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Enzyme-containing faeces of herbivores increases biogas yield of energy crops

In order to increase the biogas yield of energy crops the degradation of fibrous constituents needs to be improved. In addition to mechanical and chemical procedures of treatment, more attention is given to research regarding enzymatic treatment. Therefore, efforts are concentrating on finding inexpensive sources for enzyme production. One source could be the digestive tract of herbivores which contains microorganisms and enzymes highly specialized in fibre degradation. The influence of such microorganisms and their enzymes on the degradation of maize silage has been demonstrated in anaerobic batch digestion tests using the example of rabbit faeces.

Keywords

Biogas, rabbit faeces, enzymes, methane yield, lignocellulose

Abstract

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In the course of the conversion towards renewable energy sources, agricultural land is increasingly exclusively used for the production of energy crops. In 2011, industry and energy crops were cultivated on more than 2.5 million hectares. Of these, 810,000 hectares of energy crops in the form of maize were cultivated for use in biogas plants [1]. This is equivalent to 7 % of the agricultural crop land in Germany.

Lignocellulose forms the cell wall of plants and serves as a structural frame. The two polysaccharides hemicellulose, but mostly cellulose, form a structure, into which, during the so called process of lignification, lignin is subsequently stored. Lignin is a highly branched and difficult biodegradable macromolecule, consisting of phenolic structural units. Mainly under anaerobic conditions in biogas plants, this lignocellulose-complex in plants is difficult to break down.

Recent research aims at an enhanced decomposition of these structures, in order to improve the microbial decomposition of polysaccharides, such as cellulose, and to simultaneously increase the biogas and methane yields. Enzymatic treatment procedures have increasingly become the focus of research. Here it is essential to find cost-saving sources for suitable enzymes. One possible source is the special digestive tract of herbivores, which consume plant substrates as the principle supplier of species-specific content of crude fibre. The microorganisms and enzymes needed for this process can be found in the alimentary tract and are specialized in the decomposition of crude fibre. Microorganisms and enzymes are deposited in the faeces. Faeces of herbivores are therefore a natural carrier of enzymes.

Rabbits belong to a special group of small herbivores. They produce two different kinds of faeces; hard faeces and soft faeces. The faeces from the appendix fermentation are eaten immediately. This behaviour, called coprophagy, results in a better protein and fibre recycling of the feed by the intake of microbial protein. The rabbit's appendix measures approx. 40 % of the entire alimentary tract. It is the principal location for bacterial digestion. Bacteria living in the appendix produce for example fibrolytic enzymes, which decompose the structure of the polysaccharides in cell walls contained in herbal feed [2]. The hydrolysis of lignocellulose into simple sugars is carried out by an enzyme complex, consisting of cellulase and enzymes, which attack the non-cellulose polysaccharides hemicellulose and pectin [3]. In principle, mammals cannot produce pectin-decomposing enzymes. However, so called appendix-fermenters, such as rabbit and horse, have an appendix populated by anaerobic bacteria, which produce pectin fermenting enzymes. Research showed a concentration of pectin fermenting bacteria in the appendix of rabbits of 108 CFU (colony-forming units) per gram fresh weight [4]. In the appendix of rabbits the highest hydrolytic overall-enzyme activity of the entire digestion system was measured, which is 2 to 6 times higher than, for instance, within the colon [5].

Using the example of hard and soft faeces of rabbits, the influence of small herbivores' faeces on the decomposition of maize silage was measured in non-continuous experiments. For the experiments, the following working hypotheses were valid:

- Adding the faeces of rabbits to substrates rich in crude fibre causes an increase in its fermentation. Hard and soft faeces are carriers of enzymes. These enzymes are specialized in the digestion of feed which is rich in crude fibre.

- Because of the higher enzyme activity in soft faeces compared to hard faeces [5], a better fermentation/biogas yield can be expected.

The following questions were the central point of the studies:

- Which hydrolytic activities regarding the enzymes pectinase, cellulase and xylanase can be found in both kinds of rabbit faeces?
- Is there a faece or enzyme dependent influence on the fermentation of herbal substrates rich in lignocellulose?

Material and Methods

The hard faeces were taken from a mixed sample of approx. 200 lactating female hares (1–2 years old) and young animals (10–12 weeks old) from a rabbit breeding farm in the state of Brandenburg. The soft faeces were taken from 60 appendixes of culled young animals (12–15 weeks old) on the day of the culling in a slaughterhouse in Brandenburg. Both kinds of faeces originate exclusively from rabbits of the hybrid breed "Zika".

The following methods have been used for the chemical characterization of both kinds of faeces as well as maize silage: The identification of the dry matter and the organic dry matter followed VDLUFA Vol. III, chapter 3.1, respectively VDLUFA Vol. III, chapter 8.1 The nitrogen and ammonium analyses followed the Kjeldahl method, VDLUFA Vol. III, chapter 4.1.1 and VDLUFA Vol. III, chapter 4.8.1 [6].

For the analysis of selected enzyme activities, specific hydrolytic enzymes with high activity, such as pectinase, cellulase and xylanase, were chosen from the alimentary tract of rabbits. At the same time, these enzymes represent the main players for a natural fermentation of the lignocellulose complex. In the framework of these studies, the enzyme activities have been analysed on the basis of the generation of glucose equivalents (reduction equivalents) and have been quoted in units per gram fresh weight of faeces. One unit (U) is by definition the enzyme concentration, which releases 1 µmol of reduction equivalents per minute.

The sample preparation followed Gidene [7]. The sample aliquots of the soft and hard faeces were spiked with citric acid-phosphate-buffer (McIlvaine-buffer; pH 7.5). These dissolutions were deep frozen and then defrosted again in order to gain a primary cell disruption. Subsequently, the solutions were homogenized by ultrasound and centrifuged. The supernatant was filtered and the raw extract deep frozen for analysis.

The measurement of the enzyme activity in the faeces was carried out on the basis of colour reaction of dinitrosalicylic acid with released reducing carbohydrates from model substrates [8]. The raw extract solutions have been incubated undiluted or 1:2 diluted with the following substrate solutions pectin (Fa. Herbstreith & Fox KG; 0.25% w/v), carboxymethylcellulose CMC (Fa. Sigma-Aldrich Chemie GmbH; 1, 2 and 3% w/v) and arabinoxylan (Fa. Megazyme International Ireland Ltd.; 0.25% w/v). The incubation temperature was 38 °C, and the pH-level was 7.5. The selection of parameters was oriented on the specific value of mesophilic, single-stage biogas plants. The incubation times were 20, 40 and 60 minutes. The reaction was stopped by adding dinitrosalicylic acid and the absorption rate of the solutions measured by a spectral photometer at 530 nm.

In eudiometer devices, discontinuous fermentation experiments were carried out in order to measure the methane yield potential accordant to the regulations in VDI-Richtlinie 4630 [9]. The test substrate in the fermentation experiments was maize silage from the agricultural cooperative at Trebbin. As inoculum digested sludge from the sewage plant at Wansdorf was used. The following variants were investigated:

- Methane yield potential of maize silage (1 variant)
- Methane yield potential of maize silage after adding hard or soft faeces in two concentrations: 10 g and 50 g faeces per kg fresh matter of maize silage (4 variants)
- Methane yield potential of maize silage after adding of autoclaved hard or soft faeces in the highest concentration: 50 g of sterile faeces per kg fresh matter of maize silage (2 variants). The autoclaving at 121 °C for 30 minutes was carried out in order to inactivate the enzymes in the faeces.

The faeces were added once before the start of the experiment. All fermentation variants were carried out in three replicates. The fermentation took place at 38 ± 1 °C with a retention time of 30 days.

Results and discussion

Table 1 gives an overview of the composition of hard and soft faeces by means of selected parameters. At 20.64%, the soft faeces show a significantly lower content of dry matter than the hard faeces (27.27%). The pH-value of the soft faeces was 6.5 and 1.2 units lower than with the hard faeces.

The results of the determination of the enzyme activity are shown in **Figure 1**. In both kinds of faeces a xylanolytic, pectinolytic and cellulolytic enzyme activity could be determined. The enzyme acitivity U g-1 fresh matter of faeces varied with regard to the tested enzymes as well as the types of faeces (**Figure 1**). In general, a slightly higher overall-enzyme activity was

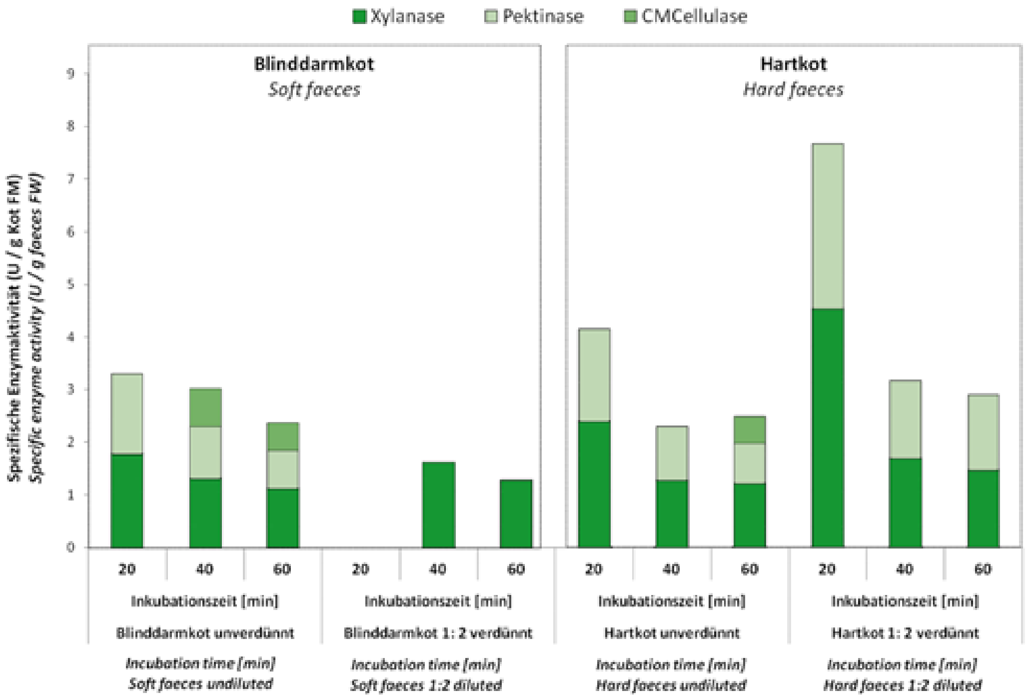
Table 1

Specific chemical parameters of hard and soft faeces

Substrat *Substrate*	TS/*DM* %	oTS/*VS* % TS/*DM*	pH	N_{ges}/N_t Kjeldahl % FM/*FW*	NH_4-N Kjeldahl % FM/*FW*
Hartkot *Hard faeces*	27.27	86.78	7.7	0.90	0.17
Blinddarmkot *Soft faeces*	20.64	83.50	6.5	1.04	0.14

TS = Trockensubstanz, FM = Frischmasse, oTS = organische Trockensubstanz, N_{ges} = Stickstoff gesamt, NH_4-N = Ammonium-Stickstoff
DM = Dry Matter, FW = Fresh weight, VS = Volatile solids , N_t = Total nitrogen, NH_4-N = Ammonium nitrogen

Fig. 1



Enzyme activities (U g-1 faeces FW) of xylanase, pectinase und CMCellulase in undiluted and 1 : 2 diluted hard and soft faeces according to different incubation times

found in the undiluted hard faeces of the 1–2 year old female hares, compared with the undiluted soft faeces of the 12–15 week old young animals. This can be explained by the increase in enzyme activity in the alimentary system with increasing age of the rabbits [5; 7].

The highest enzyme activities were measured for pectinase and xylanase in the undiluted and the diluted hard faeces after a 20 minutes incubation time. A longer incubation time led to a decrease of the specific enzyme activity in both kinds of faeces. In the case of hard faeces, a dilution caused higher specific enzyme activity regarding xylanase and pectinase. There was no pectinase activity in the diluted variant of the soft faeces. The CMCellulase activity in both kinds of faeces was low, partly preventing the measured data from being fully interpreted. Interpretable results for both kinds of faeces were only found in the undiluted variants, when the CMC solutions were more highly concentrated (2–3 %), and when the incubation times were between 40 and 60 minutes. It becomes apparent, that the ratio of enzyme to substrate plays a major role. Further investigation of the relationship between enzyme and substrate is necessary not only regarding the confirmation of activity but also the optimal ratio of faeces to biogas substrate.

Table 2 shows the specific methane yield potentials of maize silage with and without addition of hard or soft faeces. In the case of the maize silage to which different kinds of faeces were added, the innate gas production of the faeces was measured separately and taken off the full amount of methane yield. The Dunnet-T3-test did not show a significant difference between any of the treated faeces variants ($p < 0.05$) compared with the untreated variant. However, the input of faeces led by trend to a higher methane yield. Especially the hard faeces generated a 10 % increase in the methane yield of maize silage, when 50 g of faeces per kg silage were added. By the use of commercial, mostly highly concentrated enzyme supplements for biogas plants, the increase of methane yield can be between 4 and 35 % [10]. Research shows, that enzyme bearing recycling material, such as by-products from edible mushrooms, can increase the methane yield by 3 up to 24 % [11]. In these experiments the same concentration of enzyme-bearing recycling material was used, but instead of maize silage a processed substrate of straw and hay was used, which was richer in lignocellulose. Therefore, the currently demonstrated increase in yields are in the same range of those enzyme supplements, except for the variant with 10 g soft faeces per kg fresh matter of maize silage. Surprisingly, even by the addition of sterile faeces an increase in methane yield by 7.4 % (soft faeces) and 8 % (hard faeces) was found. It is possible, that, besides a non-sufficient inactivation, the autoclaving has caused a thermic disintegration of the fibre components as well as an improved microbial availability in the faeces [12]. Recent research with liquid cattle manure shows an up to 21 % higher methane yield potential [13]. Future research should therefore include the sterile faeces variants in the identification of methane potential.

Faeces differ in enzyme concentration regarding commercial substances. Products with a high enzyme concentration are presently used in biogas plants, following the recommendation

Table 2

Specific methane yields (NL kg⁻¹ VS) of maize silage with and without addition of faeces, final values after 30 days of digestion at 38 °C, average of three repetitions

	Einheit *Unit*	Maissilage unbehandelt *Maize silage untreated*	Maissilage mit *Maize silage with*					
			BK 10 *SF 10*	BK 50 *SF 50*	BK 50 Steril *SF 50 sterile*	HK 10 *HF 10*	HK 50 *HF 50*	HK 50 Steril *HF 50 sterile*
Norm-Methanertragspotenzial *Specific methane yield*	NL kg^{-1} oTS *NL kg^{-1} VS*	300	305	317	325	318	334	328
Standardabweichung der Wiederholungen *Standard deviation of the replicates*	%	3.0	13.1	0.9	7.7	3.5	2.4	9.5
Norm-Methanertragspotenzial abzüglich Eigengasproduktion des Kots *Specific methane yield minus internal methane yield of faeces*	NL kg^{-1} oTS *NL kg^{-1} VS*		304	314	323	317	330	324
Methanmehrertrag durch Kotzugabe im Vergleich zur unbehandelten Maissilage *Additional methane yield by adding of faeces to maize silage in comparison to untreated maize silage*	%		1.2	4.6	7.4	5.5	10.0	8.0

BK = Blinddarmkot, HK = Hartkot, 10 = 10 g Kot je kg Maissilage Frischmasse, 50 = 50 g Kot je kg Maissilage Frischmasse
SF = Soft faeces, HF = Hard faeces, 10 = 10 g faeces kg^{-1} maize silage fresh weight, 50 = 50 g faeces kg^{-1} maize silage fresh weight

of the producers to use 0.2 kg product t^{-1} organic dry matter (ODM). For the use of hard faeces as enzyme substance, e.g. related to the variant of 50 g faeces kg^{-1} fresh matter, converted 200 kg t^{-1} ODM respectively 50 kg per ton of fresh matter would be needed. Alternatively, enzymes could be extracted or concentrated from faeces. At present this has the advantage, that faeces are handled as recycling material. The average price of commercial substances for biogas plants is around 30 Euros per kg. These substances could be replaced by untreated faeces, which would be more cost-saving, assuming these were available near the site of the biogas plant.

Conclusions

The faeces of rabbits can be seen as a natural carrier of enzymes, which are suited for the fermentation of substrates rich in lignocellulose. The experiments have shown that the addition of soft and hard faeces of rabbits to maize silage can increase the methane yield from 4.6 to 10%. The surplus in yield during the experiments is in accordance with commercial enzyme substances. Facing the required amount of faeces either a cost-saving procedural reduction of the amount or better an increase of the enzyme concentration is needed. The results represent an encouraging approach to the topic. Further research with the faeces of rabbits is recommended. This includes discontinuous as well as continuous fermentation experiments and the processing of the available faeces. Further research should focus on the use of faeces as additive during the hydrolytic phase at two-tier plants. The present pH-levels at those plants may result in a higher enzyme effect. For this research approach the screening of faeces of other herbivores with a higher efficiency in fibre digestion appears to show promise.

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