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Effect of Adding Fibrolitic Enzymes to the Methane Yields of Rye Silage

Enzyme additives are used in practice to increase the methane yield of fibre-rich energy crops by improving the conversion of hardly degradable fractions. Batch digestion trials were conducted in the biogas laboratory of Hohenheim University in order to evaluate the influence of fibrolytic enzymes on the anaerobic degradation of rye silage. The trials did not reveal any significant increase in substrate methane yields through enzyme application in practical concentrations.

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Keywords

Biogas, enzymes, methane yield, fibres, lignocellulose

Literature

References can be called up under LT 08320 via Internet www.landtechnik-net.de/literatur.htm

In Germany there is an increasing trend towards the co-digestion of manure together with other biomass sources in on-farm biogas plants. Energy crops which are commonly used as a feedstock contain hardly degradable fibres. Those fibres are formed mainly of cellulose, hemicellulose and lignin, which are slowly or partially degraded by anaerobic bacteria. Some biogas plants rely on fibrolytic enzyme additives to increase the methane yields of energy crops by accelerating the anaerobic degradation of fibres.

The objective of the trials was to ascertain the effect of commercial cellulose, xylan and lignin-degrading enzymes on the methane yields obtained from anaerobic digestion of rye silage through batch assays in laboratory digesters.

Material and Methods

Rye (cultivar Vitalis) was harvested on 28. 6. 2006 in Dolgelin. The crops were chopped to a fibre length of 8 mm, ensiled in glass jars at IASP institute and stored at room temperature for 450 days.

Batch digestion trials for determination of the methane yield potential of rye silage were carried out according to VDI regulation 4630 and DIN 38414, part 8 [1, 2]. Each variant was run with three replicates. The digestion took place at 37°C with a residence time of 35 days.

The batch process of the Hohenheim Biogas yield Test (HBT) [3] was applied. Before

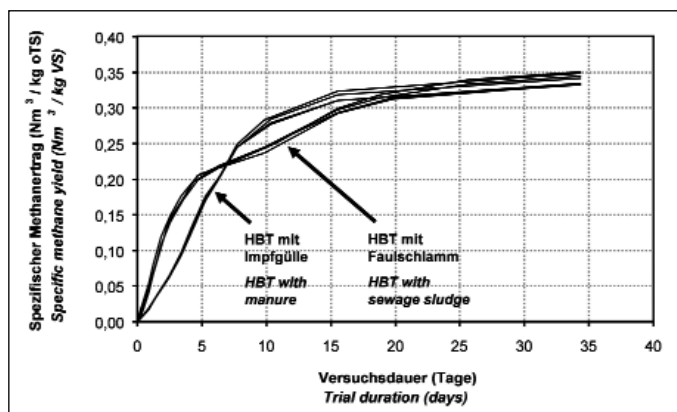
application in the HBT process, rye silage was chopped down to a particle size of 2 mm so as to provide representative samples of the substrate. At the beginning of the trial, the digesters were filled with 1 g freshly chopped rye silage and 30 g liquid inoculums.

In order to assess the influence of the inoculums source on the gas production, sewage sludge inoculums was used as an alternative to the standard inoculums of Hohenheim University ("manure inoculums"). Sewage sludge (municipal sewage plant of Wansdorf) was provided by the IASP and digested during nine days at room temperature in order to reduce its own biogas production. The standard inoculating mixture of Hohenheim University was cultivated in a digester of the biogas laboratory especially for the task of serving as inoculums to batch assays. Daily feeding of the specific inoculums digester with a mixture of dairy manure, maize silage, cereals, rapeseed oil and soybean extract (C:N ratio of the mixture 27:1) with an organic loading rate of 0.5 kg VS/m³·day ensure a sufficiently active and broadly adapted bacterial population concurrently to low biogas production from the inoculums feedstock.

Enzyme additives

The trials involved the application of three commercial enzyme products extracted from yeast (C, P and L) on rye silage (RS). The product C was a cellulase from *Trichoderma reesei*, the product P was a pectinase from *As-*

Fig. 1: Curves of cumulated methane production of all rye silage variants with and without enzyme additives— average values from three repetitions



Beschreibung der Variante Sample description	Spezifische Methanerträge (Nm ³ / kg oTS) Specific methane yields (Nm ³ / kg VS)		Standardabweichung der Wiederholungen (%) Standard deviation of the replicates (%)	
	HBT mit Impfgülle HBT with manure inoculums	HBT mit Impfschlamm HBT with sewage sludge inoculums	HBT mit Impfgülle HBT with manure inoculums	HBT mit Impfschlamm HBT with sewage sludge inoculums
Impfgülle / Inoculums	0,020	0,087	0,4	1,8
RS Kontrolle / control	0,341	0,332	0,6	1,2
RS + P	0,333	0,344	2,4	3,4
RS + P + L	0,334	0,339	3,1	0,6
RS + C + P + L	0,335	0,334	2,1	2,4
RS + C + P + L 100-fache Dosierung 100-fold concentration	0,349	0,350	2,0	3,1

Table 1: Specific methane yields of rye silage; final values after 35 days of digestion at 37°C; average values of three repetitions; RS = rye silage, C = cellulase, P = pectinase, L = laccase

pergillus niger and *Trichoderma longibrachiatum*. The product L was a laccase from *Trametes sp.*, which were aimed at degrading lignin bounds.

The enzyme products were used in different combinations: pectinase (P), pectinase and laccase (P + L), cellulase together with pectinase and laccase (C + P + L). Each product was applied with a dose of 0.07 g/kg related to the fresh mass of rye silage (i.e. 0.16 g/kg dry mass). This corresponds with the recommended dosage of the enzyme suppliers. Additionally, one variant was run with 100-fold enzyme concentration (i.e. 7 g/kg dry mass). Enzyme addition were performed in a singular step at the start of the batch assay before closing the digesters. For that purpose 1 mL of diluted enzyme product was added to the mixture of rye silage and inoculums.

Substrate composition

The biochemical composition of rye silage was determined after Weende and Van Soest analysis. The contents related to the dry mass were: NFE 44.14 %, crude protein (CP) 9.29 %, crude lipids (CL) 2.36 %, NDF (cellulose + hemicellulose + lignin) 61.37 %, ADF 37.59 % (cellulose + lignin), ADL 5.63 % (lignin).

The volatile contents of silage were determined according to HPLC analysis [5]. The dry mass (DM) of rye silage contained 3.9 % of lactic acid and 0.8 % of acetic acid.

The loss of volatile compounds during the determination of the volatile solids (VS) from silage could lead to an important deviation of the VS reference value [6]. To handle that problem, the formula of Weißbach and Kuhla [7] was employed in the trials for correcting the VS values. Because

of the very dry state of the silage (DM content of 43 %), as well as the low content in volatile substances, the proximate formula gave a VS loss during the drying of only 2.2 %.

Results of the digestion trials

The curves of the cumulated methane yields of all variants of rye silage in the HBT process with the two different inoculums are shown in Figure 1 (Average of the repetitions). The methane production curves of HBT process show different patterns according to inoculums type. After about 20 days, differences in the digestion's behaviour between both inoculums tend to disappear.

Table 1 shows cumulated values of the normalised methane yields of rye silage after a retention time of 35 days. A student test (t-test) of the methane yields did not prove any significant difference (at $p < 0.01$) towards enzyme-free variants. Standard deviations of the methane yields of the replicates of rye silage in the HBT process were comprised between 0.6 and 3.4 %.

Discussion

Neither any significant increase, nor any positive effect on the gas production velocity was measured after enzyme addition in the trials. Those results confirm the statements of former research conducted at Hohenheim University revealing that fibrolytic enzymes had only a limited effect on the anaerobic digestion of finely chopped maize in the HBT process [8].

The selection of inoculums source as well seemed to have only a limited impact on the results of the trials. Although manure inoculums had a higher pH value (8.3 instead of

7.8) as well as a higher lignin content (about 15 % instead of about 5 % of DM) compared to sewage sludge inoculums, only gas production velocity was influenced by inoculums nature, not the final values of the methane yields. According to the literature, higher pH-values [9] and increased lignin contents [10] could have a negative effect on enzyme efficiency. This affirmation could not be confirmed in the present trials.

Hence one can hypothesize that the inoculums used in the present trials had an already sufficient enzyme activity at the start of the trials for the degradation of added substrate. On this account, enzyme addition at the beginning of the trials could not bring any increase in the methane yields. However, the hardly degradable fraction of the substrate should be degraded to the end of the digestion course, whereas microorganisms would prefer to deal with easily degradable substances first (acids, starch, proteins, lipids). Enzymes as biologically degradable proteins have only a limited lifetime in the digester [11]. Therefore, a late application of enzyme products (during the digestion process) could act more efficiently. This hypothesis is currently checked in ongoing further research trials.

Contrary to the present results, in former publications of the IASP similar digestion trials run with the eudiometer process showed completely different results. With the same substrate and under similar digestion conditions, increases in methane yields of 1.0 to 12.2 % were attained [12]. According to the present data no sufficient explanation for this difference in the results could be found.

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