Improving the Efficiency of Large-Scale Biogas Processes: Pectinolytic Enzymes Accelerate the Lignocellulose Degradation

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Abstract: An enzyme preparation containing mainly pectinolytic activity was used to increase the hydrolysis of agricultural feedstock for production of biogas at lab and industrial-scale bioreactors under mesophilic conditions. Bioreactor performance was evaluated by determining lignocellulose degradation efficiency and specific methane yields over a residence time of 65 days for various substrates. Additionally, enzymatic activity assays were carried out in laboratory trials to investigate the hydrolytic potential of the enzyme preparation. During industrial-scale trials the viscosity (shear stress from torsion) was measured in fermentation media. At an applied concentration of 200 g enzyme preparation/t DM of maize silage in lab-scale biogas processes, the degradation of hemicellulose was enhanced and the specific methane production was increased by 42 l_N/kg ODM (15%) in comparison to untreated maize silage. Using enzymatically treated rye silage, the hemicellulose was utilized to a higher degree and the specific methane production enhanced by 26 l_N/kg ODM (10%). Results from the industrial trial showed that enzymatic pretreatment (100 g enzyme/t DM of substrate) of different substrate mixtures containing mainly maize silage increased the lignocellulose degradation, in particular hemicellulose and a substantial increase in specific energy production was obtained. In addition, the viscosity in the fermentation media was significantly reduced up to 18%. In conclusion, the addition of an enzyme preparation as biological treatment led to an economical operating improvement in efficiency of the biogas process.

Keywords: Lignocellulose, enzyme, biogas process, industrial biogas production, biological pretreatment.

1. Introduction

Biomass of plant origin (i.e. maize silage) can be used as substrate for production of biogas in anaerobic processes for producing electricity and heat. One of the limiting factors is the biodegradability of lignocellulose contained in these materials. Lignocellulose is a matrix of biopolymers containing mainly cellulose, hemicellulose, lignin and pectin. Cellulose is a polymer composed of glucose and hemicelluloses consisting primarily of different sugars and sugar derivatives constituting a huge energy source [1]. A pretreatment of this biomass is necessary for making these biopolymers more available for microbial conversion. Therefore, chemical, physical and biological pretreatments are in use or under investigation for implementation at industrial-scale. Addition of enzyme preparations with specific enzymatic activities is used as biological pretreatment in biogas processes. Enzyme preparations of mildews with specific enzymatic activities, mostly with cellulase or/and xylanase as main activity, are currently available on the market for improving biogas processes [2-3].

In order to investigate the potential for increasing the efficiency of biogas processes by intensifying the lignocellulose degradation, different commercially available enzyme preparations from various origins, field of applications and enzymatic activities were tested in laboratory trials at the Institute of Agricultural and Urban Ecological Projects (IASP, Germany). This study was done within the framework of the Biogas Crops Network (BCN), a network for fundamental scientific research on biogas extraction from agricultural biomass. Based on enzymatic activity assays, different enzyme preparations were selected for its application, as single preparation or as blends, during the ensiling of maize and rye, in a pre-hydrolysis of rye silage or directly in biogas processes using maize silage or rye silage as feedstock. Effects of the enzyme preparations were evaluated by measuring the biogas production or rather the methane yield and calculating the degree of lignocellulose degradation. A pectinolytic enzyme preparation (B1) with hemicellulolytic and cellulolytic side activities was found to be effective in enhancing the methane yield of maize silage and rye silage by adding it directly in the fermenter. Further, pectinolytic preparations showed improved results when adding it to raw materials before ensiling. Following the lab-scale trials, an industrial-scale trial was carried out to test the effect of the selected enzyme B1. This might be the first report on the determination of a very wide spectrum of process control parameters and analyses, including lignocellulose conversion that has been recorded for a full year of operation of industrial-scale biogas plants. Main objective of this study was the evaluation of possible enhancement on energy production by accelerated lignocellulose degradation and consequently reduction in medium viscosity due to addition of the enzyme preparation.

2. Experimental

2.1 Materials

Commercially available enzyme preparation (enzyme B1) contained predominantly pectinolytic activity with cellulase and hemicellulase as main side activities. According to the manufacturer (DSM/Netherlands), it is generally used as liquid enzyme preparation for tropical fruit and stone fruit processing. The preparation contains secreted enzymes from *Aspergillus niger* and *Trichoderma longibrachiatum*. Optimal enzymatic activity could be measured at pH range of 4 to 5 and a temperature of 50°C. The protein content was 58.12 mg/ml.

Maize or rye were used as substrates for producing biogas in laboratory fermentation trials obtained from Rhinmilch GmbH

Agrargesellschaft (Fehrbellin/Germany) or Landwirtschaftsbetrieb Martin Schulze (Dolgelin/Germany). The raw materials (8 mm theoretical cut length) were ensilaged at room temperature in 1.5 l jars for 90 days. Afterwards the silages were used as substrate for fermentation with or without addition of the enzyme preparation. Digested sludges from the wastewater treatment plant in Wansdorf (Germany) were used as inocula for laboratory fermentation tests.

Substrate mixtures containing primary maize silage were used as feedstock for the industrial fermentation experiments at KTG Biogas AG (Germany). The mixtures used for each period within a total experimentation time of one year are described in Table 1. Each bioreactor with identical configuration and size (~2000 m^3) was fed with the same substrate mixture: the control (without enzyme) and test reactor (with enzyme). The reactors had similar substrate composition with a calculated difference of less than 1% and therefore the same substrate-specific methane formation potential.

2.2 Determination of chemical composition

The methods of analyses for selected ingredients are shown in Table 2. Volatile fatty acids (VFA) measured in silages were lactic acid, acetic acid, propionic acid, butyric acid, valeric acid and caproic acid. Additionally, formic acid, phenylacetic acid, phenylpropionic acid and benzoic acid were determined in fermentation media. Ethanol, methanol, acetone, n-propanol, 1.2-propandiol, 2-butanol and 1-butanol (hereinafter uniformly referred to as: alcohols) were analysed in the silages.

2.3 Enzymatic activity assays

Pure model substrates were used for representing different components of lignocellulose in enzymatic activity tests as follows: medium viscosity carboxymethyl cellulose (CMC, Sigma-Aldrich Chemie GmbH/Germany), filter paper No.1 with a size of 1 cm × 6 cm (FP, Whatman GmbH/Germany), low viscosity arabinogalactan from larch wood (AG, Sigma-Aldrich Chemie GmbH/Germany), medium viscosity arabinoxylan from wheat (AX, Megazyme International Ireland Ltd./Ireland), high viscosity glucomannan from konjac (GM, Megazyme International Ireland Ltd./Ireland) and pectin from apple with an esterification degree of 70% (P, Herbstreith & Fox KG/Germany). These substrates were applied as 1% solutions (w/v), except of glucomannan (0.5% concentration). The release of reducing sugars from the model substrates by the enzyme preparation was determined by the DNS-method [8-9]. For this assay, the reaction mixtures were incubated at pH 4.5 and 30°C or pH 7.5 and 38°C, simulating a hydrolysis or an ensiling process as well as a biogas process. The enzyme activity was expressed as units per gram and denoted the enzyme quantity which is needed for releasing 1 µmol reducing carbohydrates (glucose equivalents) per minute. Every test was repeated six times.

2.4 Metagenome analyses

Metagenome analyses were performed at the Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB, Germany) in cooperation with the Center for Biotechnology (CebiTec, Bielefeld University, Germany). The analyses included DNA extraction and 454-pyrosequencing on a Genome Sequencer FLX Titanium System (Roche), followed by taxonomic and functional data evaluation. The interpretation of DNA sequence data allowed the determination of the hydrolytic potential of a mesophilic, biogas-producing community in a laboratory fermentation trial, similar to the trial presented in chapter 3.3. Beside the identification of time-dependent changes within microbial communities during fermentation, information about possible enzyme activities in the biogas process could be provided.

Table 1. Substrate composition and time periods for the industrial fermentation test.

	Reference period 1	Period 1	Period 2	Period 3	Reference period 2
Beginning of the period	14.12.2009	15.02.2010	17.05.2010	18.07.2010	08.10.2010
End of the period	14.02.2010	16.05.2010	17.07.2010	07.10.2010	12.12.2010
Maize silage	87.95%	90.70%	73.75 %	73.35%	72.20%
Grass silage	4.80%	0	0	0	0
Corn	7.25%	9.30%	8.50%	6.40%	7.00%
Sweet sorghum silage	0	0	17.75%	4.15%	0
Whole plant silage (Rye)	0	0	0	16.10%	20.80%

Table 2. Methods for material characterization.

Parameter	Method	Source
Dry matter	VDLUFA Bd. III, 3.1 VDLUFA Bd. III, 3.4	[4]
Organic dry matter	VDLUFA Bd. III, 8.1	[4]
Crude protein	VDLUFA Bd. III, 4.1.1	[4]
Crude fat	VDLUFA Bd. III, 5.1.1	[4]
Crude fibre	VDLUFA Bd. III, 6.1.1	[4]
NDF (Cellulose, Hemicellulose, Lignin)	VDLUFA Bd. III, 6.5.1	[4]
ADF (Cellulose, Lignin)	VDLUFA Bd. III, 6.5.2	[4]
ADL (Lignin)	VDLUFA Bd. III, 6.5.3	[4]
Cellulose	Calculated: ADF - ADL	-
Hemicellulose	Calculated: NDF - ADF	-
Water soluble carbohydrates	Continous Flow Analyzer/ Lengerken, Zimmermann 1991	[5]
Starch	Test kit/R-Biopharm AG	-
Protein	Biuret test/§ 64 LFBG 06.00-23	[6]
Ammonium	VDLUFA Bd. III, 4.8.1	[4]
Volatile fatty acids, alcohols	Gaschromatography/Flame ionization detector	-
Ammonia	Ion-selective electrode	-
VOA/TAC value (VOA: volatile organic acids/ TAC: total anorganic carbon)	Titrator/PRONOVA	-
Lactic acid	High performance liquid chromatography/ Refractive index detector	-
pH	VDLUFA Bd. III, 18.1 DIN EN 12176	[7]

2.5 Biodegradability of lignocellulose

For the calculation of the lignocellulose degradation rates, the concentration of different ingredients was analysed in the substrates, inocula and fermentation residues. Decomposition of dry matter (DM), organic dry matter (ODM), cellulose, hemicellulose and lignin was calculated via mass balance.

2.6 Laboratory fermentation trials

Batch tests with or without an enzymatic pretreatment of the maize or rye silage were performed using eudiometers in accordance to VDI 4630 [10] and DIN 38414-8 [11]. Fermentation vessels with a volume of 500 ml were filled with 5-10 g silage (depending on substrate and inocula composition), in the case of enzymatic pretreatment 0.07 g enzyme preparation/kg silage was added and at least 300 g inoculum. The fermentation vessels with maize silage or rye silage contained inoculum 1 or inoculum 2. The ratio of organic dry matter from inoculum and substrate was adjusted to 0.4 as required by VDI 4630 [10]. A digestion time of sixty-five days under mesophilic operation at 38°C was chosen. Daily biogas production amount was measured using the eudiometer. The gas was collected in a bag for analyses via sensors with a Multitec 540 (Hermann Sewerin GmbH/ Germany) for gas composition: methane, carbon dioxide, oxygen and hydrogen sulphide. For a quantitative calculation of the specific biogas and methane yields, the biogas volume was normalized, the methane content corrected (the sum of methane and carbone dioxide account for 100%) and the produced gas volume by the inocula subtracted. Results on methane production are expressed as mean values with standard deviation and coefficient of variation (CV). Differences in methane yield were statistically analysed to investigate the significant level using a Dunnett-T-Test of the program SPSS Statistics 17.0.

2.7 Industrial fermentation trials

Following the lab-scale batch tests, an industrial-scale trial was carried out in commercial continuous bioreactors with a volume of 2000 m^3 in collaboration with the company KTG Biogas AG (Germany). Configuration of bioreactors includes electricity generators that utilize the biogas (methane and CO_2) as fuel producing electricity and heat. Two identical bioreactors were fed with the same substrates, achieving a loading rate of 5-5.8 kg ODM/m^3*d. One of them was used as control (R1, no enzyme added) whereas in the test reactor (R2) the enzyme preparation, enzyme B1, was added in a concentration of 100 ppm (DM). Main objective was the determination of the impact of enzyme preparation on degradation of different substrates and on biogas production efficiency under mesophilic real-life conditions. Total evaluation time was 1 year and it was divided into different periods (Table 1). Reference Period 1 was used as initial reference to obtain data of the biogas process performance of both reactors without enzyme addition over a residence time of approximately 63 days. It was started with maize silage as primary substrate. In the Treatment Period 1 (with enzyme addition), the impact on maize silage was tested. During the following Treatment Period 2 under enzyme usage, maize silage and sweet sorghum silage were fed as substrates. During the last Treatment Period 3, the sweet sorghum silage was substituted by whole crop silage of rye. Thereafter, Reference Period 2 was carried out using no enzyme preparation to determine the effect of enzyme washing out from the bioreactor. Every period was performed over one theoretical residence time (~63 days). System performance was determined based on collection of a) bioreactor process data: pH, temperature, concentration of selected VFA (in addition VOA/TAC value), gas yields and agitator power, b) determination of concentration of ingredients like cellulose, hemicellulose, lignin and c) viscosity determination. The viscosity was measured with a torsion viscometer (System: IKA P7, IKA Viscoclick, LabJack, LabView) and determined directly at culture broth samples at the site.

3. Results and Discussion

3.1 Sample composition

Laboratory trials

Table 3 shows the composition of the used silages and inocula in the lab trials. Rye silage showed higher content of dry matter, crude ash, crude protein, acetic acid, ammonia, carbohydrates and fibres compared to those of maize silage. In contrast, the concentration of crude fat and lactic acid was higher in maize silage. Regarding lignocellulose, rye silage contained twice as much cellulose and lignin (ADL in Table 3) than maize silage. The concentration of the main constituents of lignocellulose-cellulose, hemicellulose and lignin - was 61.37% DM for rye silage and 43.19% DM for maize silage.

Composition of inocula was subject to slight changes because of differences in sampling times. The concentration of cellulose and hemicellulose was higher in inoculums 1. Inoculum 2 showed a slightly higher concentration of lignin (ADL).

Industrial trials

Figure 1 shows the variation on concentration of dry matter that was determined for substrates and fermentation media throughout beginning of Period 1 until the end of Reference Period 2 (Table 1). Maize silage had dry matter content between

Table 3. Composition of substrates and inocula for laboratory trails.

Parameter	Unit	Maize silage	Rye silage	Inoculum 1	Inoculum 2
Dry matter	[%]	36.19	43.04	2.93	3.12
Organic dry matter	[% DM]	96.31	95.61	61.07	60.58
Crude ash	[% DM]	3.71	4.92	45.34	45.53
Crude protein	[% DM]	7.79	9.29	49.15	44.23
Crude fat	[% DM]	3.79	2.36	2.05	1.50
Crude fibre	[% DM]	19.14	38.42	8.49	8.30
NDF	[% DM]	43.19	61.37	13.86	12.43
ADF	[% DM]	20.13	37.59	8.08	8.88
ADL	[% DM]	2.54	5.63	4.63	5.91
Cellulose	[% DM]	17.59	31.96	3.45	2.97
Hemicellulose	[% DM]	23.06	23.78	5.78	3.55
Water soluble carbohydrates	[% DM]	0.63	8.39	0.12	0.17
Ammonium	[% DM]	0.12	0.15	3.75	2.88
Lactic acid	[%]	1.91	1.43	-	-
Acetic acid	[%]	0.36	0.48	-	-
Alcohol	[%]	0.33	n.d.	-	-
Ammonia	[%]	0.03	0.10	-	-
pH		3.78	4.24	7.26	7.26

n.d. = not detectable, DM = Dry matter, NDF = Neutral Detergent Fibre, ADF = Acid Detergent Fibre, ADL = Acid Detergent Lignin

22.67% and 40.36% (average 31.93%). Sweet sorghum silage was used as co-substrate during Period 2 and had dry matter concentration between 23.23%and 30.24% (mean 26.09%). Dry matter content of the whole crop silage ranged between 27.69% and 36.72% (mean 31.50%).

Over the whole experimental period, the average pH of maize silage, sweet sorghum silage and whole crop silage was 3.98±0.21, 4.15±0.34 and 4.20±0.32, respectively. The average pH in reactor 1 (no enzyme) was 7.93±0.19 and in reactor 2 (enzyme B1 added) 7.95±0.20.

Figure 2 shows the average values of the variation in concentration of cellulose, hemicellulose and lignin measured during the periods of enzymatic treatment. The highest amount of cellulose was observed in Period 3 (substrates: maize silage + rye silage). Lower amounts were found in samples of maize silage during Period 1. The lowest cellulose values were measured with the mixture of maize silage and sweet sorghum silage used during Period 2. Most probably, a higher amount of hemicellulose could be determined in Period 3 (in comparison to Periods 1 and 2) because of the addition of rye silage, which is rich in lignocellulose. Over time, the concentration of lignin in the substrate mixture increased due to the type of substrate fed. The differences in lignocellulose concentration between reactor 1 and 2 are described in section 3.4. Cellulose and hemicellulose content decreased during fermentation, whereas lignin was concentrated and showed a higher content in fermentation media compared with the lignin concentration in the substrate.

3.2 Hydrolytic potential of the enzyme preparation

Figure 3 shows the enzymatic activities measured for different model substrates. As expected, the highest activity was determined for pectin. The selected enzyme preparation is able to degrade hemicellulose and cellulose at the applied operation modes. Beside these enzymatic activities, the partner DSM could measure a low hydrolytic activity using starch (results not shown).

In addition, hydrolysis of hay and straw by addition of enzyme B1 showed a 24% degradation of sugar containing polymers (e.g. cellulose). The incubation was performed at pH of 4 and 40°C. It seems that enzyme B1 has a strong effect on substrates rich in lignocellulose.

In samples from a batch test, carried out by IASP, only few pectinase coding sequences were detected using metagenome analyses. A recalcitrant mixture of hay and straw was used as fermentation substrate. This indicated that only very low levels of pectinolytic enzyme activities could be expected in the biocenosis. As a conclusion, the addition of pectinase-containing enzyme preparations, such as enzyme preparation B1, seemed beneficial for optimizing the biogas process. Nevertheless, glycosyl hydrolase coding sequences could be detected considerably and these were assigned to the taxonomic groups *Firmicutes, Bacteroidetes* und *Proteobacteria* [12]. Prospectively the named molecular biological analyses can become a tool for the analysis of functional genes in biogas processes and consequently may give indications for the enzyme or enzyme mixtures, which are needed in the process.

3.3 Laboratory fermentation trials

Table 4 shows results of the batch lab tests. Addition of enzyme B1 led to an increase in specific biogas and methane yields. Using rye silage as substrate, the percentage of methane in biogas could be enhanced by application of the enzyme preparation. Increases in methane yield of 9.2% and 6.3% were determined after fermentation of maize or rye silage for 35 days, respectively. Further, increasing the residence time to 65 days resulted in additional methane yield enhancement up to 15.3% or 10.2%, respectively.

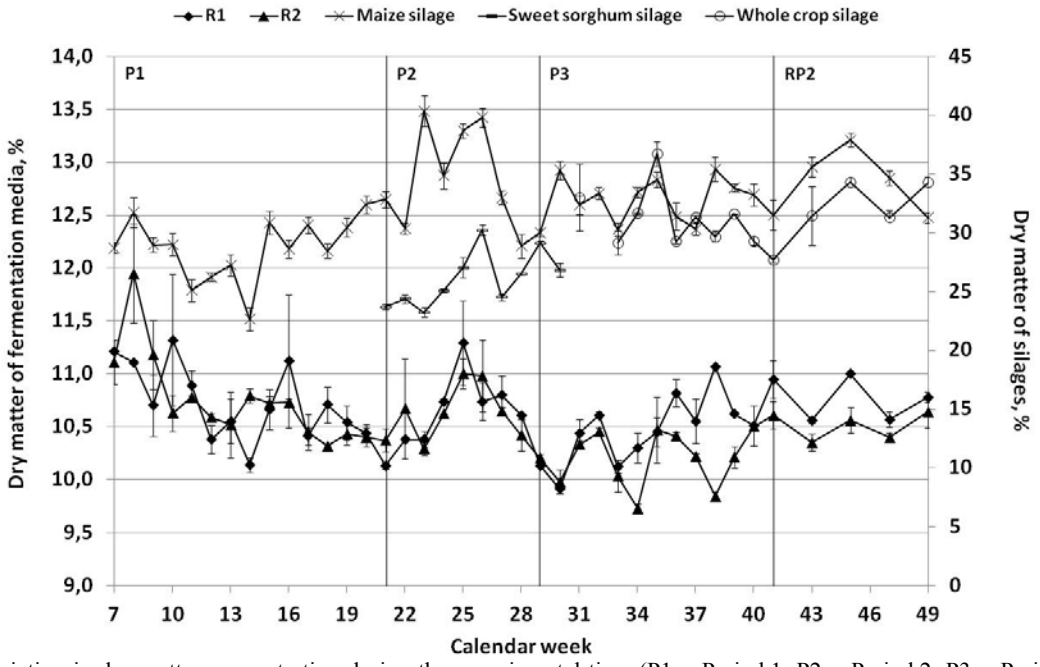

Figure 1. Variation in dry matter concentration during the experimental time (P1 = Period 1, P2 = Period 2, P3 = Period 3, RP2 = Reference Period 2, R1 = bioreactor 1, R2 = bioreactor 2).

Table 4. Specific biogas and methane yields using maize or rye silage as substrate without and with enzyme addition.

	n	Biogas	Methane	Methane	Methane	CV
		l_N/kg ODM	%	l_N/kg ODM	[m^3/t FM]	%
Maize silage	3	457 ± 64	60.0	274 ± 43	95 ± 15	15.8
Maize silage + B1	3	516 ± 25	60.5	316 ± 34	110 ± 12	10.9
Rye silage	3	439 ± 42	57.8	254 ± 27	103 ± 11	10.7
Rye silage + B1	3	468 ± 9	59.8	280 ± 7	114 ± 3	2.6

ODM = Organic dry matter, FM = Fresh matter, n = Number of samples, CV = Coefficient of variation, B1 = Enzyme

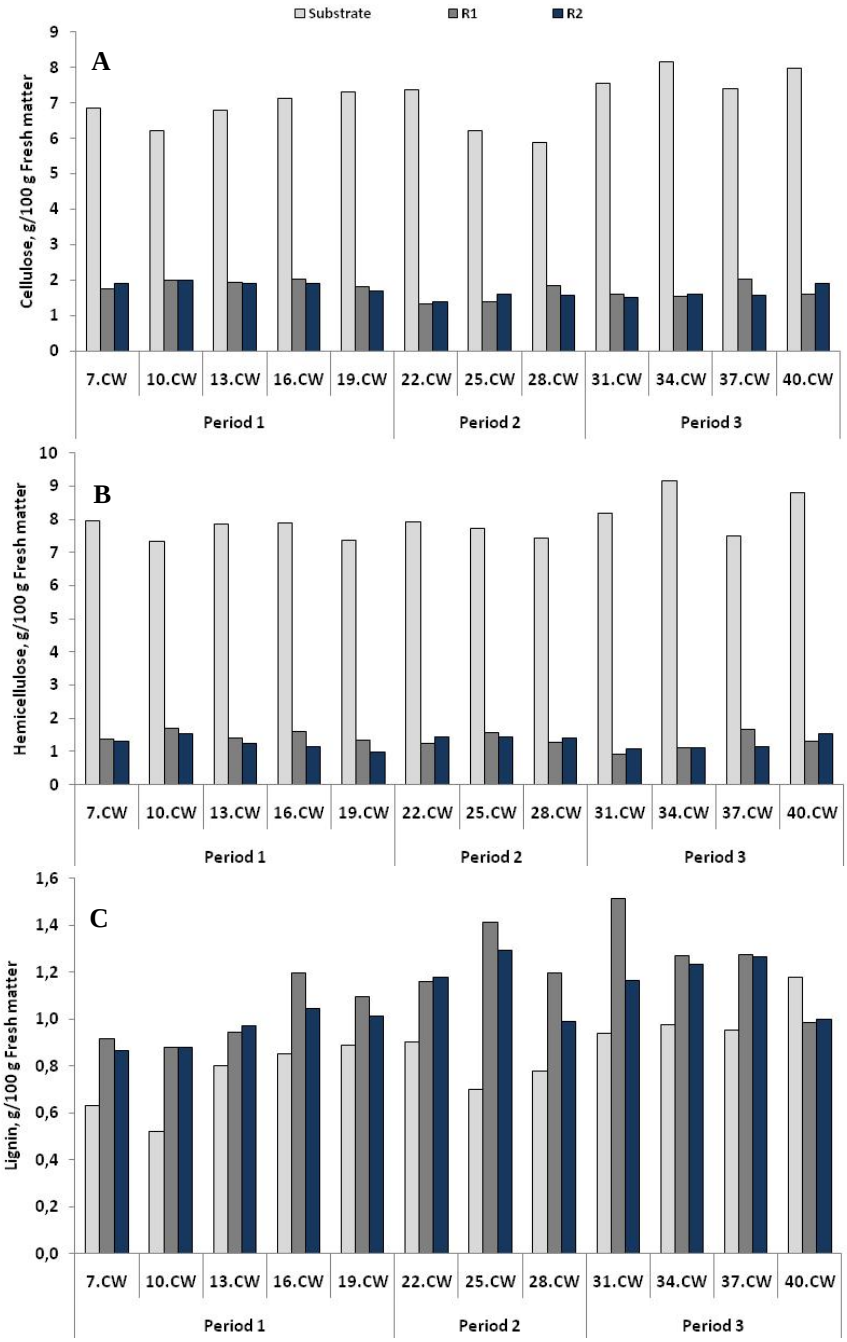

Figure 2. Variation in cellulose (A), hemicellulose (B) and lignin (C) concentration during the experimental time (R1 = bioreactor 1, R2 = bioreactor 2).

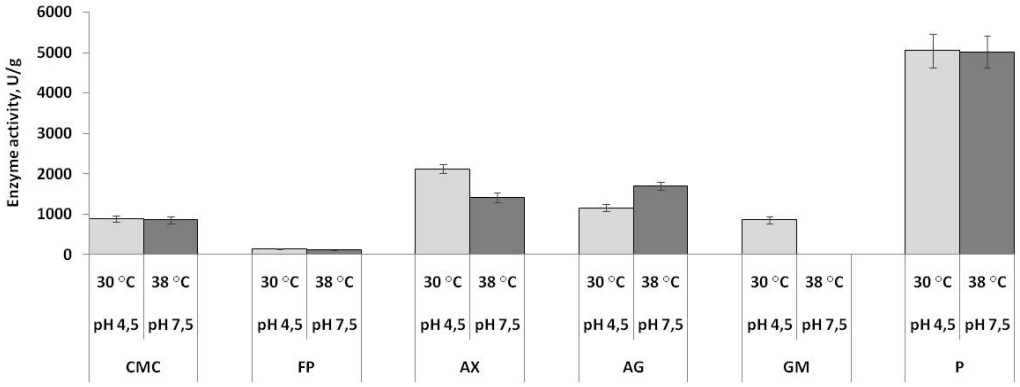

Figure 3. Selected hydrolytic activities of enzyme B1 (CMC = Carboxymethyl cellulose, FP = Filter paper, AX = Arabinoxylan, AG = Arabinogalactan, GM = Glucomannan, P = Pectin).

Figure 4 summarizes the calculated biodegradability of cellulose and hemicellulose during lab-scale fermentations of maize and rye silage using a mass balance. Utilization of organic dry matter was improved by enzyme addition, by 4%, regardless of the substrate used. Cellulose degradation seemly decreased by adding enzyme B1. The lower degradation degree of cellulose in enzymatically treated samples could be due to feedback product inhibition of enzymes through a higher release of products by addition of enzyme B1. It is known that B1 enzyme preparation contains high hemicellulase activities. As result, hemicellulose was almost completely utilized during fermentation in the enzymatically treated maize silage. The hemicellulose contained in rye silage was utilized to a higher degree using enzyme B1 (8% increase).

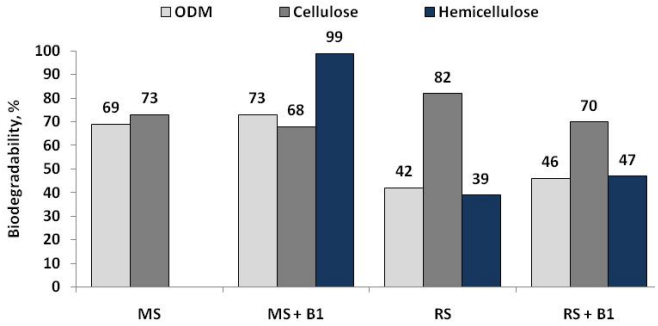

Figure 4. Biodegradability of cellulose and hemicellulose during mesophilic, lab-scale fermentation of maize and rye silage (MS = Maize silage, RS = Rye silage, B1 = Enzyme).

3.4 Industrial fermentation trials

The performance of both reactors was similar in Reference Period 1, as shown in table 5. Addition of enzyme B1 using mainly maize silage (Period 1) resulted in an electricity production increase by 2.6% at the end of that period. Process instabilities during Period 2 led to a limited evaluation of the performance. The concentration of propionic acid and the VOA/TAC value increased strongly (data not shown). No distinguished increase in energy production could be measured at the end of Period 2 between both bioreactors. Nevertheless, the highest increase in energy production was measured during Period 3 by adding the enzyme when feeding rye silage in addition to maize silage. The surplus of energy was 4.7%. The increases in energy production in Periods 1 and 3 between control and test reactor were statistically significant.

Degradation of cellulose in reactor 1 (without enzyme) and reactor 2 (enzyme added) was found to be between 68% and 82%. Hemicellulose was converted to an extent of 77-89%. As shown in Figure 5, addition of enzyme B1 showed an impact on cellulose and hemicellulose degradation. An intensified hydrolysis by enzyme B1 was measured for both polymers in Period 1. Particularly noteworthy is the enhanced hemicellulose degradation during fermentation of maize silage in Period 1. At the end of this period an increase in reduction of hemicellulose of 4.6% and for cellulose of 1.7% were calculated. Feeding sweet sorghum silage and maize silage in Period 2 a slightly higher degree of degradation regarding cellulose was observed. Starting feeding with rye and maize silage the hemicellulose was more affected. At the end of Period 3 the degradation of hemicellulose was increased by 7.2%. The degradation is comparable to that of rye silage fermented in a laboratory trial (Figure 4). Lignin was not affected by adding enzyme B1.

As shown in Figure 3 the enzyme preparation B1 contains different enzymes. Pectinolytic activities are mainly measurable; also hemicellulolytic and cellulolytic activities can be shown. The interaction of these enzymes leads to a better degradation of lignocellulose. The positive impact of the pectinolytic preparation can be primarily attributed to the degradation of pectin which is a "bonding substance" within the lignocellulose complex. The depolymerisation of the noted molecule resulted in a better availability of the components of lignocellulose. Additionally pectinases can reduce the viscosity of fermentation media by pectin degradation (Figure 6), so the microorganisms and enzymes have in aqueous systems a better access to plant constituents. These enzymes showed in determinations for liquefying maize silage the best results regarding technical and economic benefits in comparison with amylases and cellulases [13]. In nature, pectinase play a key role in lignocellulose degradation. The outermost layer of cell walls is the middle lamella which contains mainly pectin. That is why the first produced hydrolytic enzyme by plant pathogens like wood-decaying fungi is a pectinase [14]. The used preparation in our experimental work contains also high hemicellulolytic activities. Hemicellulose is beside pectin a "bonding substance" in the network of lignocellulose and could be partly degraded by the hemicellulases. The results therefore support the proposition that the degradation of bonding substances leads to an enhancement in biogas or better methane production.

Table 5. Specific energy productions [kWh/t FM] of the control (1, without enzyme) and test reactor (2, enzyme B1 added) during the individual periods.

	Reactor 1 Substrate	Confidence interval	Reactor 2 Substrate + B1	Confidence interval
Reference period 1	456	6.5	458	5.1
Period 1	467	4.6	480	4.7
Period 2	474	3.9	474	4.6
Period 3	450	5.5	473	6.9
Reference period 2	473	6.1	481	5.7

Low-molecular carbohydrates or starch were fermented almost completely in each biogas plant, yielding a degradation degree between 92% and 99% (data not shown). To this effect, no potential for an enzymatic conversion by enzyme B1 existed during fermentation of biogas substrates. An effect could be measured concerning the degradation of crude fat. Enzyme B1 intensified the conversion of crude fat during Period 1 and 3. At the end of the named periods the concentration of crude fat was reduced further by 5%, probably caused by fat-digesting enzymes in the enzyme preparation, termed lipases.

Using torsion measurements, the apparent viscosity could be determined in a laminar flow regime in 10 l of culture broth with a high reproducibility applying the power law model and 4% (w/W) carboxymethyl cellulose and 5% (w/w) pectin as calibration fluids. For monitoring the viscosity at defined enzymatic degradation the model substrates were digested with cellulase and pectinase, respectively [15]. The rheological characteristics of the biogas plants fluid phases were similar in the first phase of period 1. That is a result of increasing the enzyme concentration in the first weeks of application up to a final concentration of 100 ppm. The enzyme was concentrating in the reactors up to a final enzyme concentration of 95%. After 8 weeks of enzyme addition, the untreated process showed higher viscosities. The viscosity in the enzyme treated biogas plant could be lowered up to 6.2% (a), 6.9% (b) and 18.0% (c) at the end of the Period 1, 2 or 3. Similarly, the dry matter content of the enzyme treated biogas reactor was reduced after 8 weeks of enzyme addition. In particular, it could be observed that the dry matter content of reactor 2 in Period 3 was strongly decreased (Figure 1). A linear dependence of viscosity and dry matter was evaluated by multilinear regression at all three phases. A correlation between viscosity and agitator power was also found.

Some authors reported an increase in biogas or methane yield between 2 and 18% for enzymatic treated maize silage fermented in mesophilic laboratory or technical fermentation trials; compared to untreated maize [16-19]. For industrial

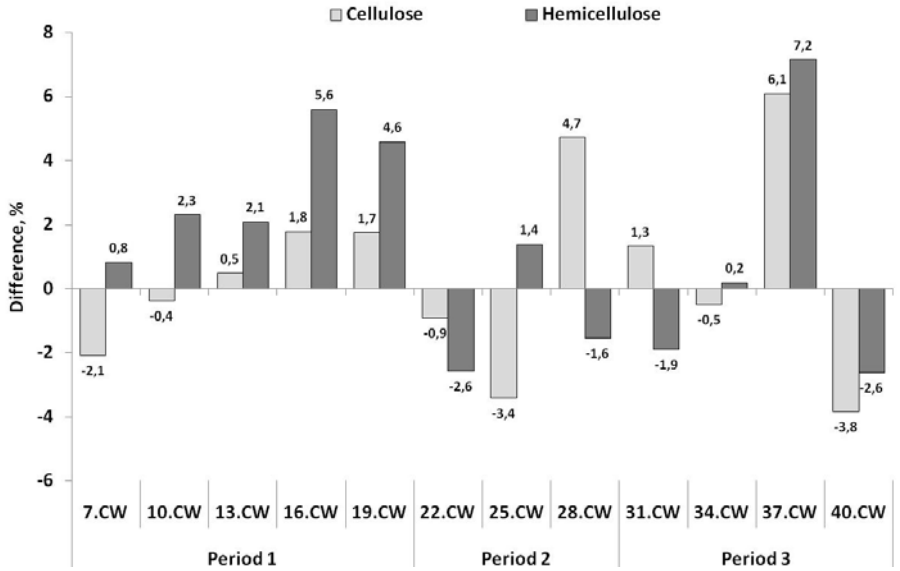

Figure 5. Differences between control and test reactor in cellulose and hemicellulose biodegradability.

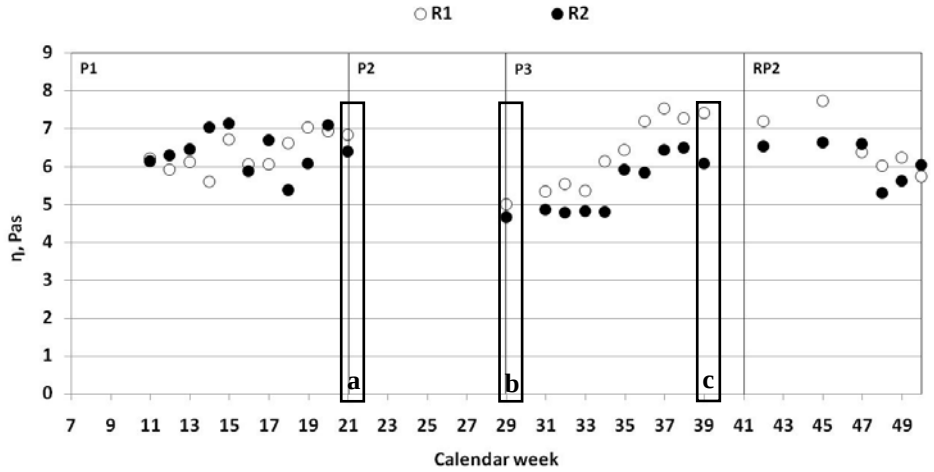

Figure 6. Variation in apparent viscosity (measured at 60 rpm) during the industrial trial (P1 = Period 1, P2 = Period 2, P3 = Period 3, RP2 = Reference Period 2, a/b/c = evaluation of the difference between reactor 1 and 2).

fermentation trials enhancements in biogas production of up to 35% were pointed out [3]. No positive impacts could be determined using rye silage treated by an enzyme mixture [20]. Cellulase, glucanase and xylanase were the most applied enzymes. 25 enzyme preparationswere used in determinations of Cordes [21]; for example pectinase preparations. Mixtures of liquid manure and co-substrates were treated with enzymes in mesophilic laboratory fermentation trials. The biogas production was calculated after a retention time of 5-7 days. A pectinase was among the most effective enzyme preparations. Not only positive impacts but also no effects or a reduction in biogas or methane production could be investigated for different substrates [22]. The impact of enzymes is depending on the selected substrate, the origin of enzyme, the type of enzyme, additional disintegrations of the substrate and trial conditions, etc. In the present study an enzyme preparation, enzyme B1, is introduced, for the first time, which could affect the methane yield of rye silage in a positive way under the mentioned trial conditions. In addition the methane yield of maize silage could be enhanced. The surplus in methane yield is in accordance with commercial enzyme preparations. It could be found in subsequent investigations that also the methane yields of substrates rich in lignocellulose like hay and straw or horse dung can be improved by enzyme B1 in laboratory fermentation trials (data not shown). The commercial enzyme preparations used in industrial biogas processes are mostly specialized on just a few substrates or an individual substrate. Enzyme B1 can be used for a higher variety of substrates. The enzyme preparation improves the conversion of the determined substrate, reduces the viscosity of the fermentation media markedly and thus enhances the efficiency of biogas processes. Furthermore, it could be shown for the first-time that the effects on lignocellulose conversion and methane yield measured in laboratory fermentation tests can be confirmed in industrial fermentation trials and that the viscosity can be directly measured in fermentation media in real-time.

4. CONCLUSIONS

The addition of enzyme preparation B1 produced positive effects on biogas enhancement under lab-scale and industrial process conditions. The resulting energy production was enhanced during fermentation of maize silage and rye silage in laboratory trials, and statistically significant during fermentation of maize silage and rye silage in industrial trials. This resulted in an increase in energy yield by addition of enzyme B1 in every case economically attractive. The greatest effect of enzymatic action and therefore the highest additional yield in energy production and reduction in viscosity was observed at a significant level using a combination of maize silage and rye silage as co-substrate as feedstock for industrial biogas plants. This increase

in efficiency can be explained by a higher degradation of organic substances like lignocellulose and crude fat.

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