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Silage effluent production from ensiled sugar beet chips

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The use of sugar beets as a biogas substrate has been increased in popularity as it offers numerous advantages such as high biomass yield and high degradation rates. A major problem for using sugar beet silage as a substrate in anaerobic digestion processes is the high storage losses due to the intense silage effluent production. Knowledge regarding produced silage effluent quantity and the influencing parameters are required for the further development of the storage process. Therefore, the influence of stack height and particle size on ensiled sugar beet chips using mass balances was the focus of this investigation. The effluent production can achieve over 50% of the ensiled fresh mass. The tests have shown variable nature of the formation of silage effluent. About half of the total amount of silage effluent was already formed during the first 3 weeks. The produced effluent was characterized by consistently extremely high COD values of around 250 g l^{-1}. In these experiments the most effluent and carbon dioxide were achieved from the 5 m columns with silage of big sugar beet chips. The variant 2 m columns with small sugar beet chips silage showed the highest amount of achieved silage based on the stored fresh mass.

Keywords
Sugar beet, silage, effluent, mass balance, preservation, biogas, substrate

Due to the finiteness and the negative environmental impact of fossil fuels, the German government aims to increase the share of renewable energies in total energy consumption by 30% by 2030 (Berlin Energy Transition Dialogue 2016). Biogas production makes an important contribution to the realization of these goals. One problem for Germany is that the production of biogas from renewable raw materials is based on corn silage, which can lead to one-sided crop rotation. In addition, a total of about 2.55 million hectares of maize cultivation in Germany grown in 2015 provided for about 0.85 million hectares of energy maize (Fachagentur Nachwachsende Rohstoffe e.V. 2016). For ecological reasons, economically equivalent substrates are desired for biogas production.

In recent years, sugar beet has increasingly been used as an alternative biogas substrate. The most important advantages are high biomass yield, high biogas yield (Mioduszewska et al. 2009, Weissbach 2009) and high degree of degradation (Krakat et al. 2010). According to Jacobs et al. (2016), sugar beet roots are an economical and ecological substrate alternative for biogas production, especially for regions where there is a need to change crop rotation or to reduce the share of maize cultivation. Sugar beet quality can be affected by various parameters, such as sugar beet variety, environment, plant cultivation, ensiling and beet storage. The cultivation and harvesting of sugar beet are well mechanized, but storage, however, needs to be improved (Bischoff et al. 2017). Various ways of storing sugar beet for anaerobic digestion are described in the literature (Bischoff et al. 2017, Deininger 1995, Heilmann

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2013, Schattschneider et al. 2011). The most advantageous conservation method could be ensiling, as the silage is available all year round and the storage period can last until the next harvest.

During ensiling of the sugar beets, silage effluent formation can be intense, which accounts for a large part of the losses. The exact knowledge about the time dependent silage effluent production and the expected quantities are therefore decisive for a successful application.

Ensiling process of sugar beet

The chemical change during the sugar beet ensiling process described Olsen (1951). It mentions that the heterofermentative formation of lactic acid lasts until the sucrose is used up. When sucrose is no longer available, acid fermentation begins and forms butyric acid, acetic acid, alcohols, carbon dioxide and hydrogen.

A different view is presented by Weissbach (2009) who states that during the ensiling process of sugar beet, mainly lactic and acetic acids are formed. Other lower fatty acids are present only in very small quantities. Vargas-Ramirez et al. (2013) confirms that under acidic conditions sucrose is hydrolyzed into glucose and fructose. According to Weissbach (2009), after the completion of lactic fermentation, the remaining sugars are fermented by yeasts to ethanol, and almost half the weight of the converted sugars escapes in the form of carbon dioxide. According to Laube (1967), this alcoholic fermentation during the ensiling process can not only reach the magnitude of the acid fermentation, but even exceed it. Low storage temperatures are favorable for alcoholic fermentation.

In literature, the alcohols found in the sugar beet silage differ. The most commonly mentioned alcohols are ethanol, methanol, propanol and butanol (Dirks et al. 2017, Weissbach et al. 2013). Erdeljan (1994), however, suspects the presence of mannitol as an additional, never previously analyzed component of sugar beet silage. There is not much information in the literature relating to the formation of mannitol during the sugar beet ensiling process, possibly due to the difficult detection of its presence. This assumption is verified by appropriate feed analyses during experiments by Deininger (1995). Wojtczak et al. (2013) also confirm the presence of mannitol in sugar beet silage effluent.

Mannitol is a polyol (sugar alcohol) which is produced in large quantities by various heterofermentative lactic acid bacteria that use fructose as an electron acceptor (Wisselink et al. 2002). These bacteria are able to completely convert fructose into mannitol from a mixture of glucose and fructose (1:2) (Saha and Racine 2011). As a result of these bacteria, glucose and fructose are finally converted to lactic acid, ethanol, carbon dioxide, acetic acid and mannitol. Mannitol dehydrogenase is a key enzyme involved in mannitol production. It is also known that a number of heterofermentative lactic acid bacteria, yeasts and filamentous fungi produce mannitol (Lee 1967, Saha and Racine 2011, Song and Vieille 2009). Homofermentative lactic acid bacteria ferment lactose into lactic acid with the only trace of other products (Carr et al. 2002, Song and Vieille 2009).

According to Buxton et al. (2003) and Herrmann (2010), the ensiling process can be divided into four phases. The first phase is referred to as the initial aerobic phase and involves the respiration of the residual oxygen trapped between the ensiled materials. As a result of the second phase of the ensiling process, the main anaerobic fermentation, gases and silage effluent are produced. The length of this phase depends on the properties of the plant and the conditions of the ensiling, and may last a week or longer than a month. The third phase, which is characterized by a slowing of the rates of change, is called “anaerobic storage”. The last phase, the extraction phase, begins after the silo opening. Due to the penetration of oxygen into the ensiled material, its chemical composition can change, thereby inducing an increase in yeast, fungi and acetic acid bacteria.

According to Weissbach and Parr (2013), the absence of oxygen results in the death of cellular tissue, which results in the release of nutrient-rich silage effluent. Mauritz (1992) describes the silage effluent production as a discontinuous process that starts shortly after filling the silos. Due to its high acidity, silage effluent is extremely corrosive (Gebrehanna et al., 2014). According to Wagner et al. (2010), the produced silage effluent is energetically just as valuable as the remaining sugar beet silage. In (Jones and Jones, 1995), it can be found that the amount of silage effluent produced during the ensiling of wet crops, such as beet tops, can reach 500 l t^{-1} of fresh mass. According to Mauritz (1992), low content of dry matter, in the case of fodder beet, has a positive effect on increasing of the silage effluent production.

Carbon dioxide is the final product of heterofermentative lactic acid fermentation and alcohol fermentation (Küntzel and Zimmer, 1972). According to Kreuger et al. (2011), a maximum of 3.3% of stored beet weight is released during the ensiling as CO_2.

Processes of sugar beet storage

The cultivation of sugar beets for biogas production does not differ from the cultivation of sugar beet for sugar production (Bischoff et al., 2017). For all storage methods, the dry cleaning process steps during loading, stone removal and washing are recommended after harvesting. Therefore, the same costs of provision are assumed. Today's most popular sugar beet storage methods are storing in a pile at the edge of the field, bunker silo, high silo, lagoon, mixed silages and silage plastic bags (Schattschneider et al. 2011, Bischoff et al. 2017). These methods differ in the form of the substrate in which it is stored (whole beet, sugar beet chips, beet pulp). For the mixed silage, whole or shredded beet with a mixing partner were stored in bunker silo (Bischoff et al. 2017). The storage losses, for example, due to large amounts of high-energy seepage juice, should be reduced by mixed silage (Bugdahl, 2013). The choice of mixing partner depends on the required consistency of the mixed silage. The most commonly used mixing partners are silage maize, ground ear maize (LKS), corn cob mix (CCM) and straw (Bischoff et al., 2017).

Bischoff et al. (2017) presented the advantages and disadvantages of different storage methods for sugar beet as well as the amount of the resulting storage losses. According to his information, the highest oDM losses occur during the storage of whole sugar beet in the bunker silo (16–40% at 30% silage effluent accumulation), while the lowest oDM losses are due to storage at the edge of the field (5–15%). In comparison, the oDM losses for storage of the sugar beet pulp are 10–18% in high silos and 13.8–23% in basins. This corresponds to the results of Vazifehkhoran et al. (2016), who found an average oDM loss of 28.63% during the preservation of sugar beet pulp in open silos. However, there are no data available on the storage of silage from chopped sugar beets and there is no known storage method that allows cheap and low-loss storage (Wagner et al. 2010).

According to Kröhl et al. (2013), silage effluent in the amount of approximately 30 to 40% or even over 50% of the fresh weight (Deininger, 1995) could be produced. Since these values vary greatly, it is important to know how much silage effluent is formed and what the influencing parameters under experimental conditions are. The aim of this study is to gain accurate knowledge about the processes that occur during the ensiling process of sugar beet chips and to carry out a mass balance of the process to determine the influence of particle size and stack height on the silage effluent production and the number of storage losses.

Material and methods

Experimental set up

The research presented in this paper was carried out in the laboratory of the State Institute of Agricultural Engineering and Bioenergy at the University of Hohenheim, Stuttgart. For this study, three upright Plexiglas columns (2 m in height and 0.3 m diameter) and six PVC columns (5 m in height and 0.3 m diameter) were constructed inside the institute building (Figure 1). At the bottom of each column, a ball valve for sample taking and effluent removal was attached. A sieve was built out of a perforated stainless steel sheet and installed at the bottom of the column to prevent clogging of the valve by sugar beet silage. At the top of each column, 500 l gas bags (Tesseraux Spezialverpackungen GmbH Bürstadt, Germany) were attached. The produced gas was accumulated in gas bags and analyzed, with the frequency of analysis depending on the actual gas production. The quality of the dried gas was measured by a gas analyzer (S710, Sick Vertriebs-GmbH, Düsseldorf, Germany) with respect to CO_2 and H_2. The quantity was measured by a drum gas meter (TG 20/5, Dr.-Ing. Ritter Apparatebau GmbH & Co. KG, Bochum, Germany) equipped with an electronic impulse device with a solution of 0.04 l pro impulse. The gas volume was always corrected to standard conditions (1,013 hPa, 273.15 K).

Figure 1: Experimental set-up for investigating the influence of stack height and particle size on the seepage formation of silage from chopped sugar beet (© Universität Hohenheim/S. Zielonka)

Tested variants

To determine the impact of stack height on the ensiling process, two different stack heights were tested: 2 m and 5 m. To determine the effect of the substrate particle size two different particle size classes of sugar beet chips were used. Three different variants were each tested in triplicate. In the first variant, the 2 m columns were filled with small sugar beet chips (abbreviation 2 s). In the second variant, the 5 m columns were filled with small sugar beet chips (5 s). As a third variant, the 5 m columns were filled with big sugar beet chips (5 b). To wash and crush the sugar beets, the beet wash “Gazelle” from Günter Schmihing GmbH (Melle, Germany) was used. Sugar beet chip sizes were varied by settings on the beet wash chopper drum. Samples of these different sized sugar beet chips of approximately 15 kg were taken in order to conduct a size distribution analysis. Each fragment was manually measured by means of a slide calliper. The mean of height, width and length served as a measure of its size. For ease of illustration, the means were assigned to size classes of 0.5 cm (Figure 2).

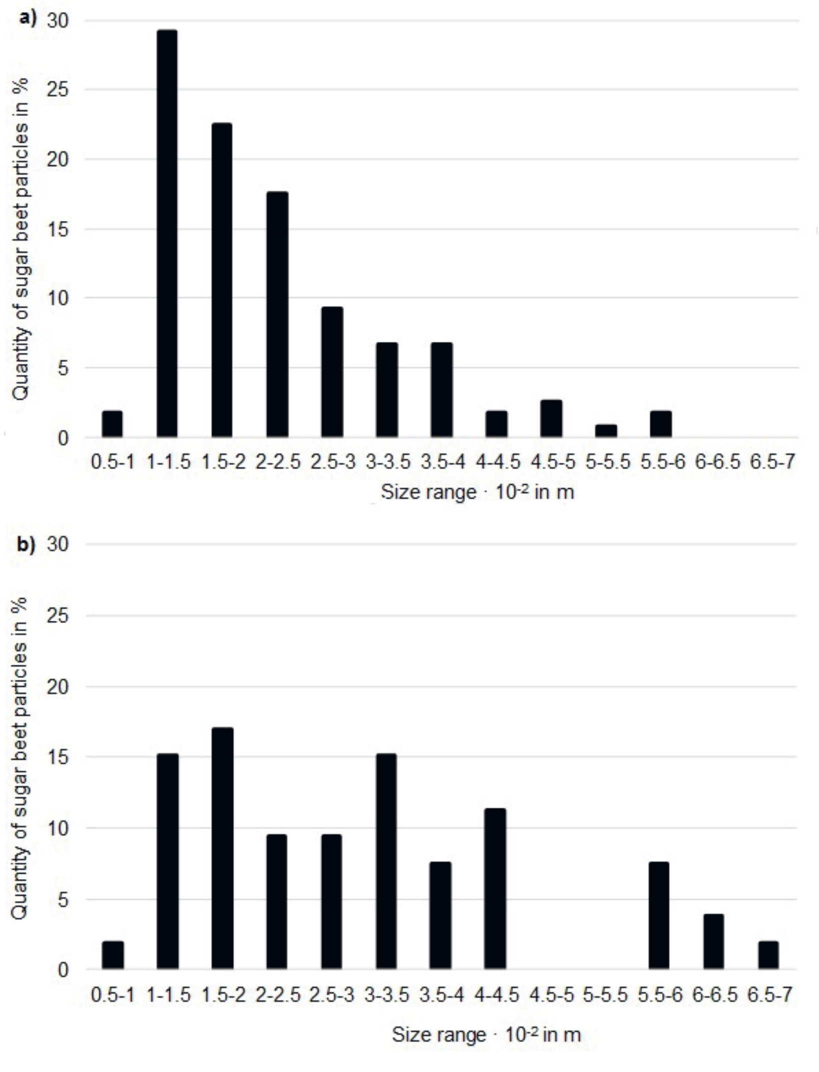

Figure 2: The size distribution of the particles of a) the small sugar beet chips (variant s) and b) the big sugar beet chips (variant b)

Experimental procedure

At the beginning of the experiment, the columns were filled with about 70 kg and about 235 kg, respectively, of sugar beet chips. For these experiments, the sugar beets from the agricultural research station Ihinger Hof (near Renningen, 50 km south west of Stuttgart) were used. After the harvest, the sugar beets were stored for about three months in a pile on a concrete floor at the research station. After filling the columns, the air was removed from their interior by a slight under pressure. The experiment was conducted for 364 days. The process was carried out without temperature control. The ambient temperature never dropped below 13° C and never exceeded 26 °C. The silage effluent was taken every day and later less frequently, depending on the amount of effluent. Each time the entire effluent collected in the column was taken and weighed, its temperature and pH value were measured. Samples of effluent were taken once a week for chemical analysis. Gas quality and gas volume were also measured. In order to determine the stack height reduction, the height of the sugar beet stack in the 2 m columns (Plexiglas) was measured once a week. In 5 m columns, the stack height was only determined after opening the columns at the end of the experiment.

Laboratory analysis

Fermentation products

Five different points in time were selected throughout the trial period when the silage effluent from each column was analyzed. Next, the average values were calculated for each variant tested. Each sample was analyzed in duplicate. The sample of sugar beet chips prior to ensiling and the silage samples from each column were analyzed. Each sample was analyzed in duplicate too. The content of acetic, propionic, butyric, valeric and caproic acids in silage effluent was determined with a gas chromatograph (GC, type CP3800 with flame ionization detector, capillary column WCOT Fused Silica, Agilent Technologies Germany GmbH, Böblingen, Germany).

For the analysis of the volatile fatty acids in solids, about 10 g of the sample material was mixed with 100 ml of distilled water and shaken for 1 hour on a sample shaker (Bühler Germany). The resulting suspension (depending on the expected fatty acid content in the samples of 2, 4, 8 ml) was acidified with 1 ml of about 17% orthophosphoric acid, then mixed with 1 ml of n-methyl-valeric acid (internal standard) and, if necessary, filled to 10 ml with the appropriate amount of distilled water.

Lactic acid and ethanol were detected with high-pressure liquid chromatography (HPLC type with RI-detector, BioRadAminex HPLC column HPX-87H, BioRad-precolumn HPX-87H, Bischoff Analysentechnik und –geräte GmbH, Leonberg, Germany). These analytical methods are described by Lindner et al. (2015). For the HPLC analyses of solids, approximately 10 g of sample material was mixed with 100 ml of double-distilled water and shaken for 1 hour on a sample shaker. The resulting suspension (depending on the expected content of sugar, alcohols and fatty acid in the samples of 2, 4, 8 ml) was acidified with 1 ml of 0.2 N sulfuric acids and, if necessary, the solution was filled to 10 ml with the appropriate amount of double-distilled water.

The determination of the sugar and mannitol contents was carried out with the HPLC-Ca method (HPLC type with RI-detector and Hyperchrome HPLC Column Repro Gel Approx, BISCHOFF Analysentechnik und –geräte GmbH, Leonberg, Germany). In order to determine the sugar and mannitol contents of the solids, they first had to undergo a non-retentive solid-phase extraction. In this way, the acids (and other interfering components) contained in the samples were filtered through or retained in the sample material using the solid phase of the sorbent as a “filter” for the sample. The total acid determination as described above must be carried out by GC and HPLC (Aminex method) in order to conduct the theoretical calculation for the capacity of the anion exchanger.

"Weender" analysis

In accordance with the European regulations for the "Weender" analysis (European Commission 2009), the analyses for crude ash (XA), crude protein (XP), crude fat (XL) and crude fibre (XF) were carried out.

Dry matter (DM)/organic dry matter (oDM)

The DM/oDM contents of sugar beet chips, sugar beet chips silage and silage effluent were determined in triplicate repetition by drying (pre-drying at 60 °C for 48 h, final drying at 105 °C for 3 h) and ashing (550 °C, for 8 h). The DM contents of the sugar beet silage and the silage effluent were subjected to a fermentation product correction. For this purpose, the methods mentioned in the literature (Weissbach and Strubelt, 2008) were not used, as they do not take into account some of the specific fermentation products. In order to maintain a more accurate result, the silages were examined for their content of fermentation products before and after drying. The DM content was directly corrected based on the mass loss of volatile fermentation products. To correct the DM content of the silage effluent, its fermentation products content was determined. From the fermentation products losses of the associated silage, a quotient of volatility was calculated, since for analysis after drying, insufficient residues of the silage effluent were left over. The DM content determined by the drying was corrected on the basis of the specific fermentation products and their volatility quotients. Based on the corrected DM content, the oDM content was determined.

Chemical oxygen demand (COD)

The COD concentration in sugar beet silage effluent was detected using the cuvette test from Hach Lange (Hach Lange Type LCK 014) with a high temperature thermostat (Hach Lange Type HT200 S) and a sensor array photometer (Hach Lange Type LASA 20). To determine the COD in sugar beets, the suspension method was used, which is described in the Annex C of DIN 15936 (Analytic Jena AG, 2010). Firstly, the samples were finely comminuted by Thermomix (TM5, Vorwerk, Germany). Finally, 200 mg of the comminuted material was suspended with 200 ml of 0.22 N hydrochloric acid and then homogenized with the aid of an disperser (T25 Ultra-Turrax, IKA Labortechnik company, Germany). As a result, the inorganic sample constituents were decomposed and expelled. The produced suspension was then analyzed by the catalytic high-temperature oxidation, similarly to a liquid sample with a TOC TNb analyzer (multi N/C 2100 S; TOC: Total Organic Carbon, TN_b: Total Nitrogen).

Biomethane potential test (Hohenheim Biogas Yield Test - HBT)

In order to determine the value of biogas and methane yields, the silage effluent and produced sugar beet silage from each column were tested in HBT. Two HBT runs were performed. In the first run, the samples of the sugar beet and all of the sugar beet silage were examined. In the second run, the samples of the sugar beet silage effluent were examined. This is a highly repetitive batch digestion test according to the VDI guideline 4630 (Helffrich and Oechsner 2003, Mittweg et al. 2012, VDI 2006). Reactors, in this case 100 ml glass syringes, were filled with 0.8 g of sugar beet or sugar beet silage or 1.5 g silage effluent sample material and 30.0 g inoculum. Each variant was examined in triplicate. As a null variant and for seeding sludge correction, seeding sludge was fermented too. In addition, two standard substrates, hay and concentrated feed, were investigated in the same HBT run. These are used to check the fermentation process, as well as to compare different batch approaches with each other. The reactors were mixed with a rotor mounted in a climate chamber. The process was carried out at 37 ± 0.5 °C for a period of 35 days. The gas volume in milliliters was determined by reading the filling level directly from the glass syringe with an accuracy of 1 ml under the condition that at least 20 ml of gas has been created. How often the measurements were taken varied between approximately once every two days and four times a day, depending on the amount of gas produced. The AGM 10 gas transducer (Pronova Analysetechnik, Berlin, Germany) was used to determine the gas quality. The measured gas volume had to be corrected to standard conditions (0 °C and 1013 hPa). The results of gas and methane yields were related to the content of volatile solids in the substrate (Helffrich and Oechsner 2003, Mittweg et al. 2012, VDI 2006).

Calculations

The conversion of the norm volume of produced carbon dioxide to kg was necessary to carry out the mass balance of the process. Taking into account the molar volume of an ideal gas (22.414 l mol^{-1}), it was possible to calculate how many moles corresponded to the amount of obtained carbon dioxide. The molar mass of CO_2 (44.01 g mol^{-1}) was used to calculate the amount of produced carbon dioxide in kg.

The statistical analyses of the data were performed using the R studio statistical software (version 3.2.3). The Statistical Procedures for Agricultural Research (R package ‘agricolae’ version 1.2-4) were used. To determine whether there were significant differences, the nonparametric Kruskal-Wallis test was used. As a post hoc test, the Tukey test was then applied. An analysis of variance was performed to test the influence of particle size and stack height on effluent and carbon dioxide production.

Results

Mass and COD balance in the ensiling process

After the columns tests, a mass balance of the process was carried out in order to estimate the potential losses that may have occurred during the storage (Table 1). To calculate the mass balance of the process, the amount of all the products produced from the ensiling process was converted to kg. For direct comparison and to implement the statistical analysis, the specific amounts of products of the ensiling process were calculated. For this purpose, the mass of product (silage or silage effluent) was divided by the mass of the input material (fresh weight of sugar beet in kg).

Based on the analyses carried out, the COD balance of the process was also performed. The COD mass of carbon dioxide was zero because CO_2 cannot be further oxidized (De Lemos Chernicharo, 2007). The amount of produced hydrogen is so small that its COD mass can be omitted from the calculations. In Table 1, the average specific methane yield (SMY) of sugar beet chips and obtained sugar beet silage, which were obtained from the batch tests (HBT), is presented.

Table 1: Results of the mass balances, the COD balance and the biomethane potential tests of all variants used (2s: 2 m columns, silage of small sugar beet chips, 5s: 5 m columns, silage of small sugar beet chips, 5b: 5 m columns, silage of big sugar beet chips; oDM values are corrected for the fermentation products)

	2 k (n = 3)	**5 k (n = 3)**	**5 g (n = 3)**
Mass balance			
Sugar beet in kg	82.09 ± 1.77	237.10 ± 2.43	240.00 ± 1.69
Silage in kg	49.75 ± 6.73	118.08 ± 16.77	101.16 ± 7.46
Silage effluent in kg	34.59 ± 0.94	112.54 ± 17.06	130.81 ± 8.92
CO_2 in kg	2.06 ± 0.31	5.34 ± 1.11	7.03 ± 1.16
Organic dry mass balance			
Sugar beet in kg oDM	20.50 ± 0.44	59.19 ± 0.61	59.92 ± 0.42
Silage in kg oDM	12.19 ± 1.65	33.89 ± 4.81	29.34 ± 2.16
Silage effluent in kg oDM	7.94 ± 0.22	25.45 ± 3.86	28.70 ± 1.96
Specific mass balance			
Silage in kg kg^{-1} FM	0.61 ± 0.07^{b}	0.50 ± 0.07^{ab}	0.42 ± 0.03^{a}
Silage effluent in kg kg^{-1} FM	0.42 ± 0.01^{b}	0.48 ± 0.07^{ab}	0.55 ± 0.04^{a}
CO2 in kg kg^{-1} FM	0.03 ± 0.004^{a}	0.02 ± 0.005^{a}	0.03 ± 0.005^{a}
COD-balance			
COD in sugar beet in kg	20.61 ± 0.44	59.39 ± 0.61	60.12 ± 0.42
COD silage in kg	17.41 ± 2.04	41.45 ± 3.98	36.63 ± 2.46
COD silage effluent in kg	8.81 ± 0.27	28.64 ± 4.73	33.62 ± 3.53
Specific methane yields from BMP tests (HBT)			
SMY sugar beet in Nl kg^{-1}oDM		257.88 ± 84.92	
SMY silage in Nl kg^{-1}oDM	361.11 ± 18.84	333.12 ± 11.08	340.61 ± 31.54
SMY Silage effluent in Nl kg^{-1}oTS	334.16 ± 22.61	318.57 ± 18.63	300.92 ± 10.90

Note: Different superscript letters indicate significant differences ($p \leq 0.05$),
Mean and standard deviations of n repetitions.

The statistical analysis did not show significant differences in the specific silage effluent production between variants of the same particle size with different stack heights and variants of the same stack height with different particle size. The statistical analysis showed significant differences in the specific silage effluent only for variants 2 s and 5 b, thus on the combination of particle size of the substrate and the stack height. However, there were no identified significant differences in the specific production of CO_2.

The specific methane yields of the obtained sugar beet silage were higher than the specific methane yield of the sugar beet. The highest SMY was obtained for variant 2 s (361.11 ± 18.84 Nl kg^{-1} oDM) and the smallest for 5 s (333.12 ± 11.08 Nl kg^{-1} oDM). The theoretical methane yield of the sugar beets was calculated and estimated according to Weissbach (2009). It was equal to 395.41 Nl kg^{-1} oDM, so it was higher than the specific methane yields obtained in BMP tests.

Sugar beets

In order to describe the properties of the sugar beet, dry matter/organic dry matter, chemical oxygen demand, concentration of VFA, sugars, alcohols in sugar beets and pH analysis were conducted (Table 2).

Table 2: The results of the DM/oDM analyses, the COD and the concentrations of fermentation products (VFA, sugars and alcohols) in the sugar beet before and after ensiling for all variants used (2 s: 2 m columns, silage of small sugar beet chips, 5 s: 5 m columns, silage of small sugar beet chips, 5 b: 5 m columns, silage of big sugar beet chips; DM and oDM values are corrected for the fermentation products)

	Sugar beets (n = 1)	**2 k (n = 3)**	**5 k (n = 3)**	**5 g (n = 3)**
DM in % FM*	25.46	25.47 ± 0.49^{b}	29.90 ± 1.42^{a}	30.39 ± 0.80^{a}
oDM in % DM*	98.06	96.22 ± 0.53^{a}	96.00 ± 0.51^{a}	95.45 ± 0.10^{a}
pH-Value**	4.01	3.55 ± 0.03^{a}	3.55 ± 0.04^{a}	3.56 ± 0.02^{a}
COD solid in g l^{-1}**	251.00	351.17 ± 27.76^{a}	356.00 ± 12.82^{a}	363.33 ± 21.39^{a}
Acetic acid in g kg^{-1} DM**	12.07	40.97 ± 1.87^{a}	40.46 ± 1.54^{a}	36.05 ± 2.59^{a}
Propionic acid in g kg^{-1} DM**	1.00	0.00	0.00	0.00
n-Butyric acid in g kg^{-1} DM**	4.96	0.00	0.00	0.00
n-Valeric acid in g kg^{-1} DM**	0.29	0.14 ± 0.12^{a}	0.12 ± 0.11^{a}	0.13 ± 0.11^{a}
Lactic acid in g kg^{-1} DM**	9.82	53.40 ± 4.64^{a}	43.03 ± 2.16^{a}	46.45 ± 6.29^{a}
Sucrose in g kg^{-1} DM**	55.36	1.19 ± 2.07^{a}	4.61 ± 0.09^{a}	1.96 ± 1.83^{a}
Glucose in g kg^{-1} DM**	28.38	37.56 ± 18.64^{b}	83.94 ± 5.88^{a}	67.74 ± 24.67^{ab}
Fructose in g kg^{-1} DM**	17.42	18.73 ± 11.60^{a}	13.40 ± 11.76^{a}	8.01 ± 4.05^{a}
Ethanol in g kg^{-1} DM**	51.45	205.34 ± 62.86^{a}	79.04 ± 9.61^{b}	115.39 ± 26.40^{ab}
Mannitol in g kg^{-1} DM**	15.54	109.41 ± 31.55^{a}	145.66 ± 22.21^{a}	118.02 ± 32.90^{a}

Note: Different superscript letters indicate significant differences (p ≤ 0.05),
Mean and standard deviation from n samples,
* Triplicate.·
** Duplicate.

Sugar beet silage

The results of the dry matter-/organic dry matter content, chemical oxygen demand and pH value analysis of the obtained sugar beet silage are presented in Table 2. Significant differences between silage stacks containing small sugar beet chips were noted only in the concentrations of glucose and ethanol. The significant differences between silage produced in 2 m and 5 m columns were also found in DM-content.

After comparing the properties of the sugar beet before and after the ensiling process, it was found that acetic acid content increased more than three times during the ensiling process and the content of lactic acid – more than six times. The sucrose during this process was almost completely decomposed. The content of glucose and fructose in the silage increased in comparison with sugar beet. The largest differences between sugar beet silage and sugar beet were noted in alcohol content. It was also noted that there was an increase of 30% in COD content during the ensiling process.

Silage effluent

At first, intensive effluent production was observed. This decreased after about 2–3 weeks. The change in the rate of silage effluent production for all variants was noted between the 14^{th} and 19^{th} days of the tests. Until then, the following results were obtained, respectively: 17 kg silage effluent, representing 49% of the total production (2 s), 55 kg silage effluent, representing 49% of the total production (5 s) and 53 kg silage effluent, representing 40.5% of the total production (5 b) In Figure 3, the average cumulative production of sugar beet silage effluent in kg kg^{-1} stored weight is presented.

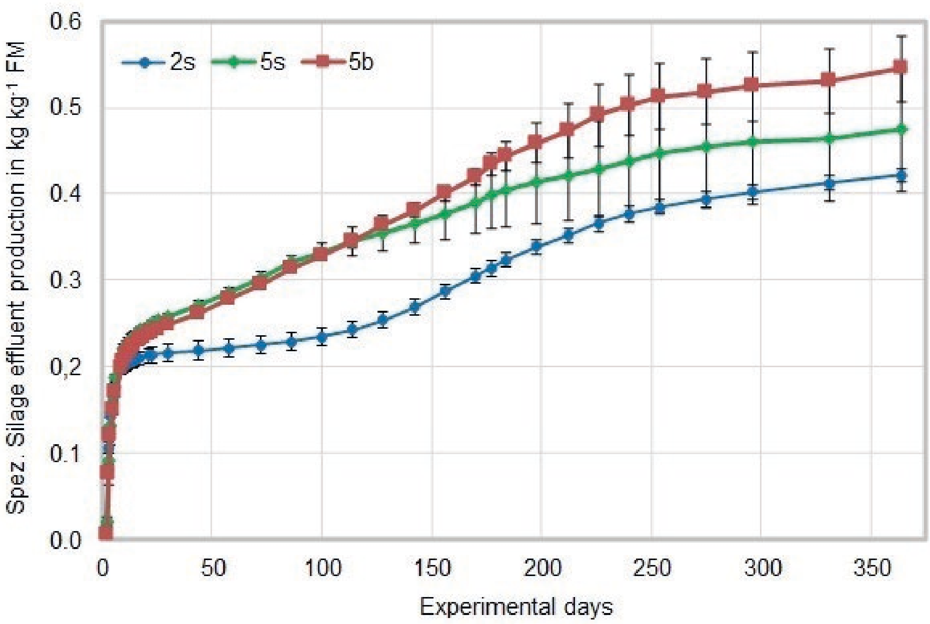

Figure 3: Specific silage effluent production from sugar beet chips silage for all variants used (2 s: 2 m columns, silage of small sugar beet chips, 5 s: 5 m columns, silage of small sugar beet chips, 5 b: 5 m columns, silage of big sugar beet chips)

Sugar beet silage effluent was characterized by extremely high and stable COD-values of 250.00 g l^{-1}. The pH value of the sugar beet silage effluent was about 3.9 and decreased more with storage time. After about 2.5 months, the rate of decrease in pH began to change. The final achieved pH value was about 3.38. Changes in the pH value of the silage effluent were accompanied by changes in its composition. Figure 4 shows the changes of the pH values and contents of acetic acid, lactic acid, sucrose, glucose and alcohols in the silage effluent for all variants used.

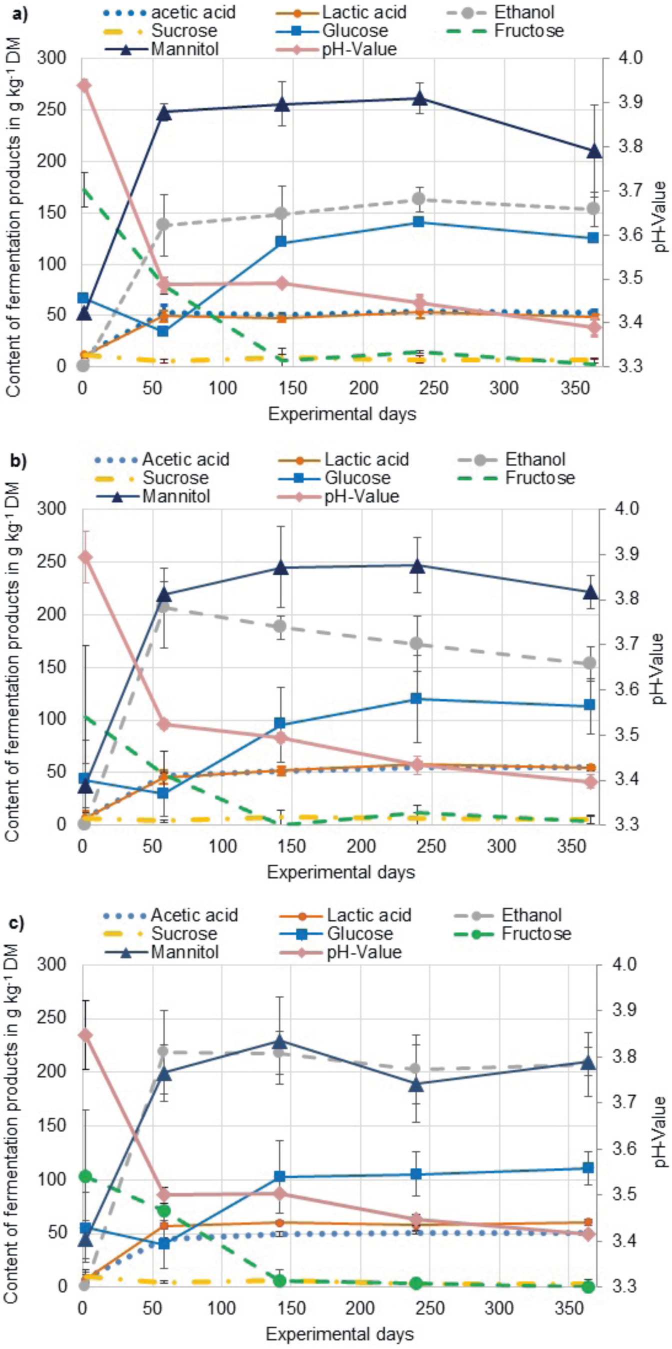

Figure 4: Changes in the composition and pH value of the sugar beet silage effluent a)b2 s: 2 m columns, silage of small sugar beet chips, b) 5 s: 5 m columns, silage of small sugar beet chips and c) 5 b: 5 m columns, silage of big sugar beet chips

At the beginning of the silage effluent production (experimental day 2), the generated effluent contained the most glucose and fructose. The process of sucrose breakdown into fructose and glucose was practically completed and sucrose was only present in small amounts. In the course of the experiments, until about the 58th day, the contents of fructose and glucose sharply dropped due to the conversion of sugars into alcohols and acids, especially into mannitol and ethanol. The amount of mannitol in the silage effluent for silage of small sugar beet chips exceeded even the amount of ethanol. Alcohol production slowed down later. Around the 58th day of the experiment, the production of acid finished too. Finally, their concentration remained practically unchanged.

The changes in the composition of silage effluent were similar for all variants used, although different values of concentration of sugars and alcohols were found. The largest differences were found in ethanol concentration, which in the case of 5 m columns were almost twice as much as in 2 m columns.

Sugar beet stack height reduction

According to the change in silage effluent production, the rate of reduction of the sugar beet stack height changed. Changes in the sugar beet stack height over time in 2 m columns are presented in Figure 5.

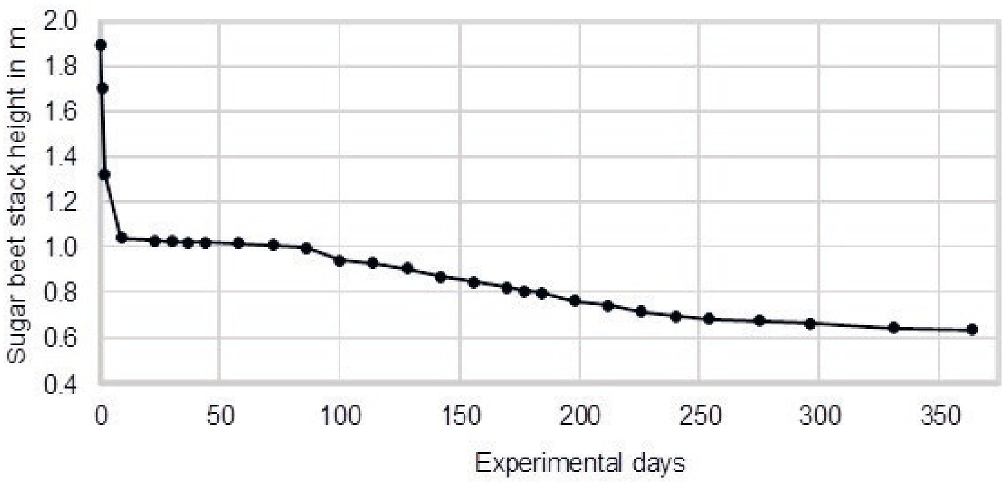

Figure 5: Reduction of sugar beet stack height in 2 m columns, silage of small sugar beet chips (variant 2 s)

After filling the columns, the stack height was 1.90 ± 0.00 m. Until the 9th day, the stack height was reduced to about 55% (1.04 ± 0.003 m). After that, the stack height decreased more slowly, and it was reduced to about 50% after 106 days (0.94 ± 0.003 m). When the experiments were complete, after 364 days, a stack height reduction to about 33% (0.63 ± 0.002 m) was observed. In the case of 5 m columns, the final reduction of the sugar beet stack height could only be determined. After filling the columns, the stack height was about 4.90±0.05 m. The average stack height was reduced to 31.67% (1.55 ± 0.21 m) for variant 5 s and to 26.50% (1.34 ± 0.1 m) for variant 5 b.

The bulk density of the stacks, calculated from the inner diameter of the columns and the stack height relative to their mass, after filling the columns for the 2 s variant was 611 ± 13 kg m^{-3}, for 5 s 714 ± 7 kg m-3 and 5 b 707 ± 5 kg m-3. After completion of the experiment, strong compaction of the stacks could be observed. The density of the silage stacks at the end of the experiment was 896 ± 14 kg m^{-3} for the variant 2 s, 1080 ± 17 kg m^{-3} for 5 s and 1065 ± 13 kg m^{-3} for 5 b. Thus, the stacks roughly reach the density of sugar beets (Kromer et al. 2004). This can be confirmed by our own observations. The stacks were almost dimensionally stable after emptying and contained only a very small pore volume.

Gas production

The vast majority of produced gas consisted of carbon dioxide with trace parts of hydrogen. The average cumulative production of carbon dioxide during the ensiling of sugar beet chips expressed in kg kg^{-1} of stored mass is shown in Figure 6.

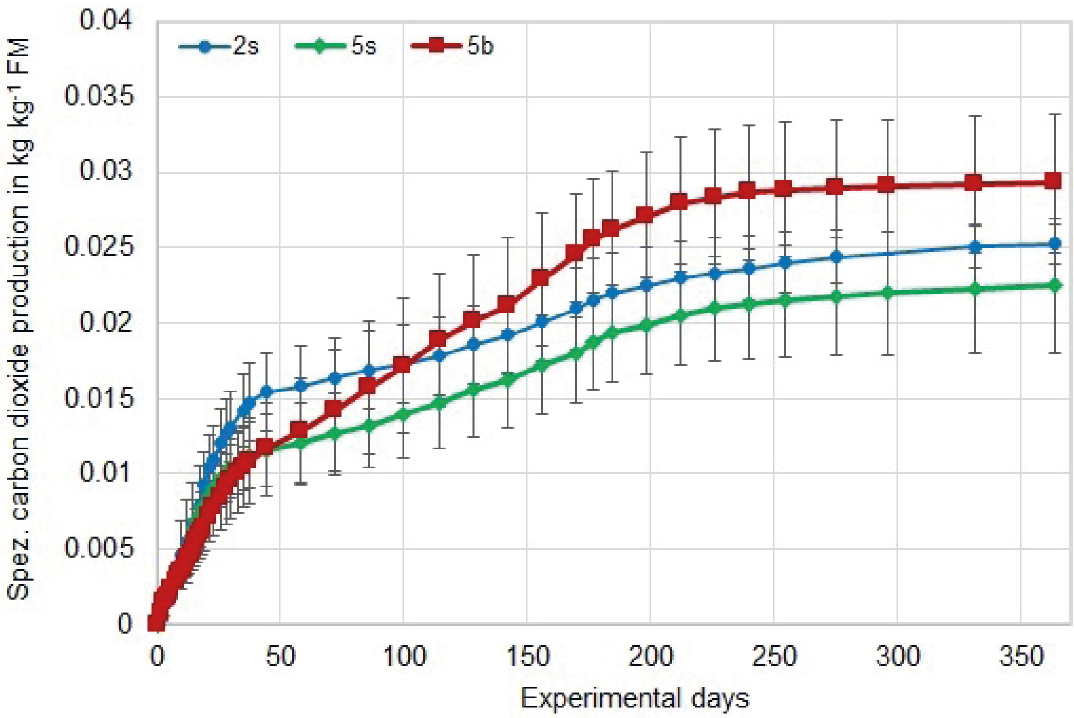

Figure 6: Specific carbon dioxide production from sugar beet silage for all variants used (2 s: 2 m columns, silage of small sugar beet chips, 5 s: 5 m columns, silage of small sugar beet chips, 5 b: 5 m columns, silage of big sugar beet chips)

At the beginning of the process, intensive carbon dioxide production was noted. Around the 44th day of the experiment, carbon dioxide production decreased in all three variants. At the beginning of the experiment, the highest specific carbon dioxide production was observed for variant 2 s. However, on day 100 of the experiment, the volume of carbon dioxide for the variant 5 b exceeded the volume obtained for variant 2 s. From the 100th day, the highest average specific carbon dioxide production was observed for variant 5 b. The lowest specific carbon dioxide production was always observed for variant 5 s.

Discussion

The average amount of produced silage effluent obtained from 2 s, 5 s and 5 b was 42.13 ± 1.00% (2 s), 47.50 ± 7.00% (5 s) and 54.50 ± 4.00% (5 b) of the stored weight, respectively, which was consistent with the literature (Deininger 1995, Kröhl et al. 2013). In this study, averages of 0.03 ± 0.004, 0.02 ± 0.005 and 0.03 ± 0.005 kg of CO_2 (for 2 s, 5 s and 5 b, respectively) were produced per kg of stored sugar beet. These values were consistent with the data presented for the ensiling of sugar beet (maximum 3.3% of stored sugar beet weight) by Kreuger et al. (2011).

An effect of particle size and stack height on specific silage effluent and on specific CO_2 production was not found. Only the combination of both parameters had an effect on specific silage effluent production. However, no statistically significant differences in specific CO_2 production could be demonstrated.

High COD values of silage effluent of around 250.00 g l^{-1}, which corresponded to high concentrations of soluble organics and low pH values, were consistent with the thesis of Wagner et al. (2010) that the produced silage effluent is just as valuable as the remaining sugar beet silage.

The highest losses were found for 5 b, which was consistent with Mauritz (1992), who found that the amount of dry matter in the material, the density of the stored material and the stack height influenced losses during the ensiling process. However, the statistical analysis showed no significant differences in the number of losses between the variants used. Low content of sucrose in sugar beet before the ensiling process, only 55.36 ± 0.41g kg^{-1} DM (Table 2), was found. It could be concluded that there were losses in sugar content during the storing of sugar beet in a pile at the edge of a field. According to Bischoff et al. (2017), the sugar content (in fresh beets, mainly sucrose) is 658.6 g kg^{-1} DM. According to Schmitz and Kromer (2004), the size of the losses in sugar content depends on the storage time, the sugar beet cover and the weather conditions in the region. Since a certain period of time between the harvest and the beginning of storage cannot be avoided, it is advantageous for the actual storage trial to take the first sample at the storage beginning, otherwise the losses between the harvest and the beginning of storage must be included in the balance. According to Herrmann (2010), reducing the particle size of the plant material directly improved the fermentation conditions associated with the rapid formation of acids and rapid reduction of the pH value. The observation of the faster pH reduction in the silage effluent from small sugar beet chips in own experiments supports these findings. The largest differences were found in dry matter contents, glucose and ethanol content.

The observed changes over time in silage effluent and carbon dioxide production could be described with logarithmic functions. The change in the intensity of silage effluent production observed within the first 20 experimental days was similar to the observations of other researches, according to which the peak of silage effluent production flows typically occurred within 10 d after ensiling (Gebrehanna et al. 2014). Küntzel and Zimmer (1972) recorded the same change in the gas formation after the 14th day of the ensiling. Under the conditions of this study, the changes described in the literature took place almost twice as long. This fact could be explained by Gebrehanna et al. (2014), according to whom several factors, such as plant factors, weather conditions and mechanical or chemical harvesting treatments, affected effluent production rates. Also, Herrmann (2010) confirmed the effect of the dry matter content of plant and the extent of its fragmentation on the silage effluent production.

The results of storage tests were confirmed partially by Mauritz (1992), who stated that the reduction of the particle size causes the destruction of plant tissues, and the larger it is, the faster the silage effluent appears. At the beginning of the study, the fastest silage effluent production was found for variant 5 s. However, this was surpassed after 114 days of the variant 5 b. This meant that more silage effluent was produced from larger stack heights and also from larger sized particles. The silage of small sugar beet chips made it difficult for the silage effluent, through its higher surface area and storage density, to permeate through the stack. The analysis of the DM/oDM contents of the obtained sugar beet chip silage confirmed this: the dry matter content was higher in the case of silage of big sugar beet chips (Table 2).

According to Schuldt et al. (2011), the silage effluent has a methane yield at the level of non-ensiled, fresh sugar beet, thanks to very high levels of ethanol and fermentation acids. The results of the HBT test have even proved that the silage effluent has a higher specific methane yield than the fresh sugar beet (Table 1). According to Buxton et al. (2003), the mixture of lactic and alcoholic fermentation during the sugar beet ensiling process lead to significant losses in dry matter, but only to a low reduction of the methane production potential due to the production of high-energy ethanol. According to Weissbach (2009), the methane production from ethanol (693 l kg^{-1}) is almost twice as high as the production of methane from lactic acid (355 l kg^{-1}). In these experiments, at least a doubling of the concentration of ethanol was observed during the ensiling process.

On the other hand, Herrmann (2010) claimed that the losses incurred during the ensiling process were caused by fermentation processes. Dirks et al. (2017) stated that the alcohol production during the ensiling process had a significant effect on the losses. This thesis confirmed the results of the ethanol content analysis in silage effluent. The highest ethanol content was found for the variant 5 b, where the highest CO_2 production was also reported. Accordingly, the lowest CO_2 production was found for the variant 5 s, of which silage effluent had the lowest content of ethanol. The opposite was observed regarding the content of mannitol in silage effluent. The lowest mannitol content in the silage effluent was found in the variant 5 b. The observed stack height reduction to 33%, 31.67% and 26.50% (for 2 s, 5 s and 5 b, respectively) corresponded to the quantity of produced silage effluent.

Conclusions

The fast effluent production and the huge share of effluent have to be recognized ensiling sugar beet chips. The high COD content and its easy digestibility make the effluent an interesting option for its use as a liquid substrate, e. g. for demand-driven biogas production.

The stack height and particle size did not show a significant influence on the mass balance. Only the combination of both had an effect on silage effluent production. The variant 2 s showed the highest amount of silage (silage: 60.50 ± 6.73% FM, effluent: 42.13 ± 1.00% FM, CO_2: 2.51 ± 0.38% FM). While the variant 5 b produced the highest amount of effluent (silage: 42.15 ± 3.00% FM, effluent: 54.50 ± 4.00% FM, CO_2: 2.93± 0.50% FM).

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