Pretreatment of poultry manure for efficient biogas production as monosubstrate or co-fermentation with maize silage and corn stover

Tamás Bőjti a, Kornél L. Kovács a, b, c, *, Balázs Kakuk a, Roland Wirth a, Gábor Rákhely a, b, Zoltán Bagi a

a Department of Biotechnology, University of Szeged, Közép fasor 52, Szeged 6726, Hungary
b Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, Temesvári krt. 62, Szeged 6726, Hungary
c Department of Oral Biology and Experimental Dental Research, University of Szeged, Tisza L. krt. 64, Szeged 6720, Hungary

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1. Introduction

The poultry industry is growing rapidly along with human consumption, which results in large quantities of animal wastes to be treated. Inappropriate management of manure may cause numerous undesirable consequences such as odor pollution, attraction of rodents, insects and other pests, release of animal pathogens, groundwater contamination, surface water runoff, deterioration of biological structure of the soil, etc. [1,2]. NH3 and greenhouse gases, CH4 and CO2, emitted from the waste storage units cause air pollution problems [3]. Anaerobic digestion (AD) is a commonly employed process for treating animal manure and biogas production is widely studied and practiced [4–7].

Chicken manure (CM) is generally considered a problematic substrate for AD [8,9]. CM has a high nitrogen content, which is in two main forms: uric acid and undigested proteins, representing 70% and 30% of the total organic nitrogen, respectively. AD of these components is accompanied with the production of inhibitory concentrations of unionized NH3 and NH4+ ions [10,11]. Accumulation of these toxic products does not allow fermentation at higher total solids (TS) loadings. The recommended substrate concentration is less than 5% TS and a decrease in biogas production rate has been observed when TS was further increased [12,13].

Stripping of the liquid phase is an efficient way to avoid the accumulation of NH3 during biogas fermentation. The removed NH3 can be reacted with sulfuric acid (H2SO4) to form ammonium sulfate ([NH4]2SO4). Addition of H2SO4 increases the operational costs but in exchange, [NH4]2SO4 can be utilized as a fertilizer [14] and accelerates the recycling of nitrogen because farms can handle the slurry or solid fertilizer more easily than the residue after wet AD [15,16].

In alternative approaches, CM was treated anaerobically after dilution with water either in a semi-solid form, i.e. 10–11.5% TS content, or in a wet form (0.5–3% TS) [17] or the high N-content was extracted with water and ammonia recovered by stripping (Nie et al., 2015). Other endeavors to reduce the inhibitory effect of NH3 involved adsorbing onto zeolite or clay [18,19].
Struvite production removes both inorganic nitrogen and phosphorus from agro-industrial and livestock wastewaters. Magnesium ammonium phosphate hexahydrate (MgNH₄PO₄ • 6H₂O, MAP) is a promising product for nutrient recovery from wastes with high nitrogen and phosphorus contents and has the general name struvite [20,21]. Struvite precipitation from anaerobic digestion effluents has great advantage due to the predominance of NH₄⁺–N and PO₄³⁻–P, so that addition of the necessary chemicals can be minimized [22–24]. MAP precipitation, combined with AD, offers important advantages in terms of renewable energy production and the recovery of MAP sludge as a valuable slow-release fertilizer for agricultural use [25]. The digestibility of nitrogen-rich wastes could also be improved by mixing them with substrates of high carbon content, thereby improving the C/N ratio [26–28]. Co-digestion has important benefits, including the balancing of the macro and micronutrients, pH, inhibitors/toxic compounds and dry matter [29–31]. C/N ratios of 20:1 and 30:1 provide optimal digestion, stable pH and low concentrations of free NH₃ and total NH₄⁻N [28]. Co-digestion of CM with other types of livestock manure such as pig waste [32], cattle manure [8] and anaerobically digested sludge [17] also improved biogas productivity.

In the present study, the removal of the majority of watersoluble inorganic and organic nitrogen compounds from CM by water extraction was tested, which is a simple and inexpensive method, carried out at ambient temperature. The insoluble particulate fraction, separated by centrifugation or simple sedimentation, became suitable substrate for biogas generation. Furthermore, co-digestion of CM with maize silage and corn stover was employed to improve the substrate C/N ratio.

2. Materials and methods

2.1. Substrates and inoculum

CM was collected from a commercial broiler poultry farm (Hungert Corp.) located at Csengele, Hungary. The free-range poultry houses use wheat straw bedding. Water extraction comprised of soaking 5 g CM in 100 mL tap water at room temperature followed by separation of the liquid and solid phases by centrifugation (10,000 rpm for 3 min). The solid fraction was air dried and stored at 20 °C. This treated chicken manure (T-CM) was used in most AD experiments.

Corn stover was obtained from University of Szeged, Faculty of Agriculture. Maize silage came from the biogas plant of Zöldforrás Ltd., Szeged, Hungary. The AD inoculum was collected freshly from the same industrial biogas plant operated with a mixture of pig slurry and maize silage at mesophilic temperature. CM and corn stover was milled and sieved with an electric grinder (Retsch SM 100, Haan, Germany).

Parameters of the biogas substrates raw chicken manure (CM), pretreated CM (T-CM), maize silage and corn stover are presented in Table 1.

2.2. Batch fermentation

Experiments were carried out in 160 mL reactor vessels (Wheaton glass serum bottle, Z114014 Aldrich) containing 60 mL liquid phase at mesophilic temperature (37 ± 0.5 °C). All fermentations were done in triplicates. The inoculum sludge was filtered to remove particles larger than 1 mm and was used according to the VDI 4630 protocol [33]. Each batch fermentation experiment lasted for 30 days in triplicates.

2.3. Fed-batch fermentation

The reactor working volumes were 5 L, the headspaces were 1 L, in continuously stirred tank fermenters (CSTR), which were designed and constructed by Biospin Ltd, Hungary [34]. The AD experiments were performed at 37 ± 0.5 °C. Inoculum sludge came from the effluent of an operating biogas plant (Zöldforrás Ltd) and was incubated in the laboratory CSTR for 7–10 days to exhaust its residual biogas potential. Afterwards the reactors were fed daily with the specific substrates/mixtures until biogas production became stabilized. The biogas production measurement started from week 5.

2.4. Carbon-to-nitrogen ratio (C/N)

To analyze C/N, an Elementar Analyzer Vario MAX CN (Elementar Group, Hanau, Germany) was used. The equipment operates using the principle of catalytic tube combustion under an O₂ supply at high temperatures (combustion temperature: 900 °C, post-combustion temperature: 900 °C, reduction temperature: 830 °C, column temperature: 250 °C). The components were separated from each other with the aid of specific adsorption columns (containing Sicapent (Merck, Billerica, USA), in C/N mode) and determined in succession with a thermal conductivity detector. Helium served as carrier and flushing gas.

2.5. NH₄⁺–N

For the determination of NH₄⁺–N content, the Merck Spectroquant Ammonium test (1.00683.0001) (Merck, Billerica, USA) was employed.

2.6. Phosphate measurement

Total phosphate content of chicken manure supernatant was measured by the standard 4500-P E ascorbic acid method (Standard Methods for the Examination of Water and Wastewater, SMWW 4000–6000).

2.7. Biochemical oxygen demand determination

To measure the biochemical oxygen demand of chicken manure supernatant (CMS) a 5-day BOD test was applied (OxiTop OC 110, Wissenschaftlich-Technische Werkstätten GmbH). In the parallel 500 mL BOD-sample bottles 0.5 mL of microorganism culture and

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CM</th>
<th>T-CM</th>
<th>Maize silage</th>
<th>Corn stover</th>
<th>T-CM + Maize silage</th>
<th>T-CM + Corn stover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic total solids (oTS) [g]</td>
<td>84.1</td>
<td>83.80</td>
<td>95.11</td>
<td>94.32</td>
<td>89.37</td>
<td>90.10</td>
</tr>
<tr>
<td>Total solids (TS)[g]</td>
<td>92.6</td>
<td>95.87</td>
<td>29.32</td>
<td>93.72</td>
<td>45.24</td>
<td>94.31</td>
</tr>
<tr>
<td>Carbon/Nitrogen ratio</td>
<td>7.5</td>
<td>19.8</td>
<td>45.3</td>
<td>52.5</td>
<td>32.55</td>
<td>36.15</td>
</tr>
<tr>
<td>Particle size [mm]</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;10</td>
<td>&lt;2</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>
43 mL of CMS solution were placed. The results were read after 5 days in mg O$_2$/L.

2.8. VOAs/TAC

5 g of sample was taken for the analysis and diluted to 20 g with distilled water. The measurements were carried out with Pronova FOS/TAC 2000 Version 812–09.2008 automatic titrator (Pronova, Berlin, Germany).

2.9. Gas chromatographic analyses

The CH$_4$ content was determined with an Agilent 6890 N GC (Agilent Technologies) equipped with an HP Molesive 5 Å (30 m × 0.33 mm × 25 μm) column and a TCD detector. The temperature of the injector was 150 °C and application was made in split mode 0.2:1. The column temperature was maintained at 60 °C. The carrier gas was Linde HQ argon 5.0, with the flow rate set at 16.8 mL/min.

3. Results

3.1. The water extraction of CM

CM has a high biogas potential, but due to its high nitrogen content the C/N ratio is only about 5–10. This leads to inhibition of the methanogenic community [35–37].

A simple water extraction pretreatment removed significant portion of the nitrogen content of the solid material which was decreased from 53.75 mg/kg to 21.99 mg/kg. The process increased the C/N from 7.5 to 19.8 (Table 1), which was close to the optimum C/N ratio of 52.5, therefore a 1:1 mixture of corn stover and T-CM, which should have provided a suitable environment for the biogas producing community and could ensure a stable long term biogas fermentation (Fig. 4). Nevertheless, the biogas yields did not improve appreciably (Fig. 3) upon addition of maize silage. This suggests that maize silage did not efficiently improve the decomposition rate and yield of the substrate mix. Apparently, the microbes chose to degrade maize silage from the daily dosage of mixed substrates and left some of the T-CM untouched. The ratio of VOAs/TAC was around 0.15–0.20, which indicated a balanced biogas fermentation. Not surprisingly, mono-fermentation of T-CM resulted the highest NH$_4^+$-N levels. The ammonium level started to accumulate and reach 3.6 g/L at the end of the experiment [10,46]. Co-digestion of T-CM with maize silage lowered the NH$_4^+$-N levels as expected. In the reactors fed with maize silage:T-CM = 1:1 ratio (at organic solid basis) NH$_4^+$-N attained 2.3 g/L at week 16, which should have provided a suitable environment for the biogas producing community and could ensure a stable long term biogas fermentation (Fig. 4). Nevertheless, the biogas yields did not improve appreciably (Fig. 3) upon addition of maize silage. This suggests that the C/N ratio may not be the best indicator of system operation when CM is a major substrate component in AD.

3.3. Batch co-fermentation of T-CM and maize silage

Improvement of the efficiency of CM fermentation frequently involves co-fermentation of the manure [10,27,45]. The results of batch fermentations are summarized in Fig. 2. To ensure the stability of the fermentation, the organic loads were decreased to VDI 1X and VDI 0.5X, the corresponding sample compositions are summarized in Table 3. Methane yields of co-digestion did not reach those of maize silage in the control reactors. Nevertheless, the mixed substrates yielded more methane than mono-fermentation of T-CM indicating the benefits of co-fermentation. The VDI 0.5X fermentation yielded 260.2 mL CH$_4$/g oTS, this specific activity slightly lower than that of VDI 1X.

3.4. Fed-batch co-fermentation of T-CM and maize silage

To test the stability and sustainability of co-digestion with maize silage we studied the system in semi-continuous fed-batch fermentation. The experiments lasted for 16 weeks and consisted of two parts. During the first 8 weeks, the dosage of the organic matter was 0.5 g oTS/L/day and then the OLR was increased to 1.0 g oTS/L/day for the second 8 weeks. In the case of co-fermentation, the ratio of maize silage to T-CM was 1:1 on oTS basis. The objective was to allow a longer period for the adaptation of the microbial community to the substrate. Fig. 3 shows the cumulative methane yields. It was apparent that the methanogenic consortium became accustomed to the increased OLR and methane yields did not change significantly from week 9. Methane productivity of mono- and co-digestion of T-CM did not reach the estimated yield assuming additive biogas production from the two substrates. This suggests that maize silage did not efficiently improve the decomposition rate and yield of the substrate mix. Apparently, the microbes chose to degrade maize silage from the daily dosage of mixed substrates and left some of the T-CM untouched. The ratio of VOAs/TAC was around 0.15–0.20, which indicated a balanced biogas fermentation. Not surprisingly, mono-fermentation of T-CM resulted the highest NH$_4^+$-N levels. The ammonium level started to accumulate and reach 3.6 g/L at the end of the experiment [10,46]. Co-digestion of T-CM with maize silage lowered the NH$_4^+$-N levels as expected. In the reactors fed with maize silage:T-CM = 1:1 ratio (at organic solid basis) NH$_4^+$-N attained 2.3 g/L at week 16, which should have provided a suitable environment for the biogas producing community and could ensure a stable long term biogas fermentation (Fig. 4). Nevertheless, the biogas yields did not improve appreciably (Fig. 3) upon addition of maize silage. This suggests that the C/N ratio may not be the best indicator of system operation when CM is a major substrate component in AD.

3.5. Batch co-fermentation of T-CM with corn stover

Corn stover is the by-product of corn derived starch production, which is usually incinerated or plough back in the soil. It had a high C/N ratio of 52.5, therefore a 1:1 mixture of corn stover and T-CM, having C/N = 19.8, yielded a biomass of C/N = 36.2. Maize silage (VDI 1X), corn stover (VDI 1X) and T-CM (VDI 0.5X, VDI 1X) were also included in these experiments as monosubstrates, and the corn stover T-CM mixtures were tested at VDI 0.5X and VDI 1X organic loads (Table 4).

The methane yields of corn stover were significantly less than that of the maize silage when the two substrates were digested in the similar range of particles sizes, i.e., about 1 cm. Slightly better biogas yields were obtained from corn stover grinded to the average size of <2 mm. Nevertheless, when complete digestion was allowed by increasing residence time, the cumulative methane production became independent of particle size (B. Kakuk et al. this

<table>
<thead>
<tr>
<th>Table 2</th>
<th>P, N and BOD values in different systems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
</tr>
<tr>
<td>CM</td>
<td>21-25 g/kg</td>
</tr>
<tr>
<td>T-CM</td>
<td>12 g/kg</td>
</tr>
<tr>
<td>CMS</td>
<td>10 mg/L</td>
</tr>
</tbody>
</table>
An additional advantage of the mechanical pretreatment of the hydrophobic, dry corn stover was that the particles did not float on the surface of the liquid phase. Co-digestion of T-CM and corn stover at VDI 0.5X yielded 250 mL CH₄/g oTS, which exceeded the specific methane production at VDI 1X and was only slightly below the yield obtained from co-digestion of T-CM with maize silage (Fig. 5).

### 4. Discussion

Partial removal of water-soluble components by simple water extraction improved methane yields of CM (Fig. 1) although the effect was pronounced at low CM concentrations. The supernatant liquid phase was separated from the solid residue by centrifugation in our experimental protocol, but in large-scale treatment sedimentation may be sufficient. Similarly, in our experiments T-CM was dried to ensure precise dosage of the substrate, which may not be necessary in practical applications. Nevertheless, this relatively inexpensive and effective pretreatment of CM generates large volume of liquid supernatant containing organic and inorganic nitrogen compounds, phosphate and BOD. Purification of this waste water can be achieved by using the supernatant for photoheterotrophic cultivation of algae [47,48]. Algae incorporate the water-soluble N and P components into their biomass. The algal
Biomass can be recycled in the AD process whereas the purified water can be reused for further pretreatment of CM. Experimental tests of the feasibility of alga cultivation on CMS are in progress.

The T-CM fraction was employed in co-fermentations in the experiments reported here. We also noted that the measured methane yield depended on the inoculum to substrate ratio and the highest biogas production was achieved at VDI 1X.

Several studies have corroborated the beneficial effect of animal manure co-digestion with agricultural crops [49]. Co-digestion of raw CM and maize silage had been carried out and specific methane production of 0.309 L CH4/g VS was achieved although only at low CM concentrations [50]. In other studies CM was co-digested with cheese whey where the average percentage of methane was around 60% [51]. Abouelenien et al. [52] described CM co-digestion with various agricultural wastes such as coconut waste, cassava waste, and coffee grounds and methane yield improvements recorded. In our batch fermentations co-fermentation of T-CM with maize silage improved biogas yield (Fig. 2), however, this improvement

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**Table 4**

Substrate compositions of batch co-fermentations of T-CM with corn stover.

<table>
<thead>
<tr>
<th></th>
<th>Maize silage [g oTS/L]</th>
<th>Corn stover [g oTS/L]</th>
<th>T-CM [g oTS/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize silage VDI 1X</td>
<td>25.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn stover VDI 1X</td>
<td>8.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-CM VDI 0.5X</td>
<td></td>
<td>3.91</td>
<td></td>
</tr>
<tr>
<td>T-CM + Corn stover VDI 0.5 X</td>
<td>2.00</td>
<td>1.96</td>
<td></td>
</tr>
<tr>
<td>T-CM VDI 1X</td>
<td></td>
<td>7.82</td>
<td></td>
</tr>
<tr>
<td>T-CM + Corn stover VDI 1 X</td>
<td>4.00</td>
<td>3.91</td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 3. Cumulative methane production in fed batch fermentations of maize silage, water extracted chicken manure (T-CM) and their 1:1 mixture. Grey columns indicate the expected methane production. Vertical line shows the time of increasing substrate load.

Fig. 4. Accumulation of ammonia in the fed-batch fermentations. Markings are the same as in Fig. 3.
disappeared under fed-batch feeding condition (Fig. 3). A possible rationalization of the results suggests that the microbial consortia utilized first maize silage and T-CM might require longer residence time as in the batch reactors. When daily supply of the fresh substrate mix was provided in the fed-batch reactors, the microbial community limited its activity primarily to the decomposition of the maize silage and did not fall back upon T-CM.

Acclimatization of methanogenic consortia to high concentration of free ammonia is an effective method to improve the process stability of anaerobic digestion and methane production from various kinds of wastes [53]. Dry fermentations of CM under mesophilic conditions at 37 °C, using repeated batch laboratory reactor systems, required a long acclimation of about 254 days. A total volume of about 4.4 L CH4/kg CM was produced, despite the presence of high ammonia concentration, i.e. in the range of 8–14 g-N/kg CM [15]. NH4–N concentrations of about 8 g/L were also tolerated in AD of monofermentations of protein-rich wastes [54]. The NH4–N level increased steadily in the fed-batch reactors containing T-CM (Fig. 4) although it did not reach very high, alarming levels. This indicates that in the chicken manure NH4–N level may not be the sole factor limiting its anaerobic degradation [55] and 112 days were not enough to acclimatize the system to this substrate.

Energy crops are one of the most preferred feedstocks to produce biogas, especially in Europe [56]. The widespread use of energy crops is due to their high biogas potential and stable operation of biogas reactors although these substrates significantly upsurge the total biogas production costs [57]. Agricultural and/or municipal wastes offer a viable alternative [58,59]. Huge quantities of corn stover, which falls in the range of 700–800 million tons/year is generated globally as agricultural waste [60–62].

The C/N ratio of this biomass is typically between 50 and 75 and therefore exceeds the optimal 20–30 value for AD. An accepted approach of nitrogen supplementation to lignocellulosic biomass is to add nitrogen-rich co-substrates, e.g. manure, food waste etc. [63,64]. Li et al. [65] investigated methane production at different corn stover and CM ratios and determined the conditions providing process stability under wet, semi-solid state and solid state conditions. A synergistic effect was observed when mixing the two substrates at ratios of 3:1 and 1:1. The highest methane yield of 218.8 mL/g VS was achieved in wet AD at corn stover:CM ratio of 3:1. The highest volumetric methane productivity of 14.2 Lmethane/ L-reactor volume was found at corn stover:CM of 1:1 in SS-AD. Their corn stover had a C/N ratio of about 63.2.

Due to the limiting cellulolytic activity and low specific growth rate of cellulolytic microbes in AD [66], hydrolysis of native lignocellulosic biomass is usually a rate limiting step [67,68]. Therefore, pretreatment of lignocellulosic biomass may improve digestion efficiency and biogas production [69].

5. Conclusions

Water extraction successfully increased the C/N ratio of chicken manure from 7.45 to 19.81 and AD of the solid fraction became sustainable when the reactors were fed with T-CM monosubstrate. In the batch reactors about 27% more methane was produced from T-CM than from CM. Co-digestion of T-CM with maize silage increased further the methane production presumably due to the improved C/N. Corn stover efficiently replaced maize silage in batch co-fermentations, which may have important ramifications for practical application. Fed-batch fermentation corroborated that T-CM was suitable monosubstrate in sustained biogas fermentation. An increase in OLR from 0.5 to 1 g/L/day did not perturb the system significantly. Interestingly methane yields of T-CM and co-fermentation with maize silage were very similar, in spite of the lower ammonium ion concentration brought about by the introduction of maize silage. This may indicate that there are components in T-CM, which hinder its AD even under acceptable C/N conditions. A conceivable consequence of this effect could be that the microbial consortium consumed first the easily biodegradable substrate component, which was maize silage in this case, and the microbes decomposed the T-CM component at low rate. Fresh maize silage was supplied daily, thus the microbes were not compelled to digest much of T-CM. This assumption is in line with the batch fermentation results but needs further experimental verification.

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