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Effect of Enzymatic Substrate Preparation on the Biogas Yield of Energy Crops

Enzyme additives are used in some agricultural biogas plants to increase the methane yield of the biomass applied by improved degradation of the fibre (cellulose, hemicellulose). For assessing the enzyme effect on anaerobic digestion of the biomass, comprehensive fermentation tests were carried out with the Hohenheim biogas yield test (HBT). The batch experiments carried out hitherto could not prove a significantly positive effect on methane yield of the tested substrates.

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Keywords

Biogas, enzymes, methane yield, fibres

Literature

Literature references can be called up under LT 07612 via internet http://www.landwirtschaftsverlag.com/landtec/local/literatur.htm.

Modified federal regulations (Renewable Energy Act) being enforced since the year 2004 lead to an increase of the number of on-farm biogas plants operating the co-digestion of manure together with energy crops in Germany.

A critical issue related to the use of fibre-rich energy crops or crop fractions (e.g. maize straw) in biogas plants is that a substantial part of the substrate (consisting mainly of cellulose, hemi-cellulose and lignin) is being slowly or incompletely degraded by anaerobic bacteria. Fibrolytic enzymes are used in some on-farm biogas plants in order to reach higher biogas yields from the energy crops, i.e. in order to accelerate their anaerobic degradation.

Enzyme are special proteins, which are acting as biological catalysts in all living beings. Enzymes are classified into different categories according to the type of reaction they catalyse. In order to shorten the long sugar chains of hardly degradable fibres (cellulose and hemi-cellulose) into smaller molecules and into soluble sugars, hydrolases (here: glucosidases) are being used. Similarly to natural organic matter, these enzymes themselves undergo bacterial degradation inside of biogas plants. It yet remains unknown, how fast the hydrolases become inactivated under the proteolytic action of anaerobic microorganisms.

The objective of the study presented in this article was to investigate the effect of commercial cellulose and xylan-degrading enzyme preparations on the anaerobic degradation of substrates using a discontinuous (batch) digestion assay in laboratory digesters.

Material and methods

Maize crops from the cultivar Gavott were harvested on September 8th, 2006 at the beginning of milk-ripe maturity stage (cob content related to the fresh mass was 39 %) at an experimental field of the university of Hohenheim (seeding date, May 3rd, 2006). The harvested product was separated into two different fractions (corncob and maize straw). The substrates were finely hatched using a laboratory mixer (particle size: about 4 mm) and ultimately stored in a freezer until the start of the digestion trials.

For determination of the methane yield of the crop substrates, the Hohenheim Biogas yield Test (HBT) was used [1]. The operating conditions of the digestion assay comply with the standards of the norms VDI 4630 and DIN 38414, part 8 [2], [3].

About 400 mg volatile solids (VS) of the crop substrates, i.e. 900 mg of freshly chopped corncob or 1 800 g of freshly chopped maize straw were brought inside of 100 mL

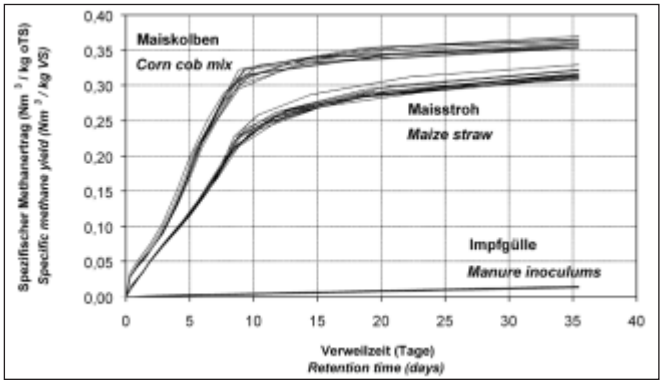

Fig. 1: Curves of cumulated methane production from all variants of maize cobs, maize straw and liquid manure inoculums; average values from three repetitions

glass syringes, together with 30 g of inoculating liquid. Inoculant cultivated at the State Institute for Farm Machinery and Farm Structures was used. The inoculums itself had a very low gas production, so that most of the gas generated during the digestion trials was generated by the substrate, not by the inoculant.

Four commercial enzyme preparations were tested (A, B, C, D). Enzyme preparations were added at the start of the batch digestion assay, with two different dosages: 0.1 g/kg VS and 1 g/kg VS. For each enzyme, an inactivated variant (inactivation of the catalytically active protein structure through heating at 95°C during 15 min) was included at the higher dosage of 1 g/kg VS.

The fermentation assay was operated at 37°C for a duration of 35 days. Over the whole trial period, the amount of gas produced as well as the methane yield were regularly measured. The methane yields of the inoculant liquid were subtracted from the methane yields of the samples containing corncob and maize straw, respectively.

Each variant was run with three replicates. Substrates were also tested without enzyme supplementation. Those "Zero variants" were used as reference for the calculation of the increments of the methane yields obtained through enzyme addition.

Description of the enzyme preparations

Two of the enzyme preparations (A and B) were developed by companies especially for the purpose of their utilisation in biogas plants. The other preparations (C and D) are used for bioethanol production or for technical applications in the food industry. Preparations A and C are enzyme mixtures composed of cellulases and xylanases originating from the yeast *Trichoderma reesei.* Preparation D is a β-glucosidase, which is produced by the yeast *Aspergillus niger*. Preparation B is a mixture of enzyme and microorganisms, which were manufactured without including any enzyme extraction step and therefore contains together with enzymes also cultivable microorganisms.

Substrate composition

The biochemical composition of the "corncob" and "maize straw" fractions were analysed according to the Weende and Van Soest analysis [4]. The contents related to the volatile solids were for maize straw: NfC 27 %, ADF 37 %, NDF 64 %, ADL 4 %, crude protein (CP) 7 %, crude lipids (CL) 2 %. The following values were determined for corncobs: NfC 63 %, ADF 9 %, NDF 24 %, ADL 1 %, CP 9 %, CL 4 %.

Zudosierung von Enzyme *Enzyme dosage*	**Zugesetzte Enzyme** *Enzyme added*	**Spezifischer Methanertrag (Nm³ / kg oTS)** *Specific methane yield (Nm³ / kg VS)*	
		Maiskolben *corn cob mix*	**Maisstroh** *Maize straw*
ohne Enzyme *Without Enzyme*	keine *none*	0,354	0,314
1 g/kg TS inaktiviert *inactivated*	A (Cellulase ; Xylanase)	n. E. / nd.	0,322
	B (Gemisch(1) - *Mixture*(1))	0,366	0,315
	C (Cellulase ; Xylanase)	0,362	0,322
	D (β-Glucosidase)	0,365 *	0,309
0,1 g / kg TS aktiv *active*	A (Cellulase ; Xylanase)	0,359	0,314
	B (Gemisch(1) - *Mixture*(1))	0,355	0,311
	C (Cellulase ; Xylanase)	0,357	0,318
	D (β-Glucosidase)	0,355	0,313
1 g / kg TS aktiv *active*	A (Cellulase ; Xylanase)	0,360	0,309
	B (Gemisch(1) - *Mixture*(1))	0,369 *	0,330 *
	C (Cellulase ; Xylanase)	0,354	0,318
	D (β-Glucosidase)	0,357	0,316

*Table 1: Specific methane yields of maize straw and maize cobs with and without enzymes added; final values after 35 days digestion at 37°C; average values from three repetitions; n.d. = not determined; (1) = enzymes + microorganisms mixture; * significant difference at a probability of error of 5 % (P<0.05) towards the variant without enzyme additive*

Results of the digestion trials

The pattern of cumulated methane generation of the different variants of corncob, maize straw and manure inoculums within the retention time of 35 days are represented in *Fig. 1*. None of the variant underwent any perturbation of the biogas production during the assay. The specific methane yield per unit of volatile solids after 35 days of digestion was reaching 0.314 Nm^3 / kg VS and 0.353 Nm^3 / kg VS for maize straw and corncob without enzyme addition, respectively.

The standard methane yields of maize straw and corncob after 35 days of digestion are shown in *Table 1*. There was no explicit increase of the biogas yield through enzyme addition relatively to the control variant.

The maximal increase of the methane yield was of 5.0 % for maize straw and of 4.6 % for corncob. Surprisingly, in some cases, the addition of inactivated enzymes had positive effect on the methane yield.

A student test (t-test) of the values of the methane yields revealed a significant ($p<0.01$), but no very significant ($p<0.001$) difference towards the enzyme-free variants.

The standard deviations of the methane yields were comprised between 0.2 and 3.6 % for corncob and maize straw, and between 0.4 and 8.5 % for the manure inoculums.

Discussion

Contrary to formerly published results from other research groups, the use of enzymes (hydrolases) brought in those trials only a very limited increase of the methane yield of 5 %, maximally. There were also no effects towards a faster gas production. A more consistent effect should be expected for maize straw than for corncob, what the results did not confirm.

Different hypotheses could explain the low efficiency of enzyme additives:

- the conditions of the medium (temperature and pH) are not optimal for the enzymes used, which are of yeast origin [5],
- the enzyme additives tested do not possess the activity range required in order to hydrolyse the fibres of the selected substrates into fermentable sugars [6],
- the enzymes added are degraded by prote-ases from living anaerobic microorganisms [7],
- the enzymes added are inhibited by high lignin contents in the medium [8].

An other explanation for the limited efficiency of enzyme additives could be that the digestion assay brings nearly optimal operating conditions for the anaerobic degradation of substrates. Therefore, the enzymes added are not able to significantly increase the methane yields. In practice, on-farm biogas plants, which are run in a suboptimal mode (e.g. high organic loading rate), the enzyme addition could relieve the process biology and bring an increase of the methane yield. This statement should be confirmed with by systematic research trials.