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The production of methane from the straw pellets with addition of enzymes

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Abstract. Biogas production requires much cheaper raw materials. The use of straw, as not always the full use of agricultural residues, increases the methane yield in pelletised form compared to non-pelletised straw. Lack is the high ratio of carbon to nitrogen content of straw, which leads to a slow and incomplete breakdown of the matter, and less producing substances from which bacteria produce methane.Variety of additives can be used to improve anaerobic digestion process. This article shows the results of the study, where the enzymes alpha amylase and xylanase and catalysts Metaferm and Melafen mixture are used for the digestion process echancement. Investigation was provided in 16 bioreactors operated in batch mode at 38 °C. Additives were filled into 14 bioreactors and only inoculum were filled into two bioreactors for control. The yield of biogas from straw pellets without additives was 0.655 L g^{-1}_{DOM} and methane 0.301 L g^{-1}_{DOM} after 34 days of anaerobic digestion. The yield of biogas from straw pellets with added alpha amylase was 0.652 L g^{-1}_{DOM} and methane 0.318 L g^{-1}_{DOM}. The yield of biogas from straw pellets with added xylanase was 0.689 L g^{-1}_{DOM} and methane 0.347 L g^{-1}_{DOM}. The yield of biogas from straw pellets with added Metaferm and Melafen mixture was 0.638 L g^{-1}_{DOM} and methane 0.254 L g^{-1}_{DOM}. The study demonstrates that the adding of enzymes increases the production of methane.

Key words: anaerobic digestion, straw pellets, methane, alpha amylase, xylanase.

INTRODUCTION

In recent years, several measures have been implemented in Latvia to reduce the support for biogas production, such as the introduction of a 9% profit margin, without taking into account large initial capital investments and high interest rates on bank loans. Raw material prices have also increased. The financial situation of the producers of biogas has deteriorated and some owners have already ceased operation of biogas plant. Therefore, the use of new, inexpensive raw biomass would be very important for them.

For the effective production of biogas from straw in typical agricultural biogas plants, it is necessary to pre-treat it. One of the rational pre-treatment methods can be granulation/briquetting which combines mechanical grinding and thermal effects. Pellets or briquettes quickly absorb moisture in the bioreactor, disintegrate, and make the biomass easily accessible to bacteria. Disgusting biomass does not float on top and does not form a floating layer as it does with chopped straw. It is known about a number of examples of successful use of briquetted/granulated straw in biogas plants, but the

experience of using significant volumes of straw for biogas production can still be considered as limited, as compared to traditional types of raw materials such as manure or maize silage (Moler & Hansen, 2014).

Straw is an abundant source of biomass that has a great potential to be used in the biogas industry, specifically in co-digestion with other substrates. Straw is poor in nitrogen and has a lignocellulosic structure giving a slow degradation. However, straw can be interesting as co-digestion material with substrates rich in easily degradable carbon and protein. One disadvantage of using straw is that it requires some kind of pretreatment, as for example reduction of particle size, prior to its use in a biogas reactor. Straw pellets and briquettes here represent an interesting alternative. These are established, easily accessible and easy-to-use products, consisting of ground and pressed straw, which can be used directly in the biogas process (Dubrovskis & Adamovics, 2012).

The results (Horwath et al., 2017) showed that the biochemical methane potential (BMP) for the straw products was 340 ± 19 NL CH_4 kg^{-1}_{DOM}. The results confirmed that, the briquetting and pelleting processes have a positive effect on the degradability of straw, higher BMP compared to virgin straw (313 ± 1 NL CH_4 kg^{-1}_{DOM}). Equal results were obtained at the two laboratories. The BMP for food waste was however significantly higher (t-test $p < 0.05$) when the test was performed at RISE, Uppsala (607 NL CH_4 kg^{-1}_{DOM}) compared at UB, Borås (445 NL CH_4 kg^{-1}_{DOM}). The difference was likely be explained by different experimental conditions in the different laboratories (Horwath et al., 2017).

Lignocellulosic residues are relatively recalcitrant to bioconversion during anaerobic digestion (AD) for biogas production. Pre-treatments with cellulolytic enzymes or diluted alkali can facilitate biomass hydrolysis and enhance the process. Both pre-treatments require low energy and chemical inputs, without accumulation of inhibitor. Milled wheat straw (Vasmara Ciro et al., 2017) was pre-treated with hydrolytic enzymes or with diluted NaOH before AD. The enzymatic pre-treatment only increased Mmax by 14%. However, the same increase was observed with heat-inactivated enzymes, thus it was merely caused by the bioconversion into methane of the organic compounds contained in the enzymatic preparations. Moreover, all the pre-treatments determined a holocellulose conversion into reducing sugars lower than 4% (Vasmara Ciro et al., 2017).

The hydrolysis of lignocellulose is assumed to be the rate-limiting step in the anaerobic fermentation process (Wellinger et al., 2013). A fungal hydrolytic enzyme mixture was used to assess the enzymatic impact on different feedstocks for biogas production. The optimal conditions for enzymatic hydrolysis of rye grain silage, maize silage, grass silage, feed residues and solid cattle manure were determined in lab-scale experiments. Finally, the effects of enhanced hydrolysis on anaerobic digestion were investigated in batch digestion tests. Enzyme treatment of substrate showed Michaelis-Menten-like behaviour and reached maximum values after 3 hours for reduced sugars as a product of hydrolysis. Methane production potential was determined for specific feedstock mixtures without enzyme, with inactivated enzyme and with active enzyme (with and without buffer). The results obtained show a clear increase in methane production after enzyme application for solid cattle manure (165 L kg^{-1}_{DOM} to 340 L kg^{-1}_{DOM}), grass silage (307 L kg^{-1}_{DOM} to 388 L kg^{-1}_{DOM}; enzyme plus buffer), feed residue (303 L kg^{-1}_{DOM} to 467 L kg^{-1}_{DOM}), maize silage (370 L kg^{-1}_{DOM} to 480 L kg^{-1}_{DOM}) and a lower increase for rye grain silage (355 L kg^{-1}_{DOM} to 413 L kg^{-1}_{DOM}) (Suarez et al., 2012).

One possibility to increase natural polymer degradation and concomitantly energy efficiency is the addition of exoenzymes to biogas facilities to enforce the primary degradation steps for biogas production. Only a marginal effect was obtained, when applying a tenfold higher concentration of added enzymes as proposed for practical use. The same result was achieved when commercially available enzymes were added to technical-scale fermentations using corn silage as monosubstrate. Therefore, these studies did not provide evidence that the addition of external enzymes into anaerobic degradation systems increases the methane yield in biogas facilities (Binner et al., 2011).

Metaferm and Melafen, created and produced in Latvia are substances, which induce biological processes. Metaferm contain multi enzymes, microelements and B group vitamins as well growing stimulators. Our previous studies shows that use of catalyst Metaferm has a positive effect on methane yield in anaerobic fermentation process of some biomass (Dubrovskis & Plume, 2016; Dubrovskis & Plume, 2017).

The aim of this study is to evaluate the suitability of straw pellets as substrate for biogas production and clarify whether the addition of enzymes alpha amylase and xylanase and biocatalysts Metaferm and Melafen (made in Latvia) in substrates leads to positive effect.

MATERIALS AND METHODS

The methodology described below and similar with German VDI 4630 (VDI 4630, 2006), Angelidaki et al. (2009) guideline and the German Methodenhandbuch Energetische Biomassenutzung (Thran, 2010) were used for the present study.

Average samples of wheat straw pellets were taken and it's the chemicals compositions were determined in the LUA laboratory according to the standardized methodology ISO 6496:1999. For each group of raw materials an average sample was taken and the total dry matter, organic dry matter and ashes content were measured.

The analysis were performed according to standard methods. Each group's raw material was thoroughly weighed carefully. All bioreactors (volume of 0.75 L) were filled with the same amount (500.0 g) of inoculums (digestate from a continuous working laboratory bioreactor with almost finished cows manure). Two bioreactors were filled with inoculums only as control. The others bioreactors were filled in with inoculums and biomass sample (10.0 g) with or without enzymes or catalyst Metaferm (see Table 1). Biomass sample 10 g is selected based on previous research experience so that the amount of biogas produced per day does not exceed 2 L. Biogas volume and composition analyzes are done once a day at about the same time. Gas from each bioreactor was directed into separate storage gas bag (2 L) located outside the heated chamber (see Fig. 1).

Amylase (EC 3.2.1.1) is an enzyme that catalyses the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. The α-amylases are calcium metalloenzymes. By acting at random locations along the starch chain, α-amylase breaks down long-chain saccharides, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β-amylase. In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0 (Silverman, 2002).

Xylanase (EC 3.2.1.8) is any of a class of enzymes that degrade the linear polysaccharide xylan into xylose, thus breaking down hemicellulose, one of the major components of plant cell walls. As such, it plays a major role in micro-organisms thriving on plant sources for the degradation of plant matter into usable nutrients. Xylanases are produced by fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insect, seeds, etc., (mammals do not produce xylanases). However, the principal commercial source of xylanases is filamentous fungi (Polizeli et al., 2005).

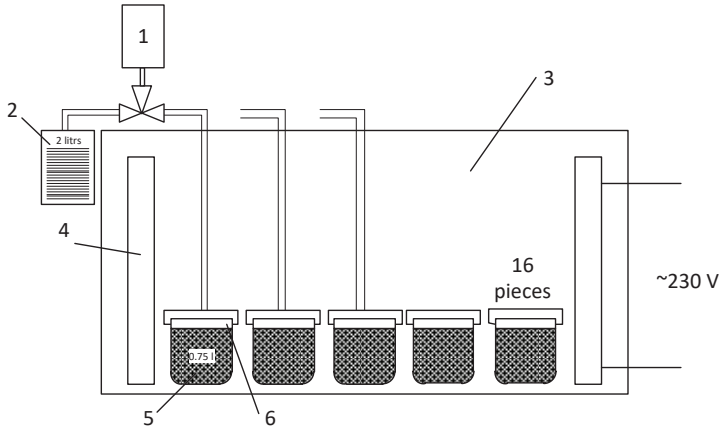

Figure 1. The schema of the experiment test bench: 1 – gas analyser; 2 – gas bag; 3 – electric stove; 4 – thermal elements; 5 – substrate; 6 – bioreactor.

Commercial applications for xylanase include the chlorine-free bleaching of wood pulp prior to the papermaking process, and the increased digestibility of silage (in this aspect, it is also used for fermentative composting) (Polizeli et al., 2005).

Wheat straw pellets (10.0 g) diameter 8 mm, length 10–20 mm were filled in bioreactors R2–R15 and in bioreactors R6–R9 added 0.5 mL alpha amylase, in bioreactors R10–R12 added 0.5 mL xylanase and in bioreactors R13–R15 added 1 mL Metaferm + Melafen mixture 1:1. Bioreactors were filled with substrate and placed in a heated chamber (Memmert model). Gas from each bioreactor was directed into separate storage gas bag (2 L) located outside the heated chamber.

Dry matter (TS) and dry organic matter (DOM) was determined by investigation of initial biomass sample weight and dry weight by using scales Shimazu at 105 °C and by investigation of ashes content help by furnace (Nabertherm model) burning the samples at 550 °C according to special heating cycle. All substrates were prepared, carefully mixed, and all sealed bioreactors were put in heated chamber in same time before anaerobic digestion. Composition of gases collected in storage bag was analysed with the gas analyser (GA 2000 model). The percentage of oxygen, carbon dioxide, methane and hydrogen sulphide were registered. Substrate pH value was measured before and after finishing of anaerobic fermentation process, using pH meter (PP-50 model) with accessories. Scales (Kern KFB 16KO2 model) was used for weighting of substrate before anaerobic processing and for weighting of digestate after finishing of fermentation process.

The accuracy of the measurements was ± 0.025 L for gas volume, ± 0.1 °C for temperature and ± 0.02 for pH. Methane (CH_4), carbon dioxide (CO_2), oxygen (O_2) and hydrogen sulphide (H_2S) content in biogas was measured periodically. Weights Kern FKB 16KO2 with accuracy ± 0.2 g was used for measurement of total weight of substrates, and the unit Shimazu with accuracy ± 0.001 g was used for weighting of biomass samples to obtain total solids and dry organic matter content.

Fermentation process was provided with single filling in batch mode until biogas emission ceases (34 days). Final digestate was weighed, and dry matter and ashes were investigated to determine organic dry matter content. Total biogas and methane production values were calculated using the biogas normal volumes and quality parameters obtained from gas collected in the gas storage bag for each bioreactor (Becker et al., 2007). Experimental data were recorded in the experimental log and also stored in computer.

RESULTS AND DISCUSSION

The data on sample analysis and on amount of biogas and methane produced was estimated for all 16 bioreactors, and average results were calculated. The results of raw material analyses before anaerobic digestion are shown in Table 1.

Table 1. Results of analysis of raw materials

Bioreactor	Biomass	Weight, g	pH	TS, %	TS, g	ASH, %	DOM, %	DOM, g
R1; R16	IN	500 ± 0.2	7.49	4.027	20.135	25.17	74.83	15.067
R2–R5	SP	10 ± 0.001		90.38	9.038	5.79	94.21	8.515
R2–R5	SP+IN	510 ± 0.2	7.5	5.72	29.173	19.16	80.84	23.582
R6–R9	IN+SP+AA	510.5 ± 0.2	7.48	5.72	29.178	19.17	80.83	23.586
R10–R12	IN+SP+XA	510.5 ± 0.2	7.47	5.72	29.177	19.16	80.84	23.586
R13–R15	IN+SP+MF1	511 ± 0.2	7.52	5.71	29.183	19.15	80.85	23.593

Abbreviations: TS – total solids; ASH – ashes; DOM – dry organic matter; IN – inoculums; SP – straw pellets; AA – alpha amylase; XA – xylanase; MF1 – Metaferm + Melafen (1:1).

Weight of raw material in Table 1 is provided with error value depending on accuracy of respective weight measuring instrument used. Weight of total solids (TS) and dry organic matter (DOM) in Table 1 is provided with accuracy ± 0.001 g. Both inoculum substrates in control bioreactors (R1, R16) have low dry matter content as almost finished digestate were used for inoculums. As it can be seen from the raw material (Table 1) straw pellets biomass has a relatively high dry matter and organic dry matter content. This is explained due to the fact that the straw are dry and pelletized. This raw material, containing a lot of organic dry matter, is well suited for biogas production. Biogas and methane yields from straw pellets and straw pellets with added enzymes are shown in Table 2.

Table 2. Biogas and methane yields from straw pellets and straw pellets with added enzymes

Reactor	Raw material	Biogas, L	Biogas, L g^{-1}_{DOM}	Methane, aver. %	Methane L	Methane, L g^{-1}_{DOM}
R1	IN500	0.4	0.026		0.029	0.002
R16	IN500	0.2	0.013		0.0008	0.0001
R1-R16	**Aver.**	0.3	0.020		0.015	0.001
R2	IN500+SP10	5.4	0.634	44.79	2.415	0.284
R3	IN500+SP10	5.3	0.622	40.22	2.132	0.250
R4	IN500+SP10	5.7	0.669	48.88	2.781	0.327
R5	IN500+SP10	5.9	0.693	49.64	2.929	0.344
Aver. R2–R5		**5.575**	**0.655**	**45.88**	**2.564**	**0.301**
± st. dev.		**± 0.29**	**± 0.033**	**± 4.39**	**± 0.360**	**± 0.042**
R6	IN500+SP10+AA	5.1	0.599	48.91	2.498	0.293
R7	IN500+SP10+AA	5.5	0.646	50.77	2.800	0.328
R8	IN500+SP10+AA	5.7	0.669	47.53	2.710	0.318
R9	IN500+SP10+AA	5.9	0.693	48.05	2.843	0.333
Aver. R6–R9		**5.55**	**0.652**	**48.82**	**2.713**	**0.318**
± st. dev.		**± 0.34**	**± 0.040**	**± 1.42**	**± 0.154**	**± 0.018**
R10	IN500+SP10+XA	6.0	0.705	48.09	2.884	0.339
R11	IN500+SP10+XA	5.7	0.669	53.96	3.071	0.361
R12	IN500+SP10+XA	5.9	0.693	49.06	2.897	0.340
Aver. R10–R12		**5.867**	**0.689**	**50.36**	**2.951**	**0.347**
± st. dev.		**± 0.15**	**± 0.018**	**± 3.15**	**± 0.104**	**± 0.012**
R13	IN500+SP10+MF1	5.6	0.658	32.37	1.819	0.213
R14	IN500+SP10+MF1	6.0	0.705	40.28	2.417	0.284
R15	IN500+SP10+MF1	4.7	0.552	47.82	2.247	0.264
Aver. R12–R15		**5.433**	**0.638**	**40.16**	**2.161**	**0.254**
± st. dev.		**± 0.67**	**± 0.078**	**± 7.73**	**± 0.308**	**± 0.037**

Note: Biogas and methane values for bioreactors 2–15 with fresh source biomass are provided with already subtracted average biogas and methane values obtained from reactors 1 and 16.

Abbreviation: L g^{-1}_{DOM} – litres per 1 g dry organic matter added (added fresh organic matter into inoculum); MF1 – mixture Metaferm: Melafen 1:1.

Specific biogas and methane gases volumes obtained from bioreactors R2 – R15 are presented in Fig. 1.

The figure shows that the least methane was obtained from the R3 and R13 bioreactors. Although all bioreactors are filled with inoculum from a single bucket and thoroughly mixed, never before will all bioreactors have the same number of different bacteria. This explains the fact that different yields of methane are extracted from bioreactors. Using alpha amylase enzyme, 0.318 L g^{-1}_{DOM} methane was obtained from straw pellets. Using biocatalysts mixture MF1, 0.254 L g^{-1}_{DOM} methane was obtained from straw pellets. More than 9.12% than from alpha amylase, methane was derived from the use of the xylanase enzyme. Surprise was the deterioration of results with the use of the MF + ME mixture of biocatalysts because of positive results with other biomasses were achieved (Dubrovskis & Plume, 2016). Methane was produced less from the first days of the study, and later on its content in these bioreactors was lower. Further studies are needed to explain why methane-forming bacteria have multiplied less.

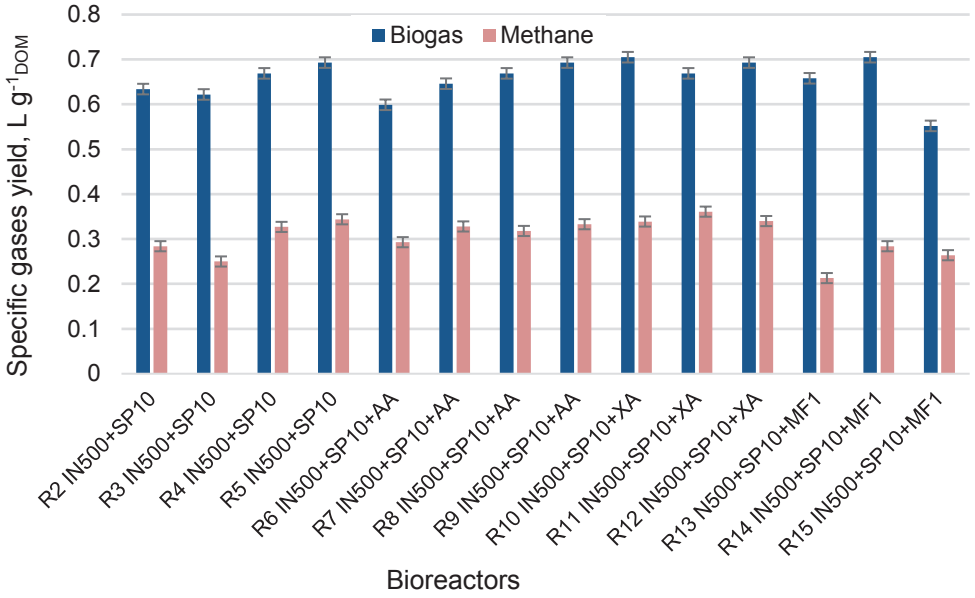

Figure 2. Specific biogas and methane gases volumes.

The relatively low average methane content in biogas can be explained by the fact that the amount of biogas was increased by the warm air and water vapour, which was released at the beginning of the process more than usual due to the use of cold inoculum. The second reason is that there is a lot of lignin and cellulose in the straw, so more CO_2 is formed. Addition of enzymes methane content increased, but MF1 decreased. MF1 contributed more to the release of CO_2.

CONCLUSIONS

The average specific methane yield from wheat straw pellets biomass was 0.301 L g^{-1}_{DOM}. The result is good, similar that obtainable from maize silage. The average specific methane yield from straw pellets is better than from manure, but expectations in improving of high methane production were not met. The addition of alpha amylase increased the specific methane yield 5.65%.

The addition of xylanase increased the specific methane yield 15.28%. It is more advantageous to use this enzyme.

The addition of Metaferm + Melafen decreased the specific methane yield 15.57%. Using these biocatalysts (mixture 1:1) for wheat straw biomass cannot be economically. Such level of methane yield in Latvian conditions do not justify the application costs of Metaferm.

The results of the study show that wheat straw pellets can be used as raw materials for the production of methane. Addition of both enzymes improved methane yield.

In future studies, it would be desirable to clarify the effect of different pre-treatment (treatment with acids, bases, and grinding degree) methods on the anaerobic fermentation of investigated biomass.

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