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ORIGINAL PAPER

Effects of Pretreatment with a Ball Mill on Methane Yield of Horse Manure

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Abstract

Lignocellulosic biomass is an abundant organic material, which can be utilised in biogas plants for sustainable production of biogas. Since these substrates usually have high lignin contents and consist of rather elongated particles, a special pretreatment is required for an economical and process-stable utilisation in the biogas plant. The mechanical pretreatment of horse manure was carried out with the prototype of a ball mill at different speeds. The aim of ball milling is to comminute the substrate and disintegrate the lignocellulosic bond. Mechanical pretreatment in the ball mill resulted in a significant increase in specific methane yield of more than 37% in anaerobic batch digestion (up to 243 L_{CH4} $kg_{VS}{}^{-1}$) of horse manure. The kinetics of the methane gas formation process was analysed by a modified Gompertz model fitting and showed a higher methane production potential and maximum daily methane production rate as well as a lower duration of the lag phase after pretreatment at 6 rpm. This was further confirmed by sieve analyses, which showed a significant reduction of particle size compared to the untreated variant. Thus, the use of the ball mill increases the specific methane yield and improves the fermentation of lignocellulosic substrates such as horse manure.

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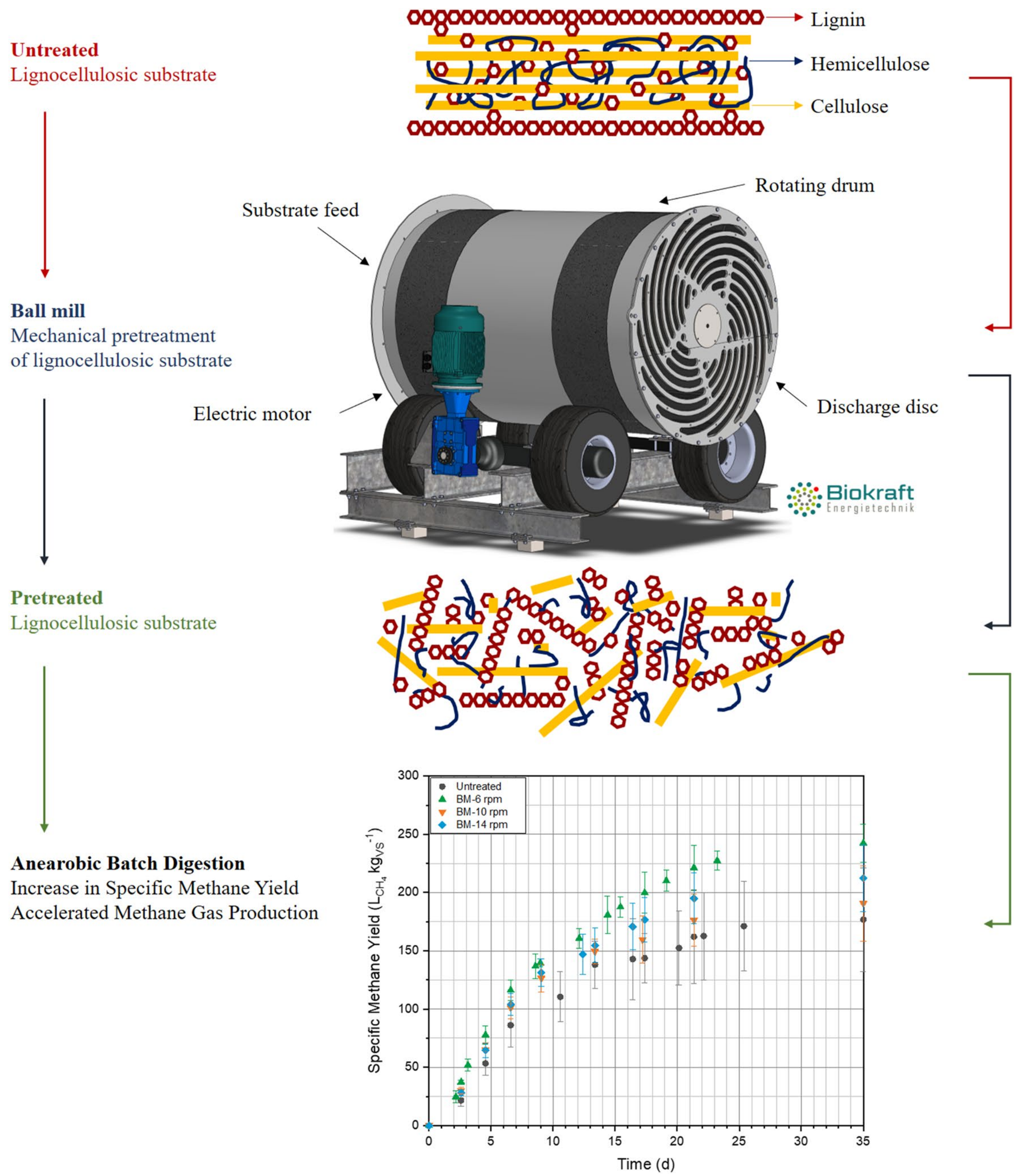
Graphical Abstract



Keywords Mechanical disintegration · Anaerobic digestion · Lignocellulose · Specific methane yield · Particle size

Statement of Novelty

The specific objective of this research was to investigate the influence of mechanical pretreatment of a new prototype ball mill on the specific methane yield and particle size distribution of horse manure. For this purpose, different rotational speeds of the ball mill were investigated, at constant filling weight. Since horse manure consists mainly of lignocellulose, which can lead to problems such as floating layers and incomplete digestion, pretreatment is necessary before the substrate is added to the fermenter of a biogas plant. The results are new and therefore of importance for the pretreatment of lignocellulosic residues such as horse manure for anaerobic digestion at plant scale.

Introduction

Lignocellulosic biomass is an abundant organic material, which can be utilised in biogas plants for sustainable production of biogas [1]. The utilisation of agricultural by-products, wastes, and lignocellulosic residues, such as horse manure, offers the possibility of reducing feedstock costs and also reducing land-use competition between food production and biogas production [2]. In addition, Angelidaki and Ahring [3] state that the use of farm wastes allows to the conversion of manure, which is normally considered to have little or no commercial value, into renewable energy. The structural characteristics of lignocellulosic substrates are a common problem affecting the efficient valorization of many agricultural wastes [4, 5]. Lignocellulose is mainly composed of cellulose, hemicellulose and lignin, which are cross-linked together and make the plant structures flexible and resistant [6–8]. This cross-linking stabilises the plant cell wall and protects it from enzymatic or microbial degradation [4, 7–9], preventing lignocellulosic biomass to reach efficient hydrolysis [6, 10]. Anaerobic digestion (AD) can convert both cellulose and hemicellulose of the lignocellulosic substrate, while lignin remains undigested [11]. In order to increase the biodegradability of lignocelluloses and to utilise agricultural waste, pretreatment is an essential element for the economic operation of biogas plants [10, 12]. Pretreatment methods can be generally categorised into physical, chemical and biological or a combination of these methods [1, 4, 12–14]. Chemical pretreatment methods cover alkaline, acid, catalyzed steam explosion, wet oxidation, oxidative pretreatment with peroxides and ionic liquids, while biological pretreatment methods can be carried out by fungi, enzymes or a microbial consortium. Physical pretreatment techniques include mechanical comminution, steam-explosion, liquid hot water pretreatment, extrusion, and irradiation like ultrasound and microwave [1, 15]. Various mechanical pretreatment processes such as grinding, shredding, crushing, or chopping are already available for the large-scale biogas process [16]. Mechanical pretreatment usually causes a particle size reduction, alteration of cell walls to increase the enzyme accessibility [17] and an increase in the surface area of the organic material, which provides a larger contact area for microorganisms to degrade the material, resulting in a higher biogas yield [18–21]. In addition, the reduction of particle size can accelerate the kinetics of the degradation and gas formation processes of lignocellulosic substrates [18, 22], reduce the risk of process-related engineering problems such as floating layers in the digester [23, 24] and recalcitrance of solids to enzymatic breakdown [25]. Fernandez et al. [17] mention energy savings of pumping and mixing operations in the digester as another objective of substrate pretreatment. Mönch-Tegeder et al. [26] investigated grinding of horse manure with a cross-flow grinder (Bio-QZ, ANDRITZ MeWa GmbH, Gechingen, Germany). A collision reactor with a rapidly rotating chain was filled with a portion of substrate which was grinded for 15 s. The batch digestion test was conducted according to the guidelines of the VDI 4630 [27] in glass bottles with a working volume of 2,000 mL for 35 days. The pretreatment led to an increase in methane yield of 9.2% compared to the control variant. Another investigation of Mönch-Tegeder et al. [24] showed an increase of 26.5% in methane production of a pretreated substrate mixture (liquid manure, horse manure, solid manure, grain silage, maize silage, grass silage, crushed grain) compared to the untreated material. This full-scale experiment was carried out in two continuous stirred tank reactors with working volumes of 800 m^3 each. During an experimental time of 160 days one digester was fed with the pretreated and the other one with the untreated substrate mixture. The specific energy consumption of the cross-flow grinder accounted to 11.3 ± 1.3 kWh $t_{FM}{}^{-1}$ for the pretreatment of the substrates during this trial.

By the application of a pretreatment machine (coarse steel roller against a steel roller at a rotating speed of 400 rpm) on meadow grass, Tsapekos et al. [28] showed an enhanced methane yield of up to 27% compared to untreated samples. Batch digestion was conducted in glass bottles of 547 mL total volume at a thermophilic temperature of 54 °C.

Gallegos et al. [29] evaluated the effect of particle size reduction on wheat straw. The substrate was pretreated by means of chopping to a particle size of 2.0 cm by a straw-mill and grinding to a particle size of 0.2 cm by a bio-extruder. The test was operated under mesophilic conditions of 38 °C. The batch digestion was conducted using eudiometer devices in accordance with VDI 4630. The mechanical pretreatment of wheat straw increased the methane yields by up to 26%.

In the present study, a prototype ball mill was investigated, which represents a new type of mechanical disintegration process for fibre-rich and lignocellulosic feedstocks in the biogas sector [30]. The objective was to analyse the effects of mechanical pretreatment on specific methane yield and particle size distribution of horse manure. The used prototype of the ball mill is novel and unique in the biogas sector.

Materials and Methods

Ball Mill

The ball mill used in this study is patented as a comminution device, especially for the mechanical pretreatment of fibre-rich biomass for biogas production, under German patent number DE102019106792A1 [31]. The prototype of this ball mill was developed and built by *Biokraft Energietechnik* (Biokraft Energietechnik GmbH, Stuttgart, Germany) and is located at the biogas plant *Heuberghof* (Bio-Energie Heuberg GmbH & Co. KG, Balingen, Germany). It consists of a cylindrical rotating drum unit of 2 m diameter and 3 m length mounted on a truck tandem axis (Fliegl Fahrzeugbau GmbH, Triptis, Germany). The ball mill is driven by a 45-kW electric motor (MOLL-MOTOR, Mechatronische Antriebstechnik GmbH, Stockerau, Austria) and is filled with about 2500 kg of grinding balls with a diameter of 80 mm.

Figure 1 shows different views of the prototype ball mill and its components. The grinding balls are made of cast iron and have an average weight of 2.1 kg. Iron rods are welded to the inner wall of the drum to carry the grinding balls up and drop them onto the substrate.

The resulting collisions and interactions of the grinding balls with the substrate lead to frictional and impact forces which grind and comminute the substrate, reduce the particle size and thus increase the particle surface area.

Mechanical comminution occurs in continuous operation and can be influenced by various parameters such as rotational speed, drum filling weight, grinding media, substrate composition and substrate feed rate.

As the ball mill is equipped with load cells (combined error $\leq \pm 0.03\%$), the actual weight of the substrate in the drum is permanently measured. The automated feed enables the feeding of fresh substrate as soon as the filling weight of the drum falls below the set value. The level control also allows the desired amount of substrate to be treated during a trial.

The substrate is treated in a continuous flow inside the drum and gets transported to the discharge disc and pushed through it. Pretreated substrate is then fed to the biogas plant via screw conveyors.

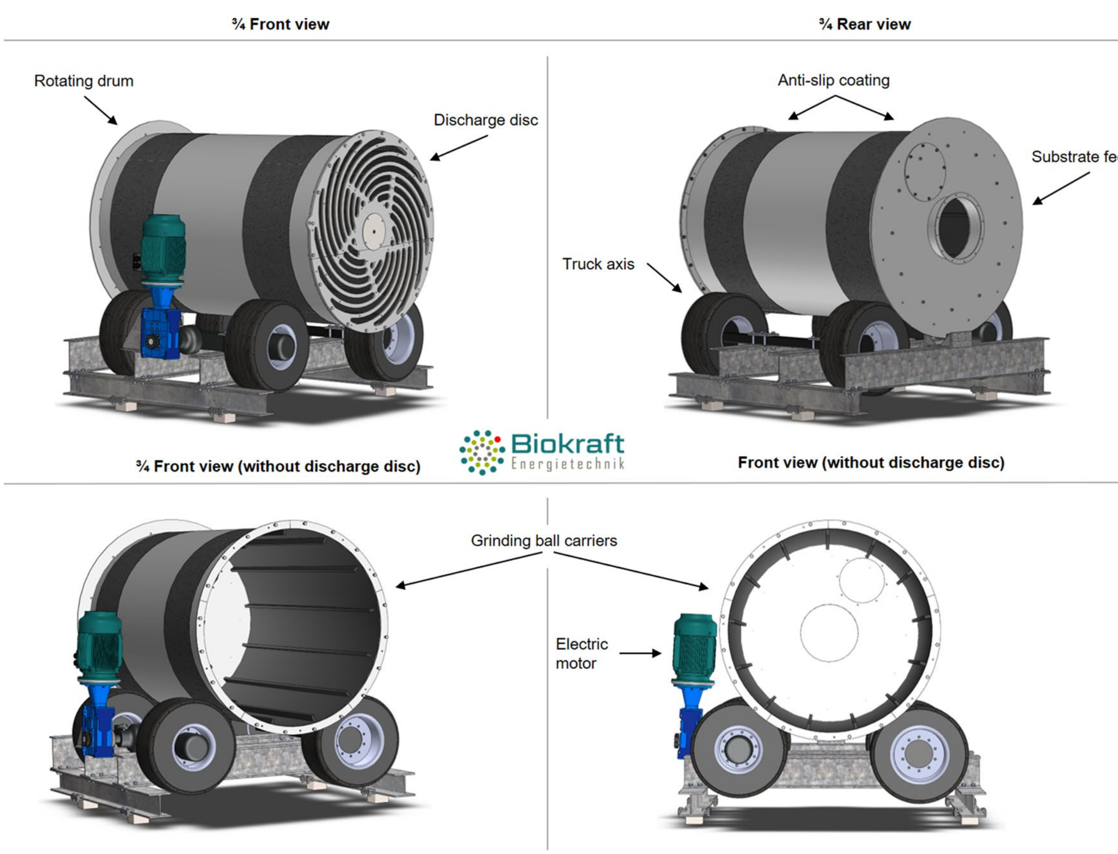


Fig. 1 Front view and rear view of prototype ball mill [32]

Substrate Properties

Substrates Investigated

The ball mill was fed with a mixture of horse manure and wheat straw during this study. The horse manure used in this study was collected from a horse farm near Balingen (Hofgut Reichenbach—EQH Egenter Quarter Horses, Balingen, Germany). The matured horse manure was 8–12 weeks old and therefore very moist and adhesive. To reduce moisture of the horse manure and the adhesion in the ball mill, wheat straw was added in the form of square bales (300 ± 30 kg) in the dosing unit prior to the ball mill. The ratio of horse manure to wheat straw was adjusted to 4:1 in all trials. Before the substrate was automatically added to the ball mill for the disintegration process, it was fully mixed in a solids dosing unit for 30 min before each trial run.

Most equine waste that can be considered for anaerobic digestion consists of manure and some type of stable bedding [33–36]. In this paper, the term *horse manure* is used to refer to horse excrement and urine mixed with straw bedding material and then removed from stalls.

Mechanical Pretreatment and Sampling

In this experiment, the rotation speed of the ball mill was varied to investigate the effect on particle size and specific methane yield. Three experiments were conducted at three different rotational speeds of 6, 10 and 14 rpm. The drum was cleaned before the trials and emptied after each trial. The substrates horse manure and wheat straw were successively added to the solids dosing unit using a wheel loader. With the start of each trial, the mixed substrates passed through the ball mill at the set speed. The filling weight of 1.5 t substrate in the drum was kept constant during the experiments. Samples of treated substrate were taken from below the discharge disc after 0.5 t of the substrate had been milled in continuous operation. For sampling, a special sample vessel was placed below the discharge disc along its entire length to obtain homogeneous samples of treated horse manure. For each treatment variation, a reference sample of the untreated substrate was taken.

In order to obtain homogeneous untreated samples of the substrate, the samples were taken after complete agitation at the outlet of the solid dosing unit. After the sampling, both the untreated and treated samples were immediately shock frozen with liquid nitrogen and then stored at − 20 °C to prevent further degradation of organic acids. The experimental data was recorded in a database connected to the ball mill to facilitate subsequent data analysis and calculations.

Batch Digestion Test

The determination of methane potential of differently pretreated horse manure in the ball mill was performed using the Hohenheim Biogas Yield Test (HBT). The HBT is a patented method with high reproducibility [37] and was performed according to the VDI Guideline 4630 [27] as described by Helffrich and Oechsner [38] and Mittweg et al. [39]. The method largely agrees with the method described by Hollinger et al. [40]. Since the HBT test provides information about the actual fermentation process and degradation kinetics, the sample preparation has to correspond as closely as possible to the subsequent practical conditions according to VDI Guideline 4630 [26]. Therefore, the substrates were assayed using the HBT without any additional laboratory pretreatment, as further comminution would have significantly biased the samples from the practical trials. High standard deviations were expected due to the small fermenter size, in particular for the untreated variant.

Horse manure was digested in 100 mL glass syringes filled with 30 g of inoculum and 0.8 g of sample material, corresponding to an inoculum-to-substrate ratio of 3.42:1, based on volatile solids. The glass syringes served simultaneously as digestion chambers and gas containers, as shown in Fig. 2.

Each treatment variant was run as a replicate of nine samples, while the untreated variant was run as a replicate of 27 samples, as horse manure is known as a heterogeneous

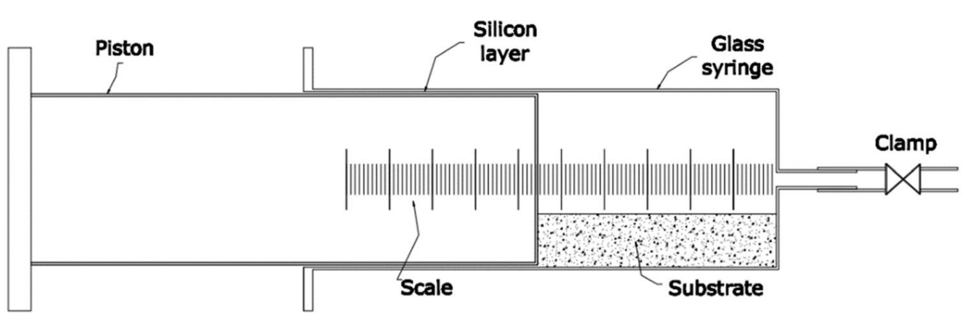


Fig. 2 Glass syringe to determine specific methane potential in HBT anaerobic batch digestion [41]

substrate. Over a period of 35 days, batch digestion was performed under mesophilic conditions at a constant temperature of 37 ± 0.5 °C.

The methane content of the dried biogas produced was measured manually using an infrared spectrometric methane gas sensor (Advanced Gasmitter, Pronova Analysetechnik, Berlin, Germany) and was related to the gas volume. The sensor was calibrated before and after each measurement with a calibration gas mixture of 60% CH_4 and 40% CO_2 (G325792 Compressed gas, Westfalen AG, Münster, Germany) [42]. Before measuring the gas volume, the gas was dried with an absorbent (SICAPENT®, Merck, Darmstadt, Germany). The measured gas volumes were corrected to standard temperature and pressure of 273.15 K and 1013.25 hPa. The procedure of gas measurements is described in detail by Mittweg et al. [39] and Hülsemann et al. [42].

For the determination of the specific methane yield of horse manure, the inoculum was tested separately in HBT syringes without co-digestion substrate. In addition, a hay and concentrate standard with known gas yields were digested to verify the accuracy of the experimental conditions according to VDI 4630. The results of the cumulative specific methane yields were related to the volatile solids content of the samples (L_{CH4} kg_{VS}^{-1}).

Inoculum

The inoculum for the HBT was obtained from the secondary digester of a biogas plant in Laupheim, Germany (Biokraftwerk Deubler GbR, Laupheim, Germany), which is operated at a mesophilic temperature of 42 °C. Fresh samples of inoculum were analysed for pH and FOS/TAC (785 DMP Titrino, Metrohm, Herisau, Switzerland) in the laboratory of the State Institute of Agricultural Engineering and Bioenergy (University of Hohenheim, Stuttgart, Germany) according to VDLUFA guidelines [43].The inoculum used had a pH of 7.92 and a FOS/TAC value of 0.169, which were in a suitable range for further use in the HBT batch digestion test [44].

Prior to the HBT, the inoculum was screened using a sieve with a mesh size of 0.7 mm to remove bigger particles. The determination of the total solids (TS) content resulted in a value of 5.46% fresh mass (FM) and a volatile solids (VS) content of 70.80% TS (3.87% FM).

Dry Sieve Analyses

Sieve analysis is one of the oldest methods of particle size analysis. A known sample weight is passed through progressively finer sieves. The amount collected on each intermediate sieve is weighed to determine the percent weight of each size fraction [45]. Sieve analysis was performed according to DIN ISO 66165–2:2016–08 [46] as dry sieving and on a *Fritsch Analysette 3 Spartan* two-dimensional vibratory sieve shaker (FRITSCH GmbH, Idar-Oberstein, Germany). Due to the vibration, the individual particles were collected in the intermediate sieves with mesh sizes of 8, 4, 2, 1, 0.5, 0.25 mm and the sieve pan at the bottom, which were arranged in descending order. The samples were dried at 105 °C for 24 h before sieving. For each sieve pass, 15 g of dried horse manure was weighed before sieving. The samples were then placed on the sieve with the largest mesh size and sieved for 10 min at a set amplitude of 2 mm. Before and after sieving, each intermediate sieve was weighted on a laboratory balance (KERN EG 4200-2NM, Kern&Sohn GmbH, Balingen, Germany). This procedure was performed in triplicate for each treatment variant (6, 10 and 14 rpm) and for the untreated reference. The particle size distributions were related to the total solids (% TS) of the substrate's testes.

Microscopy

The surface morphology of horse manure was observed using a Greenough Stereo Microscope (Zeiss Axio Zoom. V16, Carl Zeiss Microscopy GmbH, Jena, Germany), to analyse the surface destruction of the treated sample compared to the untreated reference. Pretreated substrate at 6 rpm and the untreated reference were analysed under the microscope. The microscope is equipped with a 12-megapixel microscope camera (Zeiss Axiocam 712 color, Carl Zeiss Microscopy GmbH, Jena, Germany) and was used at different magnification levels of 7x up to 40x. With the possibility of Z-stacking integrated into the analysis software ZEN core (ZEN core Version 3.3.92.00000, Carl Zeiss Microscopy GmbH, Jena, Germany), the depth of field could be extended at high magnification levels.

Chemical Composition Analysis

TS and VS

Total solids (TS) and volatile solids (VS) content of each sample were determined prior to the experimental run of batch digestion and sieve analysis. By drying the samples at 105 °C (UF450, Memmert GmbH, Schwabach, Germany) to constant weight, the total solids content was determined according to DIN EN 12880:2000 [47] and Frydendal-Nielsen et al. [48]. Subsequently, the samples were incinerated at 550 °C in a muffle furnace for 6 h according to DIN EN 12879:2000 [49] to determine the volatile solids content.

Feed Analysis

The concentrations (g kg_{TS}^{-1}) of crude protein (XP), crude fat (XL), crude fibre (XF) and nitrogen-free extract (NfE) of the samples were analysed by the analytical laboratory of Gerhardt Analytical Systems (Gerhardt GmbH & Co. KG, Königswinter, Germany) according to the European regulations for the Weender feed analysis [50, 51].

The concentrations of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were also analysed in the analytical laboratory of Gerhardt Analytical Systems (FibreBag Analysis System, Gerhardt GmbH & Co. KG, Königswinter, Germany) using the standard methods described by the Association of German Agricultural Analytic and Research Institutes (VDLUFA) [43].

Calculations and Statistical Analyses

Modified Gompertz Model

The modified Gompertz kinetic model has been commonly used to describe biogas production mathematically [52–59] assuming that the biogas production rate under batch conditions is a function of the bacterial growth rate of the predominant bacteria in the digester:

$$M = P \times exp\left\{-exp\left[\frac{R_m \times exp(1)}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

where M represents cumulative methane production (L_{CH4} kg_{VS}^{-1}) after digestion time t (d), P represents methane production potential (L_{CH4} kg_{VS}^{-1}), R_m represents maximum daily methane production rate (L_{CH4} kg_{VS}^{-1} d^{-1}), λ represents lag phase duration (d), and e equals exp(1), or 2.7182818. The kinetic constants of P, R_m and λ were determined using non-linear regression analysis (Microsoft Excel Solver Add-In, Microsoft Corporation, Redmond, USA). For each sample of the batch digestion test in the HBT the modified Gompertz fitting has been performed and subsequently the mean value and standard deviation were calculated.

Energy Balance

The energy consumption and treatment time was recorded for each trial and calculated as the specific energy consumption (SEC) per ton (t) fresh mass in kWh t_{FM}^{-1}. Additional specific methane yield (SMY_{Add}) was calculated from the difference between the yield of the pretreated sample in the ball mill and the yield of the untreated sample.

(in m^3_{CH4} t_{FM}^{-1}). Energy surplus (ES) due to treatment was calculated by following equation:

$$ES = SMY_{Add} \times LHV \times \eta_{CHP} \quad (2)$$

where, LHV is defined as lower heating value of 1 m^3 CH_4 which is 9.97 kWh [44] and η_{CHP} is the electrical energy conversion efficiency of the combined heat and power unit, which was assumed to be 38% for the conversion from methane into electrical power [26].

The energy balance (EB) was calculated by following equation:

$$EB = ES - SEC \quad (3)$$

which describes the additional energy yield obtained after subtracting the specific energy consumption needed for the pretreatment process (SEC) from the energy surplus (ES) generated by the pretreatment of horse manure.

The energy recovery rate (ER) was determined based on the ratio of methane energy (ME) recovered from digestion to the gross energy content (GE) of the substrate.

$$ER = \frac{ME_{digestion}}{GE_{substrate}} \quad (4)$$

The gross energy content (GE) of the substrates, expressed in MJ kg_{VS}^{-1}, can be estimated from the results of the Weender feed analysis and using the following equation [60]:

$$GE = 0.0239\,MJg^{-1} \times XP + 0.0398\,MJg^{-1} \times XL + 0.0201\,MJg^{-1} \times XF + 0.0175\,MJg^{-1} \times NfE \quad (5)$$

The specific energy content of the component parameters exhibits certain differences, as can be seen from the fixed coefficients of the equation [41].

Statistical Evaluation

Since the data had a normal distribution and homogeneous variance, statistical significance ($p < 0.05$) was tested by one-way ANOVA (Excel, Microsoft) followed by Tukey–Kramer post-hoc test using the Real Statistics Resource Pack software *XRealStats* for Excel [61] for statistical analyses of particle size and specific methane yields.

Outliers were determined and excluded from the data using OriginPro 2020 statistical analysis and visualisation software. The criterion for determining outliers was the interquartile range (IQR) multiplied by 1.5.

The graphs were created using OriginPro 2020.

Table 1 Chemical composition of untreated and pretreated horse manure

Determination method	Unit	Variant of treatment for horse manure			
		Untreated	BM 6 rpm	BM 10 rpm	BM 14 rpm
Total solids (TS)	% FM	49.74	53.42	50.45	48.07
Volatile solids (VS)	% TS	84.85	85.67	82.51	83.87
Crude protein (XP)	$g\ kg_{TS}^{-1}$	68.37	72.65	77.39	69.83
Crude fat (XL)	$g\ kg_{TS}^{-1}$	14.84	15.04	13.98	15.15
Crude fibre (XF)	$g\ kg_{TS}^{-1}$	423.03	398.09	406.92	403.30
Neutral detergent fibre (NDF)	$g\ kg_{TS}^{-1}$	715.54	675.79	671.33	673.53
Acid detergent fibre (ADF)	$g\ kg_{TS}^{-1}$	521.99	527.61	525.74	519.01
Acid detergent lignin (ADL)	$g\ kg_{TS}^{-1}$	122.01	139.79	139.49	134.66
Nitrogen free extracts (NfE)	$g\ kg_{TS}^{-1}$	403.40	425.51	404.34	414.12
Gross energy (GE)	$MJ\ kg_{VS}^{-1}$	15.13	15.20	14.67	14.73

BM treated horse manure in ball mill; *FM* fresh mass; *TS* total solids; *VS* volatile solids

Results and Discussion

Chemical Composition of Raw Materials

The compositions of the raw materials are listed in Table 1. Due to the weighing inaccuracy of the solid dosing unit, there were slight variations in the substrate composition of horse manure and wheat straw. The average total solids content of the substrates was 50.42% FM and varied from 48.07 to 53.42% FM. Contents of volatile solids showed a mean value of 84.23% TS and varied from 82.52 to 85.67% FM. Nevertheless, all samples had similar TS and VS content after agitation.

The average ADL fraction of the horse manure was 139.99 $g\ kg_{TS}^{-1}$, with the untreated variant having the lowest acid detergent lignin (ADL) content of 122.01 $g\ kg_{TS}^{-1}$. For NDF, the untreated variant showed the highest value with 715.54 $g\ kg_{TS}^{-1}$ compared to the mean value of 684.05 $g\ kg_{TS}^{-1}$.

The XP, XL and XF values were of the same order of magnitude for all treatment variants. The chemical compositions of the different treatment variants are comparable to the results of the stored horse manure samples studied by Mönch-Tegeder et al. [58]. The calculated gross energy content (GE) of horse manure ranged from 14.93 ± 0.27 $MJ\ kg_{VS}^{-1}$.

Pretreatment Process Data

In the studies it was shown that in continuous operation and with a constant filling weight of the ball mill, the flow rate increased as the speed of the mill increased. The retention time of horse manure in the ball mill decreased sharply with increasing speed. At a speed of 6 rpm, the milling time increased almost fivefold compared to 14 rpm. The highest flow rate was found at 14 rpm with 0.924 $t_{FM}\ h^{-1}$, followed by 0.494 $t_{FM}\ h^{-1}$ at 10 rpm and 0.201 $t_{FM}\ h^{-1}$ at 6 rpm. Clogging was observed at the discharge disc with time. Longer milling led to more intensive clogging at the discharge disc in the tests. The decisive factor for this was the matured horse manure, which was between 8 and 12 weeks old and thus adhered very strongly to the inner wall of the drum and the discharge disc. This limitation is not expected with fresh material. Fresh horse manure, which is utilised without intermediate storage, remains relatively dry due to its high straw content and can thus be processed much more efficiently in the ball mill. Adhesions on the inner wall, which lead to decreasing flow rates, can thus be largely avoided.

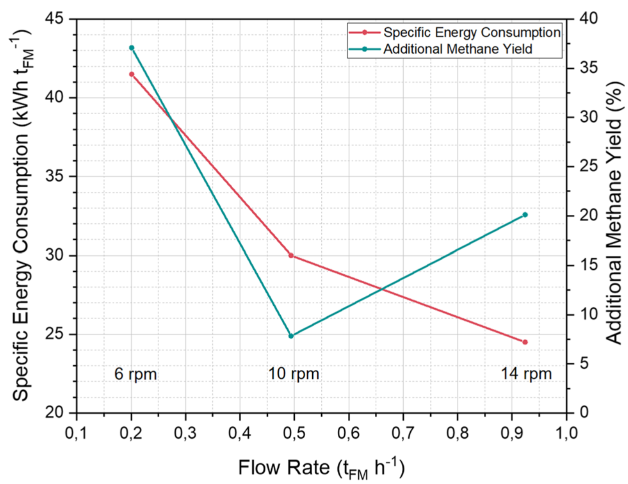


Fig. 3 Specific energy consumption and additional methane yield in relation to the flow rate at a constant filling weight of the ball mill of 1.5 t, measured during each treatment of 0.5 t of horse manure

The logged experimental data including specific energy consumption and additional methane yield as a function of flow rate are shown in Fig. 3.

It could be shown that as the speed increases, the number of revolutions and the energy consumption for the same treatment time increase. In this experiment the specific energy consumption of the ball mill varied between 24.5 and 41.5 kWh t_{FM}^{-1}. In its trials with fresh horse manure, the manufacturer of the prototype ball mill reported an average specific energy consumption of 6.5 kWh t_{FM}^{-1} at a flow rate of 4.5 t_{FM} h^{-1} which is significantly lower than the values reported in the present work [62].

Zheng et al. [1] mention that the size reduction is a very expensive operation, which can consume up to 33% of the total electricity demand for the whole process depending on the comminution method. They conclude that reducing the energy demand and increasing efficiency for grinding or milling of biomass at the same time would improve the economic performance of the whole process.

Effects of Pretreatment on the Degradation Kinetics

The experimental data of cumulative specific methane yields in the HBT were fitted to the modified Gompertz model for each sample of the respective treatment variant. The results of the modified Gompertz kinetic model show a strong correlation to the data points of the cumulative specific methane yields determined in the HBT. To assess the efficiency of the model, the adjusted coefficient of determination (Adj. R^2) was used. Overall, the values were high at over 0.9786 for all treatment variants (see Table 2) with accordingly low error values. This shows that the model is reliable and accurate in predicting the methane yield [63].

Methane production potential (P) was higher for all treatment variants compared to the untreated reference. A significant difference (significance level $p < 0.05$) in methane production potential was observed between the untreated variant and 6 rpm, and between 6 and 10 rpm. The highest value was 241.6 ± 16.0 L_{CH4} kg_{VS}^{-1} at 6 rpm, an increase of 37.16% compared to the untreated variant with 176.2 ± 44.1 L_{CH4} kg_{VS}^{-1}. Significant differences were found for the maximum daily methane production rate (R_m), between the untreated variant and 6, 10 and 14 rpm (significance level $p < 0.05$). Among the pretreated variants the value of R_m did not show much variation.

Statistical analysis by a Tukey–Kramer post-hoc test (significance level $p < 0.05$) revealed significant differences in the duration of the lag phase (λ) between the untreated variant and 6, 6 and 10, as well as between 6 and 14 rpm. With a duration of only 0.8 days, the treatment variant at 6 rpm achieved the lowest lag phase, which means that methane production started about half a day earlier compared to the untreated variant. At 10 and 14 rpm, the duration of the lag phase was in a similar range to that of the untreated variant (1.3 days). Kusch et al. [64] reported that chopping the horse manure twice with a compost chopper to a length of 4 cm significantly accelerated biogas production.

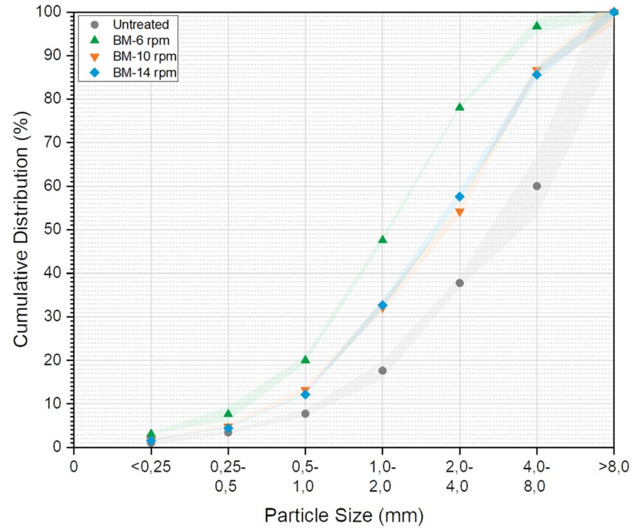


Fig. 4 Cumulative particle size distribution; data points represent mean values and shadows the standard deviations; BM = treated horse manure in ball mill

It can be concluded that the mechanical pretreatment had a significant effect on the specific methane yield as well as the degradation kinetics of the horse manure, especially for the sample treated at 6 rpm in the ball mill.

Effects of Pretreatment on Particle Size

The changes in the physical structure of the horse manure due to the disintegration process with the prototype ball mill were demonstrated by dry sieve analysis.

Table 2 Results of modified Gompertz fitting

Determination method	Unit	A variant of treatment for horse manure			
		Untreated	BM 6 rpm	BM 10 rpm	BM 14 rpm
P	L_{CH4} kg_{VS}^{-1}	176.2 ± 44.1	241.6 ± 16.0	190.4 ± 32.0	212.0 ± 28.4
R_m	L_{CH4} kg_{VS}^{-1} d^{-1}	14.3 ± 3.6	17.8 ± 1.1	17.8 ± 1.9	17.9 ± 1.6
λ	d	1.3 ± 0.3	0.8 ± 0.2	1.2 ± 0.2	1.3 ± 0.2
Adj. R^2	–	0.9791 ± 0.0060	0.9787 ± 0,0041	0.9796 ± 0,0117	0.9786 ± 0,0059

BM treated horse manure in ball mill; *VS* volatile solids; *P* methane production potential; R_m maximum daily methane production rate; *λ* lag time)

Figure 4 shows the results as a cumulative distribution curve for each treatment, with each intermediate sieve describing one data point. The curves for the variants treated in the ball mill show a remarkable reduction in particle size compared to the untreated reference. The curve at 6 rpm stands out clearly from the other treatment variants due to the intensive pretreatment and longer milling time. Graphs of the curves at 10 and 14 rpm show similar curve shapes and a significant reduction in particle size compared to the untreated variant. The overall low standard deviations within the pretreated samples show a high repeatability of the sieve analysis.

Statistical significances (significance level $p < 0.05$) of sieve fractions between treatment variants were performed as Tukey–Kramer post hoc test. All treatment variants showed a significantly lower percentage of particles larger than 8.0 mm. The strongest effect of the milling treatment on particle size was found in the 6 rpm sample, which had a mean value of 3.25% compared to 40.00% for the untreated variant. The sieve fraction of 2.0–4.0 mm was significantly higher at 6 and 14 rpm compared to the untreated variant. For particles of 1.0–2.0 and 0.5–1.0 mm, a significantly higher percentage was observed for all treatment variants compared to the untreated variant. A significant difference was also found for microparticles smaller than 0.25 mm compared to the untreated variant at 6 rpm. Fernandez et al. [17] concludes, that particle size reduction is an essential parameter on the methane yield, nevertheless other factors such as solubilisation and bioavailability of organic matter also play an important role.

Since horse manure consists mainly of irregularly shaped particles, Wills and Finch [45] point out that sieving is complicated by the fact that a particle with a size close to the nominal aperture of the test sieve can only pass if it is in a favourable orientation. Since the size of the sieve apertures inevitably varies due to the irregularity of the meshes, longer sieving results in the larger sieve apertures exerting an excessive influence on the sieve analysis. For this reason, the amplitude of the vibratory sieve shaker was set at 2 mm to compromise between the throw height that sieving generally requires and the negative effect of verticalisation and drop-through of the mostly elongated particles onto smaller intermediate screens that occurs at high amplitudes.

Effects of Pretreatment on the Surface Morphology

Figure 5 shows images at different magnifications under the Axio Zoom.V16 from Zeiss. The sample of the untreated variant consists of significantly larger particles of horse manure and straw particles (Fig. 5A and B) compared to the pretreated variant at 6 rpm (Fig. 5E), as well as substrate compounds, as shown in Fig. 5C, which cannot be separated without mechanical pretreatment. Broken particles are also present in the untreated sample (Fig. 5D) but show only minor kinks and are much less frayed.

In the pretreated variant, relatively smaller particles as well as predominantly broken and chipped particle ends are found in the sample (Fig. 5F). After treatment, fragmented and frayed particles (Fig. 5G, H) as well as microparticles smaller than 1 mm are present. It can be concluded that the physical structure of horse manure has changed due to the mechanical pretreatment in the ball mill.

Microstructural changes and the increase in particle surface area are both possible effects of the mechanical pretreatment. However, Mönch-Tegeder et al. [58] points out that increased surface area can cause an uncontrolled degradation process and energy loss. He also recommends that mechanical pretreatment should take place directly

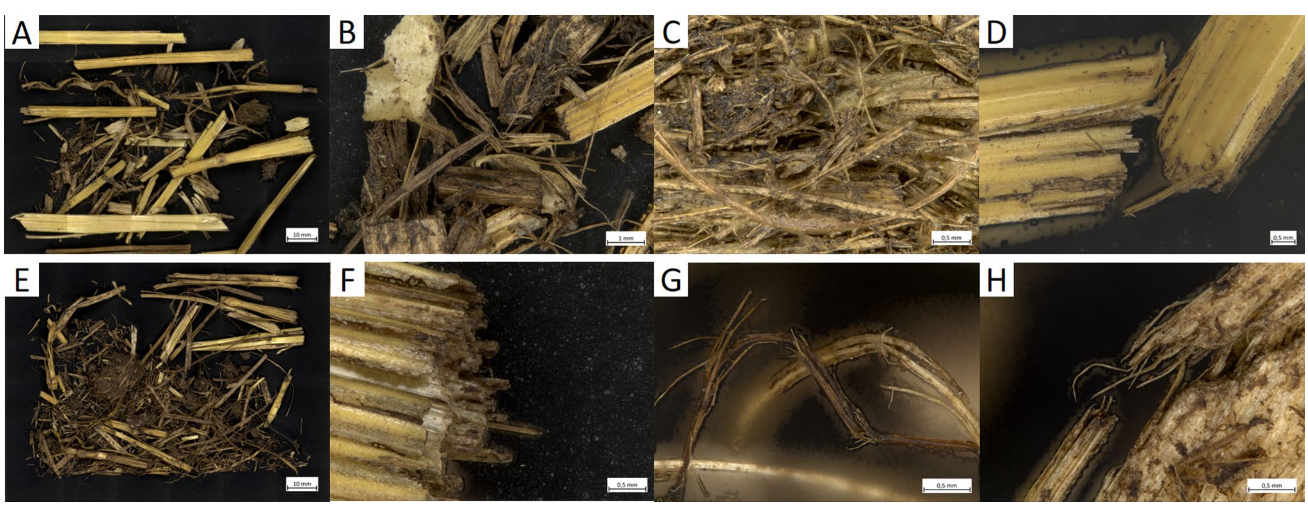


Fig. 5 Stereo microscopy images at different magnification levels of untreated horse manure (magnification levels: A = 7x; B = 16x; C = 32x; D = 40x) and pretreated horse manure in ball mill at 6 rpm (magnification levels: E = 7x; F = 32x; G = 40x; H = 40x)

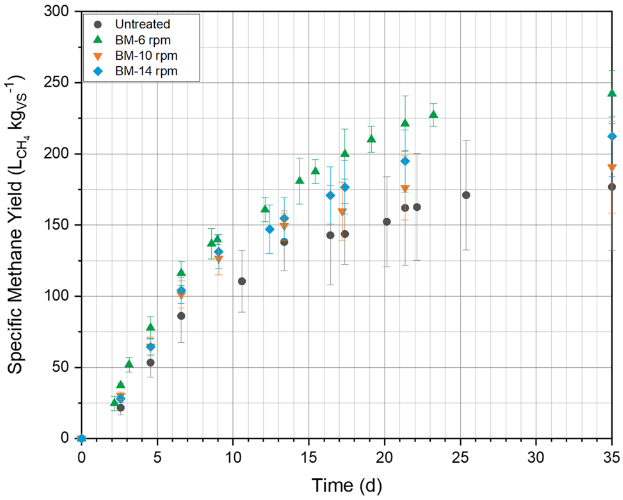


Fig. 6 Datapoints of cumulative specific methane gas measurement in HBT; BM = treated horse manure in ball mill; data points represent mean values and whiskers the standard deviations

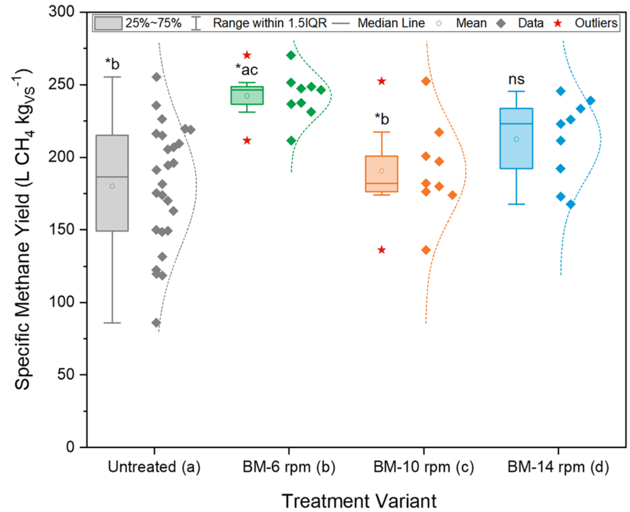


Fig. 7 Boxplots of specific methane yield of different treatment variants; significances *a, *b, *c, *d conducted by Tukey–Kramer post hoc test with p-value < 0.05; ns = not significant; BM = treated horse manure in ball mill

before processing in the digester [58]. In this regard, the ball mill offers a decisive advantage over other processing technologies, since the treated substrates are fed directly to the digester without further intermediate storage.

Effects of Pretreatment on the Specific Methane Yield

Figure 6 shows the cumulative specific methane yields and thus the development of methane formation of untreated and treated horse manure recorded in the HBT over 35 days. The untreated variant was 177 ± 45 L_{CH4} kg_{VS}^{-1}. Similar methane yields of untreated horse manure were reported by Kusch et al. [64] in batch-operated solid-phase reactors on a laboratory scale (working volume of 57 L) operated in percolation mode for 74 days and flooded mode for 46 days. Both experimental setups yielded around 170 L_{CH4} kg_{VS}^{-1}. Mönch-Tegeder et al. [58] reported a mean value of 191 L_{CH4} kg_{VS}^{-1} for fresh horse manure and 153 L_{CH4} kg_{VS}^{-1} for stored horse manure after 35 days of digestion in HBT batch digestion test.

Mechanical pretreatment in the ball mill resulted in overall increasing specific methane yields compared to untreated horse manure. The highest value of specific methane yield was obtained at a rotation speed of 6 rpm with 243 ± 8 L_{CH4} kg_{VS}^{-1}, which is a significant increase of 37.3% compared to the untreated variant. Rotation speeds of 10 and 14 rpm yielded 190 ± 16 L_{CH4} kg_{VS}^{-1} (+ 7.2%) and 212 ± 29 L_{CH4} kg_{VS}^{-1}(+ 20.1%), respectively. Mönch-Tegeder et al. [24] reported a 26.5% increase in specific methane yield after 15 s of treatment with a cross-flow grinder. In contrast, Carrere et al. [65] state that the objective of pretreatment of lignocellulosic biomass in full scale is to simplify feedstock management, digester feeding, and avoid any floating layer in the digester, rather than to increase the methane yield. Furthermore, they mention in their study that pretreatment in general not always result in higher methane production, sometimes even to lower values, due to inhibitor formation. However, they note that mechanical pretreatments are generally less sensitive to diverse substrate properties than other methods and that there is no elevated risk of recalcitrant compounds or inhibitors forming.

Standard deviation (SD) of specific methane yields for each variant show that more intensive treatment and longer treatment time result in more homogeneous samples. The untreated variant has the highest SD with 45 L_{CH4} kg_{VS}^{-1}, followed by 14 rpm (29 L_{CH4} kg_{VS}^{-1}), 10 rpm (16 L_{CH4} kg_{VS}^{-1}), and the lowest SD within the variants for 6 rpm of 8 L_{CH4} kg_{VS}^{-1}. Higher standard deviations may also be related to the small fermenter size, which ought to be compensated by a high number of replicates.

A Tukey–Kramer post-hoc test failed to show a significant increase for the 10 rpm and 14 rpm speed compared to the untreated variant. Two outliers were detected in the boxplots for a rotation speed of 6 and 10 rpm, respectively, and were excluded from the statistical analyses by multiplying the interquartile range (IQR) by 1.5 (see Fig. 7). Table 3 shows the results of the energy balance and energy recovery. It was found that energy recovery was higher for the pretreated variants. In particular, the 6 rpm treatment variant had the highest energy recovery with a value of 57.4%, compared to 42.0% for the untreated variant. The specific energy consumption for the treatment process in the ball mill varied between 24.5 kWh t_{FM}^{-1} for 14 rpm

Table 3 Electrical energy balance and energy recovery rate of pretreated horse manure compared to untreated reference

Determination method	Unit	Variant of treatment for horse manure			
		Untreated	BM 6 rpm	BM 10 rpm	BM 14 rpm
Methane energy	MJ kg_{VS}^{-1}	6.3	8.7	6.8	7.6
Energy recovery rate	%	42.0	57.4	46.4	51.8
Specific energy consumption during treatment	kWh t_{FM}^{-1}	-	41.5	30.0	24.5
Energy surplus due to treatment*	kWh t_{FM}^{-1}	-	138.3	16.6	40.8
Energy balance	kWh t_{FM}^{-1}	-	96.8	-13.6	16.6

BM treated horse manure in ball mill; *VS* volatile solids; *FM* fresh mass; assumed ηCHP = 0.38; LHV of 1 m3 CH4 = 35.9 MJ = 9.97 kWh [44]

*compared to untreated variant

and 41.5 kWh t_{FM}^{-1} for 6 rpm. The values are higher compared to the cross-flow grinder studied by Mönch-Tegeder et al. [26], which reached 13.8 kWh t_{FM}^{-1} for 15 s and 20.5 kWh t_{FM}^{-1} for 30 s treatment time. Nevertheless, a positive energy balance was found for the treatment variants of 6 and 14 rpm with 96.8 and 16.6 kWh t_{FM}^{-1}, respectively. Only for the treatment variant of 10 rpm a negative energy balance of − 13.6 kWh t_{FM}^{-1} was calculated due to higher specific energy consumption of the ball mill compared to the energy surplus of the treatment process. In contrast, Mönch-Tegeder et al. [26] achieved a positive energy balance of 12.7 kWh t_{FM}^{-1} for 15 s and negative energy balance of − 3.4 kWh t_{FM}^{-1} for 30 s treatment time. In particular, the milling process at 6 rpm showed a promising treatment effect of horse manure in terms of a high specific methane yield and thus a positive energy balance. However, Dell'Omo and Spena [15] point out that it is rather difficult to transfer the results of batch tests to continuous anaerobic digestion systems especially on a full scale. The reason is that batch digestion tests have a tendency to overestimate the methane potential resulting from the excess of inoculum, which contains important nutrients and buffer capacity in addition to an active microflora [66]. Apart from that, the experimental conditions in batch mode differ significantly from those in continuous full-scale biogas plants, where the effects of the pretreatments strongly depend on anaerobic digestion parameters such as the hydraulic retention time [15]. Hofmann et al. [67] also refer to the difficult transferability of batch to continuous systems and that the disintegration effect must be seen in the context of this limited comparability. However, additional positive effects of mechanical pretreatment may be expected in a change of rheological characteristics of the digestate and thus a lower risk of floating or sinking layers as well as a possibly lower energy requirement for mixing and pumping [17, 67].

Unfortunately, information on possibly improved substrate properties such as reduced floating and sinking layers, but also an enhancement of the rheological characteristics after pretreatment with the prototype ball mill could not be investigated within the scope of the study and require further research on these effects at plant-scale.

The additional energy demand, on the one hand, has to be considered in the economic feasibility analysis when investing in pretreatment technologies for lignocellulosic substrates such as horse manure. On the other hand, the energy demand is negligible considering the advantages such as making lignocellulosic residues available for the biogas process, the higher methane yield and faster degradation process.

Especially for the practical scale and the economic evaluation of a biogas plant, the use of low-cost residual substrates and agricultural by-products such as horse manure, straw or other lignocellulosic substrates, which are rather difficult and uneconomical to use for anaerobic digestion without pretreatment, is of great importance.

Hofmann et al. [67] consider the experiments on anaerobic digestion as a basis for a further comprehensive economical evaluation, which has to take into account costs, revenues, and energetic aspects. In their view, the disintegration technology needs to be evaluated for a profound decision on the benefits of the technology, regardless of the disintegration method applied.

In further trials with the ball mill, an optimum between milling intensity and specific energy consumption must be found in order to achieve a practical substrate flow rate and thus an economical operation of the ball mill.

The particle size analysis shows the clear reduction of particle size especially for the sample pretreated at 6 rpm compared to the untreated one. Combined with the results of the faster degradation kinetics, the higher specific methane yield in batch digestion and the changed surface morphology observed in the microscopy images, it can be concluded that the milling process of the colliding grinding media in the ball mill had a disintegrating and defibrating effect on the lignocellulosic horse manure.

Conclusion

The results show that mechanical pretreatment with the ball mill leads to a significant reduction in particle size and a significant increase in specific methane yield ($243 \pm 8\ L_{CH4}\ kg_{VS}^{-1}$), which corresponds to an increase of up to 37.3% compared to the untreated variant and thus a positive energy balance.

The lowest speed of 6 rpm in particular resulted in a longer treatment time in the ball mill and thus a more intensive treatment process, which significantly improved the kinetic performance and lead to a lower methane production lag phase (λ).

Since the experiments on specific methane yield were conducted on a laboratory scale, further trials on a full-scale are required. In particular, further investigations will be important to obtain information about the transferability of the methane yields from the laboratory scale experiments to plant-scale operation. In addition, other lignocellulosic substrates could also be tested and evaluated in terms of rheological characteristics, formation of floating layers, energy consumption and, more generally, the economical evaluation of the mechanical pretreatment process performed by the ball mill.

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Data Availability The data sets generated and analysed in this study are available upon request from the corresponding author.

Declarations

Competing interests The manuscript is the authors' original work and has not been previously submitted to Waste and Biomass Valorization. The authors declare that they have no known competing financial interests or personal relationships that could influence the work in this paper.

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