Mesophilic and Thermophilic Anaerobic Digestion of Pineapple Cannery Wastes

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Abstract

The aim of this study is to compare microbial populations and intermediate compounds in mesophilic and thermophilic anaerobic digestion of pineapple peel. Ethanol, acetic acid, propionic acid, and butyric acid were found as intermediates leading to methane formation during mesophilic digestion. Most of these compounds were found in thermophilic digester except propionic acid. The absence of propionic acid during thermophilic digestion of peel was supported by the following evidence:

a) Microbial activity tests indicated the similarity of microbial populations in mesophilic and thermophilic digesters in their ability to utilize the different substrates as sole carbon source. Exceptionally, propionic acid utilizing bacteria was found only in mesophilic digester.

b) In the presence of methanogenesis inhibitor using 2.5 mM chloroform, 32 mM phenol, and 20 mM bromoethanesulfonate, acetic and propionic acids were accumulated in mesophilic digester. Only acetic acid was detected in thermophilic digester.

c) By shifting operating temperature from 55°C to 37°C, the biochemical pathway changed in which propionic acid was one of the intermediates. No propionic acid was found during temperature shift from 37°C to 55°C.

The results suggested that β-oxidation of butyric acid to acetic acid occurred in both mesophilic and thermophilic digestion. In addition, degradation of propionic acid was another route for acetic acid production at mesophilic temperature. Propionic acid occurred in mesophilic digestion via the decarboxylation of butyric acid.

Keywords: anaerobic digestion, intermediate compounds, mesophile, microbial populations, pineapple cannery wastes, thermophile.

1. Introduction

The production of processed food in Thailand for exports is mainly frozen and canned food. Of the later, canned fruit and vegetables have become an important export commodity. Canned pineapple and pineapple juice exports were valued at US$ 963 million in 1996, contributing the equivalent of 75% of the entire pineapple export value (1). In the pineapple canning process, solid waste originates from peel and core. On an average, one ton of fresh pineapples generates approximately 0.5 ton of solid waste. Each cannery normally disposes of 100-150 tons of solid waste per day (2, 3). Although the waste is an excellent feed, the practice has not received large scale attention due to its marginal economic viability. Currently pineapple waste is dumped and left on open land for natural biodegradation, thus creating a run off problem for downstream rivers and lakes.

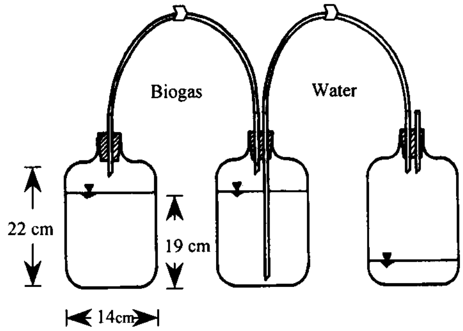
Biogas technology has been shown to be widely applicable for agro-industrial wastes (2, 3). The bioconversion of organic waste materials to methane in anaerobic digesters has potential practical and economic implications (3). The anaerobic degradation of organic compounds consisrs of several biological reactions and nutritional interaction involving several groups of microorganisms (4). Under normal conditions, propionate, butyrate and acetate are important intermediates in anaerobic degradation of complex matter. Microbial associations which produced acetate from butyrate, propionate, and hexanoate have been reported by Stieb and Schink (5) and Mucha et al (6). Generally, temperature is one of the rate limiting factors in biological reactions and in the variety of intermediate compounds that accumulate (7-11).

Pineapple cannery waste proved to be a good substrate for anaerobic digestion and biogas production. The difficulty of utilizing pineapple waste lies in the composition of waste, it is mainly cellulose. Pineapple waste has a high acid content which influences microbial activities. The process can be operated both at mesophilic (37°C) and thermophilic (55°C) temperature. Tanticharoen et al (12) reported that solids degradation and biogas production were higher in thermophilic digester compared to mesophilic digester. Moreover, soluble organic matters in the thermophilic digester was also higher than that in the mesophilic digester. It was suggested that microbial activities between these two temperatures may differ in the ability to utilize complex substrates. This investigation revealed microbial population and intermediate compounds involved in mesophilic and thermophilic anaerobic digestion of pineapple cannery wastes.

2. Materials and Methods

2.1 Anaerobic Digester

Two 3,500 cm^3 glass bottle digesters with liquid volume of 3,000 cm^3 were used for mesophilic and thermophilic digestion (Figure 1). Both mesophilic and thermophilic digesters were controlled at constant temperature by placing them in 37°C and 55°C water baths, respectively. Each digester had been operated for more than 5 years at mesophilic and thermophilic conditions and were well acclimatized with semi-continuous feeding of 30 grams of pineapple solid wastes every 2 days.



Anaerobic digester Biogas production monitor unit (using water replacement)

Figure 1 Anaerobic reactor diagram

2.2 Pineapple Solid Wastes

The collected peels and cores of solid wastes from pineapple cannery were chopped and then stored in a freezer until used. Pineapple cannery wastes is high in cellulose, hemicellulose, and sugars as shown in Table 1. Normally, each digester was fed 30 grams of fresh waste every 2 days. An equal amount of effluent was removed to keep volume of liquid constant.

Table 1 Chemical composition of pineapple solid wastes

Composition	% of fresh weight	
	Range	Average
Moisture	87.0-91.0	89.0
Glucose	0.9-1.8	1.4
Fructose	0.5	0.5
Sucrose	1.1-5.1	3.1
Cellulose	1.6-2.1	1.8
Hemicellulose	2.3-2.9	2.6
Lignin	0.4-0.5	0.4
Others	0.2-2.2	1.2

2.3 Microbial Activity Test

2.3.1 Microorganisms

The microorganisms used in this study were obtained from the mesophilic and thermophilic anaerobic digesters treating pineapple cannery wastes.

2.3.2 Digestion Fluid

Normally, the media used for growth of methanogenic bacteria contains a variety of inorganic salts and growth factors (13). To avoid complexity in preparing the media, the digestion fluid from anaerobic digestion of pineapple solid wastes was used as basal medium (14). To reduce the residual nutrient which microorganisms can use as carbon source, the basal medium was left until complete digestion after withdrawing from the digestor (2-3 months). Then, digestion fluid was filtered through whatman no. 4 and sterilized.

2.3.3 Activity test

Activity test was used to determine the ability of microorganisms from the digester in utilizing various carbon sources as substrate. The experiment was conducted using serum vial technique. This was done in anaerobic conditions in 50 ml serum vial bottles containing 10% (v/v) seed and 40 ml of digestion fluid mixed with tested substrate at pH 7.0-7.2 under N_2 gas. These vials were sealed with rubber stoppers and aluminum caps, then incubated at the required temperatures.

Microbial growths in various carbon sources were conducted in serum vials similar to activity test and incubated 10 days for microbial growth determination.

2.4 Determination of Intermediate Compounds during Mesophilic and Thermophilic Anaerobic Digestion at Different Conditions

2.4.1 Digesters Operated at Normal Condition

After the addition of pineapple cannery wastes into mesophilic and thermophilic anaerobic digesters, the effluent was drawn on various days. The samples were centrifuged at 10,000 rpm/min to remove debris. The clear supernatant was analyzed for volatile fatty acids and alcohol.

2.4.2 Addition of Methanogenesis Inhibitors

After 6 hours of treatment, the effluent containing volatile fatty acids was transferred to serum vial bottles without and with addition of various inhibitors under anaerobic conditions. The concentration of inhibitors were 20 mM 2-bromoethane sulfonate, 32 mM phenol, and 2.5 mM chloroform. Gas composition and intermediates were determined at various days and compared to the control without inhibitor.

2.4.3 Digesters Operated at Overloading Condition

30 grams per day of fresh pineapple wastes were fed to each digester. Intermediate compounds were detected by removing effluent everyday before new loading substrate. During over shock loading, high concentration of organic acids with lower pH and inhibition of methanogens were analyzed to determine the accumulation of intermediate compounds.

2.4.4 Shifting of Operating Temperature

Operating temperature in the mesophilic digester was shifted from mesophilic temperature (37°C) to thermophilic temperature (55°C). On the other hand, the thermophilic digester was shifted from 55°C to 37°C. Gas composition and intermediates were determined at various days and compared to the control without temperature shifting.

2.5 Analytical Procedures

2.5.1 Microbial growths in various carbon sources were determined by turbidity at OD_{660}.

2.5.2 Methane and carbon dioxide were analyzed on a Shimadzu gas chromatography GC 9A equipped with thermal conductivity detector (TCD) and a column (2 m x 1/3 cm) packed with Porapak-N 80/10 mesh. The carrier gas was helium and the oven temperature was 70°C.

2.5.3 Volatile fatty acids and alcohols were analyzed on a Shimadzu gas chromatography GC 14A equipped with a flame ionization detecter (FID) and a column (20m) packed with Carbo-pack B-DA 100/120 mesh, 4% carbowax. The carrier gas was nitrogen and the oven temperature was 170°C.

3. Results and Discussion

3.1 The Distribution of Microbial Populations and Intermediate Compounds in Mesophilic and Thermophilic Digesters

During the semicontinuous digestion of pineapple wastes, biogas composition consisted of 60% CH_4 and 40% CO_2; intermediate compounds were checked by removing effluent at various time intervals. Effluent from mesophilic digester (37°C) contained mainly acetic acid, propionic acid, and less in butyric acid (Figure 2). In contrast, propionic acid was not found in thermophilic digester (55°C); only acetic acid, butyric acid, and ethanol were found as the major products (Figure 3). The result corresponds with the activity tests in which microorganisms from thermophilic digester were unable to utilize propionic acid (Table 2). The study of activity tests of mesophilic and thermophilic microorganisms in utilizing various carbon sources shows the similarity of microorganisms in both digesters except for

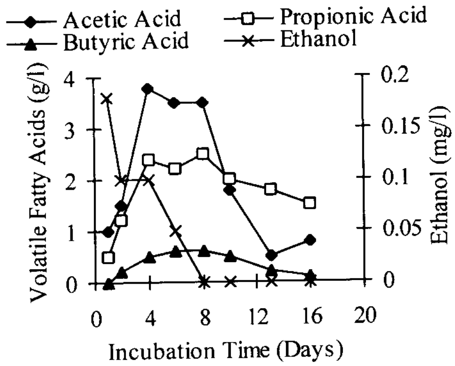


Figure 2 Intermediate compounds profile in mesophilic anaerobic digestion of pineapple solid wastes.

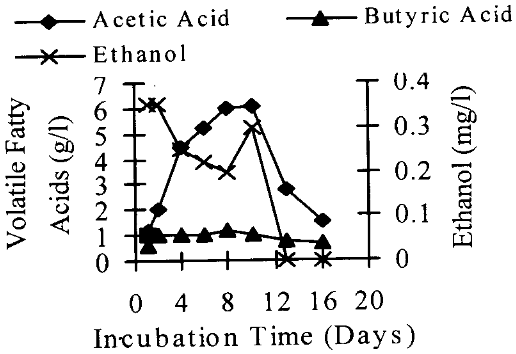


Figure 3 Intermediate compounds profile in thermophilic anaerobic digestion of pineapple solid wastes.

Table 2 Ability of microorganisms from mesophilic (37°C) and thermophilic (55°C) anaerobic digester in utilizing various carbon sources for growth and biogas composition

Substrate	Mesophile			Thermophile		
	Growth[a]	$\%CH_4$	$\%CO_2$	Growth[a]	$\%CH_4$	$\%CO_2$
1.0% α-Cellulose	+	48.5±4.1	37.5±5.0	+	60.1±2.0	30.5±5.1
1.0% Starch	+	32.3±2.6	61.0±7.0	+	20.5±3.1	79.5±3.1
1.0% Xylan	+	33.8±13.7	55.4±8.6	+	10.0±1.3	85.7±4.5
1.0% Casein	+	60.6±5.0	39.4±3.8	+	57.1±11.7	42.9±7.2
0.1% Glucose	+	67.5±0.5	14.3±1.7	+	48.7±2.1	35.1±2.6
0.1% Xylose	+	47.1±3.9	48.9±1.2	+	19.7±5.6	62.2±4.8
0.1% Formic acid	+	41.3±3.8	38.1±4.1	+	20.9±2.9	38.1±1.8
0.1% Acetic acid	+	79.0±5.0	5.0±1.4	+	50.0±10.3	22.3±3.4
0.1% Propionic acid	+	68.9±6.3	21.3±3.2	–	0	0
0.1% Butyric acid	+	8.1.1±12.1	17.9±4.9	+	68.4±3.8	9.7±1.6
0.1% Lactic acid	+	52.1±5.2	11.3±3.7	+	47.3±5.6	7.4±2.9
0.1% Methanol	+	20.0±1.9	52.3±2.6	+	50.0±2.7	28.5±4.6
0.1% Ethanol	+	50.6±6.3	40±1.1	+	43.2±3.3	37±4.2
0.1% Propanol	+	22.3±4.2	30.6±2.4	–	0	0
0.1% Butanol	+	80.6±6.1	10.0±2.2	+	45.0±8.9	28.2±1.3

[a] – = no growth and + = growth

propionic acid and propanol utilization. The presence of bacteria in mesophilic and thermophilic digesters capable of utilizing high molecular weight compounds such as starch, α-cellulose, xylan, and casein as well as glucose and xylose at temperature correspond to the inoculum's origin. To identify volatile organic acid and alcohol utilizing bacteria, microorganisms from both digesters can utilize all of the tested carbon sources, except propionic acid and propanol utilizing population were found only in the mesophilic digester. The results suggested that propionic acid and propanol utilizing bacteria was not favorable at thermophilic temperature.

3.2 Intermediate Compounds Involved During Anaerobic Digestion of Pineapple Cannery Wastes

To detect the intermediate compounds, the experiments were designed to accumulate these intermediates by setting unsuitable conditions for methane production. This can be done by either inhibition of degrading mechanism or retardation of digestion rate. In this study, we investigated the accumulation of intermediate compounds by the addition of methanogenesis inhibitors, shock loading, and alterating the incubated temperature. The experiments were supported by the following evidence:

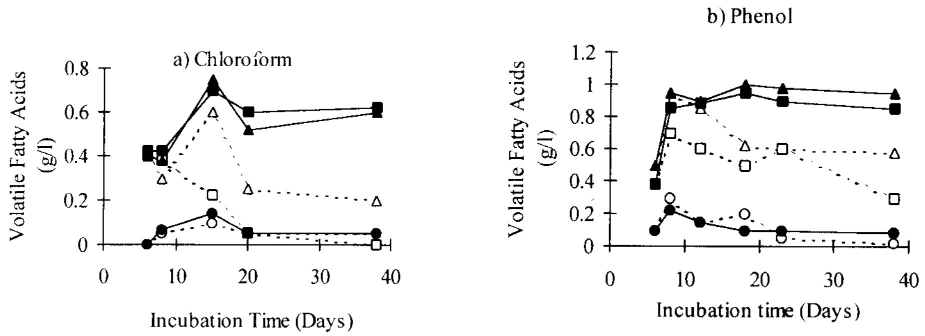
3.2.1 Methanogenesis Inhibitors

Chloroform, phenol, and 2-bromoethane sulfonate were used as inhibitors for acetoclastic methanogenesis (13, 15-16). During the anaerobic digestion, the accumulation of volatile fatty acids as intermediate compounds was observed. The results were compared to the experiment without the application of inhibitor (control).

In the mesophilic digestion, there was an increasing rate of acetic acid accumulation followed by accumulation of propionic and butyric acids as intermediates (Figure 4a-c). Whereas, acetic and butyric acids were the only intermediates found among the thermophilic digestion (Figure 5a-c). Once again in the absence or presence of inhibitors, no accumulated propionic acid was found in the thermophilic digester.

3.2.2 Overloading

The optimal operating condition of anaerobic digestion of pineapple wastes is: biogas composition contains 60% methane and 40% carbon dioxide; pH of aqueous phase is 6.5-7.0 and slight amount of volatile organic acids and ethanol accumulated in both mesophilic and thermophilic digesters. After 4 days of shock load operation, the rate of methane production decreased, indicated by the increase of carbon dioxide (Figure 6a and 7a); the increased accumulation rate of intermediate organic acid compounds caused pH rapidly to drop from 6.5 7.0 to 4.5 at day 21 and 13 in the mesophilic and thermophilic digesters, respectively (Figure 6b and 7b). Rate of digestion in the thermophilic digester was higher than that in the mesophilic digester corresponding to the higher accumulation of volatile organic acids. Due to high accumulation of volatile organic acids and low pH, methanogenic bacteria was inhibited (17-18) and methane production completely ceased at day 11 and 25 in thermophilic and mesophilic digesters, respectively. Decreased content of methane, acetic acid, propionic acid, butyric acid, and ethanol were found as intermediates in the mesophilic digester (Figure 6); whereas acetic acid, butyric acid, and ethanol were found as intermediates in the thermophilic digester (Figure 7). Similar results were found in Weigel et al (19) and Sonne-Hassen et al (20).



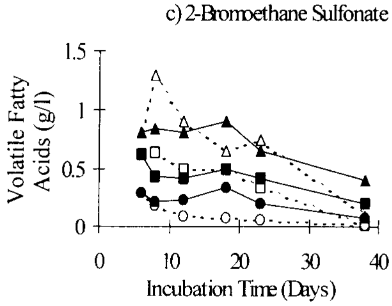
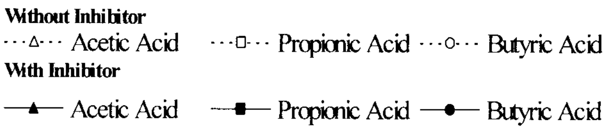
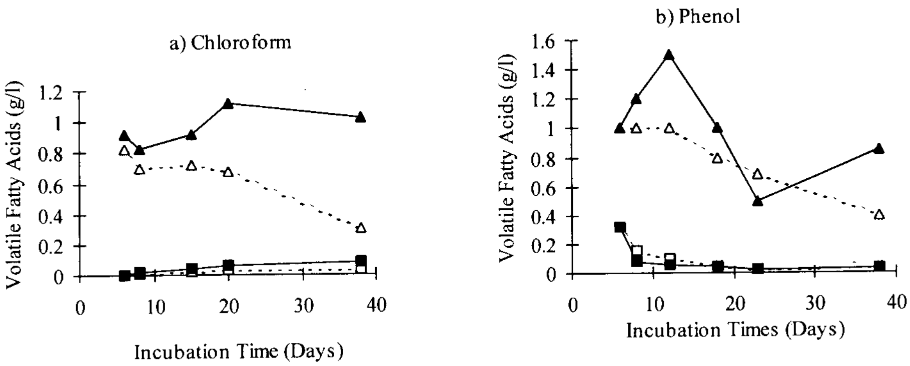


Figure 4 The accumulation of volatile organic acids in the mesophilic anaerobic digester in the addition of methanogenesis inhibitors.





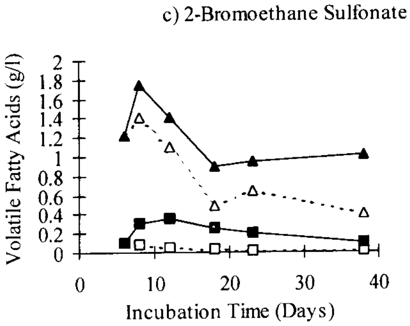


Figure 5 The accumulation of volatile organic acids in the thermophilic anaerobic digester in the addition of methanogenesis inhibitors.

Without Inhibitor

- - -Δ- - - Acetic Acid   - - -□- - - Butyric Acid

With Inhibitor

—▲— Acetic Acid   —■— Butyric Acid

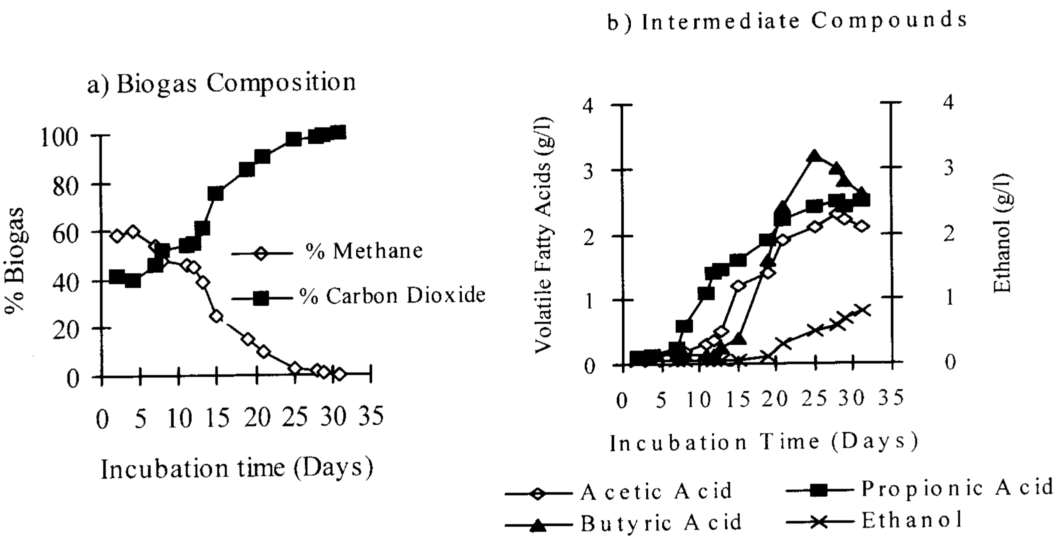


Figure 6 Biogas composition (a) and intermediate compounds (b) profiles in the mesophilic digester during pineapple wastes overloading.

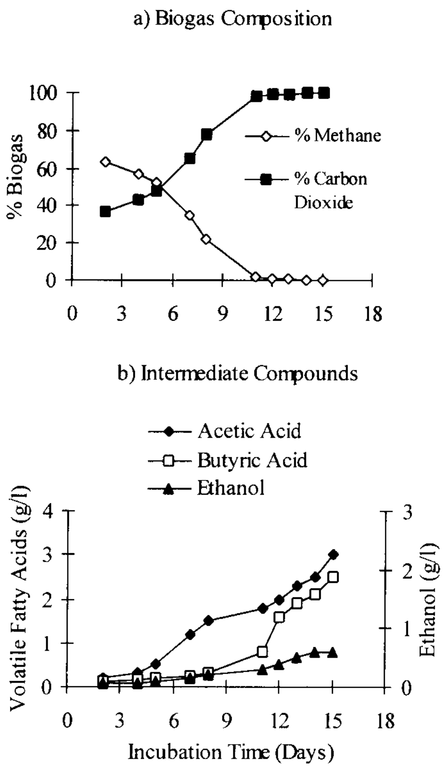


Figure 7 Biogas composition (a) and intermediate compounds (b) profiles in the thermophilic digester during pineapple wastes overloading.

3.2.3 Shifting the Operating Temperature

When the operating temperature was shifted from 37°C to 55°C, there was a time lapse for mesophilics microorganisms to grow in thermophilic temperature and adapt to such a change (Figure 8). The accumulated intermediates (acetic acid, butyric acid, and ethanol) were similarly found in 55°C digester (Figure 3). Similarly, thermophilic microorganism had a lag phase to acclimatize such impact when temperature shifted from 55° C to 37°C. However, the accumulated intermediates were similarly found in either 37° C digester (Figure 2) or 55°C shifted to 37°C digester (Figure 9).

The production of propionic acid changed after a few days at new operating temperature. After shifting from 37°C to 55°C, propionic acid production completely stopped (Figure 8). In contrast, an increase of propionic acid concentration was observed when the operating temperature shifted from 55°C to 37°C (Figure 9).

The results indicated that acetic and butyric acids were the main intermediates which occurred in mesophilic and thermophilic temperatures whereas propionic acid was found in mesophilic but not in thermophilic temperatures. It was suggested that β-oxidation of butyric acid to acetic acid occurred in both mesophilic and thermophilic digestion. In addition, propionic acid occurred in mesophilic

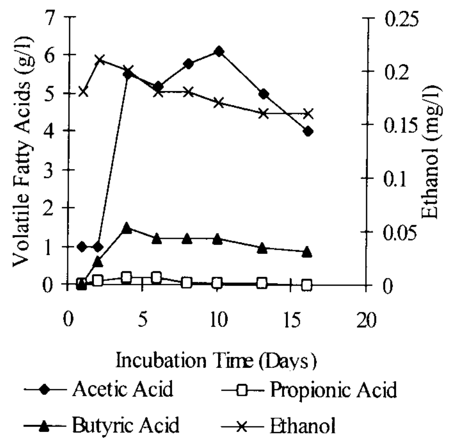


Figure 8 Intermediate compounds in mesophilic digester during shifting temperature from 37 °C to 55 °C.

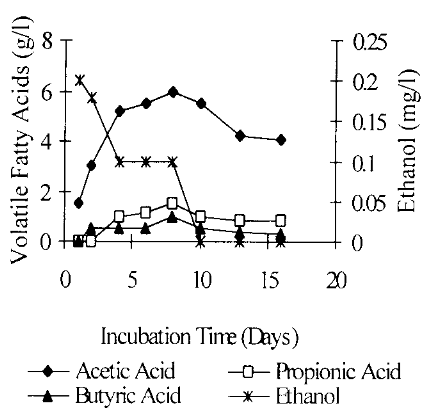


Figure 9 Intermediate compounds in thermophilic digester during shifting temperature from 55°C to 37°C.

digestion via the decarboxylation of butyric acid. In general, the degradation of butyrate even in the aerobic or anaerobic condition was found that acetate formed by the β-oxidation of butyrate (21). Gourdon et al (22) studied n-butyrate catabolism in the treatment of semisynthetic landfill leachate on anaerobic filter under sequential feeding condition and the result indicated that the pathway of butyrate degradation was not only by the reaction of β-oxidation but also by decarboxylation. Therefore, acetate and propionate were found in effluent; propionate degraded to acetate and later converted to methane.

The mixed culture in mesophilic or thermophilic digesters operating more than 5 years could adapt themselves and stabilize in its digestion to these temperatures. These results indicated that no propionic acid was found and propionic acid could not degrade in thermophilic anaerobic digestion. It might be that propionic acid was more toxic to thermophilic bacteria than mesophilic bacteria. Therefore, to avoid this toxicity no propionic acid was produced in thermophilic digester.

4. Conclusions

Each mesophilic and thermophilic anaerobic digester from pineapple cannery wastes has been operating at constant temperature of 37°C and 55°C for more than 5 years, respectively. Microbial populations should be steady in terms of selection and acclimatization. The mesophilic cultures were able to utilize various selected substrates, i.e. polysaccharides, disaccharide, mono-saccharides, volatile organic acids, and alcohol, as sole carbon source. Similar microbial populations were found in the thermophilic digester except propionic utilizing bacteria.

In the presence of methanogenesis inhibitors or overloading substrate to detect intermediate compounds, it was found acetic, propionic, and butyric acids as intermediates in the mesophilic digester. Most of these intermediate compounds were also found in the thermophilic digester except propionic acid. This might be because propionic acid was more toxic to thermophilic bacteria than mesophilic bacteria.

When culture acclimatized to thermophilic temperature (55°C) was shifted back to mesophilic temperature (37°C), ethanol, acetic acid, propionic acid and butyric acid were detected. When culture at mesophilic temperature (37°C) was shifted to thermophilic temperature (55°C), most of these intermediate compounds were also found, but not propionic acid. Therefore, temperature had an effect on intermediate compounds between mesophilic and thermophilic anaerobic digestion.

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