Comparative Study of Chemical Pretreatments of Dairy Manure for Enhanced Biomethane Production

Qiang Yang, Hui Wang, Rebecca Larson, and Troy Runge*

Dairy manure containing partially digested plant cells is an inexpensive bioenergy feedstock. The carbohydrates and lignin that remain after digestion are typically processed in an anaerobic digester to produce biomethane, but due to the remaining material's recalcitrance, the process has a low conversion efficiency. To improve the conversion of this lignocellulosic material, chemical, thermal, or biological pretreatments can be considered. This study compared several chemical pretreatments including dilute acid, sulfite, and alkali pretreatments for dairy manure as a bioenergy feedstock and analyzed their impact on biomethane production. The comparative study showed that a hot alkali pretreatment (180 °C, 30 min) can improve the methane production of dairy manure by 50%, which is more effective than dilute acid (6.8%), sulfite (26.3%), and cold or ambient alkali (19.8 to 32.8%) pretreatments. However, the ambient alkali pretreatment (23 °C, 12 h) was calculated to be more economically feasible because of the net energy production.

Keywords: Dairy manure; Cellulose; Lignin; Hemicellulose; Pretreatment; Anaerobic digestion; Biomethane

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INTRODUCTION

Animal manure is an abundant biomass feedstock, and thus is an inexpensive resource for bioenergy. Animal manure can be directly burned to produce heat and combustible syngas or pyrolyzed to bio-oil (Fernandez-Lopez et al. 2015). Animal manure contains partially digested plant cell walls and is comprised primarily of cellulose, hemicellulose, and lignin, which can be anaerobically digested to produce methane (Nasir et al. 2012; Babae et al. 2013; Arikan et al. 2015; Sun et al. 2015). However, even after anaerobic digestion (AD), animal manure still has a significant amount of unconverted carbohydrates (Yang et al. 2016), which suggests that higher conversion is possible if the carbohydrates can be processed in the AD system.

During anaerobic digestion, manure carbohydrates are converted to organic acids, which are then biologically converted to methane and carbon dioxide. Cellulose, hemicellulose, and lignin are the major carbon sources in the feedstock for anaerobic microorganisms. However, lignin is not as biodegradable as cellulose and hemicellulose and is typically not converted during AD. Moreover, lignin can inhibit the access of microorganisms to cellulose and hemicellulose by forming a physical barrier (Kobayashi et al. 2004). Cattle manure is enriched in lignin content, as the carbohydrates have been largely consumed through digestion. As a result, the anaerobic digestion process of manure is typically slow, and the achievable methane yield is generally not satisfactory (Nasir et al. 2012, 2015).

An appropriate pretreatment can facilitate the anaerobic digestion of manure, and
more importantly, can improve the methane yield (Nasir et al. 2015; Mancini et al. 2016). For example, biological pretreatments use aerobic fungi and bacteria to break down the recalcitrance of manure prior to anaerobic digestion. Physical pretreatments such as grinding and maceration can also facilitate anaerobic digestion and enhance the methane yield by increasing the available surface area of manure, which can improve the microbial access to the manure (Angelidaki and Ahring 2000). Microwave and ultrasonic-assisted thermal pretreatment also improve the anaerobic digestion performance of manure by breaking down the recalcitrant manure fiber (Jackowiak et al. 2011; Nilgun and Canan 2014). Unfortunately, the physical and thermal pretreatments of manure typically require high energy and thus may not provide net energy benefits. For example, Jiang et al. (2016) reported that the liquid hot water pretreatment obtained negative net electrical energy production from giant reed due to high energy input. In contrast, ambient alkali pretreated giant reed can achieve 27% higher net electrical energy production than that of untreated giant reed (Jiang et al. 2016).

Chemical pretreatment uses acid or alkaline additives to partially remove lignin and therefore increase the accessibility of carbohydrates to microbes (Salehian et al. 2013; Karray et al. 2015; Michalska et al. 2015; Papa et al. 2015; Jiang et al. 2016; Tsapekos et al. 2016). Combined physical and chemical pretreatment such as steam explosion also improves the methane yield of manure (Kobayashi et al. 2004; Bondesson et al. 2013). Comparative studies have shown that sulfite pretreatment is generally more effective than acid and alkaline pretreatments to improve enzymatic digestibility of lignocellulose. Such treatment works through sulfonation of the lignin to increase the hydrophilicity and allow extraction (Li et al. 2012; Yang and Pan 2012; Zhang et al. 2013). A previous study showed that sulfite pretreatment is more effective than the dilute acid and alkali pretreatments to improve the enzymatic saccharification of dairy manure (Yang et al. 2016).

For further information on pretreatments to enhance biogas production Zheng et al. (2014) provide a comprehensive review for lignocellulosic biomass and Ariunbaatar et al. (2014) for municipal solid waste. Although there has been considerable research related to pretreatment for anaerobic digestion, there have been no reported studies on the sulfite pretreatment for improving methane production. As this is a promising pretreatment for ethanol production, an investigation of this pretreatment on the methane production from dairy manure was warranted and was compared with other more well-known pretreatments. Additionally, because thermal pretreatments typically require high energy, and thus are less economically feasible, a cold alkali pretreatment was also investigated for improving the methane production from dairy manure.

**EXPERIMENTAL**

**Materials**

Undigested dairy manure samples (#1 and #2) were collected at a 4000 head dairy farm (Manitowoc County, WI, USA) and were air-dried prior to use. Two samples from this farm were acquired for these experiments to insure that fresh samples were used for each set of experiments. Fresh inoculum sludge samples after centrifugation (total solid (TS), 2.4%; total volatile solid, 1.6%; total organic carbon, 29.3% of TS; total nitrogen, 7.3% of TS; total phosphorus, 0.72% of TS; total potassium, 11.18% of TS; total sulfur, 0.29% of TS) were obtained from a different dairy that contained a digester (Dane County, WI, USA), and were used without additional nutrient addition. Sodium sulfite (SS), sulfuric
acid (SA), and sodium hydroxide (NaOH, 50% w/w) were purchased from Thermo Fisher (Waltham, MA, USA). Acetic acid, propionic acid, butyric acid, thiourea (TU), and polyethylene glycol (PEG) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Pretreatment of Manure**

The pretreatment conditions were reported previously (Yang et al. 2016) and are briefly summarized in Table 1. The hot chemical pretreatments were carried out in a 100 mL plastic vessel using a microwave reactor (Mars, CEM Corporation, Matthews, NC, USA). The cold chemical pretreatments were carried out in a 500 mL beaker. After pretreatments, the samples were washed with DI water and centrifugation until a neutral pH was achieved.

**Anaerobic Digestion of Manure**

Anaerobic digestion experiments were carried out in a 500 mL sterile glass bottle at 37 ± 0.1°C for 34 days on a continuously stirred (200 RPM), lab-scale anaerobic digester at a 5% (w/w) consistency (Jiang et al. 2016). Due to the varying treatments, which can change the N content, a constant inoculum/feed ratio of 0.64 was maintained, based on volatile solids. Batch experiments were carried out in duplicate to provide an estimate of standard error. Further replicates were not done due to equipment limitations. All experimental digestion samples were purged with nitrogen for 5 min to remove oxygen and sealed with butyl rubber stoppers. Biogas was measured through a Pulse-Flow Anaerobic/Anaerobic Respirometer (PF-8000, Respirometer Systems and Applications, Inc., Springdale, AR, USA). Biogas was collected in a Tedlar gas collection bag for composition analysis using a gas chromatograph. Volatile fatty acids in manure slurry after digestion were measured using a high-performance ion chromatograph.

The methane production potential, maximum methane production rate, and lag time were estimated according to the fit of the cumulative methane production data from the experiments to the modified Gompertz equation (Eq.1) (Lay et al. 1996),

\[
B = B_o \times \exp \left\{ -\exp \left[ \frac{R_m \times e}{B_o} (\lambda - t) + 1 \right] \right\} \tag{1}
\]

where \(B\) is the cumulative methane yield at a given time (mL CH\(_4\)/gVS), \(B_o\) is the methane production potential (mL CH\(_4\)/gVS), \(R_m\) is the maximum methane production rate (mL CH\(_4\)/gVS/day), \(e\) is the mathematical constant (2.7183), \(\lambda\) is the lag time (days) for the methane production to begin, and \(t\) is the time (days).

**Energy Calculation**

The total energy production for the manure samples through combusting the produced biomethane was calculated using Eq. 2,

\[
Total \ energy \ production = B_o \times d \times HHV \tag{2}
\]

where \(B_o\) is the measured methane production potential (L CH\(_4\)/kgTS), \(d\) is the methane density (0.000656 kg/L), and \(HHV\) is the higher heating value of methane (55 MJ/kg).

Respective energy consumptions for the manure collection and mixing and for the anaerobic digestion of manure were not considered in this study. Energy consumptions for the manure degradation reactions during pretreatments were also not considered. However, additional energy is needed for the manure pretreatment. Specifically, for the hot...
pretreatments, the manure samples (10 g, oven dried) were heated from 23 °C to 180 °C in 60 mL acidic or basic aqueous solutions; and for the ambient pretreatment, the manure sample (10 g, oven dried) was mixed with 50 mL alkali aqueous solution, and the resultant mixture was stored at 23 °C (Yang et al. 2016). The energy consumption for pretreating the manure sample was estimated using Eq. 3.

\[
\text{Energy consumption} = \frac{m_1 \Delta h + m_2 C_p \Delta T}{VS}
\]

where \( m_1 \) is the weight of water in the sample (0.060 kg), \( \Delta h \) is the change in specific enthalpy of water being heated from 23°C to 180°C (667 kJ/kg), \( m_2 \) is the weight of the manure (0.010 kg, oven dried), \( C_p \) is the specific heating capacity of manure (3.8 kJ/kg/°C) (Nayyeri et al. 2009), \( \Delta T \) is the temperature increase from 23 °C to 180 °C, and VS the amount of volatile solids in the sample (0.008 kg). This simple estimation assumes recovery of required for latent heat of vaporization, while it neglects heat losses by the reactor and heats of reaction. Although limited in scope this estimate does provide a magnitude of the energy invested in the pretreatment to compare to the additional energy obtained. Full scale pretreatment systems would need to be designed and modeled to obtain a more accurate energy return on energy invested in pretreatments.

Analytic Methods

Total organic carbon (TOC), nitrogen (N), and sulfur (S) were analyzed using a LECO CNS-2000 Carbon, Nitrogen and Sulfur Analyzer (LECO Corporation, St. Joseph, MI, USA). Phosphorus (P) was analyzed using a UV-Vis spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) at 645 nm. Potassium (K) was measured using an ICP-OES spectrophotometer (SPECTRO Analytical Instruments, Inc., Klevé, Germany). Biogas composition was analyzed by a Gas Chromatograph SRI 8610C equipped with a FID detector (SRI Instruments, Inc., Torrance, CA, USA). Ash, extractives, and acid-insoluble (Klason) lignin were analyzed according to National Renewable Energy Laboratory Analytic Procedures (Sluiter et al. 2010). Total solids (TS) and volatile solids (VS) were analyzed according to published standards (APHA 2006). Monosaccharides (glucose, galactose, arabinose, mannose, and xylose) were measured by a high performance ion chromatography (HPIC, ICS-3000, Dionex, Sunnyvale, CA, USA) equipped with an integrated amperometric detector and Carbopac guard (PA20, 3 × 30 mm) and analytic (PA20, 3 × 150 mm) columns. Acetic acid, propionic acid, and butyric acid were measured using the Dionex ICS-3000 system equipped with a UV-vis detector and Supelcogel C-610H analytic (30 cm × 7.8 mm) and guard (5 cm × 4.6 mm) columns.

RESULTS AND DISCUSSION

Effect of Pretreatment on Composition and Nutrient

The composition of manure samples is shown in Table 2. The two manure samples had similar compositions. Extractives (materials that could be extracted from exhaustive ethanol Soxhlet extraction) for manure samples #1 and #2 were about 13% and 15%, respectively. Manure sample #2 contained higher volatile solids (86.0% vs. 81.8%) but lower total organic carbon (37.3% vs. 41.3%) than manure sample #1.

As summarized in Table 1, manure sample #1 was pretreated under hot conditions, and manure sample #2 was pretreated under cold conditions. Under hot pretreatment conditions, carbohydrates can be degraded into sugars, acetic acid, furfural, and...
hydroxymethylfurfural, while lignin can be degraded into aromatic compounds (Yang et al. 2016). The produced sugars can be anaerobically digested to produce methane. Although the formed furfural, hydroxymethylfurfural, and aromatic compounds can inhibit anaerobic digestion (Mousa and Forster 1999; Hernandez and Edyvean 2008; Pekařová et al. 2017), recycling spent pretreatment chemicals for reuse is economically important. Therefore, to exclude the effects of the produced furfural, hydroxymethylfurfural, and aromatic compounds and potentially recycle the spent chemicals for reuse, the pretreated manure samples in this study were washed to allow for direct comparison. The total solids, volatile solids, cellulose, hemicellulose, and lignin contents of the pretreated manure samples are reported in Table 2.

Table 1. Pretreatment Conditions of Manure

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% SAa</td>
<td>180</td>
<td>0.5</td>
<td>4% sulfuric acid</td>
</tr>
<tr>
<td>4% SAa + 9% SSa</td>
<td>180</td>
<td>0.5</td>
<td>9% sodium sulfite + 4% sulfuric acid</td>
</tr>
<tr>
<td>8% NaOHa</td>
<td>180</td>
<td>0.5</td>
<td>8% sodium hydroxide</td>
</tr>
<tr>
<td>7% NaOHb + 2% PEGb</td>
<td>23</td>
<td>12</td>
<td>7.0% sodium hydroxide + 2% polyethylene glycol</td>
</tr>
<tr>
<td>7% NaOHb + 5.5% TUb</td>
<td>-20</td>
<td>12</td>
<td>7.0% sodium hydroxide + 5.5% thiourea</td>
</tr>
</tbody>
</table>

aChemical loading wt% based manure so oven-dry manure. bChemical loading wt% of solution. SA-sulfuric acid; SS-sodium sulfite; NaOH-sodium hydroxide; PEG-polyethylene glycol; TU-thiourea.

Table 2. Yield and Composition of Untreated and Pretreated Manure Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (%)</th>
<th>Gla (%)</th>
<th>Ara (%)</th>
<th>Xyl (%)</th>
<th>Glu (%)</th>
<th>KL (%)</th>
<th>TS (%)</th>
<th>VS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated #1</td>
<td>N/A</td>
<td>1.8</td>
<td>2.2</td>
<td>18.2</td>
<td>23.2</td>
<td>23.1</td>
<td>93.5</td>
<td>81.8</td>
</tr>
<tr>
<td>4% SA</td>
<td>46.1</td>
<td>0.7</td>
<td>0.6</td>
<td>10.4</td>
<td>35.0</td>
<td>28.2</td>
<td>93.8</td>
<td>86.9</td>
</tr>
<tr>
<td>4% SA + 9% SS</td>
<td>48.3</td>
<td>1.6</td>
<td>2.3</td>
<td>20.5</td>
<td>37.1</td>
<td>22.3</td>
<td>81.7</td>
<td>72.4</td>
</tr>
<tr>
<td>8% NaOH</td>
<td>42.6</td>
<td>1.3</td>
<td>0.9</td>
<td>18.8</td>
<td>32.4</td>
<td>20.1</td>
<td>95.8</td>
<td>79.8</td>
</tr>
<tr>
<td>untreated #2</td>
<td>N/A</td>
<td>1.6</td>
<td>1.8</td>
<td>19.0</td>
<td>24.2</td>
<td>22.6</td>
<td>94.8</td>
<td>86.0</td>
</tr>
<tr>
<td>7% NaOH + 2% PEG</td>
<td>45.4</td>
<td>1.5</td>
<td>1.0</td>
<td>19.3</td>
<td>32.6</td>
<td>20.6</td>
<td>90.3</td>
<td>82.1</td>
</tr>
<tr>
<td>7% NaOH + 5.5% TU</td>
<td>46.2</td>
<td>1.6</td>
<td>1.2</td>
<td>20.2</td>
<td>31.8</td>
<td>21.9</td>
<td>88.1</td>
<td>84.4</td>
</tr>
</tbody>
</table>

Yield: Residual manure (oven-dry) as a weight percentage of initial manure (oven-dry). Gla-galactose; Ara-arabinose; Xyl-xylose; Glu-glucose; KL-Klason lignin; TS-total solid; VS-volatile solid;

Table 3. Effects of Pretreatment on Hemicellulose and Lignin in Manure Sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Removal of Hemicellulose (%)</th>
<th>Removal of Lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% SA</td>
<td>75.7</td>
<td>43.7</td>
</tr>
<tr>
<td>4% SA + 9% SS</td>
<td>46.9</td>
<td>53.4</td>
</tr>
<tr>
<td>8% NaOH</td>
<td>59.7</td>
<td>62.9</td>
</tr>
<tr>
<td>7% NaOH + 2% PEG</td>
<td>55.8</td>
<td>58.6</td>
</tr>
<tr>
<td>7% NaOH + 5.5% TU</td>
<td>52.5</td>
<td>55.2</td>
</tr>
</tbody>
</table>

As expected, the chemical pretreatments noticeably changed the composition of the manure samples. In particular, the monomers associated with hemicellulose were reduced, suggesting hemicellulose degradation and removal during washing. As summarized in Table 3, the dilute acid pretreatment removed the most hemicellulose, followed by the
alkali pretreatment and then the sulfite pretreatment. Therefore, the dilute acid pretreated manure sample has less hemicellulose than the original sample (12%), the sulfite (24%), hot alkali (21%), and cold alkali (22 to 23%) pretreated manure samples. Lignin was also greatly removed from the manure during the chemical pretreatments under both hot and cold conditions with the alkali pretreatments, removing more lignin than the acid pretreatments (Table 3). Therefore, the alkali pretreated manure exhibited lower (20 to 22%) lignin content than the acid pretreated sample (22 to 28%). Due to the partial removal of both lignin and hemicellulose, the manure sample was enriched in cellulose. Considering higher lignin and hemicellulose removal, the hot alkali pretreatment gave manure a relatively lower (43%) substrate yield than the dilute acid (46%), sulfite (48%), and cold alkali (45 to 46%) pretreatments. The pretreatments also changed the volatile solids of the manure samples, with a decrease observed for all chemical pretreatments except the dilute acid pretreatment. The decrease in volatile solids is primarily attributed to the removal of both lignin and hemicellulose that occurred from the pretreatment and washing. Interestingly, the dilute acid pretreatment increased the volatile solid of the manure sample from 82% to 87%. The pretreatments also changed the total organic carbon of the manure samples. The acidic (dilute acid and sulfite) pretreatments increased the total organic carbon, while the basic pretreatments decreased the total organic carbon.

Manure usually contains nutrients such as nitrogen, phosphorus, potassium, and sulfur, which were also characterized along with the Total Organic Carbon (TOC). As summarized in Table 4, nitrogen and potassium were partially removed from the manure samples during the pretreatments and washing, with the greatest loss in soluble potassium. Nitrogen and potassium are essential nutrients for the growth of anaerobic bacteria, but they can be inhibitory or toxic at high concentrations (Wang et al. 2014; Wu et al. 2016). The loss of nitrogen and potassium may influence the biomethane production. However, possibly due to accumulation, the inoculum sludge samples in this study contained relatively high contents of nitrogen and potassium, which likely provided sufficient nutrients for the anaerobic digestion of pretreated samples (Steinmetz et al. 2016; Wu et al. 2016). The loss of nitrogen and potassium during biomethane production in pretreated manure samples require further study.

Table 4. Nutrients of Manure Sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>TOC (%)</th>
<th>Total N(%)</th>
<th>NH₄-N(%)</th>
<th>Total P(%)</th>
<th>Total K(%)</th>
<th>Sulfur(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated #1</td>
<td>41.3</td>
<td>1.96</td>
<td>0.10</td>
<td>0.40</td>
<td>1.52</td>
<td>0.31</td>
</tr>
<tr>
<td>4% SA</td>
<td>44.3</td>
<td>1.97</td>
<td>0.10</td>
<td>0.38</td>
<td>0.06</td>
<td>0.26</td>
</tr>
<tr>
<td>4% SA + 9% SS</td>
<td>42.4</td>
<td>1.61</td>
<td>0</td>
<td>0.49</td>
<td>0.04</td>
<td>0.34</td>
</tr>
<tr>
<td>8% NaOH</td>
<td>39.6</td>
<td>0.80</td>
<td>0</td>
<td>0.51</td>
<td>0.07</td>
<td>0.24</td>
</tr>
<tr>
<td>untreated #2</td>
<td>37.3</td>
<td>1.50</td>
<td>0.20</td>
<td>0.52</td>
<td>0.82</td>
<td>0.23</td>
</tr>
<tr>
<td>7% NaOH + 2% PEG</td>
<td>35.7</td>
<td>0.60</td>
<td>0.30</td>
<td>0.38</td>
<td>0.09</td>
<td>0.19</td>
</tr>
<tr>
<td>7% NaOH + 5.5% TU</td>
<td>34.7</td>
<td>0.60</td>
<td>0.10</td>
<td>0.40</td>
<td>0.11</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Effect of Pretreatment on Biomethane Production

Cumulative and daily methane productions of the manure (#1) samples before and after the dilute acid, sulfite, and hot alkali pretreatments are presented in Fig. 1. Their estimated methane production potentials, maximum production rates, and lag times are summarized in Table 5. The cumulative methane production was characterized by a
sigmoid shaped curve, having a slow initial methane production with a significant increase, followed by a decline and eventual halt. As shown in Fig. 1 and Table 5, the time lag (the minimum time to produce methane, $\lambda$) was the greatest at approximately 4 days for the untreated manure sample. In contrast, after the chemical pretreatments, the time lag was significantly reduced, indicating that the pretreated dairy manure is more readily digestible.

![Graph showing cumulative and daily methane production curves](image)

**Fig. 1.** Cumulative and daily methane production curves for untreated and pretreated dairy manure samples by dilute acid, sulfite, and hot alkali

**Table 5.** Estimated Methane Production Potential, Maximum Production Rate and Lag Time

<table>
<thead>
<tr>
<th>Sample</th>
<th>$B_0$ (mL CH$_4$/g VS$^{-1}$)</th>
<th>$\lambda$ (d)</th>
<th>$R_m$ (mL CH$_4$/g VS$^{-1}$. d$^{-1}$)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>predicted</td>
<td>measured</td>
<td>predicted</td>
<td>measured</td>
</tr>
<tr>
<td>untreated #1</td>
<td>181 (0.3)</td>
<td>190 (0.9)</td>
<td>6.0</td>
<td>4.2</td>
</tr>
<tr>
<td>4% SA</td>
<td>206 (0.1)</td>
<td>203 (1.2)</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>4% SA + 9% SS</td>
<td>239 (0.2)</td>
<td>240 (1.2)</td>
<td>2.0</td>
<td>1</td>
</tr>
<tr>
<td>8% NaOH</td>
<td>277 (0.2)</td>
<td>285 (1.4)</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>untreated #2</td>
<td>212 (0.5)</td>
<td>192 (0.6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7% NaOH + 2% PEG</td>
<td>281 (0.3)</td>
<td>255 (0.9)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7% NaOH + 5.5% TU</td>
<td>253 (0.9)</td>
<td>230 (0.8)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$B_0$: Methane production potential (mL CH$_4$/g VS) from biomethane production test. $\lambda$: Lag time (days) for methane production to begin. $R_m$: Maximum methane production rate (mL CH$_4$/g VS/day). All parameters are estimated according to the modified Gompertz equation through nonlinear regression using JMP Pro 11. Parenthesis are standard derivations.

Interestingly, all three of the 2nd set of experiments (untreated #2, and the NaOH experiments) predicted higher gas yields than measured. This was presumably due to the lack of lag time and initial high gas production of these samples, possibly due to the nature of inoculum use. However as both control manure samples produced approximately 190 mL of methane per gram of volatile solid (mL/g VS) after 35 days of digestion, comparison between studies can still be made, though with caution as the two sets of experiments show different biomethane production curves.

The chemically pretreated manure samples produced more methane mL/g VS over
the same time periods, and thus exhibited greater digestibility and energy conversion. Particularly, the hot alkali pretreated manure sample produced approximately 285 mL/g VS methane, and the dilute acid and sulfite pretreated manure samples produced about 203 mL/g VS and 240 mL/g VS methane, respectively. Thus, the hot alkali, dilute acid, and sulfite pretreatments improved the methane production by 50%, 6.8%, and 26.3%, respectively. The hot alkali pretreated manure sample had the least lignin content (about 20%), which is likely the primary reason that sample produced the most methane. Similarly, because of the reduced lignin and enriched carbohydrates, the sulfite pretreated manure sample produced more methane than the dilute acid pretreated one. Additionally, the methane production rates of manure samples were much faster after the chemical pretreatments. For example, during the 35 days of digestion, the observed maximum methane production rates for the untreated, dilute acid, sulfite, and alkali pretreated manure samples were 7.7, 9.8, 12.0, and 22.0 mL CH₄/g VS/d, respectively.

Because the chemical pretreatments operate at high temperatures, especially the hot alkali, which can improve the accumulative methane production of manure up to 50%, they require high energy input and therefore may not be economically feasible. Thus, a pretreatment carried out at ambient or even a low temperature is preferred to improve the methane production of manure. Cold alkali pretreatments (e.g., 7% sodium hydroxide aqueous solution containing 5.5% thiourea or 2% polyethylene glycol) greatly improve the enzymatic digestibility of manure as the treatment can partially remove lignin and reduce the crystallinity of cellulose via disrupting its hydrogen bond (Yang et al. 2016). Cold alkali pretreatments can have a similar effect though usually to a lesser degree. A 119% increase in the methane yield was observed for pine after a cold alkali pretreatment at 0 °C (Salehian et al. 2013). Mohsenzadeh et al. (2012) also reported that the methane yields of spruce and birch were improved by 56% and 600%, respectively, after a cold alkali pretreatment. Therefore, the manure samples in this study were pretreated by 7% alkali aqueous solution containing 5.5% thiourea (or 2% polyethylene glycol) at -20 °C (or room temperature, 23 ºC) for 12 h.

![Cumulative and daily methane production curves for untreated and pretreated dairy manure samples by cold alkali](image)

**Fig. 2.** Cumulative and daily methane production curves for untreated and pretreated dairy manure samples by cold alkali

Cumulative and daily methane production curves for untreated and pretreated manure samples by cold alkali are compared and presented in Fig. 2. The results show that the cold alkali pretreatment can greatly improve the methane production of manure. For
example, after treatment with 7% alkali aqueous solution with 2% polyethylene glycol at 23 °C for 12 h, the methane production of the manure sample increased from about 192 to 255 mL/g VS. The approximately 33% improvement in the methane production is likely attributable to the lignin removal (about 59%) and possibly the disruption of crystallinity of cellulose (Salehian et al. 2013). The manure sample pretreated with 7% sodium hydroxide containing 5.5% thiourea at -20 °C showed contained lignin and less cellulose (Table 2), compared with the manure sample pretreated by the alkali aqueous solution with polyethylene glycol at 23 °C for 12 h. As a result, the alkali pretreatment with 7% sodium hydroxide containing 5.5% thiourea at -20 °C improved the methane production of manure by 19%, which is lower than that (33%) by the alkali pretreatment with 7% sodium hydroxide containing 2% polyethylene glycol at 23 °C for 12 h.

![Graph showing reduction in solids and nitrogen](https://example.com/graph.png)

**Fig. 3.** Changes in total solids, volatile solids, organic nitrogen, and pH value after the 35-day anaerobic digestion period. A- untreated #1; B- untreated #2; C-8% NaOH; D-7% NaOH + 2% PEG; E-7% NaOH + 5.5% thiourea; F-4% SA; G-4% SA + 9% SS.

After anaerobic digestion, the total and volatile solids of the manure samples were greatly reduced, as shown in Fig. 3, with the pretreated manure samples generally having a greater reduction in the total and volatile solids than the untreated manure samples. During anaerobic digestion, the nitrogen in amino acids can be converted to ammonia and lost as a gas. Phosphorus and potassium were not significantly changed (data not shown), as expected (Aguirre-Villegas et al. 2014). As the anaerobic digestion proceeds, the volatile solids are reduced to intermediate volatile fatty acids (VFAs). The initial pH value decreases because of the accumulation of VFAs and then increases after conversion of VFAs to...
methane. In this study, the initial pH value of the manure sample solution after digested for 35 days generally decreased from 8.5 to 7.5, as shown in Fig. 3.

To estimate whether the chemical pretreatments used in this study are economically feasible, the respective energy productions and consumption were simply estimated, and the results are summarized in Table 6. The hot pretreated manure samples generated less net energy than the untreated controls, while the ambient pretreated manure sample achieved 33.1% higher net energy than that of the untreated manure sample.

Table 6. Estimated Net Energy Production for Untreated and Pretreated Manure Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Energy Production (kJ/kg TS)</th>
<th>Pretreatment Energy * (kJ/kg TS)</th>
<th>Net Energy (kJ/kg TS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated #1</td>
<td>6052</td>
<td>0</td>
<td>6052</td>
</tr>
<tr>
<td>4% SA</td>
<td>6847</td>
<td>0</td>
<td>1845</td>
</tr>
<tr>
<td>4% SA + 9% SS</td>
<td>7743</td>
<td>5002</td>
<td>2741</td>
</tr>
<tr>
<td>8% NaOH</td>
<td>8643</td>
<td>5002</td>
<td>3641</td>
</tr>
<tr>
<td>untreated #2</td>
<td>6341</td>
<td>0</td>
<td>6341</td>
</tr>
<tr>
<td>7% NaOH + 2% PEG</td>
<td>8440</td>
<td>0</td>
<td>8440</td>
</tr>
</tbody>
</table>

* The pretreatment energy input is estimated from a simple model of the heat energy required to heat the material from 23 °C to 180 °C, as described in the methodology section.

Based on the results from this study, which compared several chemical pretreatments including dilute acid, sulfite and alkali pretreatments for dairy manure, it appears that the alkali pretreatments are more effective than the dilute acid or sulfite. Additionally, the ambient temperature pretreatment seems to provide similar benefits as hot pretreatment, but is likely more feasible because of the lower energy inputs required.

CONCLUSIONS

1. A chemical pretreatment can greatly improve the methane production of dairy manure, primarily through partial removal of lignin.

2. The sulfite pretreatment is better than the dilute acid (with same amount of sulfuric acid) pretreatment, while the hot alkali pretreatment outperforms the cold alkali, dilute acid, and sulfite pretreatments.

3. The ambient alkali pretreatment was the most promising treatment when considering the net energy production of the system.

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