Co-Digestion Of Cow Manure And Empty Fruit Bunches: Effect Of C/N Ratio And Kinetic Studies

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Abstract

Cow manure (CM) and empty fruit bunches (EFB) are waste that can be managed properly by anaerobic co-digestion. This study examines the possibility of increasing biogas yield from anaerobic co-digestion of CM and EFB, based on C/N ratio. The digesters were carried out in a batch system and operated at room temperature for 80 days with an initial substrate concentration of 30 g/L based on volatile solids (VS). Co-digestion of CM and EFB performed better in biogas potential than mono-digestion. The results showed that the optimal C/N ratio was 27.5. The biogas yield of 189.28 mL/gVS was higher than that of the digestion of CM or EFB alone by 12.15% and 81.76%, respectively. Co-digestion enabled to maintain a stable pH value to avoid a sharp decrease in pH value. The modified Gompertz model can be used to make a better prediction with a lower difference between the measured and predicted biogas yields.

Keywords: co-digestion, cow manure, empty fruit bunches, biogas yield, C/N ratio, kinetic study

INTRODUCTION

The greatest challenges of human life in the 21st century are the availability of energy and the environmental pollutions. The mitigation of CO₂ emission and related global warming demands the exploration of alternative energy in order to reduce the dependency on fossil fuel [1]. Energy congestion, fluctuating oil prices, climate change issues, and forecasts will further deplete reserves of non-renewable energy sources are urgent concerns related to exploration of alternative energy sources [2]. Therefore, efforts are needed to increase the use of renewable energy sources as alternative energy in human life. Biogas is a promising alternative energy source that can be used for different applications in cooking, electricity production, vehicle fuels, and heating. Animal manures, lignocellulosic residues, wastewater sludge, and municipal organic wastes are potential sources of biogas production [3]. Biogas is an alternative source of energy that can be developed with the appropriate and relatively simple technology. Biogas is a gas mixture resulting from the activity of methanogenic bacteria in anaerobic conditions or fermentation of organic materials [4].

Animal manures for biogas with anaerobic digestion process is one of the most promising in the utilization of biomass waste as a source of energy as well as a solution of ecological and agrochemical issues and does not reduce its benefits as a fertilizer because the content of nitrogen and other substances is still present in the resulting sludge [5,6]. Although the anaerobic digestion can be an alternative to the final processing of animal manures, the low carbon to nitrogen (C/N) ratios in animal manures cannot fully satisfy the anaerobic digestion requirements. Therefore, to make the anaerobic digestion more effective, there should be another carbon-rich substrate to co-digestion with manure to optimize the carbon deficiency [7].

The characteristics of co-substrate utilized for co-digestion is important; wastes with high carbon and low nitrogen contents are favored. Among different feedstocks potent to be used as co-substrate in anaerobic digestion, lignocellulosic residues seem promising as they are rich in carbon and abundantly available at low cost [7]. Oil palm waste is one of the major sources of the biomass. Empty fruit bunches (EFB) are the most widely generated waste of oil palm biomass. A fresh fruit bunch produces 21-23% EFB, 12-15% fiber, 6-7% shell of its weight [8]. EFB is a lignocellulosic residue containing cellulose (42.7-65% of weight), hemicelluloses (17.1-33.5% of weight) and lignin (13.2-25.31% of weight) [9].

While choosing substrates for co-digestion, it is obvious that ratio of carbon to nitrogen (C/N) is one of the main factors for improving biogas production performance. The optimum value of C/N ratio for the anaerobic digestion process is in the range of 20-30 [1,10]. When the C/N ratio becomes higher or lower than the optimal value, it can cause instability, system failure, and decreased biogas production. Substrates with high C/N ratio have poor buffer capacity but produces large amounts of volatile fatty acids (VFAs) during the fermentation process. In contrast, substrates with low C/N ratio have a high buffer capacity, but during the fermentation process, ammonia concentration increases and will inhibit the system of microbial growth [1].

Co-digestion of animal manures and lignocellulosic materials offers a solution to balance the C/N ratio of feedstock for anaerobic digestion. This study aims to investigate the effect of C/N ratio on co-digestion of cow manure and EFB for the possibility of increasing biogas production. Furthermore, the data obtained was used to make kinetic model equation by comparing the modified Gompertz equation and the first-order kinetic.
MATERIALS AND METHODS

Substrates

The cow manure (CM) used in this experiment was obtained from the cattle farm in Semarang city, Indonesia. The CM was then stored in the refrigerator at 4°C prior to use. The EFB was obtained from an oil palm mill in West Kalimantan, Indonesia. It was further prepared into a smaller diameter of less than 2 cm and stored in a sealed plastic bag at room temperature prior to use. The characteristics of the substrates were analyzed and the results are summarized in Table 1.

Table 1. Characteristics of CM and EFB

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>CM</th>
<th>EFB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (TS)</td>
<td>%</td>
<td>21.47</td>
<td>98.34</td>
</tr>
<tr>
<td>Volatile organic carbon (VS)</td>
<td>%</td>
<td>82.30</td>
<td>97.23</td>
</tr>
<tr>
<td>Total organic carbon (TOC)</td>
<td>%</td>
<td>45.72</td>
<td>54.02</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen (TKN)</td>
<td>%</td>
<td>2.13</td>
<td>1.14</td>
</tr>
<tr>
<td>C/N</td>
<td></td>
<td>-</td>
<td>21.44</td>
</tr>
</tbody>
</table>

Note: * Percent of TS

Experimental Design

The digesters used in this experiment were made of polyethylene bottles having a volume of 2 L, with working volume of 1.5 L. The digesters were covered with a rubber stopper and the center of the rubber plug is hollowed to insert a hose in the digester. Hose function to drain the biogas that formed. The digesters were done in the batch system, in which the substrates were fed at the beginning of the process, and operated at room temperature.

Before the start of co-digestion, mono-digestion of each substrate (CM and EFB) was prepared and fed into digesters with ratio 1.0:0.0 and 0.0:1.0 (gVS CM: gVS EFB) marked as CM neat and EFB neat, respectively. In the co-digestion experiments, the CM and EFB were mixed at different ratio to reach the C/N ratio to 22.5; 25; 27.5; 30; and 35, respectively marked as C/N 22.5; C/N 25; C/N 27.5; C/N 30; and C/N 35 (Table 2). Tap water was added to bring the final volume to 1.5 L. All of the digesters were started at an initial substrate concentration of 30 g VS/L and were run in duplicate. Measurement of biogas volume is performed every day during the anaerobic digestion process, while the pH measurement is done every two days to find the pH profile of digesters.

Table 2. Experimental design

<table>
<thead>
<tr>
<th>Digesters</th>
<th>Feed ratio (%VSadded)</th>
<th>CM (g)</th>
<th>EFB (g)</th>
<th>Water (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM neat</td>
<td>100</td>
<td>0</td>
<td>254.7</td>
<td>0.0</td>
</tr>
<tr>
<td>EFB neat</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>C/N 22.5</td>
<td>91.4</td>
<td>8.6</td>
<td>232.6</td>
<td>4.1</td>
</tr>
<tr>
<td>C/N 25</td>
<td>73.9</td>
<td>26.1</td>
<td>188.3</td>
<td>12.3</td>
</tr>
<tr>
<td>C/N 27.5</td>
<td>59.7</td>
<td>40.3</td>
<td>152.0</td>
<td>19.0</td>
</tr>
<tr>
<td>C/N 30</td>
<td>47.8</td>
<td>52.2</td>
<td>121.7</td>
<td>24.6</td>
</tr>
<tr>
<td>C/N 35</td>
<td>29.1</td>
<td>70.9</td>
<td>74.2</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Analytical Methods

The TS and VS of CM and EFB samples were measured using the standard methods [11]. TS was determined by heating at 105°C while VS was determined by heating at 550°C. Digital pH meter is used to measure the value of pH. The nitrogen-Kjeldahl method was used for determination of total nitrogen [12]. Ash method was used for the determination of total organic carbon [13]. The volume of biogas formed was measured by water displacement method which is also used by other researchers [5,14,15].

C/N Ratio Measurements

Total carbon and total nitrogen content on the weight basis of both samples were used to calculate the carbon to nitrogen ratio. The calculation for the C/N ratio of the substrates mixture is as shown below Eq. (1),

\[
\frac{C}{N} = \frac{CM(TS \times TKN) + EFB(TS \times TKN)}{CM(TS \times TKN) + EFB(TS \times TKN)}
\]

where CM is the weight of cattle manure fresh matter (g), EFB is the weight of empty fruit bunches fresh matter (g), TS is total solid (% fresh matter), TOC is total organic carbon (% TS), and TKN is total Kjeldahl nitrogen (% TS).

Kinetic Model

Bacterial growth often showed a phase in which the specific growth rate starts at zero and then accelerates to a maximum value over a given period of time, resulting in lag time. Assuming the rate of biogas production in the batch system related to the specific growth rate of methanogenic bacteria in the digester, the kinetic of biogas production was modeled by modified Gompertz equation [15,16]. The modified Gompertz model is presented in Eq. (2),

\[
M(t) = A \exp \left( - \exp \left( \frac{R_{\max} e}{A} (\lambda - t) + 1 \right) \right)
\]

where \(M(t)\) is the cumulative biogas yield at time t (mL/gVS), A is the biogas potential maximum production (mL/gVS), Rmax is maximum rate of biogas production (mL/gVS.day), \(\lambda\) is lag phase time (days), \(t\) is duration of time (days), and e is Euler’s constant (2.718282).

The first-order kinetic model was used to determine the biogas production rate constant [17], the cumulative biogas yield can be described by Eq. (3),

\[
M(t) = M_0 \times (1 - \exp(-kt))
\]

where \(M_0\) is the cumulative biogas yield at time t (mL/gVS), \(M_0\) is the cumulative biogas yield in 80 days (mL/gVS), k is the biogas production rate constant (1/day), t is the duration of time (days). The parameters (A, Rmax, \(\lambda\), and k) were estimated by the least squares method using the Solver Function of Microsoft® Office Excel [18].
RESULTS AND DISCUSSION

Biogas Production

The effect of C/N ratio on biogas production was investigated by varying C/N from 22.5 to 35. Data obtained for 80 days are presented in the cumulative biogas yield that is the volume of biogas production per total VS (Figure 1). Generally, digesters contain substrates of CM and CM with EFB (C/N of 21.4 to 35) exhibit higher cumulative biogas yield than substrate just contain EFB (C/N 47.3).

Figure 1 shows that the biogas yield exhibited the same gradual increasing trend for all the samples from the beginning to the 6th day which biogas production is very low or indeed do not formed yet due to the lag phase microbial growth, which indicates that the C/N ratio has not had a significant effect at the beginning of the AD process. Observation on 6 to 20 days, the biogas yield results also show the same average upward trend for all digesters. After the 20th day, biogas yield increased rapidly for C/N 22.5; C/N 25; C/N 27.5; and C/N 30. By contrast, the biogas yield for EFB neat and C/N 35 was the lowest. According to Zhang et al. [19], lack of nitrogen can inhibit the growth of microorganisms, resulting in low biogas yield. The substrates are characterized by the high C/N ratio, which has the poor buffer capacity and produces large amounts of volatile fatty acids (VFAs) during the fermentation process and consequently low biogas yield [1].

In the 80 days observation, the cumulative biogas yield of CM neat; EFB neat; C/N 22.5; C/N 25; C/N 27.5; C/N 30; and C/N 35 were 168.78; 104.13; 186.13; 174.42; 189.28; 185.78; and 164.46 ml/gVS, respectively. Compared to CM neat and EFB neat, the cumulative biogas yields C/N 22.5; C/N 25; C/N 27.5; and C/N 30 are higher, whereas the cumulative biogas yield of C/N 35 is lower than CM neat. This suggests that co-digestion CM and EFB with C/N of 22.5 to 30 can increase biogas yield compared to mono-digestion. The highest cumulative biogas yield on C/N 27.5 increased by 12.15% and 81.76%, respectively compared to the mono-digestion CM neat and EFB neat. Significant increases in EFB neat indicated that substrate with high C/N ratio and high lignocellulose content lead to slow biodegradability and low biogas production.

Several studies have also shown that co-digestion of livestock manure with a high C/N substrate ratio provides stable operating performance and higher biogas yields than monodigestion [19,20,21]. Zhang et al. [19] studied co-digestion of sorghum stem and cow manure significantly accelerated the AD process, improved the digestion of sorghum stem and maintained a stable pH value, where the best C/N ratio was obtained at a C/N ratio of 25. The co-digestion of palm pressed fiber and cattle manure showed relative higher process stability with regard to VFA/TIC and no process inhibition by either VFAs or ammonium observed [20].

From Figure 1 it can also be seen that after 80 days of the experiment there is still a tendency to increase biogas yield and no signs to stop. It is predicted that the carbon contained in the substrates is not simultaneously degraded or converted into biogas by AD process because of the lignin content in the substrates[22].

pH Profile

After gas production, pH is the best indicator of future digester instability [21]. The pH optimum of the methane-forming microorganism is at pH = 6.7–7.5 [23]. While acidogenesis microorganisms had a tolerance of pH values 4.0-8.5 [1].

In the 80 days observation, the pH value for all digesters

Figure 2 shows the variation of pH value of each digester during 80 days observation. The initial pH conditions in each of the digesters in this experiment were not adjusted with the initial pH values of CM neat; EFB neat; C/N 22.5; C/N 25; C/N 27.5; C/N 30; and C/N 35 are 8.19; 7.11; 7.47; 7.45; 7.37; and 6.96, respectively. The pH value for all digesters
decreased in the first 6 days indicating the occurrence of the acidification (fermentation) process which biogas production is very low or indeed do not formed yet due to the lag phase microbial growth. In the range of 6 and 10 days observation, there is an increase in pH value for all digesters and the start of biogas. Digester EFB neat had the most significant pH decrease with a pH value of about 4.7. The substrate with high C/N ratio is more susceptible to the fermentation process by producing large VFAs which cause the pH value to decrease easily and result in the process of methanogenesis unoptimal [20].

Digester C/N 22.5; C/N 25; C/N 27.5; and C/N 30 have the same pH value trends as CM neat indicating that cow manure has good buffering capacity in co-digestion process of cow manure and EFB. From Figure 2, it can be seen that the final value of pH CM neat; C/N 22.5; C/N 25; C/N 27.5; C/N 30; and C/N 35 has almost the same value of 6.80; 6.72; 6.76; 6.62; 6.53; and 6.37, respectively. Otherwise, the final value of pH EFB neat is 4.70. This is predicted that in the co-digestion process, the addition of cow manure could increase the total nitrogen, which will result in the higher ammonia concentration neutralized the VFAs formed in acidification process. According to Zhang et al. [24], the ammonia and VFAs may have ionized in the liquid phase. The reaction can be defined as follows:

\[
\text{C}_x\text{H}_y\text{COOH} \rightleftharpoons \text{C}_x\text{H}_y\text{COO}^- + \text{H}^+ \quad (4)
\]

\[
\text{NH}_3\cdot\text{H}_2\text{O} \rightleftharpoons \text{NH}_4^+ + \text{OH}^- \quad (5)
\]

where \text{C}_x\text{H}_y\text{COOH} is the VFAs. Combine Eq. (4) and (5), Eq. (6) was obtained

\[
\text{C}_x\text{H}_y\text{COOH} + \text{NH}_3\cdot\text{H}_2\text{O} \rightleftharpoons \text{C}_x\text{H}_y\text{COO}^- + \text{NH}_4^+ + \text{H}_2\text{O} \quad (6)
\]

More VFAs will be neutralized at the higher ammonia concentration. Thus, the buffer system is formed in co-digestion, allowing the high VFAs concentrations without pH decrease. Therefore, higher ammonia concentrations may be the reason for increasing the buffer capacity in co-digestion [24].

**Kinetic Model of Biogas Production**

Kafle and Kim [17] reported that the term \( k \) was a measure of the rate of biogas production with time. The more positive the value of \( k \), the faster the rate of biogas production. From Table 3, the values of \( k \) for CM neat; EFB neat; C/N 22.5; C/N 25; C/N 27.5; C/N 30; and C/N 35 are 0.0168; 0.0212; 0.0166; 0.0183; 0.0181; 0.0170; and 0.0166, respectively. The greatest \( k \) value was obtained mono-digestion EFB neat (C/N 47.3) while the co-digestion of CM with EFB has varying \( k \) values (0.0166-0.0183). Having a higher biogas production rate so that the biogas yield produced by EFB neat is higher than the others at the beginning of the digestion process.

According to Budiyono et al. [22] and Syaichurrozi et al. [25], the experimental data obtained in 60 days was sufficient to make the biogas production prediction using the kinetic model of the modified Gompertz equation. In this study, the digesters were operated in 80 days (> 60 days). Figure 3 shows that the modified Gompertz curves of all the digesters can be classified into lag-phase, growth phase, and stable phase. The experiment period in this study (80 days) only covered lag-phase and growth phase. Lag-phase (\( \lambda \)) is an important parameter to determine the initial time of biogas formation. The smaller the kinetic constant value \( \lambda \) the less time it takes to produce biogas [15]. In Table 3, the shortest \( \lambda \) was in EFB neat (4.56 days), while the longest was in CM neat (17.45 days).

The short \( \lambda \) indicates that the resulting intermediate (VFA) is converted rapidly into biogas, whereas the long \( \lambda \) in CM neat can be attributed to the accumulation of VFA and ammonia [17]. Co-digestion of CM with EFB can shorten the time to produce biogas when compared to mono-digestion CM neat, where the smallest value of \( \lambda \) is obtained C/N 25 and C/N 27.5 (12 days).

The value of \( R_{\text{max}} \) in co-digestion has the same trend value as the mono-digestion CM neat is about 2.6-3.0 mL/gVS.day, but for EFB neat has a smaller value of 1.53 mL/gVS.day. This suggests that co-digestion of CM with EFB can increase the maximum biogas production rate by nearly twice that of mono-digestion EFB, which indicates that mono-digestion EFB is easier for acidification resulting in large amounts of VFAs and a decrease in pH that inhibits methanogenic activity. From Table 3 and Fig. 3, variable with C/N of 27.5 was the best variable because it had the highest value of \( R_{\text{max}} \) and the lowest value of \( \lambda \).

Table 3 shows that the first-order kinetic model had the value of the difference between the predicted and measured biogas yield higher than the modified Gompertz model. The value of first-order kinetic is 18.36-25.98%, while the modified Gompertz model is only 0.36-2.81%. So the modified Gompertz model gave the better-predicted result with a range of \( R^2 \) values of 0.995-0.999. The better result provided by the modified Gompertz model than the first-order kinetic showed that the biogas yield from co-digestion of CM and EFB was a sigmoid curve thus implying that it may not increase linearly with time due to the lag phase. The similar result was also shown by co-digestion of apple waste with swine manure by Kafle and Kim [17].
Table 3. Parameters of the first-order kinetic model and the modified Gompertz model

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CM neat</th>
<th>EFB neat</th>
<th>C/N 22.5</th>
<th>C/N 25</th>
<th>C/N 27.5</th>
<th>C/N 30</th>
<th>C/N 35</th>
</tr>
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**First-order kinetic model**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CM neat</th>
<th>EFB neat</th>
<th>C/N 22.5</th>
<th>C/N 25</th>
<th>C/N 27.5</th>
<th>C/N 30</th>
<th>C/N 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$ (1/day)</td>
<td>0.0168</td>
<td>0.0212</td>
<td>0.0166</td>
<td>0.0183</td>
<td>0.0181</td>
<td>0.0170</td>
<td>0.0166</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.823</td>
<td>0.924</td>
<td>0.842</td>
<td>0.871</td>
<td>0.870</td>
<td>0.848</td>
<td>0.860</td>
</tr>
<tr>
<td>Measured biogas yield (mL/gVS)-80 days</td>
<td>168.78</td>
<td>104.13</td>
<td>186.13</td>
<td>174.42</td>
<td>189.28</td>
<td>185.78</td>
<td>164.46</td>
</tr>
<tr>
<td>Predicted biogas yield (mL/gVS)-80 days</td>
<td>124.94</td>
<td>85.01</td>
<td>136.83</td>
<td>134.00</td>
<td>144.86</td>
<td>137.91</td>
<td>120.70</td>
</tr>
<tr>
<td>Difference between measured and predicted biogas yield (%)</td>
<td>25.98</td>
<td>18.36</td>
<td>26.49</td>
<td>23.18</td>
<td>23.47</td>
<td>25.76</td>
<td>26.61</td>
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</table>

**Modified Gompertz model**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CM neat</th>
<th>EFB neat</th>
<th>C/N 22.5</th>
<th>C/N 25</th>
<th>C/N 27.5</th>
<th>C/N 30</th>
<th>C/N 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda$ (days)</td>
<td>17.45</td>
<td>4.56</td>
<td>16.58</td>
<td>12.13</td>
<td>12.13</td>
<td>15.19</td>
<td>16.72</td>
</tr>
<tr>
<td>$R_{\text{max}}$ (mL/gVS.day)</td>
<td>2.86</td>
<td>1.53</td>
<td>2.99</td>
<td>2.77</td>
<td>2.96</td>
<td>2.95</td>
<td>2.58</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.999</td>
<td>0.995</td>
<td>0.998</td>
<td>0.998</td>
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<tr>
<td>Measured biogas yield (mL/gVS)-80 days</td>
<td>168.78</td>
<td>104.13</td>
<td>186.13</td>
<td>174.42</td>
<td>189.28</td>
<td>185.78</td>
<td>164.46</td>
</tr>
<tr>
<td>Predicted biogas yield (mL/gVS)-80 days</td>
<td>169.86</td>
<td>101.25</td>
<td>185.47</td>
<td>169.52</td>
<td>184.88</td>
<td>183.59</td>
<td>162.76</td>
</tr>
<tr>
<td>Difference between measured and predicted biogas yield (%)</td>
<td>0.64</td>
<td>2.77</td>
<td>0.36</td>
<td>2.81</td>
<td>2.32</td>
<td>1.18</td>
<td>1.03</td>
</tr>
</tbody>
</table>
CONCLUSION

The C/N ratio had the effect of increasing the biogas production by co-digestion of cow manure and EFB with maximum C/N ratio of 27.5 and biogas yield of 189.28 mL/gVS. Cow manure that has a good buffering capacity helped maintain a stable pH value in order to avoid a sharp decline in pH value. Co-digestion also stepped up hydrolysis to shorten the lag phase. The kinetic data was better adapted to the modified Gompertz model, which showed the sigmoid performance of biogas production, which may not increase linearly over time due to lag phase.

ACKNOWLEDGMENTS

Authors thank Department of Chemical Engineering, Diponegoro University for all facilities to do this research.

REFERENCES


Figure 3. Comparison of experimental data with the first-order kinetic model and the modified Gompertz model


