BIOMETHANATION POTENTIAL OF ANIMAL FLESHING AND PRIMARY SLUDGE AND EFFECT OF REFRACTORY FRACTION OF VOLATILE SOLIDS

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Batch studies on anaerobic digestion were performed in 100ml bench top digesters (serum bottles) with solid wastes (limed fleshing) generated from tannery and primary sludge from effluent treatment plant of tannery liquid waste. Digestion was carried out under mesophilic condition. Batch reactors were run at two different organic loading rates. Gas production was higher in test reactor 1 (volatile solids load of 51.9 g/l) than test reactor 2 (volatile solids load of 15.8 g/l). VFA concentration in test reactors 1 and 2 had reduced by 11,970 mg/l (43%) and 2115 mg/l (28.4%) respectively. As biodegradability fraction present in the tannery solid wastes was lower, lag time for the onset of maximum gas production was longer. Based on experimental observation it is concluded that it is more appropriate to evaluate biomethanation potential of solid wastes in terms of biodegradable fraction of volatile solids present in the wastes.

Key Words: refractory fraction, biomethanation, biodegradability, kinetics, animal fleshing

1. INTRODUCTION

Development of alternative renewable source of energy is becoming increasingly important due to rapid depletion of conventional energy sources.

In view of increasing energy demand, engineers and scientists have initiated studies focusing more specifically on anaerobic digestion of animal wastes and industrial wastes.

Tanneries produce enormous quantities of solid wastes containing high concentration of soluble and insoluble organics, amenable to anaerobic digestion. The organic fractions present are mostly lipids and proteins.

The energy recovery aspect in anaerobic digestion process depends mainly on the physico-chemical process parameters such as pH, temperature, particle size, biodegradable volatile solids concentration and volatile fatty acids concentration. Mesophilic condition is preferable especially in tropical regions for maximum utilization of available biogas using efficient energy conversion devices such as boilers or gas engines.

1) Stress Inducing Parameters

Under natural conditions microorganisms may be associated with one another to form microbial consortia capable of carrying out hydrolysis and methanogenesis. The consortia in anaerobic
ecosystem comprise of different groups of microorganisms (Chartain and Zeikus 1986), which are able to convert organic matter into methane (CH₄) and carbon dioxide (CO₂) (Van Andel and Breure 1984; Van der Berg 1984).

With increasing full-scale application of anaerobic digestion for waste treatment and biogas production, there is a need to develop reliable methods for the evaluation and control of the anaerobic digestion process with suitable parameters reflecting the metabolic state of the process.

Several parameters have been suggested as stress indicators. Some of the most commonly used indicators include pH, gas production, gas composition, volatile solids destruction, alkalinity and VFA concentration. In general, most of these indicators are suitable for detecting gradual changes in the reactor. However, pH, volatile solids reductions and gas composition are often too slow for detection of sudden changes precisely (Angelidaki and Ahring 1994). An important feature of a good process indicator is its ability to reflect directly the metabolic state of the system causing imbalance at an early stage.

For a long time it has been recognized that VFA concentration is one of the most important parameters for the accurate control of anaerobic digestion process (Fischer et al 1981; Hill and Bolte 1989). Volatile fatty acid accumulation reflects a kinetic uncoupling between acid producers and consumers and typically indicates stress development in the system. In this paper the impact of stress developed due to high organic loading especially of refractory fraction of feed containing animal tissue and primary sludge from the effluent treatment plant on the anaerobic digestion process is discussed.

2. MATERIALS AND METHODS

(1) Equipment design

A simple methanogenic activity test procedure (Fig.1a and 1b) as proposed by Isa (1993) was adopted with suitable modifications to the requirement of this study. The schematics of experimental set-up is shown in Fig.1a. The experimental set-up is shown in Fig.1b. A known amount of waste mixture was transferred into a 130 ml serum bottle (working volume 100 ml and 30 ml head space). No additional nutrients were supplemented to enhance the growth of biomass during the test period as nutrient sources present in the feed are adequate to meet the anaerobic process requirements (Soto et al, 1993). Methane gas production was measured by means of the liquid displacement method at an interval of 24 h after 3 - 5 days of startup period. Contents of the serum bottle were mixed by swirling manually, after every gas measurement. Daily gas production was recorded. All the tests were conducted at room temperature (30 ± 3°C) for a period of 5 weeks.

(2) Substrate

The substrates used were limed fleshing from tannery and primary sludge from the treatment plant of tannery liquid waste. The fleshing was ground up to 6 mm diameter in a meat grinder (Make: Wolfking) and mixed with primary sludge in the ratio of 1:1 (weight basis) to act as a source of organic matter and various microorganisms required for anaerobic digestion process, maintain fleshing solids in suspension and improve the flow properties of the feed mixture.
(3) **Inoculum**

Pre-digested material containing all the essential microbes (hydrolyzing, fermentative, acetogenic and methanogenic bacterial consortium) was used as an Inoculum for early start up of anaerobic digestion process. This predigested material was synthesized using cow dung, limed fleshing and primary sludge in equal weight. After incubation for a period of 30 days, digested residues were used as Inoculum for the study.

(4) **Experimental procedures and sampling schedules**

a) **Substrate composition**

The average composition of limed fleshing, primary sludge and inoculum used in all the experiments are given in **Table 1**.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Limed Fleshing</th>
<th>Primary Sludge</th>
<th>Pre Digested Sample Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>12.29</td>
<td>7.33</td>
<td>7.63</td>
</tr>
<tr>
<td>TS</td>
<td>10%</td>
<td>6.36%</td>
<td>9.02%</td>
</tr>
<tr>
<td>VS</td>
<td>61.34%</td>
<td>52.71%</td>
<td>46.74%</td>
</tr>
<tr>
<td>Oil and grease (crude lipid)</td>
<td>4.79%</td>
<td>2.57%</td>
<td>10.1%</td>
</tr>
<tr>
<td>Protein (crude)</td>
<td>56.5%</td>
<td>28.39%</td>
<td>34.47%</td>
</tr>
<tr>
<td>Volatile fatty acid (as acetic acid)</td>
<td>-</td>
<td>4770 mg/l</td>
<td>7065 mg/l</td>
</tr>
</tbody>
</table>

b) **Startup of the digester**

Two sets of reactors having 5 bottles in each set were constructed and inoculum, limed fleshing, primary sludge were added in a definite proportion (**Table 2**) to obtain two different VS loading to study their effect on performance of the process.

<table>
<thead>
<tr>
<th>A: Test reactor 1</th>
<th>B: Test reactor 2</th>
<th>C: Test reactor 3</th>
<th>D: Test reactor 4</th>
<th>E: Test reactor 5</th>
<th>F: Test reactor 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>30</td>
<td>40</td>
<td>-</td>
<td>5.19</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
<td>70</td>
<td>1.58</td>
<td></td>
</tr>
</tbody>
</table>

Standard procedures recommended in the standard methods for examination of water and wastewater (APHA, 1992) was used for determining total solids (TS), volatile solids (VS) and total kjeldahl nitrogen (TKN). The concentration of total VFA was determined by titration of distillate obtained by steam distillation of digested samples. Oil and grease was determined by using soxhlet apparatus.

One bottle each of test reactors was sacrificed every week for estimating above mentioned parameters. Daily gas production from reactors was monitored by water displacement method. The volume of water displaced was equivalent to volume of gas generated. The gas production was recorded everyday for a period of five weeks.

### 3. RESULTS

(1) **Total gas production**

The cumulative gas production for a period of five weeks was 145 ml from test reactor 1 and 35 ml from test reactor 2 (**Fig.2**). The specific gas production was 27.9 l/kg VS in from test reactor 1 and 22 l/kg VS in from test reactor 2 (**Table 3**).

(2) **Concentration of VFA**

Toxic effects of high VFA concentration on the anaerobic digestion process have been studied and reported by several authors (Ahring and Westermann 1988; Gorris et al 1989). VFA concentration was 20,385 mg/l in test reactor 1, and 7,425 mg/l in test reactor 2 after one week. After five weeks VFA concentration reduced to 14,355 mg/l in test reactor 1 and 5,310 mg/l in test reactor 2. Weekly variation of VFA and cumulative gas production are shown in **Fig.3**.
Time required for conversion of biodegradable organic fraction of substrates into products of hydrolysis and fermentation and subsequently into that of acetogenesis varies from one substrate to another. During methanogenesis, conversion of certain volatile fatty acids (propionate, butyrate) is very slow, resulting in accumulation of fatty acids in the digester. When their concentrations exceeded a particular level they become toxic thereby causing cessation of gas production.

(3) Discussion

Anaerobic digestion is a complex process consisting of a series of microbial reactions catalyzed by different enzymes (McInerney and Bryant1981). The inter-dependent nature of microbial consortia on substrates and their reactions is a key factor in biogas generation process. Under conditions of unstable operation, intermediates such as volatile fatty acids (VFA) and alcohols accumulate (Gujer and Zehnder 1983) at different rates depending on the substrate and type of perturbation causing instability (Allison 1978). Most common disturbances causing process imbalance are hydraulic or organic overloading, presence of inorganic or organic toxins or fluctuations in process conditions such as temperature and substrate composition (Robins and Switzenbaum 1990).

The composition of methanogenic population in an anaerobic digester and their response to changes in the organic loading and physical and chemical factors have been evaluated (Visser et al, 1991). However, microbial interactions with complex substrates and mechanism of growth of microbial consortia are still not completely known.

(4) VFA Conversion

The experiments were conducted on bench-top digesters (Serum bottle) using substrates obtained from a single source to maintain consistency in the feed characteristics. In test reactor 1 with higher loading rate (5.19g/100ml), VFA concentration was very high (>10,000 mg/l) whereas in test reactor 2 (1.58g/100ml), it was always less than 10,000 mg/l (Table 3).

From the many different levels of VFA reported in the literature (Young-Chae et al, 2004; Chulhwan Park et al, 2005) for different substrates, it can be concluded that it is not feasible to define an absolute VFA level indicating the state of the process. Different anaerobic systems have their own “normal” levels of VFA, according to the nature of the constituents of the substrates digested or the operating conditions (Angelidaki and Ahring 1994). The residual level of VFA indicates the production of the other organic acids (C3 - C6) during the process.

(5) Biodegradability and Kinetics

The experimental results were evaluated under mesophilic conditions in terms of biodegradability of mixture of wastes and digester performance. In the biodegradability estimation of the mixture of wastes, refractory fraction was determined from the portions of VS that remained in the reactor as HRT approached infinity (Morris, 1977). The refractory fraction was determined graphically from the intercept of the plot of (S/S0) vs (S0.HRT)-1 for the two initial substrate concentrations studied.

Table 3 Comparison of volatile fatty acids (VFA) concentration, cumulative and specific gas production in the test reactors

<table>
<thead>
<tr>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1</td>
<td>51.9</td>
<td>145</td>
<td>20385</td>
<td>14355</td>
<td>35</td>
<td>27.9</td>
<td>80</td>
</tr>
<tr>
<td>Test 2</td>
<td>15.8</td>
<td>35</td>
<td>7425</td>
<td>5310</td>
<td>23</td>
<td>22.0</td>
<td>95</td>
</tr>
</tbody>
</table>

G: Reactors
H: VS load, g/l
I: Cumulative gas, ml
J: VFA, I week, mg/l
K: VFA V week, mg/l
L: VSd, %
M: Sp. gas production, l/kg Volatile solids in the feed
N: Sp. gas production, l/kg Volatile solids destroyed

(6) VS Destruction

VS destruction was 35% in test reactor 1 and 23% in test reactor 2. Total gas production per gram of VS destroyed was 80 ml in test reactor 1 and 95 ml in test reactor 2 (Table 3). Weekly variation of VS destruction and cumulative gas production are shown in Fig.4.
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The refractory fraction was as 0.6443 in test reactor 1 and 0.7469 in test reactor 2. The biodegradable fraction present in the mixture of wastes studied was ranging between 25 - 35% of the influent VS concentration.

According to first order kinetic model for biomethanation process

1) rate of substrate conversion is directly proportional to substrate concentration and
2) volume of gas generated is proportional to mass of substance destroyed.

Hence, 
\[ \frac{dS}{dt} = -kS \]  -- (1)

\[ G = CV.(S_0 - S) \]  -- (2)

\[ G = \text{cumulative gas production, l} \]
\[ V = \text{volume of reactor, l} \]
\[ C = \text{yield constant, l/g} \]
\[ S_0 = \text{initial substrate concentration, g/l} \]
\[ S = \text{final substrate concentration, g/l} \]
\[ k = \text{rate constant, d}^{-1} \]
\[ t = \text{time, d} \]

Integrating (1),
\[ \frac{S}{S_0} = \exp\{-k(t-t_0)\} \quad t > t_0 \]  --- (3)

\[ t_0 = \text{lag time, d} \]

This model (Fulford, 1988) describes average reactor behavior at a longer retention time. Cumulative gas production can be correlated with first order reaction rate constant by substituting S from Equation (3) in Equation (2) as given below.

\[ G = CV.S_0[1-\exp\{-k(t-t_0)\}] \]  --- (4)

Rearranging (3) by taking natural logarithm gives
\[ \ln(1-\frac{G}{CVS_0}) = -kt + kt_0 \]  --- (5)

Regression analysis of the experimental data gives k & t₀ for the mixture of waste used in the study. The rate constant k and lag time t₀ determined from the Fig.5 & 6 are shown in Table 4.

4. CONCLUSION

Mixture of fleshing and primary sludge is found to contain very low concentration of readily biodegradable volatile solids. This is confirmed by the observed VS destruction of 23 – 35% and very low specific gas production. Rapid increase of VFA in test reactor 1 is attributed to higher VS load applied in the reactor. However, presence of higher fraction of biodegradable VS in test reactor 1 contributed towards higher gas production as compared to test reactor 2. It was also observed that higher the refractory matter content in the substrate,
longer was the lag time observed for the onset of maximum gas production. Predicted and observed lag time were found to be closely related to biodegradable fraction present in the substrates. Anaerobic digestion of fleshing and primary sludge fits in first order kinetic model.

Based on the above experimental results, it is more appropriate to consider available biodegradable fraction of VS than total VS present in the waste for evaluating biomethanation potential of the substrates. In simple terms, substrate with high VS concentrations need not yield higher biogas production.

REFERENCES

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