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Abstract
Biogas digesters are commonly used in livestock farming in China. The digestion process converts large amounts of raw liquid manure (RLM) to biogas digester effluent (BDE). The BDE is then stored on the farm for some time before land application as crop or orchard nutrients. Storage of RLM or BDE is associated with gas emissions, although little information is available concerning comparison of air emissions between the two handling practices. This study was conducted to compare methane (CH\(_4\)), carbon dioxide (CO\(_2\)), nitrous oxide (N\(_2\)O), and ammonia (NH\(_3\)) emissions from RLM and BDE storages using dynamic emission vessels (DEVs). Both media were stored in closed vessels (50 L) at a 40 cm storage depth, a constant storage temperature of 30°C, and a headspace air exchange rate of 15 to 17 air changes per hour (ACH) for 22 days. The results showed that the average daily gas emission rates for RLM and BDE, in mg L\(^{-1}\) d\(^{-1}\), were, respectively, 102.9 and 125.3 CO\(_2\) (p < 0.05), 0.72 and 3.33 N\(_2\)O (p < 0.05), 29.2 and 0.32 CH\(_4\) (p < 0.05), and 1.21 and 0.66 NH\(_3\) (p = 0.08). The total greenhouse gas (GHG = CH\(_4\) + CO\(_2\) + N\(_2\)O) emissions were similar for RLM and BDE, 1.05 and 1.12 g CO\(_2\)-eq L\(^{-1}\) d\(^{-1}\), respectively (p = 0.26). Nitrous oxide (N\(_2\)O) emissions accounted for 88.2% of the CO\(_2\)-eq GHG emissions for BDE, whereas CH\(_4\) emissions accounted for 69.7% of the CO\(_2\)-eq GHG emissions for RLM. The high N\(_2\)O emissions from BDE likely resulted from the lower COD/N ratio in BDE than RLM under the storage conditions. Differences in gaseous emission characteristics between RLM and BDE were attributed to the differences in methanogen species and the population of ammonia-oxidizing bacteria (AOB).

Keywords
Ammonia, Biogas digester effluent, Greenhouse gas, Raw liquid manure

Disciplines
Agriculture | Bioresource and Agricultural Engineering

Comments
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ABSTRACT. Biogas digesters are commonly used in livestock farming in China. The digestion process converts large amounts of raw liquid manure (RLM) to biogas digestor effluent (BDE). The BDE is then stored on the farm for some time before land application as crop or orchard nutrients. Storage of RLM or BDE is associated with gas emissions, although little information is available concerning comparison of air emissions between the two handling practices. This study was conducted to compare methane (CH₄), carbon dioxide (CO₂), nitrous oxide (N₂O), and ammonia (NH₃) emissions from RLM and BDE storages using dynamic emission vessels (DEVs). Both media were stored in closed vessels (50 L) at a 40 cm storage depth, a constant storage temperature of 30°C, and a headspace air exchange rate of 15 to 17 air changes per hour (ACH) for 22 days. The results showed that the average daily gas emission rates for RLM and BDE, in mg L⁻¹ d⁻¹, were, respectively, 102.9 and 125.3 CO₂ (p < 0.05), 0.72 and 3.33 N₂O (p < 0.05), 29.2 and 0.32 CH₄ (p < 0.05), and 1.21 and 0.66 NH₃ (p = 0.08). The total greenhouse gas (GHG = CH₄ + CO₂ + N₂O) emissions were similar for RLM and BDE, 1.05 and 1.12 g CO₂-eq L⁻¹ d⁻¹, respectively (p = 0.26). Nitrous oxide (N₂O) emissions accounted for 88.2% of the CO₂-eq GHG emissions for BDE, whereas CH₄ emissions accounted for 69.7% of the CO₂-eq GHG emissions for RLM. The high N₂O emissions from BDE likely resulted from the lower COD/N ratio in BDE than RLM under the storage conditions. Differences in gaseous emission characteristics between RLM and BDE were attributed to the differences in methanogen species and the population of ammonia-oxidizing bacteria (AOB).

Keywords. Ammonia, Biogas digester effluent, Greenhouse gas, Raw liquid manure.

China is the largest pig producer in the world. According to FAO (2011) statistics, China had 471 million pigs, accounting for 55% of the total domestic large livestock population (i.e., cattle, buffaloes, pigs, sheep, and goats) and nearly 50% of global pig production. The increase in pig production has led to the generation of more pig manure. In 2007, the amount of pig manure reached approximately 2.0 billion Mg, accounting for 50.6% of the total animal manure production in China (Zhang et al., 2009). Understanding of gaseous emissions from pig manure storage is important for determining the gas emissions from the entire livestock farming sector in China.

To reduce pollution caused by raw manure, researchers and farmers alike have concentrated on finding more integrated manure management systems. The anaerobic digestion system is an attractive method for producing energy, reducing odors, recycling organic nutrients, and improving utilization of the manure as fertilizer (Umetsu et al., 2005; IPCC, 2006). Consequently, biogas digesters are commonly used in livestock farming as a method for energy production in China. By the end of 2009, there were 39,510 agricultural biogas plants in China, with a total digester volume of 4.51 million m³ (Chinese Renewable Energy Industry Association, 2010). In the meantime, large amounts of anaerobic fermentation byproducts are produced from the biogas plants, leading to on-farm effluent storage.

Several studies have indicated that both raw liquid manure (RLM) and biogas digestor effluent (BDE) storage in livestock farming are important sources of ammonia (NH₃), methane (CH₄), nitrous oxide (N₂O), and carbon dioxide (CO₂) emissions (Clemens et al., 2006; Hansen et al., 2006; Gioelli et al., 2011). The extents of these emissions depend on various parameters, such as volume and manure composition, type and duration of manure storage, and weather conditions (i.e., temperature, wind velocity, and air humidity) (Loyon et al., 2007).

Amon et al. (2006) studied gas emissions from raw cattle slurry storage and subsequent land application and found that 90% of all CH₄ emissions occurred during slurry storage. Loyon et al. (2007) examined 180 days of raw piggy slurry storage and found that the annual carbon emissions in terms of CH₄ and CO₂ accounted for 30% of the raw slurry carbon. Large amounts of NH₃ can be emitted from lagoons. For swine waste storage, the available...
data suggest that NH\textsubscript{3} emissions are between 0.18 and 7.02 g m\textsuperscript{-2} d\textsuperscript{-1} (Aneja et al., 2000; Petersen et al., 2013). Ammonia-N emissions from lagoons were estimated to account for 33% of the total NH\textsubscript{3}-N emissions from commercial hog operations under the production and climatic conditions of North Carolina (Aneja et al., 2000). Nitrous oxide is another strong greenhouse gas (GHG), with a global warming potential (GWP) of 298 (IPCC, 2007). Most studies have indicated low or nearly no N\textsubscript{2}O emissions from animal waste water storage (Park et al., 2006; Li et al., 2008). A few studies have indicated that N\textsubscript{2}O emissions increase from livestock slurry storage under ventilated conditions (Amon et al., 2006; Molodovskaya et al., 2008).

Several studies have shown that biogas effluent storage in livestock farming is an important source of GHG emissions (Sommer et al., 2000; Clemens et al., 2006; Hansen et al., 2006; Gioelli et al., 2011; Menardo et al., 2011). Moreover, the anaerobic fermentation process always leads to increased ammonium ion (NH\textsubscript{4}\textsuperscript{+}) concentration in the BDE, causing abundant NH\textsubscript{3} emissions during the storage process (Sommer, 1997). Ammonia emissions from stored anaerobically digested animal slurry were even higher than from raw animal slurry (Sommer, 1997; Sommer et al., 2000).

Furthermore, N\textsubscript{2}O is produced during both the nitrification and the denitrification processes (Groenestein and Van Faassen, 1996). Because NH\textsubscript{4}\textsuperscript{+} acts as a nitrification substrate, the increased NH\textsubscript{4}\textsuperscript{+} concentration would lead to higher N\textsubscript{2}O emissions in anaerobically digested animal slurry storage compared with raw animal slurry (Sommer et al., 2000; Amon et al., 2006).

The differences between air emissions from RLM and BDE storages need to be further investigated. Some data are available that directly compare air emissions between raw animal waste and fermented animal waste storages. Sommer et al. (2000) compared CH\textsubscript{4} and N\textsubscript{2}O emissions from raw cattle slurry and fermented cattle slurry storage during summer and winter. The results showed that the gas emission differences were more pronounced in summer due to higher temperatures (15°C to 25°C) compared with winter (0°C to 10°C). Umetsu et al. (2005) compared CH\textsubscript{4} emissions from stored dairy slurry and digested slurry under controlled temperatures of 5°C, 10°C, 15°C, and 20°C. Their results indicated that the slurry characteristics and storage temperature had large effects on CH\textsubscript{4} emissions. However, limited data are available for the four major polluting gases (i.e., NH\textsubscript{3}, CH\textsubscript{4}, N\textsubscript{2}O, and CO\textsubscript{2}) from RLM and BDE stored on pig farms at warm temperatures. Thus, the primary objective of this study was to compare gas (NH\textsubscript{3}, CH\textsubscript{4}, N\textsubscript{2}O, and CO\textsubscript{2}) emissions from RLM and BDE under controlled laboratory conditions, which would provide insight into mitigation of gas emissions from storages. We also profiled methanogens and ammonia-oxidizing bacteria (AOB) during the study period to further understand the gaseous emissions based on the microbial characteristics of RLM and BDE.

**MATERIALS AND METHODS**

**RAW LIQUID MANURE (RLM) AND BIOGAS DIGESTER EFFLUENT (BDE)**

Raw liquid manure and BDE were collected from a commercial swine farm near Beijing. Flush water and the solid manure (collected with scrapers) from the pig barn were mixed in an average volume ratio of 40:1 and used as an influent for the biogas digester. The biogas digester at the pig farm operated under mesophilic conditions with a hydraulic retention time of 15 to 20 days. The RLM and BDE used in this experiment were the biogas digester influent and the effluent, respectively. The RLM and BDE were transported to the storage lab in Beijing within 2 h after collection at farm. After homogenizing of each media, the two media were pumped into their respective storage vessels.

**DYNAMIC EMISSION VESSELS (DEVs)**

Six Plexiglas cylindrical DEVs (50 cm outside diameter × 66 cm height each) were designed and built (fig. 1). Each DEV consisted of five parts: the main internal bucket (40 cm diameter × 66 cm height), a water jacket, inlet and outlet ports, sampling ports, and an air distribution unit. The air distribution unit was cross-shaped using four PVC pipes. Each pipe of the distribution unit was 7.5 cm in length and had five 1 cm dia. holes facing the lid. Fresh air was introduced into each vessel through the air distribution unit near the top of each vessel and was exhausted above the liquid manure surface without disturbing the liquid surface. A submersible electrical heater was used to heat the water jacket. A 2 cm layer of thermal insulation foam was applied to the exterior of the storage vessel to better maintain the desired storage temperature (30°C) of the DEV. Six RLM and BDE samples were stored in individual DEVs for 22 d. The air exchange rate of each vessel was set at 15 to 17 air changes per hour (ACH) based on Li and Xin (2010), who reported that ACH of 10 or 20 did not cause differ-

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**Figure 1. Schematic of the dynamic emission vessel (DEV).**
ences in GHG emissions from poultry manure storage. A schematic representation of the experimental setup is shown in figure 2.

A photoacoustic multi-gas analyzer (model 1312, Innova AirTech Instruments, Ballerup, Denmark) and a multichannel sampler (fabricated at the Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing, China) were used to sample and analyze air samples from the six exhaust outlets and the fresh air supply. Before measurements, the multi-gas analyzer was checked and calibrated, as needed, using individual CO₂, CH₄, N₂O, NH₃, and nitrogen (N₂) standard calibration gases procured from the National Standard Material Center in Beijing, China. The gas analyzer was checked every 1 to 2 weeks during the experiment with the span gases to ensure that deviations in measured readings were less than 5%; otherwise, the gas analyzer would be recalibrated. For each of the seven air samplings (i.e., six exhausts and one inlet), five 2 min measurement cycles were completed by the INNOVA gas analyzer, with the first four cycles for stabilization and the fifth as the measured value. Thus, it took a total of 70 min to complete one system sampling cycle. A total of 20 measurements were made per day for each vessel during the entire storage monitoring period.

**DETERMINATION OF GAS EMISSION RATE**

Concentrations of CO₂, CH₄, N₂O, and NH₃ of the fresh and exhaust air were automatically measured and recorded. With a known airflow rate and surface area, the gas emission rate from the storage vessel was calculated using the following equations:

\[
ER_A = \frac{(C_o - C_i) \times VR}{A}
\]

(1)

\[
ER_V = \frac{(C_o - C_i) \times VR}{V}
\]

(2)

where \(ER_A\) and \(ER_V\) are the emission rates (mg m⁻² h⁻¹ and mg L⁻¹ h⁻¹, respectively), \(C_i\) and \(C_o\) are the inlet and outlet gas concentrations, respectively (mg m⁻³), \(VR\) is the ventilation rate (m³ h⁻¹) of the storage vessel, \(A\) is the surface area of the DEV (m²), and \(V\) is the volume of the stored material (L).

**SAMPLE COLLECTION AND ANALYSIS**

A 500 mL sample was collected from each of the two sampling ports on each storage vessel before and after the storage period. Total solids (TS), volatile solids (VS), total nitrogen (TN), total ammoniacal N (TAN), chemical oxygen demand (COD), and nitrite plus nitrate-N (NO₂⁻N + NO₃⁻N = NOₓ⁻N) were determined according to standards of the China National Environmental Protection Agency (2002).

The pH and dissolved oxygen (DO) levels were measured with a calibrated pH meter (Easy pH, WTW GmbH, Weilheim, Germany) and a digital oxygen meter (HI9146, Hanna Instruments, Woonsocket, R.I.), respectively. Total solid and VS contents were determined by oven-drying the samples at 105°C and using a muffle furnace at 550°C, respectively. The COD level was determined using the dichromate method according to China National Standard GB11914-89. The TAN concentration was determined using the distillation-neutralization titration method according
to China National Standard HJ 537-2009 with a distillation unit (B-324, Büchi Labortechnik AG, Flawil, Switzerland). The TN content was measured using the alkaline potassium persulfate digestion UV spectrophotometric method with an ultraviolet spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). The collected sample was centrifuged and filtered through 0.45 μm membrane filters (Gelman type Supor-450, Pall Corp., Ann Arbor, Mich.) and then analyzed for NOX-N and dissolved organic carbon (DOC). The NOX-N concentration was monitored with a flow injection analyzer (FIAnalyst 5000, Foss, Hillerød, Denmark). Lastly, DOC was measured with the non-dispersive infrared absorption method using a total organic carbon (TOC) analyzer (Apollo 9000, Teledyne Tekmar, Mason, Ohio).

**DNA EXTRACTION, PCR-DGGE ANALYSIS, AND QUANTITATIVE REAL-TIME PCR ANALYSIS**

The DNA and PCR analyses were conducted to study the microbial changes in the two media. The liquid media samples were collected at the beginning and end of storage and were immediately transported to the laboratory for further treatments. Total DNA was extracted according to the manufacturer’s instructions using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The DNA concentration and quality were determined at A260 nm and A280 nm using a spectrophotometer (Bio-Rad, Hercules, Cal.). The PCR amplification was performed in a C1000 thermal cycler (Bio-Rad, Hercules, Cal.). The partial 16S rDNA of methanogens was amplified using universal primer pairs of 519F and 915r with a GC-clamp, as described by Coolen et al. (2004). The AOB gene was amplified using amoA-1F with a GC-clamp and amoA-2R primer pairs, as described by Rotthauwe et al. (1997). Denaturing gradient gel electrophoresis (DGGE) was performed using the Decode system (Bio-Rad, Hercules, Cal.). The PCR amplicons were separated using a 6% polyacrylamide gel in 0.5 x TAE buffer with a linear denaturing gradient from 30% to 60%. The gel was run at 60°C and 85 V for 16 h and stained with ethidium bromide (0.5 mg L⁻¹) for 20 min. The gel was then washed with 400 mL distilled water for 10 min and photographed using the MiniLumi gel documentation system (DNR, Jerusalem, Israel). Real-time PCR was performed using an IQ5 system (Bio-Rad, Hercules, Cal.). The uniMet1-F and uniMet1-R primer pairs were used to amplify the 16S rDNA gene of methanogens, as described by Zhou et al. (2009). The amoA-1F and amoA-2R primer pairs were used to amplify the AOB gene. The PCR efficiency (E) was calculated using the equation E = (10⁻¹/slope – 1) × 100, with efficiencies near 100%.

**STATISTICAL ANALYSES**

Statistical analyses of gas emission data, media properties, and real-time PCR data were performed using analysis of variance (ANOVA) with SAS 9.2 (SAS Institute Inc., Cary, N.C.). Pearson’s correlation analysis between gas emissions was performed with SAS 9.2. Similarities among the DNA fingerprints were detected using Fingerprinting II software (Bio-Rad, Hercules, Cal.). Furthermore, clustering was performed using the unweighted pair group method with arithmetic averages (UPGMA) according to Nei (1987). Microorganism diversity information was calculated using the Shannon-Wiener index, which combines two components of diversity: richness and evenness of individuals among the species (Krebs, 1985). Pearson’s correlation analysis was used to determine relationships between AOB and methanogens with bootstrap values of 1000. Differences among means were considered significant at the p < 0.05 level.

**RESULTS AND DISCUSSION**

**PROPERTIES OF RLM AND BDE**

Table 1 shows that organic matter content indices of RLM, including COD, DOC, TS, and VS, were significantly higher than those of BDE at the beginning of the experiment (p < 0.05). After storage, these organic components in RLM clearly decreased. The COD and DOC indices of BDE also decreased, but to a lesser extent. However, the TS and VS contents of BDE remained constant during the storage, which is possibly attributable to a lower reduction in decomposed organic matter. Water evaporation could have also contributed to the basically constant percentages of TS and VS in BDE. During storage, a larger TAN decrease and NOX-N content increase were observed with BDE as compared to RLM, indicating occurrence of nitrification and/or denitrification in the BDE storage.

**CH₄ AND CO₂ EMISSIONS FROM RLM AND BDE**

Emission rates and cumulative emissions of CH₄ and CO₂ from RLM and BDE are shown in figure 3. The CH₄ emissions from RLM were significantly higher than those from

<table>
<thead>
<tr>
<th>Constituent</th>
<th>End</th>
<th>Start</th>
<th>End</th>
<th>Start</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.9 ±0.0 ab</td>
<td>7.7 ±0.5 b</td>
<td>7.7 ±0.1 ab</td>
<td>8.2 ±0.2 a</td>
</tr>
<tr>
<td>COD (mg L⁻¹)</td>
<td>809 ±59 b</td>
<td>5290 ±587 a</td>
<td>5153 ±577 b</td>
<td>752 ±73 b</td>
</tr>
<tr>
<td>DOC (mg L⁻¹)</td>
<td>167 ±2 c</td>
<td>747 ±23 a</td>
<td>200 ±17 b</td>
<td>159 ±4 c</td>
</tr>
<tr>
<td>TN (mg L⁻¹)</td>
<td>561 ±21 c</td>
<td>788 ±91 a</td>
<td>701 ±50 ab</td>
<td>602 ±20 bc</td>
</tr>
<tr>
<td>TAN (mg L⁻¹)</td>
<td>538 ±27 c</td>
<td>661 ±12 a</td>
<td>614 ±29 b</td>
<td>398 ±22 d</td>
</tr>
<tr>
<td>NOX-N (μg L⁻¹)</td>
<td>22.3 ±10.1 b</td>
<td>4.7 ±8.1 b</td>
<td>38.1 ±11.9 b</td>
<td>201,893 ±3,678 a</td>
</tr>
<tr>
<td>TS (% FM)</td>
<td>0.19 ±0.00 b</td>
<td>0.38 ±0.05 a</td>
<td>0.21 ±0.01 b</td>
<td>0.22 ±0.00 b</td>
</tr>
<tr>
<td>VS (% FM)</td>
<td>0.06 ±0.00 b</td>
<td>0.20 ±0.03 a</td>
<td>0.08 ±0.00 b</td>
<td>0.08 ±0.00 b</td>
</tr>
<tr>
<td>Methanogenic abundance (10⁶ copies mL⁻¹)</td>
<td>4.78 ±0.21 a</td>
<td>5.29 ±0.37 a</td>
<td>4.97 ±0.11 a</td>
<td>4.36 ±0.48 a</td>
</tr>
<tr>
<td>AOB abundance (10⁶ copies mL⁻¹)</td>
<td>0.86 ±0.02 a</td>
<td>1.00 ±0.10 a</td>
<td>1.52 ±0.21 b</td>
<td>1.13 ±0.01 b</td>
</tr>
</tbody>
</table>

[¹] Values are means ±SD (n = 3). Within a row, means followed by different letters are significantly different (p < 0.05).

[²] FM = fresh matter basis.
The RLM CH4 emissions increased during the early storage stage and decreased afterward (fig. 3a). The CH4 emission rate of 486.8 ± 12.9 mg m⁻² h⁻¹ from RLM was considerably lower than the literature values. Park et al. (2010) reported a CH4 emission rate of 5940.0 mg m⁻² h⁻¹ for liquid swine manure storage in a tank with a storage depth of 2.5 m during the summer. Todd et al. (2011) reported a CH4 emission rate of 4262.4 mg m⁻² h⁻¹ from a dairy wastewater lagoon with a storage depth of generally 1 to 2 m. The lower CH4 emission rate observed in the current study could have been due to the high air exchange rate and the shallow storage depth, which may have resulted in a more aerobic environment. The DO level of RLM increased from 0.34 to 4.03 mg L⁻¹ during the last week of storage. For the BDE storage, the CH4 emission rate was 26.4-28.0 mg m⁻² h⁻¹ during the first three days, decreasing to nearly zero afterward (fig. 3a). The DO level of BDE ranged from 2.2 to 6.3 mg L⁻¹ during the entire storage process, which largely inhibited the methanogens, thereby causing low CH4 emissions. Sommer et al. (2000) reported a similar CH4 emission pattern, with high emission first and then decreasing afterward, from fermented dairy slurry storage. Furthermore, Sommer et al. (2000) indicated that fermentable organic material decomposition remaining in the fermented slurry was the primary reason for the relatively high CH4 emission rate during the early storage stage. After decomposition of the remaining organic matter and continuous ventilation during storage, the CH4 emissions from BDE were nearly negligible. In particular, the BOD5/COD ratio of BDE in this experiment was 0.177, suggesting low bio-degradability, while the BOD5/COD ratio of RLM was 0.624. The primary factor for the nearly negligible CH4 emissions from BDE could be its low fermentable organic matter concentration.

The CH4 and CO2 emissions showed a strong positive relationship (fig. 3) during the first ten days of RLM storage (r = 0.915, p = 0.0002). However, during the later storage stage, CH4 emissions steadily decreased with time but the CO2 emission pattern was relatively stable (fig. 3). The anaerobic degradation of organic components produced CH4 and CO2 simultaneously (Steed and Hashimoto, 1994). Moreover, significant amounts of CO2 can also be produced at the liquid-air interface via aerobic microbial degradation processes (Møller et al., 2004). The decreased organic matter but increased aerobic surface due to the continuous inlet air jointly affected CO2 emissions from RLM, causing the CO2 emissions to decrease at a lower rate than CH4. The CO2 emissions from BDE were significantly higher than from RLM, with higher cumulative CO2 emissions in BDE in the 22-day storage. The difference in cumulative CO2 emissions between RLM and BDE would have been smaller had the storage time been extended because the emission rate from RLM started to exceed that of BDE and became quite stable during the latter storage period.
N₂O and NH₃ Emissions from RLM and BDE

Emission rates and cumulative emissions of N₂O and NH₃ from RLM and BDE are shown in figure 4. The N₂O emission rate from BDE was significantly higher than that from RLM (table 2), first increasing and then decreasing (fig. 4a). In RLM, the N₂O emissions showed a slowly increasing trend during the entire storage period (fig. 4a). The N₂O emission rates from both RLM and BDE were higher than the emission values reported in the literature under different manure storage conditions. Moset et al. (2011) reported a N₂O emission rate of 1.98 mg m⁻² h⁻¹ for pig slurry storage (TS = 37,100 mg kg⁻¹). Park et al. (2006) observed negligible N₂O emissions from non-aerated liquid swine manure storage (TS = 6,610 to 30,519 mg kg⁻¹).

Compared with RLM, the BDE had substantially higher N₂O emissions. The N₂O-N loss was 6.6% of the initial TN for BDE storage and only 1.3% for RLM storage. Molodovskaya et al. (2008) reported a N₂O-N loss of 4.2% from aerated dairy slurry storage (TS = 1.4% fresh matter basis). Some studies also indicated that fermented manure had higher N₂O emission potential than untreated manure. The N₂O emissions from digested cattle slurry storage were 26% higher than for raw cattle slurry (Sommer et al., 2000). Amon et al. (2006) found that N₂O emissions were 41% higher from digested dairy slurry storage than from unprocessed slurry.

Nitrous oxide can be produced from the process of either nitrification and/or denitrification. Transformation from NH₄⁺ to NO₃⁻ via nitrification is a source of N₂O when NO₃⁻ undergoes denitrification. The denitrification process

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### Table 2. Gas emission rates from raw liquid manure (RLM) and biogas digester effluent (BDE) stored for 22 days at 30°C.

<table>
<thead>
<tr>
<th>Media</th>
<th>Units</th>
<th>Statistic</th>
<th>CO₂</th>
<th>CH₄</th>
<th>N₂O</th>
<th>NH₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg m⁻² h⁻¹</td>
<td>Maximum</td>
<td>2540.0</td>
<td>945.8</td>
<td>28.56</td>
<td>51.4</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>1137.0</td>
<td>137.5</td>
<td>0.12</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>1714.6 ±112.2</td>
<td>486.8 ±12.9</td>
<td>12.03 ±2.83</td>
<td>20.2 ±6.8</td>
<td></td>
</tr>
<tr>
<td>RLM</td>
<td>mg L⁻¹ d⁻¹</td>
<td>Mean ±SD</td>
<td>102.9 ±6.8 b</td>
<td>29.2 ±20.8 a</td>
<td>0.72 ±0.17 b</td>
<td>1.21 ±0.41 a</td>
</tr>
<tr>
<td></td>
<td>mg m⁻³ h⁻¹</td>
<td>Maximum</td>
<td>3700.3</td>
<td>28.0</td>
<td>108.7</td>
<td>56.1</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>1082.8</td>
<td>0</td>
<td>6.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>2088.4 ±55.2</td>
<td>5.3 ±2.3</td>
<td>55.4 ±5.3</td>
<td>11.00 ±1.59</td>
<td></td>
</tr>
<tr>
<td>BDE</td>
<td>mg L⁻¹ d⁻¹</td>
<td>Mean ±SD</td>
<td>105.3 ±3.3 a</td>
<td>0.32 ±0.14 b</td>
<td>3.33 ±0.32 a</td>
<td>0.66 ±0.10 a</td>
</tr>
</tbody>
</table>

[a] Within a column, means of the gas emission rate followed by different letters are significantly different (p < 0.05).

![Figure 4](image-url)

**Figure 4.** (a) Daily N₂O emission rate (mean ±SD) from raw liquid manure (RLM) and biogas digester effluent (BDE), (b) cumulative N₂O emissions (mean ±SD) from RLM and BDE, (c) daily NH₃ emission rate (mean ±SD) from RLM and BDE, and (d) cumulative NH₃ emissions (mean ±SD) from RLM and BDE during 22-day storage at a constant temperature of 30°C.
means biological reduction of NO\textsubscript{3}^{-} to N\textsubscript{2} gas, where N\textsubscript{2}O is an important product of incomplete denitrification (Chadwick et al., 2011). During denitrification, DO, low pH, or low carbon content will lead to incomplete denitrification and an increase in N\textsubscript{2}O emissions (Hynes and Knowles, 1984; Bernet et al., 1996; Béline et al., 1999).

Ventilation rate is a major factor influencing N\textsubscript{2}O emissions. Previous studies have reported positive relationships between N\textsubscript{2}O emissions and ventilation rate (Park et al., 2006; Molodovskaya et al., 2008). In a well-ventilated environment, N\textsubscript{2}O was produced at the boundary between the anaerobic and aerobic environments (Osada et al., 1995; Sommer et al., 2000; Fukumoto et al., 2003). Amon et al. (2006) found that N\textsubscript{2}O emissions from aerated dairy cattle slurry increased by 144.1% compared with untreated slurry. Willers et al. (1996) reported that for continuous high ventilation rates and a lack of readily degradable organic matter, N\textsubscript{2}O was primarily produced during the nitrification process, especially in the presence of NO\textsubscript{X} accumulation, which occurred in this experiment (table 1). However, little data were available regarding the relationship between N\textsubscript{2}O emissions and storage temperature. It has been suggested that high storage temperatures (30°C to 35°C) increase the nitrification rate (Antoniou et al., 1990). The high ventilation rate and high temperature (30°C) in the current experiment were expected to create an environment more conducive to N\textsubscript{2}O emissions.

The low depth of stored media could be another factor contributing to the high N\textsubscript{2}O emissions. IPCC (2006) reported that a shallow septic tank (less than 1 m deep) often possesses an aerobic environment. In this experiment, the storage depth was 0.4 m; the DO level in each media type increased at the end of the storage period with continuous ventilation. Moreover, the DO level increased from 2.2 to 6.3 mg L\textsuperscript{-1} in BDE storage. With nitrification or denitrification intensification during storage, higher N\textsubscript{2}O emissions occurred during the first half of the storage period. In the later stage of BDE storage, N\textsubscript{2}O production decreased. A possible explanation for the decreasing trend was a high DO level in the later period. Von Schultheiss et al. (1994) and Molodovskaya et al. (2008) reported that low DO levels (<4 mg O\textsubscript{2} L\textsuperscript{-1}) favored N\textsubscript{2}O formation. During the final few days of the current experiment, the DO level reached 6.3 mg O\textsubscript{2} L\textsuperscript{-1}.

At the same storage temperature, ventilation rate, and storage depth, the much lower COD/N ratio of 1.6:1 in BDE might be the primary reason for the higher N\textsubscript{2}O emissions, as compared with RLM, which had a much higher COD/N ratio of 6.8:1. The results of this experiment were consistent with the observations of Wu et al. (2009) and Kishida et al. (2004), who reported that N\textsubscript{2}O emissions depended fundamentally on the influent COD/N ratio. Wu et al. (2009) compared the effects of five COD/N ratios (0:1, 2:1, 5:1, 10:1, and 20:1) on N\textsubscript{2}O emissions from microcosm wetlands. Their results showed that the total N\textsubscript{2}O emissions for COD/N ratios of 0:1 and 2:1 were 4 to 6 times greater than for COD/N ratios of 5:1 and 10:1. Moreover, Itokawa et al. (2001) found substantially higher N\textsubscript{2}O emissions with an influent COD/N ratio of 3.5:1 compared with wastewater with an influent COD/N ratio of 5:1.

In this study, the N\textsubscript{2}O emissions from BDE storage were 4 times higher than for RLM.

There was no significant difference in NH\textsubscript{3} emissions between RLM and BDE. The NH\textsubscript{3} emissions observed in the current study were lower than those reported in the literature under different storage conditions. For instance, the NH\textsubscript{3} emission rates reported by Balsari et al. (2007) were 33.3 to 104.2 mg m\textsuperscript{-2} h\textsuperscript{-1} for pig slurry storage and 26.3 to 152.5 mg m\textsuperscript{-2} h\textsuperscript{-1} for cattle slurry storage over the course of one year. Petersen et al. (2013) reported that the mean NH\textsubscript{3} emission rate from pig slurry storage was 36.9 mg m\textsuperscript{-2} h\textsuperscript{-1} in winter and 129.5 mg m\textsuperscript{-2} h\textsuperscript{-1} in summer. One possible reason for the lower NH\textsubscript{3} emissions was related to the low initial TAN in this experiment. Initial TAN concentration ranged from 1.0 to 1.4 g kg\textsuperscript{-1} in Balsari et al. (2007). In Petersen et al. (2013), the TAN ranged from 2.0 to 2.5 g kg\textsuperscript{-1} in winter and from 3.8 to 4.4 g kg\textsuperscript{-1} in summer. However, for the digested cattle slurry storage with an initial TAN content of 2.13 g kg\textsuperscript{-1}, the mean NH\textsubscript{3} emission rate was 10.5 mg m\textsuperscript{-2} h\textsuperscript{-1} (Amon et al., 2006), which was comparable to the NH\textsubscript{3} emissions from BDE storage in this study. Another possible reason for the low NH\textsubscript{3} emission rate could be the high ventilation rate. Loyon et al. (2007) reported nearly no NH\textsubscript{3} emissions during the biological aerobic treatment of piggery slurry using intermittent aeration.

The NH\textsubscript{3} emission pattern of BDE had a decreasing trend during the entire storage process (fig. 4e). However, for RLM, low NH\textsubscript{3} emissions were observed during the first storage stage; the emissions gradually increased thereafter (fig. 4c). The large gap in initial pH values between RLM and BDE contributed to the large differences in their NH\textsubscript{3} emissions during the early storage stage (Dewes, 1996). The decreased TAN and pH values at the end of the BDE storage seemed to be the primary reasons for the decreasing NH\textsubscript{3} emission trend in BDE. For RLM, the CO\textsubscript{2} emissions might have caused pH to increase (Blanes-Vidal and Nadimi, 2011), which in turn increased NH\textsubscript{3} emissions during the later storage stage.

**MICROBIAL ANALYSIS OF STORED MEDIA**

**Analysis of Methanogenic Community**

The pattern and number of bands showed clear differences in the two media types and storage stages (fig. 5a). Based on hierarchical cluster analysis of the microbial diversity, it was clear that the methanogenic communities differed between RLM and BDE (fig. 5b). These findings suggest that the microbial communities varied between the media types and with storage time. The Shannon-Wiener index is useful for evaluating the species and abundance in a sample. It showed that methanogenic diversity in the RLM sample was initially relatively low (H = 0.476) (table 3) compared to the corresponding BDE sample (H = 0.730) (p < 0.05). The methanogen diversity in both media increased at the end of the storage, with only a small difference in the Shannon-Wiener index (i.e., H = 0.887 for RLM and 0.885 for BDE, p = 0.631). Furthermore, the real-time PCR analysis showed no difference in methanogenic abundance between BDE and RLM at the start time (p = 0.795) and the end time (p = 0.472) (table 1). However, the species evenness was different in bands b and c in BDE and...
The initial properties of the media were important factors in determining the selection of the methanogenic species; different species might be associated with CH4 production. Furthermore, real-time PCR analysis showed that the methanogenic population in both RLM and BDE decreased during storage (table 1), consistent with the decline in CH4 emissions from RLM storage. The PCR analysis also showed a somewhat higher level of methanogenic abundance in RLM than in BDE, albeit not significantly different (p = 0.472). Methane emissions from RLM were obviously higher than from BDE. Therefore, the methanogenic abundance was not the driving factor for the higher CH4 emissions from RLM. Considering the DGGE pattern, we hypothesize that there might be some potential genera of methanogens that were associated with the differential emissions from the two liquid storages, such as bands a, b, and c (fig. 5a), which were dominant and specific in the BDE and RLM samples. Based on the result of the lower fermentable organic matter accompanied with the lower CH4 emissions in BDE, the different methanogenic communities might be caused by the different fermentable organic matter concentrations in the two media.

### Analysis of AOB Community

The pattern and number of bands showed differences between the two types of media (fig. 6a). Similarly, hierarchical cluster analysis suggested distinct clusters of AOB diversity in the two media (fig. 6b). The Shannon-Wiener index showed significant changes in the diversity of RLM and BDE samples from beginning to end of storage (p < 0.05) (table 3). However, the AOB species composition was relatively scarce compared to methanogens, with only one band in the final RLM sample and two bands in the final BDE sample. As shown in figure 6a, band c appeared...
only in the initial samples (RLM1 to RLM3). However, band c of the RLM1-RLM3 samples disappeared during storage, and band a occurred in the final RLM4-RLM6 samples as well as the BDE1-BDE3 and BDE4-BDE6 samples. The change in bands c and a indicates that the initial RLM samples contained a different AOB composition from that of the final RLM samples and BDE samples. The AOB composition change might be the result of adaptation of different AOB to the storage conditions. We hypothesize that the AOB species represented by band a might be associated with N2O emissions. Furthermore, real-time PCR analysis showed that AOB abundance in the BDE samples was significantly higher than in the RLM samples (p < 0.05) (table 1). Hence, it could be concluded that the higher N2O emissions from BDE vs. RLM could have been associated with the AOB abundance in the media.

Interaction Analysis of AOB and Methanogens

Ammonia oxidation is driven by DO in that a sufficient supply of DO leads to highly efficient NH3 oxidation, whereas low DO results in CH4 synthesis. With the influence of DO in the animal wastewater storage, there may be competition between NH3 oxidation and CH4 synthesis. To reveal the differences between CH4 and N related gas emissions, Pearson correlation analysis on the abundances of AOB and methanogens was conducted using the bootstrap method. The result showed that clear change occurred in the correlation between AOB and methanogens. In the earlier storage stage, the Pearson correlation index was 0.204 (p = 0.698), decreasing to -0.777 (p = 0.069) at the later stage, suggesting a negative relationship that was nearly statistically significant between AOB and methanogens. The results showed that differences in CH4 and N2O emissions during controlled storage were related to the competition among microbial communities. The DO level of the stored animal wastewater was also an important factor.

CONTRIBUTIONS OF INDIVIDUAL GHGS TO OVERALL GLOBAL WARMING POTENTIAL (GWP)

Based on 100-year GWP for GHGs, total GHG emissions were 1.05 ±0.04 g CO2-eq L-1 d-1 from RLM and 1.12 ±0.09 g CO2-eq L-1 d-1 from BDE (p = 0.26). This study demonstrated the important contribution of N2O emissions to the total GHG emissions from BDE storage, where N2O emissions accounted for 88.2% of the total GHG emissions as compared with only 0.7% for CH4 emissions (fig. 7). In contrast, the GHG emissions from RLM storage were dominated by CH4, with a relative contribution of 69.7%. Berg et al. (2006) reported that for pig slurry statically stored in a vessel (65 L) for 162 days at an ambient temperature of 18°C to 20°C, the relative contributions of CH4 and N2O emissions to the total GHG emissions were approximately 85% and 10%, respectively. Li et al. (2008) indicated that the relative contribution of CH4 emissions to the total GHG emissions from a raw pig slurry lagoon was 95% (i.e., negligible N2O emissions). The storage depth was 2 m, and the slurry temperature ranged from 23.4°C to 29.3°C in the study by Li et al. (2008). On the contrary, Külting et al. (2002) found that N2O emissions constituted 32% to 61% of GHG emissions during 14-week storage of 40 m3 of dairy manure at 20°C with a ventilation rate of 0.33 m3 s-1. The diverse aeration schemes and storage depths, which affected O2 availability, contributed to the different patterns of GHG emissions.

Figure 7. Contributions of individual GHGs to the total GHG emissions from raw liquid manure (RLM) and biogas digester effluent (BDE).

SUMMARY AND CONCLUSIONS

Emissions of CH4, CO2, N2O, and NH3 from RLM and BDE storages were quantified using dynamic emission vessels during a 22-day storage period at an air exchange rate of 15 to 17 ACH, constant storage temperature of 30°C, and storage depth of 40 cm. The mean (±SD) emission rates over the 22-day storage period for RLM and BDE were, respectively, 29.2 ±0.8 and 0.32 ±0.14 mg CH4 L-1 d-1, 102.9 ±6.8 and 125.3 ±3.3 mg CO2 L-1 d-1, 0.72 ±0.17 and 3.33 ±0.32 mg N2O L-1 d-1, and 1.21 ±0.41 and 0.66 ±0.10 mg NH3 L-1 d-1. Throughout the entire storage period, the AOB population of BDE was significantly higher than that of RLM, but methanogens showed different diversities between RLM and BDE.

Based on 100-year GWP, the total GHG emissions were 1.05 ±0.04 g CO2-eq L-1 d-1 for RLM and 1.12 ±0.09 g CO2-eq L-1 d-1 for BDE. The 6.7% higher total GHG emissions for BDE storage was primarily due to its higher N2O emissions. Under the storage conditions of this study, the low COD/N ratio in BDE seemed to be the primary reason for the high N2O emissions.

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REFERENCES


Loyon, L., Guiziou, F., Beline, F., & Peu, P. (2007). Gaseous...
Emissions (NH₃, N₂O, CH₄ and CO₂) from the aerobic treatment of piggy slurry—Comparison with a conventional storage System. *Biostems Eng.*, 472-480. doi: [http://dx.doi.org/10.1016/j.biosystemseng.2007.03.030](http://dx.doi.org/10.1016/j.biosystemseng.2007.03.030)


