC		
185.37	48.48	96.39	29.21	25.13	16.12	98.77

Note: OC = organic carbon; IC = Inorganic carbon

The summary of performance data of high solid food waste treatment is shown in Table 3.11 and Figure 3.26. As can be seen in Figure 3.26, the stepwise increase of pH contributed a prolonged acid buildup leading to the generation of a substantial amount of VFA. The methane yield was calculated by dividing the final (210 day) STP methane volume by the weight of food waste added. The two-phase digesters exhibited a methane yield of 0.40 l CH<sub>4</sub>/g VS<sub>added</sub> or 0.67 l CH<sub>4</sub>/g COD<sub>added</sub>. In an experiment involving the co-digestion of food waste in semi-continuously fed laboratory reactors, Nayono *et al.* (2009) achieved an average methane content of 62-66%, which is equivalent to a methane yield around 0.33 l CH<sub>4</sub>/g COD<sub>added</sub>. Both dilution and neutral pH application were unable to reduce undissociated acid concentration to meet the threshold level for methanogenesis. The stepwise increase pH manner suffers from a drawback in that the final methanogenesis stage appeared to be delayed. Failure to operate 15% TS under a single phase reactor was probably related to a high concentration of TVA released from the high solid content of 15%TS food waste. Under high solid condition, the coupling of acid digester with the methanogenic reactor established a well balanced pH, total VFA, and undissociated VFAs, and improved the biogas production in the acidogenic reactor.



**Figure 3.26** Changes in liquid and gas phase of high acid digester by inhibitory product control and phase separation

**Table 3.11** Summary of treatment high solid food waste (15 %TS) results

Performances	Values
Hydrolysis yield (g/g VS <sub>added</sub> )	0.40
Acid yield (g HAc/g VS <sub>added</sub> )	0.29
Total VFA generation rate (g HAc/l d)	1.26
VFA composition (%)	
Acetic acid	36
Propionic acid	21
Butyric acid	34
iso-Butyric acid	2
Valeric acid	7
COD of CH <sub>4</sub> produced (g)	347.1
Ultimate CH <sub>4</sub> yield (l CH <sub>4</sub> /g VS <sub>added</sub> )	0.40
COD removal (%)	90.2
VS reduction (%)	88.2

### 3.4 Summary of Anaerobic Food Waste Treatment

Rapid VFA formation was the main cause of poor digester performance of high biodegradable substrates like food waste. A digester in an upset condition or 'a sour digester' is a problem often encountered particularly in the initial digestion phase that leads to greater imbalance and biogas process inhibition. Anaerobic digestion of food waste was started at 10%TS food waste as the feedstock. First, the batch run was carried out to verify the optimum pH value for VFA production of food waste digestion under different pHs tested (5, 6, and 7). The VFA concentration at pH 7 was higher than those at other pH conditions, achieving the maximum concentration of 34.9 g HAc/l on day 45 indicating an extended acidification by neutral pH control. Product inhibition focusing in term of undissociated acid showed a quite low level, 13.5 mg/l, showing the high efficiency in the reduction of undissociated acid by controlling pH at 7. However, the pH fluctuation observed at pH 7 indicated alkalinity consumption and reflected the requirement for an external alkalinity source.

A relatively low pH of 3.98, on average, in the control reactor was responsible for the hindrance of conversion of organic material characterized by a relatively low VS reduction of 33.4%. The corresponding undissociated VFA at maximum TVA concentration, as represented by inhibition levels for acidogenesis of food waste, were 3230, 994, and 73 mg/l at pH 5, 6, and 7, respectively. The production of VFA of the neutral pH control was 455.2 g HAc/kg VS (not including

the VFA conversion toward gas phase), which was 2.8 times higher than that of uncontrolled pH (165.4 g HAc/kg VS). The leachate dilution improved the VFA production signified by an increase of the amount of TVA of 73% compared to before the dilution. But the increase of the amount of VFA by the dilution technique was around 4.5 fold lower than that of neutral pH, with the VS reduction of 53% compared to 76% of the controlling neutral pH.

The dilution after the neutral pH control had positive effects on the methanogenesis as the methane was 84% of biogas compared to 63% under the neutral pH control; however, this positive effect was not found for the high solid digestion (15%TS food waste). The stepwise pH control could solve an accumulation of organic acid which is often found during the start-up of anaerobically high solid waste by controlling the rate of VFA generated, resulting in an extension of the acidification. The production of VFA and methane was 479 g HAc/kg VS and 0.16 l CH<sub>4</sub>/g VS, respectively, under single-phase process.

The digestion of large amounts of solids creates high concentrations of end products that inhibit the anaerobic decomposition. The results obtained in this work showed that food waste is highly biodegradable with a two-phase reactor. Production of biogas from high solid food waste was enhanced by integrating neutral pH adjustment for encouraging VFA production coupled with a phase-separated two stage anaerobic process for supportive VFA conversion to the gas phase. An increase of the loading rate (under reduced HRT phenomenon) from 1 to 3 g COD/l d resulted in a drop of methane yield and deteriorating COD removal efficiency from almost 100% to 91%, on average. Nevertheless, the pH value remained at 7.99, on average, in the methanogenic fixed film and the methane levels were also normal, at about 69% by volume. The average COD removal at 1-3 g COD/l d was 94% with a HRT of 11.2-24 hours. High efficiency for conversion of high solid food waste into biogas with 98.8% recovery was attained using product inhibition control technique and phase separation, as the remaining organic solid carbon was only 7%.