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Water Electrolysis

Ammonia rich waste streams

$\text{H}_2$

Biogas reactor

Thermophilic/Mesophilic

Biomethanation

Yield ✓

Biogas composition ✗
Submission to Water Research

Ammonia inhibition on hydrogen enriched anaerobic digestion of manure under mesophilic and thermophilic conditions

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Abstract

Capturing of carbon dioxide by hydrogen derived from excess renewable energy (e.g., wind mills) to methane in a microbially catalyzed process offers an attractive technology for biogas production and upgrading. This bioconversion process is catalyzed by hydrogenotrophic methanogens, which are known to be sensitive to ammonia. In this study, the tolerance of the biogas process under supply of hydrogen, to ammonia toxicity was studied under mesophilic and thermophilic conditions. When the initial hydrogen partial pressure was 0.5 atm, the methane yield at high ammonia load (7 g NH$_4^+$-N L$^{-1}$) was 41.0% and 22.3% lower than that at low ammonia load (1 g NH$_4^+$-N L$^{-1}$) in mesophilic and thermophilic condition, respectively. Meanwhile no significant effect on the biogas composition was observed. Moreover, we found that hydrogenotrophic methanogens were more tolerant to the ammonia toxicity than acetoclastic methanogens in the hydrogen enriched biogas production and upgrading processes. The highest methane production yield was achieved under 0.5 atm hydrogen partial pressure in batch reactors at all the tested ammonia levels. Furthermore, the thermophilic methanogens at 0.5 atm of hydrogen partial pressure were more tolerant to high ammonia levels ($\geq$ 5 g NH$_4^+$-N L$^{-1}$), compared with mesophilic methanogens. The present study offers insight in developing resistant hydrogen enriched biogas production and upgrading processes treating ammonia-rich waste streams.

Keywords

Anaerobic digestion; Ammonia inhibition; Hydrogenotrophic methanogens; Hydrogen; Wastewater treatment
1. Introduction

Anaerobic digestion (AD) is a sustainable technology that has been used for the treatment of various waste streams such as animal manure, food waste and sludge. However, AD treatment of the substrates containing high total ammonia (ammonium ion and free ammonia) concentration can be seriously inhibited by the ammonia which is produced during the biodegradation of proteins, urea and nucleic acids. There are two principal forms of inorganic ammonia nitrogen in aqueous solution: Ammonium ion ($\text{NH}_4^+$) and free ammonia ($\text{NH}_3$). $\text{NH}_3$ has been considered to be the main inhibitor (Rajagopal et al., 2013; Yenigün & Demirel, 2013). $\text{NH}_3$ molecules diffuse into the microbes’ cells freely which can cause proton imbalance, increase maintenance energy requirements, change intracellular pH and inhibit specific enzyme reactions (Gallert et al., 1998; Sprott & Patel, 1986). $\text{NH}_3$ concentration mainly depends on temperature, pH and total ammonia concentration in anaerobic digestion process (Hafner & Bisogni, 2009). For example, the concentration of $\text{NH}_3$ increases with an increase in pH and/or temperature which causes the enhanced ammonia toxicity on the AD process (Nielsen & Angelidaki, 2008).

The AD process can be described by four distinctive steps namely: hydrolysis, acidogenesis, acetogenesis and methanogenesis. In detail, with the exception of the initial solubilisation of complex particulate material, methanogenesis seems to be the rate-limiting step. Moreover methanogens are the most vulnerable to ammonia compared to other groups of microorganisms involved in AD process (Angelidaki et al., 2011). There are two distinct methanogenic pathways for converting acetate to methane, which has been well described in previous studies (Fotidis et al., 2013; Wang et al., 2015). There are many papers referring on the sensitivity of the meth-
anogens to ammonia (Fotidis et al., 2013). It was reported that acetoclastic meth-
anogens (i.e. *Methanosarcinaceae* spp. and *Methanosaetaceae* spp.) are more vul-
nerable to ammonia toxicity compared to hydrogenotrophic methanogens (i.e.
*Methanomicrobiales* spp., *Methanococcales* spp., *Methanocellales* spp., *Methano-
bacteriales* spp. and *Methanopyrales* spp.) (Angelidaki & Ahring, 1993; Yenigün &
Demirel, 2013).

Recently, an innovative AD process, which introduces hydrogen produced by water
electrolysis using excess electricity from wind mill into anaerobic digester and sub-
sequently converts it together with carbon dioxide in biogas into methane has been
developed for simultaneous H\(_2\) utilization and in-situ biogas upgrading (mainly re-
fers to reduction of CO\(_2\) content), giving synergistic advantages for both wind mills
and biogas plants. (Deng & Hägg, 2010; Luo & Angelidaki, 2012; Luo et al., 2012).

Such process has several advantages over conventional AD process: (1) low cost for
further biogas upgrading since CO\(_2\) content was reduced; (2) increase of methane
production; (3) fully use of the wind mill capacity. Though promising, the H\(_2\) en-
riched AD process is just emerging from a technology perspective. There are sever-
al challenges to be addressed for being able to develop a sustainable feasible tech-
nology. One important aspect is the resistance of the process to ammonia inhibition,
which is the very aspect that is unclear so far. Considering that most of the feed-
stocks (e.g., cattle manure) in biogas plants (especially in Denmark) contain high
level of ammonia, it is of outmost important to reveal the sensitivity of the process
to high level of ammonia in order to accelerate the wide application of the technol-
gy. The outcome of such investigation will also help to find suitable strategy to
counteract the ammonia inhibition.
During this process, enrichment of hydrogenotrophic methanogenic cultures in ana-
aerobic biogas reactors is occurring. In Luo and Angelidaki (2012)’s study, hydro-
gen was injected into anaerobic reactors to achieve a hydrogen partial pressure of
0.8 atm. After two months cultivation with H₂, the hydrogenotrophic methanogenic
activities increased to 198 mL CH₄ (g VSS h⁻¹) under mesophilic and 320 mL CH₄
(g VSS h⁻¹) under thermophilic condition, from around 10 mL CH₄ (g VSS h⁻¹) of
the original inoculum. This indicated that hydrogenotrophic methanogens were suc-
cessfully enriched by long term injection of hydrogen. Thus, it would be obvious to
assume that this process would be more resistant or tolerant to ammonia toxicity
due to the enrichment of hydrogenotrophic methanogenesis compared to the con-
ventional AD processes (Luo & Angelidaki, 2013b; Luo & Angelidaki, 2012; Luo
et al., 2012). So far, information about the effect of ammonia toxicity on this inno-
vative AD process is still lacking. Therefore, in this study, the effect of different
ammonia levels on hydrogen enriched biogas upgrading process (different hydrogen
partial pressure were included in the current study) in anaerobic reactors at both
mesophilic and thermophilic temperature was explored.

2. Materials and methods

2.1 Inoculum and feedstock

The mesophilic and thermophilic inoculum were obtained from mesophilic and
thermophilic anaerobic reactors in Hashøj Biogas plant (Denmark) and Snertinge
Biogas Plant (Denmark), respectively. Both biogas plants use a mixture of manure
(pig and cattle) and organic waste (fat and flotation sludge from food industries) as
feedstock. As feedstock, dairy manure taken from Hashøj municipality (Denmark)
was used in this study. The dairy manure was mixed in one plastic barrel and was
sieved, in order to remove the large solid particles, and then kept at -18 ºC. Before use as substrate in the batch experiment, the frozen manure was thawed and stored at 4ºC for 2-3 days. The basic characteristics of the inoculum and feedstock were analyzed and shown in Table 1.

Table 1 is here

2.2 Experimental setup

Both mesophilic and thermophilic inocula were incubated under four different ammonia concentrations (1, 3, 5 and 7 g NH₄⁺-N L⁻¹) with NH₄Cl as ammonia source. As batch reactors, vials with 118 mL total and 40 mL working volume, respectively were used. The working volume contained 10 mL inoculum, 10 mL dairy manure and 20 mL distilled water. After filling the content into the vials, butyl rubber stoppers and aluminum crimps were used to seal them. Then all the batch reactors were flushed with nitrogen (flow rate 290 ml/s) for 10 min. Before the hydrogen injection, the same volumes as the injected hydrogen of gas were extracted from the batch reactors to make sure the total pressure of all the batch reactors was the same. After that, 19.5, 39 and 78 mL of hydrogen were introduced with syringes into batch reactors to obtain different hydrogen partial pressure (0.25, 0.5, and 1 atm) for each ammonia level. Moreover, batch reactors without hydrogen addition, were also included. Additionally, reactors only with inoculum were used as blanks to evaluate the residual methane production. Two shaking incubators (37±1 ºC and 55±1°C, 180 rpm) were used for mesophilic and thermophilic batch reactors respectively and each condition was evaluated in triplicates (n=3).
2.3 Analytical methods

Total solids (TS), volatile solids (VS), pH, total ammonia and total Kjeldahl nitrogen (TKN) were measured according to APHA’s Standard Methods (Federation & Association, 2005). The pH level of the batch reactors was determined by using PHM99 LAB pH meter which was connected to the Gel pH electrode (pHC3105-8, Radiometer analytical). The electrode was filled with a gel containing KCl. Before measuring samples, the pH meter was calibrated at the temperature of the corresponding batch reactors. Shimadzu-14A gas chromatograph (GC) equipped with a thermal FID detector with hydrogen as a carrier gas (Shimadzu, Kyoto, Japan) was used to measure methane accumulation in the headspace of batch reactors. Hydrogen concentration in batch reactors was measured by using GC-TCD fitted with a 4.5 m×3 mm stainless column packed with Molsieve SA (10/80). Moreover, a gas-chromatograph (GCTCD) equipped with a column of 1.1 m × 3/16 “Molsieve 137 and 0.7 m × 1/4” chromosorb 108 (MGC 82-12, Mikrolab A/S, Denmark) was used to determine the biogas composition in the headspace of batch reactors. The bottles were not vented during the whole experiment. The methane concentration (in percentage) in the headspace was measured by GC with pressure. Thus, the accumulated methane was obtained by multiplying headspace volume of the batch reactors (78 ml) and the methane concentrations measured by GC. Additionally, the accumulated volatile fatty acids (VFA) concentration of the batch reactors were determined by using a gas-chromatograph (HP5890 series II) equipped with a flame ionization detector and a FFAP fused silica capillary column, (30 m × 0.53 mm i.d., film thickness 1.5 µm), which uses nitrogen as carrier gas.
2.4 Calculations

2.4.1 Calculation of methane production

The hydrogen injected into the batch reactors was consumed by hydrogenotrophic methanogens to produce methane. Thus, the reactors with hydrogen addition had higher average methane yield compared to the reactors without hydrogen injection. Therefore, the calculation of subtracting the theoretical methane production from the introduced hydrogen in the batch reactors was made.

2.4.2 Statistical analysis

OriginLab program (OriginLab Corporation, Northampton, Massachusetts) was used for all the statistical analyses. For statistical analysis, one way Analysis of Variance (ANOVA) at 0.05 level was used. The effects of two factors (ammonia concentrations and hydrogen pressure) on methane production rate, methane production yield, VFA, pH level and carbon dioxide content were analyzed. All values presented are the means of independent triplicates (n=3)±SD.

3. Results and discussion

3.1 Accumulated methane yield of the reactors

In general, the methane yield decreased significantly (p<0.05, P was ranging from 4.4×10⁻⁸ to 1.3×10⁻⁵) with the increase of ammonia levels under all different hydrogen partial pressures tested (Figure 1a). In detail, for the reactors without hydrogen injection, when ammonia concentration increased from 1 to 7 g NH₄⁺-N L⁻¹, a decrease of 65.0% in the methane yield was observed at mesophilic condition. For the mesophilic reactors adding hydrogen (0.25, 0.5 and 1 atm), inhibition caused by increasing ammonia level was also detected. However, the inhibition was less pronounced when H₂ was added. More specifically, the methane yields at ammonia
level of 7 g NH$_4^+$-N L$^{-1}$ were 42.7%, 41.0% and 48.3% lower compared 1 g NH$_4^+$-N L$^{-1}$ for hydrogen additions of 0.25, 0.5 and 1 atm respectively (Figure 1a).

**Figure 1 is here**

Similarly, at thermophilic condition, the methane yield decreased by 44.2% in the reactors without hydrogen injection, when ammonia was increased from 1 to 7 g NH$_4^+$-N L$^{-1}$ (Figure 1a). Likewise the mesophilic conditions, inhibition was also less serious for the reactors with hydrogen. In addition, the highest methane yield at ammonia concentration of 7 g NH$_4^+$-N L$^{-1}$ was observed under 0.5 atm initial hydrogen partial pressure both at mesophilic and thermophilic conditions. An interesting observation was that the methane yield in the thermophilic reactor was higher than that in the mesophilic reactors with ammonia concentration of 7 g NH$_4^+$-N L$^{-1}$ regardless of the initial hydrogen partial pressure. This is in particular noticeable as thermophilic methanogenesis is in general considered more ammonia sensitive.

At high ammonia concentration (7 g NH$_4^+$-N L$^{-1}$) even after subtracting the theoretical methane production from the introduced hydrogen (which was completely consumed in all the reactors) in the batch reactors higher methane production was observed, indicating that the tolerance to ammonia toxicity was promoted by hydrogen addition. Therefore, the results confirmed that the hydrogen enriched biogas upgrading process was more resistant to high ammonia levels compared to the conventional AD processes.

Ammonia is considered as an inhibitor of slowing down the growth and metabolic rates, therefore we calculated the methane production rates at different initial hydrogen partial pressures (0, 0.25, 0.5 and 1 atm) under 1 and 7 g NH$_4^+$-N L$^{-1}$ in
mesophilic and thermophilic conditions (Figure 1b). The length of time for calculating methane production rate was from the beginning to the day that stable accumulated methane production was obtained (26 days, the whole length of the process was 48 days). The same tendency as for the methane yields, were shown for the methane production rates with ammonia concentration increase. In detail, the most serious inhibition occurred in the reactors without hydrogen injection at 7 g NH$_4^+$-N L$^{-1}$ both in mesophilic (56.7% lower) and thermophilic (53.4% lower) conditions, which was in agreement with the methane yield result. Furthermore, at 7 g NH$_4^+$-N L$^{-1}$, the highest methane production rate was also achieved under 0.5 atm initial hydrogen partial pressure both at mesophilic (7.7 mL CH$_4$ (L · h)$^{-1}$) and thermophilic (13.4 mL CH$_4$ (L · h)$^{-1}$) conditions.

**Figure 2 is here**

In general, the methane yield decreased with the increase of ammonia levels (Figure 2). In detail, when ammonia concentration was increased to 5 and 7 g NH$_4^+$-N L$^{-1}$, the accumulated methane yield decreased significantly (p<0.05, p=8.7×10$^{-7}$) in mesophilic condition. In thermophilic condition, the methane yield was affected less by the increasing ammonia levels compared to the mesophilic reactors. Both the results of methane yield and production rate indicated that the hydrogen based biogas upgrading process can still function at high ammonia level and was more tolerant compared to the conventional AD processes, though ammonia inhibition occurred. The highest methane production yield was achieved under 0.5 atm hydrogen partial pressure in batch reactors at high ammonia levels. However, introducing hydrogen to anaerobic biogas reactors could also lead to negative effect at least at the initial phase, until the hydrogen consumption rate by hydrogentrophic
methanogens is equal or greater compared with the hydrogen production and injection rate which may make a balance process again (Luo & Angelidaki, 2013a). Based on theoretical considerations but also by experimental proof, the increase of the hydrogen partial pressure in biogas reactors could cause decreased degradation of VFA, leading to process disturbance or break down (Fukuzaki et al., 1990; Luo et al., 2012; Siriwongrunson et al., 2007). Thus, the relatively lower methane yield and methane production rate at 1 atm (compared with 0.5 atm) indicated that the threshold of hydrogen partial pressure could be between 0.5 and 1 atm for causing disturbance of the process in the current study. Furthermore, an interesting observation was that thermophilic batch reactors were more resistant under high ammonia levels (5 and 7 g NH$_4^+$-N L$^{-1}$), compared with mesophilic reactors (0.5 atm). Free ammonia (NH$_3$) has been considered to be the main toxic compound causing ammonia inhibition and high temperature will increase the free ammonia levels. Therefore, the result of the current study was contradictory to some previous studies which reported that mesophilic methanogenesis is more resistant to high ammonia loads compared to the thermophilic process due to the lower free ammonia concentrations (Chen et al., 2008; Fotidis et al., 2013). However, it was also (Wang et al., 2015a) previously reported that hydrogenotrophic thermophilic methanogens can tolerate higher ammonia and free ammonia concentrations compared to mesophilic methanogens, which was in agreement with the result of this study. Moreover, in a previous study, thermophilic hydrogenotrophic methanogenic enrichment cultures were shown to be more efficient for methane production (122 mL CH$_4$ (g VSS h)$^{-1}$ higher) compared to mesophilic enrichment cultures due to the higher rates of digestion, which could be another explanation (Luo & Angelidaki, 2012).
The discrepancy on the ammonia tolerance at mesophilic or thermophilic conditions could very well be explained by the mechanism of ammonia inhibition. As it is assumed that free ammonia concentration (NH₃) is the active form for inhibition, which of course would constitute the thermophilic processes more susceptible for inhibition. However, this does not exclude the possibility that the thermophilic organisms are more tolerant to free ammonia (NH₃) levels. This could also be supported by the evolutionary pressure in thermophiles to develop tolerance to free ammonia levels.

The results of the current study that high ammonia concentration can inhibit the hydrogen enriched biogas production and upgrading processes by lowering the methane yield should be noticed especially when substrates containing high ammonia levels are used. Moreover, one of the challenges that the innovative AD process has is the increasing of pH due to the consumption of carbon dioxide, which subsequently will increase the free ammonia concentration and enhance the ammonia inhibition. Therefore, some sustainable and practical methods for counteracting ammonia inhibition on such processes are needed in the future. Controlling pH levels by co-digestion with appropriate low pH substrates could be an optional solution. For example, in a previous study, Luo and Angelidaki (2013a) maintained the pH level in an optimal range for anaerobic digestion in the biogas reactor with addition of hydrogen by co-digestion of manure and acidic whey.

### 3.2 VFA Accumulation and pH levels

Generally, the total VFA concentrations of the reactors increased with the increasing ammonia levels. The reactors with initial hydrogen partial pressure of 0.5 atm had the lowest VFA concentrations, indicating a healthy AD process without VFA
accumulation and inhibition of methanogenesis, which was in agreement with the results of the methane yield (Figure 3). Specifically, under mesophilic condition and ammonia levels of 7 g NH$_4^+$-N L$^{-1}$, the VFA concentrations were 1.3 and 1.6 g L$^{-1}$ at 0 and 1 atm of hydrogen partial pressure respectively, which were significantly (p<0.05, p=1.7×10$^{-8}$) higher compared with ones at 0.25 and 0.5 atm (Figure 3a). Additionally, at 0.5 atm of hydrogen partial pressure, total VFA at all tested ammonia levels were below 0.4 g L$^{-1}$ (Figure 3a) and similar results were obtained under thermophilic condition.

**Figure 3 is here**

High hydrogen partial pressure is considered to cause inhibition of propionate and butyrate degradation (Fukuzaki et al., 1990; Siriwongrungson et al., 2007). However, at shaking speed of 100 rpm under 1 atm of hydrogen partial pressure, no inhibition of either propionate or butyrate degradation was observed (Luo et al., 2012). The hydrogen’s slow mass transfer from gas to the liquid phase combined with the fast consumption rate of the dissolved hydrogen by the hydrogenotrophic methanogens, was the procedure for keeping dissolved hydrogen level low for efficient degradation of propionate and butyrate (Fukuzaki et al., 1990). On the contrary in the current study, the relatively higher shaking speed (180 rpm) applied may cause fast hydrogen transfer to the liquid phase resulting in more dissolved hydrogen in liquid, and along with the high ammonia level (7 g NH$_4^+$-N L$^{-1}$) could be the reason of the increase of the VFA concentrations at 1 atm of hydrogen partial pressure. On the contrary for the middle hydrogen partial pressures (0.25 and 0.5 atm), lower VFA concentrations were obtained. The reason for the less VFA accumulation at
0.25 and 0.5 atm could be the lower dissolved hydrogen level in the liquid and also
the resistance to ammonia toxicity.

The pH levels in both mesophilic and thermophilic batch reactors were shown in
Figure 4. At 0 and 1 atm of hydrogen partial pressure, the pH decreased from 7.95
to around 7.80 (7 g NH$_4^+$-N L$^{-1}$), while at 0.5 atm, the pH levels under different
ammonia concentrations increased from 7.95 to around 8.10. During anaerobic di-
gestion of cattle manure, several substances such as ammonia, bicarbonate, and
VFA could affect pH levels (Batstone et al., 2002). Therefore, at ammonia concen-
tration of 7 g NH$_4^+$-N L$^{-1}$, the significant increase (p<0.05, p=6.9×10$^{-5}$ at mesophilic,
p=2×10$^{-5}$ at thermophilic) of pH at 0.25 and 0.5 atm was caused by the consumption
of bicarbonate which was used by hydrogenotrophic methanogens for methane
production (Luo & Angelidaki, 2013a; Mu et al., 2006). However, the relatively
lower pH at 1 atm and 7 g NH$_4^+$-N L$^{-1}$, was due to the accumulation of VFA.

**Figure 4 is here**

### 3.3 Biogas composition

In the mesophilic reactors, the carbon dioxide content decreased with the increasing
of hydrogen partial pressure at ammonia concentration of 7 g NH$_4^+$-N L$^{-1}$ (Figure
5a). Nevertheless, no further decrease was observed when hydrogen partial pressure
was higher than 0.5 atm. It was consistent with previous observation in hydrogen
enriched biogas production and upgrading process at low ammonia load (≤ 2 g
NH$_4^+$-N L$^{-1}$) (Luo et al., 2012). Although similar trend was observed in the thermo-
philic reactors for initial hydrogen partial pressures of 0 and 0.5 atm, the carbon
dioxide content further decreased at higher hydrogen partial pressure (1 atm) (Fig-
ure 5a). This could be due to the higher conversion rates and activity of the micro-
organisms at thermophilic temperature which permits a faster removal of the hydrogen and avoids accumulation of VFA. The results again confirmed that the hydrogen enriched biogas upgrading processes can still function at high ammonia concentration.

**Figure 5 is here**

Comparatively, with fixed hydrogen pressure, the ammonia concentration had no significant influence on carbon dioxide content, both in mesophilic and thermophilic conditions (p>0.05, p=0.135 at mesophilic, p=0.138 at thermophilic) (Figure 5b). In detail, the carbon dioxide content was 38.1% and 40.0% (mesophilic and thermophilic, respectively) at 7 g NH$_4^+$-N L$^{-1}$ without adding hydrogen which was in accordance with previously reported (Lindeboom et al., 2012). Meanwhile the methane content was around 80% when hydrogen was added, as it was reacting with carbon dioxide to produce methane. According to Figure 1a and 1b, the methane yield had a significant (p<0.05) decreasing at high ammonia levels. On the contrary, at the same ammonia concentration (7 g NH$_4^+$-N L$^{-1}$) the methane content increased (or carbon dioxide content decreased) in the reactors with hydrogen injection, which indicated that hydrogenotrophic methanogens might be more resistant to high ammonia levels compared to acetoclastic methanogens in the hydrogen enriched biogas production and upgrading processes.
4. Conclusions

The results of the current study indicated that high ammonia concentration can inhibit the hydrogen enriched biogas production and upgrading processes by lowering the methane yield. Nevertheless, the ammonia concentration had no significant effect on the biogas composition in such processes. It also implied that the hydrogen enriched production and upgrading processes was more tolerant to high ammonia concentrations compared with conventional AD process. Moreover, thermophilic methanogens seemed to perform better compared with mesophilic methanogens under high ammonia levels (5 and 7 g NH₄⁺-N L⁻¹). The current study was the first time to quantify ammonia toxicity for the hydrogen enriched biogas production and upgrading processes. Therefore, some sustainable and practical methods for counteracting ammonia inhibition on such processes (e.g., pH control or co-digested with low pH substrate) are needed in the future.

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References


Table and figure captions

Table 1. Characteristics of the inoculum and the dairy manure.

Figure 1. Methane yield (a) and methane production rate (b) as a function of hydrogen partial pressure.

Figure 2. Methane yield as a function of ammonia concentrations at hydrogen partial pressure of 0.5 atm.

Figure 3. Total VFA accumulation under different hydrogen partial pressure (a) and under different ammonia concentrations (b).

Figure 4. pH levels at different hydrogen partial pressure under 7 g NH$_4^+$-N L$^{-1}$ (a) and pH under different ammonia concentrations at hydrogen partial pressure of 0.5 atm (b).

Figure 5. Carbon dioxide content under different hydrogen partial pressure (a) and under different ammonia concentrations (b).
### Table 1. Characteristics of the inoculum and the dairy manure

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Mesophilic Inoculum</th>
<th>Thermophilic Inoculum</th>
<th>Dairy manure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (g·L⁻¹)</td>
<td>1003 ± 0.17</td>
<td>1003 ± 0.52</td>
<td>1002 ± 0.78</td>
</tr>
<tr>
<td>TS (g·L⁻¹)</td>
<td>48.04 ± 0.24</td>
<td>31.24 ± 0.17</td>
<td>86.93 ± 0.00</td>
</tr>
<tr>
<td>VS (g·L⁻¹)</td>
<td>28.42 ± 0.00</td>
<td>16.70 ± 0.00</td>
<td>63.30 ± 0.01</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen (g N L⁻¹)</td>
<td>4.61 ± 0.21</td>
<td>4.23 ± 0.16</td>
<td>3.51 ± 0.13</td>
</tr>
<tr>
<td>Ammonia (g NH₄⁺-N·L⁻¹)</td>
<td>3.63 ± 0.09</td>
<td>3.04 ± 0.05</td>
<td>2.10 ± 0.08</td>
</tr>
<tr>
<td>Total VFA (mg L⁻¹)</td>
<td>705.6±27.91</td>
<td>900.8 ± 24.40</td>
<td>3781 ± 137.14</td>
</tr>
<tr>
<td>pH</td>
<td>7.78</td>
<td>7.83</td>
<td>8.06</td>
</tr>
</tbody>
</table>

“±” means standard deviation and all values presented are the means of independent triplicates (n=3)
Figure 1. Methane yield (a) and methane production rate (b) as a function of hydrogen partial pressure.
**Figure 2.** Methane yield as a function of ammonia concentrations at hydrogen partial pressure of 0.5 atm.
Figure 3. Total VFA accumulation under different hydrogen partial pressure (a) and under different ammonia concentrations (b).
Figure 4. pH levels at different hydrogen partial pressure under 7 g NH$_4^+$-N L$^{-1}$ (a) and pH under different ammonia concentrations at hydrogen partial pressure of 0.5 atm (b).
Figure 5. Carbon dioxide content under different hydrogen partial pressure (a) and under different ammonia concentrations (b).
Highlights

• High ammonia concentration inhibited hydrogen enriched biogas upgrading processes.

• High ammonia concentration can lower the methane yield.

• The ammonia concentration had no significant effect on the biogas composition.

• Hydrogenotrophic archaea were more resistant to ammonia toxicity.

• The ammonia toxicity was alleviated at thermophilic condition.