Development of the Manure Gas Research Facility (MANGAS-RF) for modelling greenhouse gas emissions from stored manure:

*Phase I- evaluation of dietary manipulation effects*

**Final report**

**DEVELOPMENT OF MANGAS-RF AND ONGOING EXPERIMENT**

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Executive Summary

Methane (CH₄) is the main GHG emitted from liquid manure storage systems. Appropriate manure management and treatment practices have been encouraged to achieve the goal of reducing the net GHG emission. This study focused on the effect of swine diet manipulation on GHG emission from liquid manure storage. Ten 25 L anaerobic digesters were constructed for this study. Each digester was constructed with sealed digesta sampling and flow cell portions for direct measurement of pH, conductivity, and oxidation reduction potential of digesta. The digesters also had feeding portion, gas portion for concentration determination by gas chromatography, and connection to water replacement for biogas flow rate determination. A semi-automatic gas flow determination system was set up by connecting automatic data acquiring system with a GC and 10 anaerobic digesters, 3 standard gas tanks along with water replacements. Fifteen pigs were randomly grouped into three pens, each of which enclosed 5 pigs. The pigs in each pen were fed diets varying in fibre contents (12%, 16%, and 20% for low, medium and high fibre treatments, respectively). A manure collection facility was developed to make certain that manure from each pen was collected separately. Manure (faeces and urine) from each pen washed into a corresponding trough once a week. Then the manure sludge in the trough was collected and fed to anaerobic digesters once a week. Manure from each pen was used for feeding three assigned digesters and a litre of liquid manure (3% total solids) was fed to each digester once a week. Before feeding digesters, a 250 ml digesta sample was collected from each digester. The biogas production of each digester was recorded daily. Methane (CH₄) and carbon dioxide (CO₂) concentrations of biogas produced from each digester were recorded hourly. The experiment run for 20 weeks in T. K Cheung Animal Science Centre, University of Manitoba. After 20 weeks, the manure accumulated in each digester reached 20 L. In 20 weeks, 154, 146 and 178 L of CH₄ was emitted from digesters fed with manure from low, medium and high fibre diets, respectively. Manure collected from high fibre diet had significantly higher fibre content than manures from low and medium fibre diets. Consequently, under liquid manure storage condition, manure from high fibre diet produced significantly higher amount of CH₄ than manures from low and medium fibre diets. There were no significant differences in fibre content and therefore in CH₄ productions between manures from medium and low fibre diets. There was a large variation in CH₄ emission rate over time. On average, manures from low, medium, and
high fibre diet had a CH$_4$ emission rate of 95, 90, and 113 ml L$^{-1}$ (Digesta) d$^{-1}$ respectively. Manure from high fibre diet has significantly higher CH$_4$ emission rate than other two manures. This study demonstrated three factors contributing to CH$_4$ emission rate variation: temperature, feeding schedule, and length of storage period. In 20 weeks, a digester fed with manure from low, medium, and high fibre diets emitted 109, 100, and 127 L CO$_2$ respectively. Manures from low, medium, and high fibre diets had an emission rates of 69, 62, and 81 ml L$^{-1}$ (digesta) d$^{-1}$ respectively. Manure from high fibre diet produced significantly higher cumulative CO$_2$ production and emission rate than the manures from low and medium fibre diets. There was no significant differences in both cumulative CO$_2$ production and emission rate between manures from low and medium fibre diets.
Background

Methane (CH₄) and nitrous oxide (N₂O) are major contributors to global warming, and the agricultural sector produces about 50 and 70% of total anthropogenic emissions of these gases respectively (Cole et al. 1997). Enteric fermentation, flooded rice field, and manure management systems are three primary sources for greenhouse gas (GHG) emissions from the agricultural sector (Patty et al. 2005; Cole et al. 1997). In 2002, the GHG emissions from agricultural sector contributed 8% to total national emissions in Canada. More than half of Canadian agricultural emissions came from animal production (Kebreab et al. 2006), and 17% from manure management (Martin et al. 2004). It is possible to reduce CH₄ emission from manure storage systems by up to 25 - 80%, using appropriate manure management and treatment practices (Cole et al. 1997). Diet strongly influences animal growth and their excretions. Additives or feeding strategies have been used by some Canadian farmers to reduce nutrient content in manure (Kebreab 2006). However, very little is known about the effects of diet on GHG emissions from manure storage. While current diet manipulations have focused on nutrient efficiency and digestion, they also relate to GHG reduction (Clark et al. 2006). It has also been reported that low protein content in diet may increase CO₂ and CH₄ emissions from excretions of swine (Clark et al. 2005) and dairy cattle (Kulling et al. 2001). However, the emission of N₂O from dairy cattle manure was higher with higher dietary protein content (Kulling et al. 2001). In contrast, a Dutch study showed that CH₄ emissions decreased with lower manure protein content due to decreasing VFAs (Velthof et al. 2005). More research is needed to clarify the effect of diet manipulation on GHG emission from manure and the potential for reducing GHG emission through diet manipulation.

Objectives

During the reporting period, the objective of the project was to develop a manure gas research facility (MANGAS-RF) to measure greenhouse gas (GHG) emissions from stored manures and use the facility to evaluate engineered manures. Subsequently, the project aims to (1) quantify the effect of diet on manure composition and on GHG emission from liquid manure storage (2) recommend appropriate diet manipulation practices for reducing GHG emission from liquid
manure storage and (3) use the stored manure for future work on evaluation of dietary manipulation on GHG emissions from the manure applied soil. In subsequent phases, we aim to develop a mechanistic model for predicting GHG emission from liquid manure storage that will simulate a lagoon.

Project progress

The initial phase of the project was set to meet 5 objectives: 1) construct and test manure digesters, 2) build animal holding pens and manure separation, collection, and processing facilities, 3) formulate three different diets fed to a total of 15 pigs 4) collect manure from each group separately and incubate in the digesters, and 5) measure greenhouse gas emissions from digesters and recorded data.

Objective 1 – Construct and test digesters

The project started in May 2009 and objective 1 was met at the end of August 2009. Ten digesters were built for this project. The 24 L Better-Bottle fermentation carboys were modified to become digesters. A gas sampling portion for gas chromatographic system, a pipe line portion for water replacement, a valve-controlled opening for feeding, two openings for sampling manure slurry (bottom and middle), two openings for Flowcell measurement (bottom and middle) were added (Fig. 1). The Flowcell measurement is in situ measurement of pH, temperature, conductivity, and oxidation reduction potential (ORP). The specification of each openings are listed in Table 1.

The water replacement bottles are used to monitor gas production. Two bottles were connected with a pipe and each contained half volume of an acid-salt solution to prevent gases from dissolving into the liquid. The produced biogas enters one bottle and pushes the liquid on to the other bottle. The volumetric gas productivity can be determined by the liquid level changes in the bottle.
Figure 1. Digester and water replacement bottles

Table 1. The specifications of modifications made to the fermentation carboys
<table>
<thead>
<tr>
<th>Openings</th>
<th>Quantity</th>
<th>Diameter (Inch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC analysis</td>
<td>1</td>
<td>1/4</td>
</tr>
<tr>
<td>Water replacement</td>
<td>1</td>
<td>1/4</td>
</tr>
<tr>
<td>Feeding</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Manure slurry sampling</td>
<td>2</td>
<td>1/2</td>
</tr>
<tr>
<td>Flowcell measurement</td>
<td>2</td>
<td>1/2</td>
</tr>
</tbody>
</table>

**Objective 2. Pens and manure collection facility**

Three pens each with an area of 1.15×3.00 m² are used in this experiment. On both sides of each pen, a PVC board is glued on the ground for separating one pen's manure from the other (Fig. 2). Behind each pen, a trough with dimension of 0.4×0.4×2 m³ was set in the channel for collecting manure from each pen (Fig. 3).

![Figure 2. Glued PVC board for separating manure from different pens](image)
Objective 3. Diet formulation

As organic matter substrate is important for GHG emission during manure digestion, three diets that vary in their fiber contents were formulated for this experiment. The diets were formulated to contain 236, 197 and 154 g crude fibre/kg DM for high, medium, and low fiber diets, respectively (Table 2).

Figure 3. Trough used for collecting manure from each pen
Table 2. Ingredient and chemical composition of the experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>High fibre</th>
<th>Medium fibre</th>
<th>Low fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>27.50</td>
<td>46.00</td>
<td>66.35</td>
</tr>
<tr>
<td>Soybean meal, 48% CP</td>
<td>15.20</td>
<td>16.88</td>
<td>18.10</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>38.00</td>
<td>24.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Corn starch</td>
<td>10.4</td>
<td>6.00</td>
<td>1.90</td>
</tr>
<tr>
<td>Canola oil</td>
<td>6.10</td>
<td>4.00</td>
<td>1.20</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.80</td>
<td>1.60</td>
<td>1.40</td>
</tr>
<tr>
<td>Biofos</td>
<td>-</td>
<td>0.52</td>
<td>1.05</td>
</tr>
<tr>
<td>Vitamin and mineral premix</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Calculated composition1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>3268</td>
<td>3279</td>
<td>3267</td>
</tr>
<tr>
<td>CP, %</td>
<td>15.5</td>
<td>15.6</td>
<td>15.5</td>
</tr>
<tr>
<td>Dietary fibre, g/kg DM2</td>
<td>236</td>
<td>197</td>
<td>154</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
</tr>
<tr>
<td>P, %</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
</tr>
</tbody>
</table>

1Based on the NRC (1998) feed composition table except dietary fibre content

2Based on the data reported by Bach Knudsen et al. (1997)

Objective 4. Collection of manure and incubation in the digesters

During this stage, five pigs per treatment (total of 15) were raised in pens and fed with a specific diet (Figure 4). The pigs in each pen were fed with the corresponding diet as of August 22, 2009. Manure collection began on August 24, 2009. A 10 L sample of each manure was collected from each trough and kept in a large container to soak overnight. In the meantime, two sub-samples from each manure were collected and put in the 1050°C oven for dry matter (DM) determination.
Following soaking, each 10 L manure sample was diluted to 3% DM content by adding water. Then the anaerobic bacteria seed, which was a waste water sludge collected from a Winnipeg waste water treatment plant, was mixed with each diluted manure at a ratio of 1:5 to act as ‘starter’ bacterial colony. Finally, five liter of each seeded manure was fed to three randomized digesters. The experiment had 10 digesters in total, nine were used for manure storage and one fermenter acted as a control (tap water). The digester containing water was also used for counting the effect of atmospheric pressure on the readings from equalization bottle. After this time, the manures under each pen were washed out to corresponding trough and then a 10 L sample was collected once a week (Tuesday’s). Meanwhile, two 200 ml subsamples from each manure were collected to determine DM. Every week (Wednesday’s), each manure was diluted to 3% DM based on the DM measured on the previous day and a 1000 ml diluted manure was fed to the corresponding digesters. Before feeding, a 250 ml digesta sample was collected from
the digesters. Therefore, the amount of the digesta in the digester increased 750 ml every week. This pattern of feeding was employed to simulate the feeding of natural lagoon.

Figure 5 shows the monitoring greenhouse gas emission section of the experiment. It includes digesters, biogas production monitoring system (water replacement), and methane (CH$_4$ and CO$_2$) concentration analysis system. The portable gas chromatograph (GC) used in this experiment sequentially measures sampled gas and passes the information to the computer that automatically logs data. The auto-acquiring system was set to analyse and record CH$_4$ and CO$_2$ concentration every 10 minutes for every digester, standard gas tank, and room air. Five temperature sensors were attached to 5 digesters to monitor temperature every 10 minutes.

A flowcell equipment was used to monitor pH, temperature, conductivity, and ORP weekly (Fig. 6). Up to now, the experiment was running well and data have been collected for two months.
Objective 5. Results of greenhouse gas emissions monitored for 139 days

The experiment run for 20 weeks (139 days) and was completed on January 22, 2010. After the experiment was finished, the digesters was emptied and tested for gas leakage. The leakage testing was carried out as following processes:

1) Each digester was rinsed with tap water several times and filled with 5L tap water.
2) Liquid levels of two equalized bottles with a difference more than 20 cm were set.
3) Every portion of the digester was closed and connected to equalized bottles. Liquid level of the bottle with graduation was recorded.
4) Liquid level change over time was recorded. The liquid level will not change if a setup does not leak. Otherwise, leakage rate was calculated.

The leakage test found the leakage in digester #3 and #7. Therefore, the gas data obtained from those two setup was not included in the analysis.

**Objective 5.1 Methane**

**The effect of diet on CH₄ emission**

Figure 7 shows the accumulated CH₄ emissions from the digesters fed three different manures. Effect of diet on CH₄ emissions was not apparent in the first 45 days. After 45 days, however, the digesters fed with high fiber contented manure started to produced more CH₄. There was no obvious difference in accumulated CH₄ emission between the digesters fed medium and high fiber content manure (Fig. 7).

![Figure 7](image-url)
Based on the data of accumulated CH$_4$ emissions for 139 days (Table 2), there was significant difference between the three different manures at 10% level of significance (Table 3). The multiple comparison showed that manure with high fiber content had a significantly higher CH$_4$ production than manure with medium fiber content at 5% and significantly higher CH$_4$ production than manure with low fiber content at 10%. There is no significant difference in CH$_4$ production between manure with medium and low fiber contents (Table 5). The high fiber diet also resulted in a significantly high fiber content in manure, and this may be the reason that manure from high fiber diet produce significantly high CH$_4$ than other manures. The manure analysis haven't finished now and the results will be present later.

**Table 3 Cumulative methane emissions from three different manures**

<table>
<thead>
<tr>
<th>Diets</th>
<th>Amount of CH$_4$ emission (ml)</th>
<th>Average (ml)</th>
<th>STD (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replicate 1</td>
<td>Replicate 2</td>
<td>Replicate 3</td>
</tr>
<tr>
<td>Low fibre content</td>
<td>161304.99</td>
<td>---</td>
<td>146309.40</td>
</tr>
<tr>
<td>Medium fibre</td>
<td>158625.99</td>
<td>145031.52</td>
<td>134519.86</td>
</tr>
<tr>
<td>content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High fibre content</td>
<td>179755.69</td>
<td>---</td>
<td>177122.12</td>
</tr>
</tbody>
</table>

**Table 4. ANOVA analysis of the effect of three manures on accumulated CH$_4$ emission**

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>1.30x10$^8$</td>
<td>2</td>
<td>6.48x10$^8$</td>
<td>6.36</td>
<td>0.057</td>
</tr>
<tr>
<td>Within Groups</td>
<td>4.08x10$^8$</td>
<td>4</td>
<td>1.02x10$^8$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.7 1x10$^9$</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Multiple Comparisons of accumulated methane emissions between three manures

<table>
<thead>
<tr>
<th>(I) Diet</th>
<th>(J) Diet</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fiber</td>
<td>Medium fiber</td>
<td>7748.07</td>
<td>9219.98</td>
<td>0.448</td>
<td>-17850.69</td>
</tr>
<tr>
<td>High fiber</td>
<td></td>
<td>-24631.71</td>
<td>10099.98</td>
<td>0.071</td>
<td>33346.83</td>
</tr>
<tr>
<td>Medium fiber</td>
<td>Low fiber</td>
<td>-7748.07</td>
<td>9219.98</td>
<td>0.448</td>
<td>-33346.83</td>
</tr>
<tr>
<td>High fiber</td>
<td></td>
<td>-323 00*</td>
<td>9219.98</td>
<td>0.025</td>
<td>17850.69</td>
</tr>
<tr>
<td>High fiber</td>
<td>Low fiber</td>
<td>24631.71</td>
<td>10099.98</td>
<td>0.071</td>
<td>-3410.33</td>
</tr>
<tr>
<td>Medium fiber</td>
<td></td>
<td>32379.781*</td>
<td>9219.98</td>
<td>0.025</td>
<td>52673.75</td>
</tr>
</tbody>
</table>

*: The mean difference is significant at the 0.05 level.

**Methane concentration**

Similar to reported data, biogas emitted from anaerobic digestion is mainly composed of CH₄ and CO₂. Methane comprised of 60-70% and the rest is mainly CO₂. The CH₄ concentration increased for 35 days and becomes relatively stable around 60 - 65% afterwards (Fig. 8). The diet effect on CH₄ concentration has not been observed from this study. There is variation in CH₄ concentration over time, especially a "valley" appeared by the end of the experiment (Fig. 8). This may be due to testing error by the GC because this phenomenon was observed in all digesters.
Methane emission rate

In this study, methane emission rate was defined as the volumetric CH₄ emitted from one litre digesta in one day. Manure from low fiber diet has an average emission rate of 95 ml L⁻¹ (Digesta) d⁻¹, manure from medium fiber diet has an average emission rate of 90 ml L⁻¹ (Digesta) d⁻¹, and manure from high fiber diet has an average emission rate of 113 ml L⁻¹ (Digesta) d⁻¹. The statistical results show that the manure from high fiber content diet has significant higher emission rate than the manures from low and medium fiber content diets. There is no significant difference in CH₄ emission rate between manures from low and medium fiber contents.

The methane emission rates vary greatly over time (Fig. 9). Temperature variation, time of feeding manure, and time storage period were the three major factors contributing to variation of CH₄ emission rate. Figure 9 shows that temperature variation corresponds to CH₄ emission rate variation.
Table 6. Average methane emission rates from three different manures

<table>
<thead>
<tr>
<th>Diets</th>
<th>CH₄ emission rate (ml L⁻¹ (Digesta) d⁻¹)</th>
<th>Average (ml L⁻¹ (Digesta) d⁻¹)</th>
<th>STD (ml L⁻¹ (Digesta) d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replicate 1</td>
<td>Replicate 2</td>
<td>Replicate 3</td>
</tr>
<tr>
<td>Low fibre content</td>
<td>100.18</td>
<td>---</td>
<td>89.96</td>
</tr>
<tr>
<td>Medium fibre content</td>
<td>98.78</td>
<td>90.15</td>
<td>80.98</td>
</tr>
<tr>
<td>High fibre content</td>
<td>113.91</td>
<td>---</td>
<td>112.04</td>
</tr>
</tbody>
</table>

Table 7. ANOVA analysis of the effect of three manures on CH₄ emission rate

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>659.11</td>
<td>2</td>
<td>329.55</td>
<td>6.20</td>
<td>.059</td>
</tr>
<tr>
<td>Within Groups</td>
<td>212.44</td>
<td>4</td>
<td>53.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>871.55</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Multiple Comparisons of methane emissions rate between three manures

<table>
<thead>
<tr>
<th>(I) Diet (J) Diet</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
</tr>
<tr>
<td>1 2</td>
<td>5.10</td>
<td>6.65</td>
<td>.486</td>
<td>-13.3709</td>
<td>23.57</td>
</tr>
<tr>
<td>1 3</td>
<td>-17.90</td>
<td>7.28</td>
<td>.070</td>
<td>-38.1388</td>
<td>2.32</td>
</tr>
<tr>
<td>2 1</td>
<td>-5.10</td>
<td>6.65</td>
<td>.486</td>
<td>-23.57</td>
<td>13.37</td>
</tr>
<tr>
<td>2 3</td>
<td>-23.00*</td>
<td>6.65</td>
<td>.026</td>
<td>-41.47</td>
<td>-4.53</td>
</tr>
<tr>
<td>3 1</td>
<td>17.90</td>
<td>7.28</td>
<td>.070</td>
<td>-2.32</td>
<td>38.13</td>
</tr>
<tr>
<td>3 2</td>
<td>23.00*</td>
<td>6.65</td>
<td>.026</td>
<td>4.53</td>
<td>41.47</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.
**Figure 9. Time course of CH₄ emission rates of digesters fed with three different manures**

**The relationship between CH₄ emission rate and digester feeding**

Feeding digester with manure once a week is one of the reasons that variation of CH₄ emission rate is observed over time. If temperature remained relatively constant, after anaerobic digester is fed with fresh manure, the CH₄ emission rate increases over next 2 or 3 days and then decreases until next feeding time. This pattern is shown clearly in Fig. 10, which is an amplified time series of CH₄ emission rate that occurred from day 60 to 93. The feeding effect is more clear than other period because the digester temperature was maintained relatively constant than other periods and thus temperature effect was not a confounding factor. The fresh feeding manure for digesters contained easily digestible substrates for methanogens and other microbes. Fresh organic matter would be used by microbes to produce a large amount of CH₄. After the easily digestible substrates were exhausted, other more resistant substrate in the manure started to be used and produce less CH₄.
**Effect of temperature on methane emission rate**

Temperature effect on CH$_4$ emission rate can be identified by comparing CH$_4$ emission rate time courses with temperature recordings (Fig. 9). However, more clear temperature effect on CH$_4$ emission rate can be shown after removing feeding effect on the variation of CH$_4$ emission rate. Figure 11 shows each data point an average emission rate over its feeding period. For example, digesters were fed with fresh manure in day 48 and had next feeding at day 55. From day 48 to day 55, the period is entered as day 52 (median between days 48 and 55). Its emission rate was an average emission rate over days 48 and 55. Thus, the time course of the feeding period emission rate does not include a variation caused by feeding effect, and the temperature effect can be seen more clearly (Fig. 11). The correlation coefficients of CH$_4$ emission rate for three manures vs. temperature were 0.71, 0.78, and 0.81 for low, medium and high fibre diets, respectively.
Fig. 11. Methane emission rate courses of three manures. Each data point represents an average emission rate over its feeding period.
Fig. 12. Plot of methane emission rates vs. temperature.

The feed stage averaged data showed a power relationship between CH$_4$ emission rate and temperature (Fig. 12). However, the data used to generate the above figure is confounded with length of storage time. Additional data need to be produced for generating a reliable relationship between CH$_4$ emission rate and temperature.

**Effect of storage time on methane emission rate**

The emission rate of a litre of digesta increased for 50 days after the experiment begun. It reached an average value of 133 ml L$^{-1}$ (digesta) d$^{-1}$ at day 50. Afterwards, the emission rate declined lineally and became 74 ml L$^{-1}$ (digesta) d$^{-1}$ at day 130. The pattern of CH$_4$ emission rate course can be explained by bacterial activity and substrate supplement. In the first 50 days, the bacteria were supplied with sufficient substrates and their population increased continuously and reached a maximum at 50 days. Afterwards, the bacteria exhausted all the readily digestible
substrates and start to utilize more resistant materials. The available substrates were not enough to support a large population of bacteria, so their numbers might have decreased with concomitant decrease in CH$_4$ emission rate.

![Graph showing CH$_4$ emission rate over time](image)

*Figure 13. The change of CH$_4$ emission rate over time*

**Object 5.2. Carbon Dioxide**

**Effect of diet on CO$_2$ emission**

Figure 14 shows the cumulative CO$_2$ emissions from the digesters fed with three different manures. Similar to CH$_4$ emission, effect of diet on CO$_2$ emissions was not apparent in the first 45 days. After 45 days, however, the digesters fed with high fiber content manure started to produce more CO$_2$. Figure 14 also shows that manure from low fiber diet emitted less CO$_2$ than the manure from high fiber content and more CO$_2$ than the manure from medium fiber content diet. However, the statistical analysis showed that there was no significant difference in cumulative CO$_2$ emission between manure from medium and low fiber content diets.
Based on the data of cumulative CO$_2$ emissions for 139 days (Table 9), there was a significant difference between the three different manures at 5% significance level (Table 10). The multiple comparison showed that manure from high fiber content diet had a significantly higher CO$_2$ production than both manures from medium and low fiber content diets. There was no significant difference in cumulative CO$_2$ production between manure from medium and low fiber content diet (Table 11).
Table 9. Cumulative CO₂ emissions from three different manures

<table>
<thead>
<tr>
<th>Diets</th>
<th>Amount of CO₂ emission (ml)</th>
<th>Average (ml)</th>
<th>STD (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replicate 1</td>
<td>Replicate 2</td>
<td>Replicate 3</td>
</tr>
<tr>
<td>Low fibre content</td>
<td>114844.16</td>
<td>---</td>
<td>102237.85</td>
</tr>
<tr>
<td>Medium fibre content</td>
<td>108039.73</td>
<td>98800.05</td>
<td>92819.43</td>
</tr>
<tr>
<td>High fibre content</td>
<td>126054.96</td>
<td>---</td>
<td>127624.57</td>
</tr>
</tbody>
</table>

Table 10. ANOVA analysis of the effect of three manures on accumulated CO₂ emission

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>1.04x10⁹</td>
<td>2</td>
<td>5.22 x10⁸</td>
<td>10.50</td>
<td>.026</td>
</tr>
<tr>
<td>Within Groups</td>
<td>1.99 x10⁸</td>
<td>4</td>
<td>4.97 x10⁷</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.24x10⁹</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 11. Multiple Comparisons of accumulated CO₂ emissions between three manures

<table>
<thead>
<tr>
<th>(I) Diet (J) Diet</th>
<th>Mean Difference</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(I-J)</td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Low fiber Medium fiber</td>
<td>8654.60</td>
<td>6433.99</td>
<td>.250</td>
<td>-9209.02</td>
</tr>
<tr>
<td>High fiber</td>
<td>-20639.40</td>
<td>7048.08</td>
<td>.043</td>
<td>-40208.01</td>
</tr>
<tr>
<td>Medium fiber Low fiber</td>
<td>-8654.60</td>
<td>6433.99</td>
<td>.250</td>
<td>-26518.22</td>
</tr>
<tr>
<td>High fiber</td>
<td>-29294.00</td>
<td>6433.99</td>
<td>.010</td>
<td>-47157.62</td>
</tr>
<tr>
<td>High fiber Low fiber</td>
<td>20639.39*</td>
<td>7048.08</td>
<td>.043</td>
<td>1070.77</td>
</tr>
<tr>
<td>Medium fiber</td>
<td>29293.99*</td>
<td>6433.99</td>
<td>.010</td>
<td>11430.36</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.
Carbon dioxide concentration

Similar to reported data, biogas emitted from anaerobic digestion is mainly composed of CH$_4$ and CO$_2$. Methane composed of 60-70% and the rest mainly is CO$_2$. The CO$_2$ concentration increased for 30 days and reached around 45%. Then it dropped a little and became relatively stable at 40% (Fig.15). The diet effect on CO$_2$ concentration was not observed from this study. Corresponding to a "Valley effect" of CH$_4$ concentration observed at the end of experiment, a "Peak" of CO$_2$ concentration appeared by the end of the experiment (Fig. 8). This kind of gas concentration variation may come from GC testing error.

![Figure 15. Time course of CO$_2$ concentration of the digesters fed with three different manures](image-url)
**CO₂ emission rate**

Manure from low, medium and high fiber diets had an average CO₂ emission rates of 69, 62 and 81 ml L⁻¹ (Digesta) d⁻¹, respectively (Table 12). The statistical analysis shows that the manure from high fiber content diet had significantly higher emission rate than the manures from low and medium fiber content diets. There was no significant difference in CO₂ emission rate between manures from low and medium fiber contents (Table 13 and 14).

The CO₂ emission rates vary greatly over time (Fig. 16). Similar to CH₄ emission rate variation, temperature, time of feeding manure, and length of storage period were the three major factors contributing to the variation of CO₂ emission rate.

**Table 12** The 139 days' averaged CO₂ emission rates from three different manures

<table>
<thead>
<tr>
<th>Diets</th>
<th>CO₂ emission rate (ml L⁻¹ (Digesta) d⁻¹)</th>
<th>Average (ml L⁻¹ (Digesta) d⁻¹)</th>
<th>STD (ml L⁻¹ (Digesta) d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replicate 1</td>
<td>Replicate 2</td>
<td>Replicate 3</td>
</tr>
<tr>
<td>Low fibre content</td>
<td>73.19</td>
<td>---</td>
<td>65.24</td>
</tr>
<tr>
<td>Medium fibre content</td>
<td>67.28</td>
<td>61.81</td>
<td>55.81</td>
</tr>
<tr>
<td>High fibre content</td>
<td>80.94</td>
<td>---</td>
<td>81.29</td>
</tr>
</tbody>
</table>

**Table 13. ANOVA analysis of the effect of three manures on CO₂ emission rate**

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>455.506</td>
<td>2</td>
<td>227.753</td>
<td>9.345</td>
<td>.031</td>
</tr>
<tr>
<td>Within Groups</td>
<td>97.490</td>
<td>4</td>
<td>24.372</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>552.996</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 14. Multiple Comparisons of CO₂ emissions rate between three manures

<table>
<thead>
<tr>
<th>(I) Diet</th>
<th>(J) Diet</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fibre</td>
<td>Medium fibre</td>
<td>7.58</td>
<td>4.50</td>
<td>.168</td>
<td>-4.93</td>
<td>20.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High fibre</td>
<td>-11.90</td>
<td>4.93</td>
<td>.074</td>
<td>-25.60</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td>Medium fibre</td>
<td>Low fibre</td>
<td>-7.58</td>
<td>4.50</td>
<td>.168</td>
<td>-20.09</td>
<td>4.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High fibre</td>
<td>-19.48*</td>
<td>4.50</td>
<td>.012</td>
<td>-31.99</td>
<td>-6.96</td>
<td></td>
</tr>
<tr>
<td>High fibre</td>
<td>Low fibre</td>
<td>11.90</td>
<td>4.93</td>
<td>.074</td>
<td>-1.80</td>
<td>25.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium fibre</td>
<td>19.48*</td>
<td>4.50</td>
<td>.012</td>
<td>6.96</td>
<td>31.99</td>
<td></td>
</tr>
</tbody>
</table>

*. The mean difference is significant at the 0.05 level.

---

**Figure 16.** Time course of CO₂ emission rates of digesters fed with three different manures
Further study

This project successfully developed a setup for monitoring CH₄ and CO₂ emission from anaerobic manure storage. From last 6 months' experience of running the setup, we are confident that the setup is an accurate and convenient device for investigating CH₄ and CO₂ emission from liquid manure management systems. In this project, we used the setup successfully to quantify the effect of fiber content in diet on CH₄ and CO₂ emissions from stored swine manure. This setup will be useful for obtaining many other information related to GHG emission from manure management system. For example, B₀ values of many local materials, temperature effect on GHG emission from liquid manure storage, and the effect of loading rate on GHG emission from lagoon and tank can be determined. In the next phase we recommend further work on effect of diet (for example crude protein content) and manure loading rate on GHG emission from liquid manure storage.