Defining the best pretreatment method for biomethanisation of sewage sludge and corn straw.

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Keywords: biogas, steam explosion, waste activated sludge, lignocellulose-rich biomass
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Biomethanisation is a common strategy to generate environment-friendly biogas and represents an expanding field in the renewable energy sector. While some substrates used for energy production are readily used by the microbiota of the inocula, other substrates require a treatment prior to their introduction into biogas plants. A pretreatment (PT) can either be necessary for recalcitrant materials such as lignocellulose-rich biomass (LCB) or for low-energy materials such as waste activated sludge (WAS) accruing at wastewater treatment plants. Both substrate types have been studied regarding different PT strategies, but neither has the resulting net-energy output been calculated, that considers the energy demand by the PT itself, nor have the microbial communities been investigated in order to depict significant changes in their compositions and resulting functions.

Here we first optimise five different PT methods (I-V) for two representative co-substrates, LCB (corn straw) and WAS. We subsequently test the methane potential of the optimally pretreated substrates according to a standardized procedure (VDI 4630, 2006) and compute an energy balance considering the energy input for PTs to identify the best method for each co-substrate. In addition, we aim to track the changes in the microbial communities caused by the different PTs in order to draw conclusions on the optimum conditions for high methane production. The PTs include three traditional approaches (I) enzymes (II) steam explosion and (III) thermo-chemical PT as well as two innovative methods, (IV) the hydro-thermal carbonisation (HTC) and (V) the implementation of active rumen microorganisms and –enzymes. Of these five PT methods the three traditional approaches are well studied and deliver robust results. Approaches IV and V, however, are less studied but are bearing additional potential. During HTC, an energy-rich liquid as well as a HTC-char are generated, of which the liquid fraction could be used as a substrate for biomethanisation, while the HTC-char may be used for soil conditioning, increasing the benefits retrieved from HTC. The use of rumen microorgansims, however, is known to increase biomethanation through their production of potent (exo)enzymes, efficiently breaking down recalcitrant (hemi)celluloses.

In order to optimise the mentioned PTs, each PT was investigated individually for both substrates. For (I), a mixture of the respective substrate and citrate buffer (15 % of total solids) was pretreated using 1.5 FPU/mL of commercially available enzymes (Accellerase 1500) at 50 °C for 96 h, maintaining a pH of 5 under continuous stirring. (II) For the steam explosion, a mixture of corn straw and deionised water (1:1) and the WAS in its pure form were investigated at temperatures of 180, 190 and 200 °C for 15 and 5 min, respectively. The resulting biogas-potential (BGP) and concentrations of structural carbohydrates served as criteria to define the optimum PT conditions. (III) The best thermo-chemical PT for WAS was defined over a range of 39-200 °C using three alkaline agents (NaOH, aluminate and ash from a biomass power plant) at different concentrations (0.02, 0.04 and 0.08 M) for a duration of 1 h, and additionally the dewaterability of the pretreated sludge was measured (Nagler *et al* 2018). For corn straw, PTs with NaOH over a range of 0.0125-1.85 M for a duration of 2 or 4 h at 70 °C were investigated. The most promising thermo-chemical PT was determined measuring chemical oxygen demand (COD) as well as dissolved potassium and -organic C which served as alternative disintegration parameters. (IV) HTC was performed on corn straw at 200, 220 and 240 °C and optimal conditions were defined measuring calorific value, COD and phenol-concentrations of the resulting energy-rich liquid and bio-char. (V) Finally, the use of rumen liquid was investigated for the corn straw substrate only. Survival of the highly adapted microbial community is best possible in a digester driven with (cattle) manure and treating LCB and not assumed in WAS. An amendment with 20 or 40 % (v/v) of cattle rumen to a bioreactor driven with cattle manure and corn straw (16 g L^{-1}) was evaluated by measurement of BGP and (hemi)cellulose degradation efficiencies.

Results on the various PT-strategies for both substrates are summarized in table 1: (I) While for the enzymatically pretreated substrates, no significant effect on the BGP of WAS was detected, BGP of corn straw was found to be significantly increased by 15.7 %. (II) A PT with steam explosion had a negative effect on the methane potential of WAS, while a treatment at 190 °C for 15 min was found to be ideal for corn straw, resulting in a 61.3 % increased methane potential. Furthermore, this PT yielded a structurally highly degraded corn straw, decreased amounts of hemicellulose and increased lignin-content. (III) For the thermo-chemical PT of WAS, a concentration of 0.04 M NaOH at 70 °C and 1 h resulted in the highest cell-disintegration in combination with lowest energy demands and yielded 22.4 % more methane than the untreated WAS. Furthermore, such a pretreated sludge showed an improved dewaterability hence decreasing WAS disposal costs. (IV) Testing HTC, the most promising products were generated by applying a temperature of 240 °C. The obtained energy-rich liquid exhibited a similar methane potential as the untreated corn straw. Together with an additional use of the HTC-produced HTC-char, both benefits could subsequently sum up to a positive net-energy-balance. (V) The addition of 40 % rumen liquid to the biogas-reactors resulted in a 66.9 % increase in methane potentials of corn straw. The monitoring of the microbiota showed not only a survival of anaerobic fungi, which is a group of microorganisms responsible for LCB-breakdown in ruminants, but also increasing numbers of bacteria and methanogenic archaea with increasing amounts of rumen liquid. Next to an increased degradation of cellulose and hemicellulose, an investigation of the methane production and abundance of methanogens also suggested that the efficiency of methane production is positively affected by the addition of rumen liquid.

Table 1. Defined optimum PT strategies and resulting methane productions compared to the untreated substrates in NL $kgVS^{-1}$.

		Pretreatment	optimised method	Untreated	Pretreated	% change
WAS	I	Enzymes	Accelerase, as recommended	187	203	8.6
	II	Steam explosion	200 °C, 5 min	187	171	-8.6
	III	Thermo-chemical	0.04 M NaOH,70 °C, 1 h	174	213	22.4
Corn straw	I	Enzymes	Accelerase, as recommended	204	236	15.7
	II	Steam explosion	190 °C, 15 min	204	329	61.3
	III	Thermo-chemical	1.85 M NaOH, 70 °C, 2 h	235	287	22.1
	IV	HTC	240 °C	323	305	-5.6
	V	Rumen	Addition of 40 % (v/v) rumen liquid	260	434	66.9

Ongoing experiments aim to subject all optimally pretreated substrates to a simultaneous methane potential test using the automated methane potential testing system (AMPTS, Bioprocess Control, Lund, Sweden) following a standardised procedure (VDI 4630). Samples are retrieved at several time points for next-generation sequencing as well as qPCR to investigate microbial community shifts owed to the different PTs. Degradation efficiencies will be investigated, measuring total solids (TS), volatile solids (VS), volatile fatty acids (VFA) and (hemi)cellulose concentrations. Finally, the best PT for both substrates will be defined calculating the net-energy output of each treatment and interpreting results of (hemi)cellulose-, TS-, VS- and VFA degradation potentials.

Using this approach, we aim to unravel the best PT strategies for two typical biomethanisation substrates and give statements on their positive or negative effects on the microbiota, substrate degradation rates and net energy productions.

Acknowledgements

We kindly thank Gerda Gritsch, Sebastian Waldhuber and Ljubica Begovic for their analytical and professional help, Josef Kreiser and Rudolf Häusler from the WWTP Zirl for facilitating on-site experiments and sampling, the Kläranlagenberatung Kopp for the dewaterability measurements, the power plant Bioenergie Kufstein for providing ash and the slaughterhouse J. Mayr and the Abenthum farm for supplying us with rumen liquid and cattle manure. This work has been supported by the Fund for Hochschulraum-Strukturmittel (HRSM) of the Federal Ministry of Science, Research and Economy (BMWFW).

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