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Effect of enzyme addition on the methane yields of effluents from a full-scale biogas plant

Biogas plants fed with a high share of fibre-rich energy crops (e.g. ensiled grass) often resort to enzyme additives in order to increase substrate degradation as well as the resulting methane yield. In order to evaluate the effect of enzyme additives on the digestion process, effluents were collected from the first and second reactor of a full-scale on-farm biogas plant. The sampled effluents were digested again in batch anaerobic digestion assays at the biogas laboratory of the University of Hohenheim. Enzyme addition even at high dosage could not yield any significant increase of the methane yields of effluents.

Keywords
Biogas, enzymes, methane yield, fibres, lignocellulose

Abstract
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Since the reinforcement of the German Renewable Energy Act in year 2004, most on-farm biogas plants operate with a co-digestion of manure together with energy crops in the country. Fibrous fractions of plant products, mainly cellulose and hemicellulose, are degraded only slowly or partially by anaerobic bacteria. It is common practice to feed fibrolytic enzymes into the reactors of on-farm biogas plants in order to accelerate substrate degradation and thereby reach higher methane yields.

Batch anaerobic digestion trials were performed in laboratory reactors in order to evaluate the effect of cellulose and hemicellulose-degrading enzyme products on the methane yields of effluent from a full-scale on-farm biogas plant.

Characteristics of the full-scale biogas plant

The on-farm biogas plant was operated as a two-stage process and consisted in two completely stirred reactors (first reactor and second reactor). The substrate fed into the biogas plant had on average the following composition: dairy manure 33 %, cow dung 16 %, ensiled maize 27 %, ensiled grass 22 %, and other whole crop silages 3 %. The key parameters of both digesters are described in **table 1**.

Characteristics of the effluents

Effluents were sampled from the first and second digester of the biogas plant, respectively. The biochemical composition of the effluents was determined according to Van Soest analysis [1]. The following contents were measured, as related to the volatile solids (VS), for the sample from the first digester: NfE (nitrogen-free extract) 2 %, RP (raw protein) 21 %, RL (raw lipids) 3 %, oNDF (cellulose + hemicellulose + lignin, organic fraction) 73 %, oADF (cellulose + lignin, organic fraction) 62 %, ADL (lignin) 27 %. The sample from the second digester had following composition: RP 24 %, RL 4 %, oNDF 72 %, oADF 66 %, ADL 33 %.

Determination of the methane yields

Batch anaerobic digestion trials were performed using the Hohenheim Biogas Test (HBT) [2]. The fermentation took place at 37 °C for a duration of 87 days. The effluents were homogenized with a kitchen mixer prior to entering the assay. At the beginning of the experiment, reactors were fed with each 50 g effluent sample. Each variant was run in three replicates.

Characteristics of enzyme products

Two enzyme additives were tested, each in two dosages (i.e. normal and 10-fold dosage). The additives were three commercial enzyme products of fungal origin (A, B and C). Product A was a mixture of cellulase and xylanase from *Trichoderma reesei*. Product B was a cellulase from *Humicola sp*. Product C was a xylanase from *Bacillus sp.* Product A had often been used in biogas plants, but had highest enzyme activity at low pH values. Products B and C had a higher enzyme activity at neutral till alkaline pH values. Product A and a 1/1 (v/v) mixture of products B and C were used in the trials. Hence product A as well as the mixed product B+C were expected to display both cellulase and

Table 1

Effluent characteristics and process parameters of the digesters

Eigenschaften und Bedingungen/ *Characteristics and parameters*	Hauptfermenter/ *First reactor*	Nachgärer/ *Second reactor*
TS-Gehalt [%]/ *Total solids content [%]*	10.36	8.51
oTS-Gehalt [% TS]/ *Volatile solids content [%]*	77.70	72.90
pH-Wert/ *pH value*	7.8	8.2
Gärtemperatur [°C]/ *Digestion temperature [°C]*	41	39
Verweilzeit [d]/ *Hydraulic retention time [d]*	35	35
Raumbelastung [kg oTS/(m³ × d)]/ *Organic Loading Rate (OLR) [kg VS/(m³ × d)]*	6.5	3.3[1)]
Faulraumvolumen [m³]/ *Digester volume [m³]*	1250	1250

[1)] Gesamtraumbelastung der Biogasanlage (Hauptfermenter + Nachgärer)
Overall OLR of the biogas plant (first reactor + second reactor).

xylanase activities. Control variants containing inactivated enzyme (treatment: 121 °C, 30 min) were applied to each enzyme additive and each dosage.

Enzymes were added repeatedly on a regular basis during the digestion process, in order to prevent the decrease of enzyme activities due to degradation or inactivation. For this purpose enzyme solutions were prepared by diluting 20 and 200µL enzyme product or product mixture into 20 mL distilled water, for the normal and 10-fold dosage, respectively. From these enzyme solutions 0.5 mL aliquots were taken with a pipette and added into the reactors on 3-day intervals after the start of the experiment. Thus, enzyme addition occurred 20 times within a period ranging from day 1 till day 57. The same protocol was applied to enzyme-free variants, by replacing the solutions containing diluted enzyme products with distilled water.

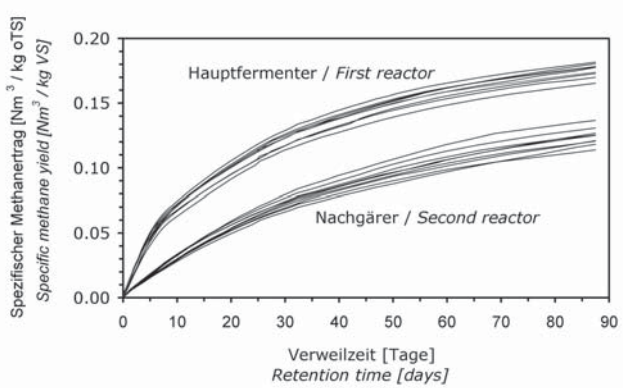
Over the whole digestion period, a total amount of 10 mL diluted enzyme or distilled water was fed into each digester. The cumulated amount of enzyme over the whole duration of the experiment was 0.2 and 2 g per kg fresh weight of the effluent for normal and 10-fold dosage, respectively. These amounts correspond to 1.9 and 19 g/kg TS for the effluent of the first reactor or 2.3 and 23 g/kg TS for the effluent of the second reactor, respectively.

Results of anaerobic digestion trials

Figure 1 represents the curves of cumulated methane yields of all variants with and without enzyme addition. Though to the end of the experiment methane was produced at a lower rate, it appears that methane production was not completely terminated after 87 days of anaerobic digestion. The increasing trend of the curve shows that the maximal methane yield had yet not been reached by this time.

The final methane yields of enzyme-free variants after the 87-day digestion period at 37 °C were 0.170 Nm³/kg VS for the effluent from the first reactor and 0.118 Nm³/kg VS for the effluent from the second reactor, respectively. Standard deviations of the final methane yields ranged between 0.6 and 8.2 %. **Table 2** shows the effect of enzyme addition on the final methane yields of all variants as compared to enzyme-free variants. A Student-test (t-test) of the final methane yields could not reveal any significant difference ($P < 0.05$) of enzyme addition as compared to inactivated enzyme addition. A significant increase of the final methane yields ($P < 0.05$) relatively to the enzyme-free variant was only noticed for the effluent sample from the second reactor at a high dosage of the enzyme mixture B+C. The possibility that trace elements contained within the enzyme products may have a positive effect on digestion performance can not be excluded.

Fig. 1



Curves of the cumulated methane production of all variants with and without enzyme addition; average values out of three repetitions

Table 2

Percent change of the final methane yields of reactor samples through enzyme addition as compared to samples without enzyme addition

Fermenter/ ***Reactor***	**Enzymzustand/** ***Enzyme state***	**Zugabe von Präparat A/** ***Addition of product A***		**Zugabe der Mischung B+C/** ***Addition of the mixture B+C***	
		Geringe Dosierung 0,2 g/kg FM/ ***Low dosage 0.2 g/kg DM***	**Hohe Dosierung 2 g/kg FM/** ***High dosage 2 g/kg DM***	**Geringe Dosierung 0,2 g/kg FM/** ***Low dosage 0.2 g/kg DM***	**Hohe Dosierung 2 g/kg FM/** ***High dosage 2 g/kg DM***
Hauptfermenter/ *First reactor*	aktiv/ *active*	+1.8	-2.7	+5.3	+6.2
	inaktiviert/ *inactivated*	+4.6	+6.9	+4.7	+4.9
Nachgärer/ *Second reactor*	aktiv/ *active*	+2.9	+7.3	+2.1	+15.7[1)]
	inaktiviert/ *inactivated*	-3.6	+6.0	+2.4	+10.7

[1)] Signifikanter Unterschied (P<0,05) zur enzymfreien Variante, jedoch nicht zu der Variante mit inaktivierten Enzymen
Significant difference (P<0.05) from the variant without enzyme addition but not from the variant with inactivated enzyme.

Conclusions

Enzyme addition performed on effluent samples in anaerobic digestion trials involving one-step fermentation at pH values around 8 could neither induce a significant increase of the final methane yields nor a clear effect on the methane production rate.

Earlier works of the University of Hohenheim were showing only limited effects of fibrolytic enzyme additives on the digestion of finely chopped maize [3] and rye silage [4] in the HBT-process.

One can hypothesize that the process biology of the sampled biogas reactors was not appropriate for enzyme addition. The biogas plant investigated was chosen according to the relatively high organic loading rate and short retention time of the digesters. However, other parameters may play a key role to guarantee the success of enzyme additives. According to the literature, most enzymes extracted from fungi are efficient at low pH values [5]. Enzyme efficiency can also be negatively affected by high lignin contents [6].

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