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Prof. Pascal Boeckx
Prof. Peter Bossier
Prof. Guy Smagghe
Prof. Els Van Damme
Prof. Niko Verhoest
Bjorn Vandekerkhove

EDITORIAL ADDRESS

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Special issue

Advances & Trends in Biogas and Biorefineries

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PREFACE

The current special issue is the fruit of a collaboration between the European Biogas Association (EBA) and Ghent University in frame of the BioRefine Cluster Europe (BCE).

This cluster stimulates the interaction between European and national projects involved in R&D on biorefinery processes, aiming to produce and recover chemicals, materials and energy from biomass sources. It is coordinated from the Ghent University, Faculty of Bioscience Engineering.

The issue contains a summarized overview of (a selection of) involved projects as well as contributions that were made to recent conferences organized by Ghent University or EBA. In addition, attention is paid to policy recommendations that stem from scientific and industrial research in the form of formulated policy papers. Finally, the special issue also wishes to highlight some awarded research with one hand the nominees and winner of the best Poster of the 3rd European Biogas Conference (Ghent, Sept. 2016) and the Ivan Tolpe Award 2017. Ivan Tolpe was a Belgian farmer / agro-manager that has defined and shaped technological innovation in the agro-sector over the last decades until his untimely demise in 2013. The award was created thereafter as a homage to his work and an inheritance to future generations.

The international scientific peer-review process was conducted by a board of 10 international experts from 9 different institutes. The editorial board, consisting out of EBA and Ghent University staff members wishes you a pleasant and informative read. We hope that the current collaboration will be the first in a longer series of topical issues.



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Contents

Contents..... 1

I. Projects – Drivers of Innovation 5

 Biogas Action - Removing the Non-Technical Barriers to Contribute to Creating Better Frameworks for the Widespread Production of Biogas and Biomethane 6

 TransBio: Transition to a More Cost-Effective Biogas Sector in Flanders..... 9

 Pocket Power! – Extending Small-Scale Anaerobic Digestion in Flanders..... 13

 DIGESMART – Digestate from Manure Recycling Technologies 17

 ReciDigest - Implementation of Digestate Treatment and Recirculation - Influence on Anaerobic Digestion..... 21

 INEMAD - Reconnecting Livestock and Crop Production..... 25

 AGROCYCLE – for a Circular Economy..... 29

 P-REX - Sustainable Sewage Sludge Management fostering Phosphorus Recovery and Energy Efficiency 31

 SYSTEMIC - Systemic Large Scale Eco-Innovation to Advance Circular Economy and Mineral Recovery from Organic Waste in Europe (H2020 project) 34

 KASAV - Cascaded Utilisation of Food Waste for the Production of Liquid and Gaseous Biofuels 38

 GR3 - GRass as a GReen Gas Resource..... 41

 BIOSURF - BIOMethane as SUSTainable and Renewable Fuel 44

 ERGaR – The European Renewable Gas Registry 50

 GoBiGas – First Full-Scale Demonstration of Biomethane from Forest Residues 53

 Recovery and Use of Nutrients, Energy and Organic Matter from Animal Waste (ReUseWaste) 57

II. Extended Abstracts – Research in Motion 63

 Grass Supply Chains for Biogas Production..... 64

 Bioaugmentation of the thermophilic anaerobic biodegradation of lignocellulose-rich substrates 70

 Opportunities of Co-Digesting Manure with Grass..... 76

 Energy from Waste Biomass: Grass from Roadsides and Nature Management 78

Key Parameters for the Assessment of Biogas Yields from Manures in Commercial-Scale Biogas Plants.....	80
Simple Pretreatment of Poultry Manure for Efficient Biogas Production and Co-Fermentation with Maize Silage.....	84
Wastewater Stabilisation Pond Train System as Ecotechnological Tool for Bioregenerative Resource Recycling: Showcasing an Indian Experience	91
Combining Phototrophic Polyhydroxybutyrate Production by Cyanobacteria with Anaerobic Digestion for Providing Nutrients and Utilizing Residual Biomass	96
The Fate of Heavy Metals in Anaerobic Digestion Process.....	99
Nutrient Recovery from Wastewaters as High-Valued Hydroponic Fertilizer and Non-Fertilizer Products using Hybrid Ion Exchange Nanotechnology	102
Energetic Valorisation of Cheese Whey using UASB Technology: a case-study	106
Two-stage Anaerobic Digestion: Towards Pipeline-Quality Biogas Production	111
Comparison of Biogas Upgrading Systems with different Biomethane usage Paths and decentralised Biogas Usage in CHP Units.....	115
Liquid Fraction of Digestate as good as Chemical Fertiliser? – Observations from a Three-Year Field Experiment	118
The Issues of Nitrogenous Components during Anaerobic Digestion	122
Greenhouse Gas Mitigation and Renewable Energy Production through Farm-Scale Anaerobic Digestion – Potential for Flanders and the European Union	127
Molecular Analyses of Microbial Communities Reveal an Optimization of the Anaerobic Fermentation Process for Improved Biogas Production during Calcium-Nitrate Treatment.	129
Microbial Conversion of H ₂ and CO ₂ into Liquid and Gaseous Energy Carriers.....	134
Power-to-Gas in Fed-Batch Reactors Using Biogas Digestate Catalyst.....	137
Biomass to SNG via Woodroll® process – Results from syngas preparation for catalytic conversion	141
Comparison of Biogas Plant Financial Indicators between different Regions as a Mean to deduce the Impact of regional Framework Conditions	146
Social acceptability of innovative nutrient management strategies to recycle nutrients.....	148
Comparison of different Digestate Processing Systems Concerning environmental Efficiency	154
III. Policy Recommendations based on Research & Development	159
Meeting the Paris Agreement Objectives requires a Forward-Looking Circular Economy Approach	160
EU Waste Policies: Getting the Right Measures to Recycle Biowaste	164

Economic Opportunities for the German Biogas Market within the Framework of the EEG 2017	169
IV. In the Spotlight – Awarded Research	171
Nominee - Tolpe Award 2017	172
GENIAAL - from Manure to green Minerals and Clean Water	172
Winner - Tolpe Award 2017	175
Manure Valorisation – Turning a Problem into a Commodity.....	175
Nominee for EBA Conference Poster Award 2016	178
Techno-Economic Analysis of Biogas Plant Repowering Measures – Effects of an Extended Digestate Storage Capacity	178
Nominee for EBA Conference Poster Award 2016	183
Power-to-Gas by Biomethanation– From Laboratory to Megawatt Scale	183
Winner of EBA Conference Poster Award 2016	188
Underpinning green energy: Reference standards and methods for the measurement of siloxanes in biogas.....	188

Simple Pretreatment of Poultry Manure for Efficient Biogas Production and Co-Fermentation with Maize Silage

Böjti, T.^a, Kovács, K. L.^{a,b,c}, Kakuk, B.^a, Wirth, R.^a, Rákhely, G.^{a,b}, Bagi, Z.^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

Contact: kovacs.kornel@bio.u-szeged.hu

SUMMARY

Water extraction of raw chicken manure elevated the carbon-to-nitrogen ratio 2.7-fold, i.e. from 7.48 to 19.81. The treated chicken manure (T-CM) became suitable for biogas fermentation as monosubstrate. Improved methane production was achieved in co-fermentations with maize silage (24% more methane) relative to T-CM monosubstrate. The standardized biogas potential assay indicated that the methane yields varied with the organic loading between 160 and 250 mL CH₄/g organic total solid (oTS). Co-fermentation with maize silage was sustainable in continuous anaerobic digestion for at least 4 months.

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 problem, attraction of rodents, insects and other pests, release of animal pathogens, groundwater contamination, surface water runoff, deterioration of biological structure of the soil, etc. (Kocak-Enturk et al., 2007). NH₃ and greenhouse gases, CH₄ and CO₂, emitted from the waste storage units cause air pollution problems (Yetilmezsoy and Sakar 2008). Anaerobic digestion (AD) is a commonly employed process for treating animal manure and biogas production from manure is widely studied and practiced (Mackie and Bryant 1995, Huang and Shih 1981, Nishio and Nakashimida 2007, Kovács et al., 2014).

Chicken manure (CM) is generally considered a problematic substrate for AD (Güngör-Demirci and Demirer 2004). CM has 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 (Chen et al., 2008, Salminen and Rintila 2002). Accumulation of the 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 (Sakar et al., 2009, Kelleher et al., 2002).

The digestibility of nitrogen-rich wastes could be improved by mixing them with substrates of high carbon content, thereby improving the C/N ratio (Ahring et al., 1992, Kaparaju et al., 2002, Wang et al., 2012). Co-digestion has important benefits, including the balancing of the macro and

micronutrients, pH, inhibitors/toxic compounds and dry matter. C/N ratios of 20:1 - 30:1 provide optimal digestion, stable pH and low concentrations of free NH₃ and total NH₄⁺-N (Wang *et al.*, 2012).

In the present study, the removal of the majority of water-soluble inorganic and organic nitrogen compounds from CM by water extraction at ambient temperature was tested, which is a simple and inexpensive method. 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.

MATERIALS AND METHODS

Substrates and inoculum

CM was collected from a commercial broiler poultry farm (Hungerit Corp.) located at Csengele, Hungary. The free-range poultry houses use wheat straw bedding. Water extraction comprised of soaking 5g 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.

Table 1: Parameters of the substrates used

Parameter	CM	T-CM	Maize Silage	Corn Stover
Organic total solids (oTS) [g]	84.1	83.80	95.11	94.32
Total solids (TS)[g]	92.6	95.87	29.32	93.72
Carbon/Nitrogen ratio	7.5	19.8	45.3	52.5
Particle size [mm]	<2	<2	<10	<2 <10

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 (VDI 2006). Each batch fermentation experiment lasted for 30 days in triplicates.

Fed-batch fermentation

The working volumes of custom-made reactors were 5 L, the headspaces were 1 L, the continuously stirred tank fermenters (CSTR) were designed and constructed by Biospin Ltd, Hungary (Kovacs et al., 2013). The AD experiments were performed at 37 ± 0.5 °C. Inoculum sludge came from the effluent of an operating biogas plant (Zöldforrás Ltd, Szeged, Hungary) 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 after the beginning of daily feeding.

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.

NH₄⁺-N

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

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).

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 43 mL of CMS solution were placed. The results were read after 5 days in mg O₂/L.

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).

Gas chromatographic analyses

The CH₄ content was determined with an Agilent 6890 N GC (Agilent Technologies) equipped with an HP Molesive 5 Å (30 m × 0.53 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.

RESULTS AND DISCUSSION

The water extraction of CM

CM has a high biogas potential, but due to its high N-content the C/N ratio is only about 5–10. This leads to inhibition of the methanogenic community (Yenigün and Demirel 2013).

A simple water extraction pretreatment removed significant portion of the nitrogen content of the solid material, which 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 range of 20-30 (Yadvika et al., 2004). After the pretreatment the phosphate, nitrogen and BOD contents of the supernatant (CMS) were 10 mg/L, 21.3 mg/L and 5.9 g/L, respectively. The C/N ratio of CMS was 4.7. The water extraction technology may therefore be considered a suitable approach to pretreat CM as biogas substrate.

Batch AD of pretreated and untreated CM using two organic concentrations

According to previous experience, at higher TS loadings, i.e. >50 g/L, raw CM cannot be subjected to AD efficiently (Bujoczek et al., 2000, Dalkilic and Uruglu, 2015). Batch measurements at two lower organic load concentrations were tested using twice the substrate concentration prescribed by the standard VDI test protocol (VDI 2X) (VDI 2006), which contained 16.26 g oTS/L of CM and 15.65 g oTS/L of T-CM; and VDI 4X, which contained 32.52 g oTS/L and 31.29 g oTS/L of CM and T-CM, respectively.

The reactor fed with T-CM produced 209.5 mL CH₄/g oTS, which is considerable if one takes into account that the “golden standard” maize silage yields around 250-280 mL CH₄/g oTS under the same conditions. The difference between the T-CM and raw CM was about 24% in favor of the T-CM substrate. Doubling the substrate concentration decreased specific methane production.

Batch co-fermentation of T-CM and maize silage

Improvement of the efficiency of CM fermentation frequently invokes co-fermentation of the manure (Chen et al., 2008, Kaparaju et al., 2002, Heiermann et al., 2007). To ensure the stability of the fermentation, the organic loadings were decreased to VDI 1X and VDI 0.5X. Methane yields of co-digestion did not reach those of maize silage in the control reactors. Nevertheless, the mixed substrates yielded more CH₄ than mono-fermentation of T-CM indicating the benefits of co-fermentation. The VDI 0.5X fermentation yielded 260.2 mL CH₄/g oTS, this specific activity slightly lower than that of VDI 1X.

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 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. Figure 1 shows the cumulative methane yields. It was apparent that the methanogenic consortium became accustomed to the

increased OLR and CH₄ yields did not change significantly from week 9. CH₄ 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 in the highest NH₄⁺-N levels. The ammonium level started to accumulate and reached 3.6 g/L at the end of the experiment (Salminen and Rintala 2002, Nielsen and Angelidaki 2008). Co-digestion of T-CM with maize silage lowered the NH₄⁺-N levels as expected. In the reactors fed with maize silage:T-CM=1:1 ratio (at oTS) NH₄⁺-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. Nevertheless, the biogas yields did not improve appreciably 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.

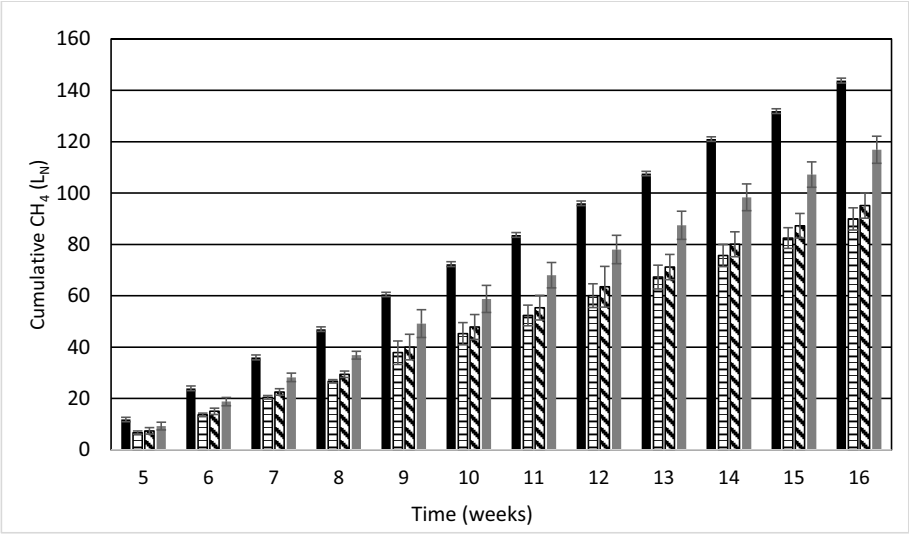


Figure 1: Cumulative biogas productions in fed-batch AD reactors using various substrate compositions: maize silage (black columns), the solid fraction of water extracted chicken manure (T-CM) (horizontally striped columns), co-fermentation of maize silage and T-CM (diagonally striped columns), and the estimated yield assuming additive biogas productivity of the co-fermentation partner substrates (grey columns)

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 CH₄ was produced from T-CM relative to raw CM. Co-digestion of T-CM with maize silage increased further CH₄ 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 a 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 CH₄ 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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