

## Variety of End Products at Different Ph Conditions and Thermokalite Pretreatment Cooperate with Ph Controlling During Anaerobic Digestion

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**Abstract:** In this study, we show that the acetic acid was mainly product, accounting for 59.83% of total VFA, propionic acid only accounting for 3.94%, composition of end products is most reasonable. Moreover, when the pH was 4~5 in anaerobic digestion, it was beneficial to following methanogenic process. In addition, we show that the method that thermokalite pretreatment cooperated with pH controlling could promoted efficiency of acidification and optimize composition of acidification production at same time, cumulative concentration of total VFA increased by 68%, composition of end products was reasonable. Results of analysis from SEM showed that microstructure of kitchen wastes had changed obviously after thermokalite treatment. Results of kinetics analysis showed that acidogenic rate of the kitchen wastes was faster after thermokalite treatment.

**Keywords:** Acidification products, anaerobic digestion, pH controlling, thermokalite pretreatment

### INTRODUCTION

The acidogenic process is necessary for anaerobic digestion of organic matter. During this period, previous hydrolysates such as dissoluble carbohydrates, aminophenol, long chain fatty acid etc are further biodegraded to acetic acid and H<sub>2</sub> used by methanogen. Some scholars think that there are three types in acidogenic process, they are acetic acid-type or ethanol-type fermentation, butyric acid-type fermentation and propanoic acid cumulation type fermentation (Cohen *et al.*, 1984; Ren *et al.*, 1995; Liu, 2008). But, butyric acid is not easy be used by methanogen, propanoic acid is poisonous for methanogen. So it is detrimental to subsequent methanogenesis process if concentration of butyric acid and propanoic acid is high. Hanaki's research result (Hanaki *et al.*, 1994) also prove the speed propanoic acid translate into acetic acid is slowest. Thus propanoic acid is most poisonous for methanogenic system. The end products of acetic acid-type or ethanol-type fermentation is mainly acetic acid or ethanol, they all is easy be used by methanogen, the fermentation type is optimum for methanogenic process. Base on the above-mention reason, optimization of end products is a key problem for increasing utilization rate of methanogen for end products, reducing pernicious effects for mehanogen.

Ren *et al.* (1995) analyzed the physiological process in acidogenic fermentation of organic waste water, then put forward the engineering strategy how optimize

technological conditions in acidogenic phase reactor (Li and Ren, 1998). Elefsinotis and Oldham's (1991) research result proved as well proportion of acetic acid was highest in end products. Moreover, other's research results (Lin and Lay, 2004; Liu *et al.*, 2004) also showed difference of C/N had obvious effects on type of end product. However, in these previous studies, the discussions about influence of key controlling factors on composition of end products have been absent yet, actual controlling method has not yet been put forward. In this study, with the shortcoming of previous studies, on the basis of study influence of pH on composition of end products, will discuss in detail the feasibility of the method pH cooperate with thermokalite pretreatment optimize composition of end products and promote efficiency of acidification at the same time. Besides, the mechanism of controlling was analyzed.

### MATERIALS AND METHODS

**Experimental device:** The device of acidification experiment was showed by Fig. 1. The acidogenic reactor was a glass vessel with volume of 3L, temperature of digestion was controlled by homothermal water bath. The feedstock port was sealed with rubber plug after kitchen wastes was loaded into glass vessel, then keep fermenting under anaerobic circumstance after nitrogen purge. Gas was collected by gas collector with saturate NaCl solution.

**Experimental materials:** The kitchen wastes samples was collected from student canteen in Chongqing

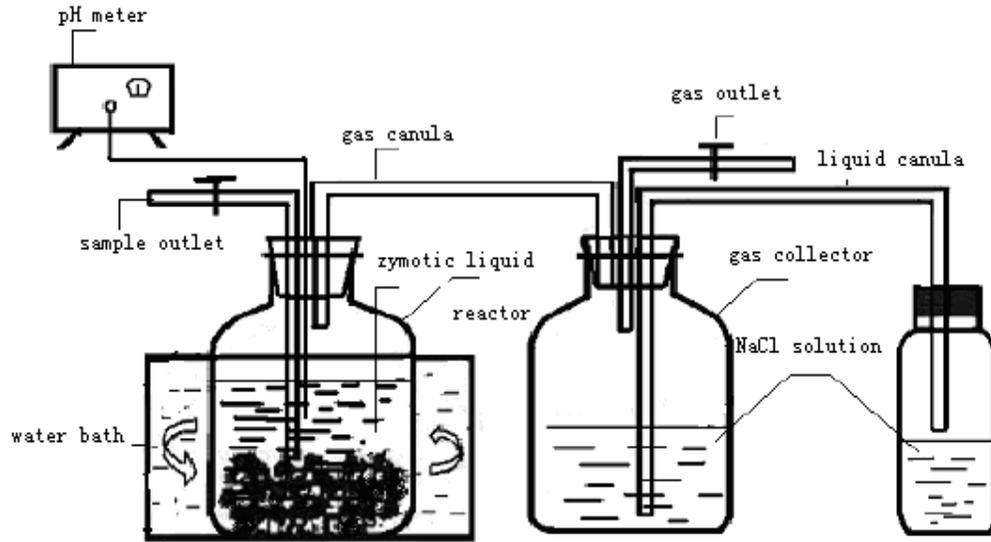


Fig. 1: The device of anaerobic digestion

technology and business university. The sundries of sample such as paper scrap, wood, glass, rock, metal and plastic etc were cleared out, then sample was pulverized into small fragments with grain diameter about 20 mm and stored in refrigerator at 4°C. Solid composition of kitchen wastes sample is 21.47%, this volatile solid is 90.87%. Carbohydrate content is 70.21%, protein content is 12.85%, fattiness content is 13.65% in solid composition. The active sludge come from the anaerobic digestion pool in Chongqing tangjiaqiao sewage water plant. Water content of sludge is 94.23%, MLSS was 47.36 g/L, VSS was 32.48 g/L, the domestication sludge was used as seed sludge.

**Operation methods:** According to literature (Zhao *et al.*, 2006; Li *et al.*, 2009), the proportion of seed sludge accounting for kitchen wastes is different from 10 to 100%. Base on the previous experimental result from our research teams (Wu *et al.*, 2009), digestion and gas generation was better when proportion of seed sludge was 30%. Consequently, in this experiment, 30% was selected as proportion of seed sludge. That was to say 1.5 kg kitchen wastes and 0.45 kg seed sludge was loaded into the reactor, then the water was added until it was about 2.5 L. the temperature of system was controlled at about 35°C by homothermal water bath. Leachate collected on the same day was loaded into reactor per day, then the water was added to reactor until it was about 3 L. The pipe was purged with nitrogen. The pH of system was adjusted as 4~5~5~6~6.5~7.5~7~8 with 2 mol/L HCl or NaOH solution during test period, respectively. acidification experiment lasted 15 d.

In thermokallite pretreatment test, kitchen wastes was soaked in 5 mol/L NaOH solution for 6 h, then loaded into

reactor after rinsing with water, adjusted pH of system to 4~5 with 2 mol/L HCl solution.

**Test methods:** Activity of Acetokinase (AK) was measured with method of Ross (1955), activity of Phosphotransferase Acetyl (PTA) was measured with method of Andersch's *et al.* (1983), activities of Butyrate Kinase (BK) and Phosphotransferase Butyryl (PTB) were measured with method of Zhu and Yang's (2003), activity of Methylmalonyl CoA carboxyltransferase (MCM) was measured with method of Kellermeyer and Wood's (1969). Volatile fatty acids were determined by gas chromatography (HP-6820). zymotic fluid was collected from acidogenic reactor and disposed for 10 min by centrifugal machine at 6800 rpm, filtrated with micropore membrane Filters, other steps was same with disposal method of standard sample. The test condition of gas chromatography: FID detector, PEG-20M capillary column (30 m×0.32 mm×0.5 μm), rose the temperature with one step temperature-programmed, initial temperature was 80°C, keep 3 min, then rose the temperature to 210°C at the rate of 15°C/min, keep 2 min. Injector and detector temperature was 250°C. Apparent structure of kitchen wastes samples was observed and analyzed by SEM (Hitachi-S4700). In order to convenience of results discussion, formic acid was seen to acetic acid, isobutyric acid was seen to butyric acid, common valeric acid was seen to valeric acid.

## RESULTS AND DISCUSSION

**Influence of pH on production and composition of Volatile Fatty Acids (VFA):** Table 1 showed that cumulative concentration of total VFA was the lowest,

Table 1: Cumulative concentration of individual VFA at different pH

| Range of pH | Cumulative concentration of volatile fatty acids (g/L) |                |              |              |                 |
|-------------|--------------------------------------------------------|----------------|--------------|--------------|-----------------|
|             | Acetic acid                                            | Propionic acid | Butyric acid | Valeric acid | Total VFA (g/L) |
| 4~5.0       | 13.38                                                  | 0.88           | 5.93         | 2.17         | 22.36           |
| 5~6.0       | 13.71                                                  | 3.62           | 9.06         | 2.26         | 28.65           |
| 6.5~7.5     | 10.98                                                  | 3.39           | 28.51        | 3.35         | 46.23           |
| 8~9.0       | 11.63                                                  | 2.99           | 19.91        | 2.25         | 36.78           |

Table 2: Maximum cumulative concentration of individual VFAs produced after pretreatment

| pH = 4~5            | Variety of acid |                |              |              |                 |
|---------------------|-----------------|----------------|--------------|--------------|-----------------|
|                     | acetic acid     | propionic acid | butyric acid | valeric acid | Total VFA (g/L) |
| Concentration (g/L) | 21.40           | 1.45           | 12.21        | 3.37         | 37.68           |
| Percentage (%)      | 56.79           | 3.84           | 32.42        | 8.95         |                 |

Table 3: Activity of key enzymes at different pH condition

| Range of pH | Activity of key enzymes (U/mg) |      |      |      |      |
|-------------|--------------------------------|------|------|------|------|
|             | AK                             | PTA  | MCM  | BK   | PTB  |
| 4~5         | 2.36                           | 0.66 | 0.86 | 0.36 | 0.46 |
| 5~6         | 1.62                           | 0.72 | 3.22 | 0.68 | 0.82 |
| 6.5~7.5     | 2.89                           | 0.58 | 2.86 | 1.86 | 1.62 |
| 8~9         | 0.86                           | 0.21 | 1.23 | 2.76 | 3.62 |

only 22.36 g/L when pH was 4~5. Cumulative concentration of total VFA was 28.65 and 36.78 g/L when pH was 5~6 and 8~9, respectively. Cumulative concentration of total VFA was the highest, reached to 46.23 g/L when pH was 6.5~7.5, the result was identical with Zhao's test observation (Zhao *et al.*, 2006).

Mainly acidification production was acetic acid, accounting for about 59% of total VFA and propionic acid was the smallest when pH was 4~5. Propionic acid increased obviously, accounting for about 12.64% when pH was 5~6. Mainly acidification production was butyric acid, accounting for about 61.66% of VFAs when pH was 6.5~7.5. Mainly acidification production was also butyric acid, accounting for about 54.13% of VFAs when pH was 6.5~7.5. So proportion of end products in VFA was remarkably different at different pH range.

The above-mention results showed proportion of acetic acid was highest and proportion of propionic acid was lowest in VFAs, the composition of end products was most reasonable when pH is 4~5, it was advantage for following methanogenic process. However, efficiency of acidification was lower obviously at low pH circumstance, cumulative concentration of total VFA was smallest. Consequently, in order to solving the contradiction above, the method pH cooperate with thermokalite pretreatment will be discussed. It will be actual valuable for the two phase anaerobic digestion.

**Influence of pH controlling cooperate with thermokalite pretreatment on VFAs:** Table 2 showed that cumulative concentration of total VFA reached to 37.68 g/L, increased by 68% when pH was 4~5. Mainly acidification production was acetic acid yet, accounting for about 57% of total VFA, propionic acid was also the smallest, only accounting for 3.84% of total VFA. The results illustrated the method of thermokalite pretreatment cooperate pH controlling could promote efficiency of acidification, composition of end products was also reasonable.

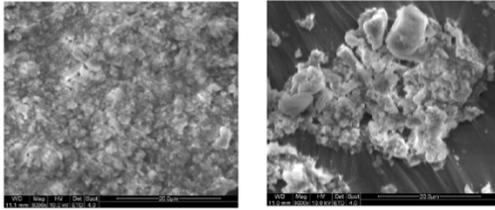
Metabolic Mechanism analysis of pH control composition of acidification products

Some research result have proved (Ramsay and Pullamm, 2001) growth of acetogen would be inhibited by alkaline circumstance, it caused content of butyric acid increase rapidly. Fu's research result (Fu and Mathews, 1999) also showed the best pH condition promoted growth of lactobacillus was between 5 and 6, because lactic acid was optimum substrate for *Bacterium acidipropionici*, so propionic acid would cumulate when pH was 5~6. Another research result also showed predominant bacterium crowd in system would be *Fusobacterium bacterium* at low pH condition, it was not necessary that the bacterium crowd coupled with *Bacterium acidipropionici* in acidogenic process, so production of propionic acid would reduce.

In this study, because of carbohydrate content accounting for over 70% of organic matter in kitchen wastes, so mainly intermediary metabolite in anaerobic digestion of kitchen wastes was glucose. The glucose was converted to pyruvic acid through EMP, HMP or ED path, then pyruvic acid was further converted to various end products by anaerobe. In the above-mention metabolic process, Acetokinase (AK), Phosphotransferase Acetyl (PTA), Butyrate Kinase (BK), Phosphotransferase Butyryl (PTB) and Methylmalonyl CoA carboxyltransferase (MCM) would work together, the activity of these key enzyme would be affected by pH, caused composition of end products was different at different pH conditions.

Table 3 showed the activity of key enzymes at different pH range after fermenting 72 h. It could be seen that activity of AK was higher, activity of MCM was the lowest when pH was 4~5. Activity of MCM was higher obviously when pH was 5~6. Activity of BK and PTB was stronger under alkaline conditions. Variety of activity reflected the influence of pH on key enzymes.

**Microcosmic mechanism analysis of thermokalite treatment:** The observations from SEM showed solid structure of kitchen wastes was broken up and become loose, its surface become concavo-convex, there were



(a) Before treatment (b) After treatment

Fig. 2: Microphotograph of the kitchen wastes before and after pretreatment

many micropore after thermokalite treatment. Test result also showed solid content decreased by 27.26% after thermokalite treatment. Figure 2 gives the microphotograph of the kitchen wastes before and after pretreatment.

Some scholar thought (Xu and Wu, 2006) heat treatment could make grain of organic wastes bulge, enlarged aperture of outermost layer of organic matter, was advantage for releasing of macromolecule in grain of organic wastes. On the other hand, heat treatment caused organic matter in wastes fluidify, so promoted degradation of large molecular organic matter.

Neyens and Baeyens's (2003) research also proved the heat treatment at 60~180°C could dissolve outermost layer of organic matter, quickened release of inner organic matter. Moreover, in process of alkali treatment, OH<sup>-</sup> could destroy structure of organic matter, hydrolyzed and saponified protein, fattiness and carbohydrate, promoted them to dissolve and transfer to liquid. So these matter would be utilized by anaerobe in earlier stage of digestion.

**Kinetics analysis of thermokalite treatment:** Generally, acid-producing kinetics was base on Monod equation. Consequently, in this study, acid-producing kinetics model of kitchen wastes treated by thermokalite in anaerobic digestion was also base on Monod equation. The Eq. (1) and (2):

$$V = \frac{V_{\max} S}{K_s + S} \quad (1)$$

$$V = \frac{-dS / dt}{X} \quad (2)$$

In equations above, S is concentration of organic matter (g/L), V is specific degradation rate ratio of organic matter (d<sup>-1</sup>), V<sub>max</sub> is maximal degradation rate ratio of organic matter (d<sup>-1</sup>), X is concentration of anaerobe (g/L), K<sub>s</sub> is half-saturation constant (g/L).

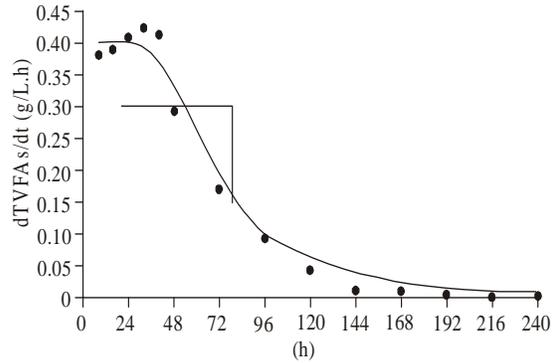


Fig. 3: Curve of acid-producing rate

Supposed all organic matter to translate into VFAs and that VFAs was intermediate products in degradation of organic matter, then the Eq. (3) could be induced:

$$(dS_{TVFAs} / dt) = -kdS/dt \quad (3)$$

In equation above, S<sub>TVFAs</sub> is the concentration of total VFAs (mg/L); k is proportion coefficient less than 1.

Consequently, Eq. (1) could be convert to acid-producing kinetics Eq. (4), but significance of parameter in the equation have changed:

$$V = \frac{V_{\max VFAs} S}{K_s + S} \quad (4)$$

In equation above, S is concentration of unacidogenic substrate (g/L), K<sub>s</sub> is half-saturation constant (g/L), V<sub>max,VFAs</sub> = RV<sub>max</sub>, R is proportion coefficient.

Stated thus, acid-producing rate could be calculated approximately by Eq. (5):

$$dS_{TVFA}/dt = (S_{TVFAs2} - S_{TVFAs1})/(t_2-t_1) \quad (5)$$

According to data from previous test and calculation of Eq. (5), acid-producing rate curve as showed Fig. 3 could be obtained. It could be seen from Fig. 3, there was a section of flat curve, this could be seen to zero order reaction stage. In the stage, acid-producing bacterium was not affected by concentration of substrate, acid-producing rate was the fastest. So average acid-producing rate in the stage could be seen to maximal value of acid-producing rate. through calculating, dS<sub>TVFAs</sub>/dt = 0.402 g/Lh, through testing, average concentration of anaerobe in reactor was 2.79 g/L. So according to Eq. (2), maximal value of acid-producing rate as showed below could be obtained:

$$V_{\max, VFAs} = (1/X) \times (dS_{TVFAs}/dt) = (1/2.79) \times 0.402 \times 24 = 3.46(d^{-1}) \quad (6)$$

Because of  $K_s = S$  when  $V = (1/2) \times V_{max}$ , so a line intersected to curve could be drawn base on  $dS_{TVFAs}/dt = 0.201$  (half of 0.402), then draw a line base on the point of intersection perpendicular to X axis, obtained a point of intersection, it was residence time, 69.82 h. Through testing, residual organic matter was 83.29 g/L when residence time was 70 h, that was to say  $K_s = 83.29$ . Consequently, cid-producing kinetics Eq. (6) could be obtained:

$$V = (3.46 \times S) / (83.29 + S) \quad (7)$$

The results through calculating acid-producing kinetics constants of unpretreatment kitchen wastes showed  $V_{max, VFAs} = 2.02 \text{ d}^{-1}$ ,  $K_s = 96.35 \text{ g/L}$ . It illustrated acidogenic process of kitchen wastes after thermokalite treatment was faster obviously than unsettled kitchen wastes.

### CONCLUSION

- Proportion of acetic acid was highest in VFAs, reach to 59.83%, proportion of propionic acid was lowest in VFAs, only was 3.94%, the composition of end products was most reasonable when pH is 4~5, it was advantage for following methanogenic process. However, efficiency of acidification was lower obviously at low pH circumstance, cumulative concentration of total VFA was smallest in this time.
- The method of thermokalite pretreatment cooperate pH controlling could promote efficiency of acidification, composition of end products was yet reasonable.
- The observation by SEM showed microcosmic structure of organic matter had changed profitably after thermokalite treatment. The result of kinetics analysis showed acid-producing kinetics constants  $V_{max, VFAs}$  and  $K_s$  of kitchen wastes after thermokalite treatment was 3.46 and 83.29, respectively. acid-producing rate was faster obviously than unsettled kitchen wastes.

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