**Inhibition of thermophilic anaerobic digestion of waste food by long chain fatty acids and propionate**

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**Abstract**

Different initial concentrations of slurry waste food (discarded food), with and without the overlying layer of fat derived from the waste were anaerobically digested at 55°C. At solids concentrations less than 20 g/l no significant difference was observed in terms of volatile fatty acids and methane production between samples containing fat layer and samples without it. However, at higher concentrations, differences became more obvious. Biogas released from a 50 g/l fat excluded sample was around 100% more than the gas generated from the fat included sample with the same initial solids concentration. Inhibition by propionate was not significant in concentrations less than 2000 mg/l. In the absence of fats, the inhibition caused by accumulation of propionate could be overcome partially by the methanogenic bacteria. Based on the energy generated in the form of methane, it was found that thermophilic anaerobic digestion of waste food could be an autothermal process for fat excluded feeds.

**Keywords:** Thermophilic anaerobic digestion; Waste food; Volatile fatty acids; Biogas.

**INTRODUCTION**

Thermophilic anaerobic digestion (TAD) of biodegradable organic fraction of solid wastes appears to be an attractive method for environmental protection and energy saving (Cooney and Wise, 1975; Cecchi et al., 1993 and Sosnowski et al., 2003). This is, however, due to its merits in destruction of organic solids, improvement in solid-liquid separation, and inactivation of pathogen organisms better than mesophilic anaerobic digestion (Buhr and Andrews, 1977; Rinkus et al., 1982). Waste Food (WF) – anaerobic digestion of which is emphasized in this work – contains hygienic substances amenable to anaerobic digestion free from disturbance of common inhibitors (Dohanyos and Zabranska, 2001). However, known inhibitors in this regard are long-chain fatty acids (LCFA) and propionate (Pr). These two can specifically inhibit the activity of methanogenic bacteria; resulting in overall prevention and decrease the total biogas achievable (McCarty and McKinney, 1961; Hobson and Shaw, 1976; Koster and Carmer, 1987; Fukuzaki et al., 1990; Angelidaki and Ahring, 1992; Rinzema et al., 1994; Ayithi and Sreekrishnan, 2001; Ghanem et al., 2001). Since the biogas produced by an anaerobic digestion is usually used for its own heat requirements, any kind of inhibition on methanogens would increase the energy consumption from external sources. As a result, it could be concluded that by controlling the total biodegradable solid content and simple deleting the fat contents from the WF slurry, the inhibitory effects of LCFA and Pr on methanogens will be reduced considerably. This may cause to receive enough methane to compensate heat and energy requirements during the period of anaerobic digestion. However, there is no experimental study showing the effects of LCFA and Pr on TAD of food slurries. In this
regard, the present research contributes to the following objectives: a) determination of the effect of fat and total biodegradable solid content and b) presentation an energy balance (audit) on TAD of WF.

MATERIALS AND METHODS

Anaerobic digester: A bench scale batch insulated anaerobic digester (2.5 liters) as illustrated in figure 1 was set up for the studies. Gas produced by the digestion, can vent out from the top of the digester through a connecting pipe and is collected by water displacement method. The gas collector was not insulated and it was exposed to an ambient having a temperature of 20°C. In each experiment, 2 liters of WF slurry were digested anaerobically. In order to avoid gas pressure build-up inside the vessel, the collected gas was measured and discharged frequently.

Waste food (WF): WF samples were collected from the student residences of the campus of Indian Institute of Technology (I.I.T.), New Delhi. This contained edible things like bread, rice, potato, grains, green vegetables, and edible oils. The samples were mixed and ground well to get a thick paste. It contained, on an average, 13.3% fats on dry weight basis. Table 1 shows the WF analysis. Total solid (TS), total volatile solids (TVS), chemical oxygen demand (COD), total organic carbon (TOC), total Kjeldahl number (TKN), fats, and proteins were experimented according to standard methods (APHA, 1992). For a more simple and practical pretreatment process, the WF was ground, made into a slurry with water, and the fat layer at the top was separated by skimming [fat excluded (FE) samples]. Fat skimming procedure was repeated 3 times on a single sample. Final FE samples contained maximum 0.75% fats on dry weight basis. Sample without fat layer skimming process, i.e. fat included (FI), was also prepared. The WF samples (2 mother samples: FI and FE) were then diluted to a concentration of 110 g/l. Each batch then was inoculated with 10 volume percent of filtered fresh cow dung slurry (TS=10 g/l; pH 7.0) in order to produce anaerobic seeded sample having TS of 100 g/l. Different initial concentrations of FI and FE samples, ranging from 10 to 50 g/l TS (10 g/l in interval) were prepared and digested anaerobically. Two other FE samples containing 75 and 100 g/l of TS were also prepared and digested separately to study the effects of Pr in absence of LCFA.

Measurements: An electrical heating tape (200 cm and 200 Ohms) was wound on the outside surface of the digester vessel and a thick layer of glass wool was provided to insulate it from the surroundings. This was augmented with a layer of thermocel. Finally a thin layer of aluminum foil was provided on the outside surface. The tape was energized and controlled through an auto-transformer and a wattmeter. For keeping the digester’s temperature at thermophilic condition (55°C), power input to the tune of 0.75 watts was required. This power, however, shows the overall heat loss rate from the digester. The total energy consumed to perform anaerobic digestion could be calculated by multiplying the digestion time in to this electrical power (0.75 watts). Also, heat equal to 219 kJ was necessary to reach the digester at thermophilic condition ($E_{pre-heating}$).

Twice a day, 2-3 ml of slurry was taken and immediately acidified by adding a drop of concentrated HCl (1M) to stop further anaerobic digestion. The VFAs were measured by gas chromatography (Nucon, India, Series 5765) equipped with a flame ionization detector (FID).

![Figure 1. Schematic of the bench scale batch thermophilic anaerobic digester.](image-url)

Table 1: Waste food analysis.

<table>
<thead>
<tr>
<th>TS</th>
<th>TVS</th>
<th>Ash</th>
<th>pH</th>
<th>COD</th>
<th>TOC</th>
<th>TKN</th>
<th>C/N</th>
<th>Fats</th>
<th>Proteins</th>
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<tr>
<td>(g/l)</td>
<td>(g/l)</td>
<td>(g/l)</td>
<td>(g/l)</td>
<td>(g/l)</td>
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<td>199</td>
<td>162</td>
<td>13</td>
<td>4.0</td>
<td>280</td>
<td>81</td>
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<td>28</td>
<td>26.5</td>
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<td>±1</td>
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<td>±0.1</td>
<td>±2</td>
<td>±1</td>
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<td>±1</td>
<td>±0.5</td>
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</table>
Biogas production was measured by the water displacement method. The biogas composition was determined using a Gas Chromatograph (Nucon, India, Series 5700) equipped with a thermal conductivity detector (TCD). Biogas samples were collected using a gas-tight syringe and a sample of 100-200 µl was used for each run.

RESULTS

Table 2 presents results of TAD of different samples with respect to their biogas production and TS reduction (TSred). Figures 2 to 10 show the individual profiles of released biogas and corresponding concentrations of VFAs for some of the samples in table 2.

The energy audit for TAD is presented in table 3 (heat of combustion of methane is 50 kJ per gram in dry basis). The total energy lost by heater (Elost) to provide thermophilic condition is demonstrated in table 3 also. To distinguish whether the total biogas can compensate digester’s energy requirements, the energy loss (Elost) and preheating energy (Epre-heating) should be subtracted from methane energy (Emethane). The results are presented in the last column of table 3 (Ebalance). Positive results are belonged to those digestions that could be operated autothermally.

Relatively large amounts of gas were observed in the first two days of digestion process. This is related to the process of initial fermentation and acidogenesis, which are believed to be much faster than the methanogenesis part (Ayithi and Sreekrishnan, 2001). Gas quality and quantity analysis showed that the early-produced biogas was quite poor with respect to methane content (Max. 30%). The volumes of gas produced in the first 36h (V36h) are reported in table 2. Bar graphs were used for interpreting the volume of measured biogas at each 12h interval starting from 36th hour. Bar summations (total gas volume after 36th h) were calculated and shown on each bar graph (available for FI and FE samples). The volume percent of methane (not in the first two days) for all samples fluctuated in the range of 64 to 70. No significant and stable difference has been seen in methane purity for FI and FE samples. Durations of anaerobic digestion experiments (tbatch) were taken as the time up to which biogas production was observed. The ultimate volumes of gas produced (Vf) are reported in the last column of table 2. The concentrations of the Final total solids (TSbatch), at the end of each batch, are also shown in the table.

No significant differences in biogas production as well as VFAs profiles were observed between FI and FE samples with initial TS less than 20 g/l. However, obvious differences in these two regards were seen at higher TS concentrations. The TS reductions in concentrated FE samples (75 g/l and 100 g/l) were less

<table>
<thead>
<tr>
<th>Feed</th>
<th>TS0 (g/l)</th>
<th>TSbatch (g/l)</th>
<th>TSred (%)</th>
<th>V36h (l)</th>
<th>Vf (l)</th>
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<tr>
<td>FI</td>
<td>10</td>
<td>4.6</td>
<td>54</td>
<td>1.4</td>
<td>9.4</td>
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<td>10</td>
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<td>55</td>
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<tr>
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<td>68.0</td>
<td>32</td>
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Table 2: Biogas production and solids reduction in the waste food during thermophilic anaerobic digestion process.

![Figure 2](image_url). Profiles of produced biogas from thermophilic anaerobic digestion of a 10 g/l waste food (FI and FE).
than 50% (Table 2). Profiles of biogas released from a 10g/l TS sample and their corresponding VFA concentrations are shown in figures 2 and 3 respectively. Error bars represents a little difference (Max. 5%) between VFAs in FI and FE samples (Fig. 3). Figure 4 shows that in 20 g/l sample, the difference between the amount of biogas in FI and FE samples is still negligible. However, in figures 5 and 6 (40 and 50 g/l TS samples), the differences become more prominent. The amount of total biogas produced by the FI 50 g/l sample is half of that released from the FE sample (Table 2). Figures 7, 8, and 10 show that large amount of VFAs, especially butyrate (Bu), are produced during anaerobic digestion of WF. Initially, the rate of production of Bu was clearly more than the other two VFAs, and it increased with increasing the initial concentration of TS.

<table>
<thead>
<tr>
<th>Feed</th>
<th>TS0 (g/l)</th>
<th>t_batch (d)</th>
<th>E_pre-heating (kJ)</th>
<th>E_loss (kJ)</th>
<th>E_methane (kJ)</th>
<th>E_balance (kJ)</th>
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<td>10</td>
<td>219</td>
<td>648</td>
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<td>13</td>
<td>219</td>
<td>842</td>
<td>1475</td>
<td>+414</td>
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</table>

Table 3: The energy audit for thermophilic anaerobic digestion of waste food.

**DISCUSSION**

It is widely believed that Pr biodegradation pathway involves unusual and complicated enzyme reactions and its oxidation is thermodynamically unfavorable (Hanaki et al., 1987; Gijzen et al., 1988; Ozturk, 1991 and van Lier et al., 1993). The process of Pr degradation by anaerobic bacteria is also inhibited by acetate (Ac) and Bu (Dohanyos and Zabranska, 2001). Consequently, one can conclude that in a high VFA concentrated media, such as WF slurries, which is rich in Ac and Bu, the Pr degradation is always under inhibition. This phenomenon, however, causes the accumulation of Pr and its maximum possible concentration in the reactor, which is harmful for methanogenic bacteria (Wang et al., 1999; Ozturk, 1991 and van Lier et al., 1993). Pr itself, as mentioned, can inhibit the activity of methanogens (Hobson and Shaw, 1976). In the present work also, no significant decrease in Pr concentration was observed during the TAD. In other word, the concentration of Pr almost remained constant during the anaerobic process (Fig. 3, 7, 8, and 10). Consequently, FI samples enhance Pr accumulation and provide higher level of Pr than FE sample (compare figures 7 and 8).

In the 50 g/l FE sample, the ultimate concentration of Pr was 1800 mg/l; no inhibition of methanogenic bacteria, as seen by the biogas production, took place and the process proceeded well up to 50% reduction of TS (Fig. 7). In the 50 g/l FI sample (Fig. 8), Pr reached to a concentration of 3800 mg/l and the biogas production was found to have stopped soon after (Fig. 6). In 100 g/l FE sample (Fig. 10), Pr concentration increased up to 5000 mg/l, however, in the absence of long chain fatty acids biogas production continued to reduce TS to 32% (Table 2 and Fig. 9). Compare to the biogas released from a FE 100 g/l sample, almost equal volume of biogas was released from a FE sample with 75 g/l initial TS (Table 2); Pr in that case did not exceed 3300 mg/l.

![Figure 3](image-url)  
Figure 3. VFA concentrations during thermophilic anaerobic digestion of a 10 g/l waste food (FE and FI).
Figure 4. Profiles of produced biogas from thermophilic anaerobic digestion of a 20 g/l waste food (FI and FE).

Figure 5. Profiles of produced biogas from thermophilic anaerobic digestion of a 40 g/l waste food (FI and FE).

Figure 6. Profiles of produced biogas from thermophilic anaerobic digestion of a 50 g/l waste food (FI and FE).

Figure 7. VFA concentrations during thermophilic anaerobic digestion of a 50 g/l waste food (FE only).
The existence of fats in the form of edible oil in WF resulted in higher concentrations of Pr. The accumulation of un-oxidized form of LCFA, as Ayithi and Sreekrishnan (2001) showed, can inhibit the overall anaerobic digestion severely. In highly concentrated FI samples, these two factors together exert a severe inhibitory action and stop the methanogenesis step; this was exactly what happened to the 50 g/l FI waste (Fig. 6); after 8 days, an un-stabilized and greasy waste remained which was neither able to produce biogas nor could be disposed because of very bad odor emitted. In the 100 g/l TS sample of FE waste, even a Pr concentration of 5000 mg/l (Fig. 10) did not stop the methanogenesis step completely. Therefore, it can be concluded that the main reason for inhibition of the methanogenesis process in 50 g/l FI sample was existence of LCFA. Inhibitors of methanogenesis, naturally cause accumulation of Ac followed by deterioration in the rate of degradation of Bu to Ac. This, plus the almost permanent inhibition condition for Pr, causes an overall accumulation of VFAs in the reactor (Fig. 8 and 10). A successful anaerobic digestion process, even during the digestion of a thick WF, needs to keep the intermediate or final concentrations of VFAs at low levels. The key is separating the fat contents from the feed. Thermophilic anaerobic digestion of FE samples concentrated up to 50 g/l provides a total solids reduction of 50%. However, solids reduction of more concentrated samples (fat excluded) will be less than this amount due to the partial inhibitory effect of Pr on methanogens.

In view point of energy consumption and generation, FI samples could not be operated as an autoheated process (Table 3). Inhibition on methanogens causes to receive insufficient energy to compensate all heat requirements. When energy audit is done for the 50 g/l FI sample, it owes an energy around 163 kJ for being an autothermal process. However, the 50 g/l FE sample provides 88 kJ more than its own consumption. Table 3 shows that all fat excluded samples concentrated more than 40 g/l could be operated autothermally. Even the 100 g/l FE sample - which was under partial inhibition of Pr and was not digested sufficiently - resulted in generation of an extra energy equal to 414 kJ.

TAD in autothermal condition may be more feasible in pilot or full-scaled plants digesting concentrated FE feeds. Since digester’s frequent pre-heating is not necessary, and the heat loss from the body is decreased proportional to the specific area of the digester (specif-
ic area decrease by increasing the volume of the digester), TAD in continuous condition and in full scaled projects seems to be more feasible and attractive.

CONCLUSIONS

1. High concentrations of VFAs, specially Bu, is produced during the TAD of WF, followed by the concentrations of Ac and Pr.
2. Because of the inhibition of Pr degrading bacteria, concentration of Pr stays almost unchanged during a TAD.
3. In a TAD process, WF samples with high solids content, with its fat-content included, could not be digested due to the inhibitory effects of higher fatty acids (long chain fatty acids) and Pr on the methanogenic bacteria.
4. In a TAD, simple and easy pretreatment of WF, like dilution and fat separation, can enhance the biogas production by as much as two folds.
5. Both fats and Pr can inhibit TAD process. In the absence of fats, the inhibition caused by accumulation of Pr alone could be overcome partially by the methanogenic bacteria.
6. TAD of FE feeds could be made to operate as an autothermal process.

NOMENCLATURES

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Ac</td>
<td>Acetate or acetate concentration (mg/l)</td>
</tr>
<tr>
<td>Bu</td>
<td>Butyrate or butyrate concentration (mg/l)</td>
</tr>
<tr>
<td>C/N</td>
<td>Carbon to nitrogen weight ratio</td>
</tr>
<tr>
<td>d</td>
<td>Day</td>
</tr>
<tr>
<td>E_balance</td>
<td>Energy difference between sources and sinks (kJ)</td>
</tr>
<tr>
<td>E_lost</td>
<td>Energy lost from digester during anaerobic digestion period (kJ)</td>
</tr>
<tr>
<td>E_methane</td>
<td>Colorific energy reachable after methane combustion (85% eff.) (kJ)</td>
</tr>
<tr>
<td>E_pre-heating</td>
<td>Energy required to pre-heat sludge to thermophilic temperature (kJ)</td>
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<tr>
<td>F_E</td>
<td>Fat excluded</td>
</tr>
<tr>
<td>F_I</td>
<td>Fat included</td>
</tr>
<tr>
<td>t</td>
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</tr>
<tr>
<td>t_batch</td>
<td>Retention time batch reactor (d)</td>
</tr>
<tr>
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<td>Thermophilic anaerobic digestion</td>
</tr>
<tr>
<td>TKN</td>
<td>Total Kjeldal number (g/l)</td>
</tr>
<tr>
<td>TOC</td>
<td>Total organic carbons (g/l)</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids (g/l)</td>
</tr>
<tr>
<td>TS_0</td>
<td>Initial total solids (g/l)</td>
</tr>
<tr>
<td>TS_red</td>
<td>Total solids reduction (percent)</td>
</tr>
<tr>
<td>TVS</td>
<td>Total volatile solids (g/l)</td>
</tr>
<tr>
<td>V_36h</td>
<td>Volume of released biogas within the first 36h of anaerobic digestion (l)</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acid, or concentration of volatile fatty acid (mg/l)</td>
</tr>
<tr>
<td>VFA_s</td>
<td>Volatile fatty acids, or concentration of volatile fatty acids (mg/l)</td>
</tr>
<tr>
<td>V_t</td>
<td>Total volume of released biogas in anaerobic digestion (l)</td>
</tr>
<tr>
<td>WF</td>
<td>Waste food</td>
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</tbody>
</table>

References


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