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# Conservation of sugar beet for substrate supply of biogas plants – Procedure of ensiling and losses in 12 varieties

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Due to the criticism of the intensive cultivation of maize in Germany as well as the problem of more frequently occurring pests in crop rotation sugar beet has been used successfully as a substrate for biogas plants in recent years. Varieties with a high sugar yield proved to be particularly suitable. However, sugar beet has to be conserved and stored in order to integrate it into the process of biogas production throughout the entire year. Neither the success of conservation nor potential losses over the storage period have been documented so far. Batch tests have been used to investigate the course of conservation, watching the pH value and the losses while ensiling twelve different sugar beet varieties. The sugar beets were ensilaged as pulp under anaerobic conditions in a fourfold repetition for 90 days. In all variants, an almost parallel development of the pH value was observed. Although there were significant differences between the pH values of the different variants all pH values were sufficiently low to ensure safe conservation. Losses of methane forming potential after 90 days of storage fluctuated between approximately 10 and 20%. Alcoholic fermentation was identified as the reason for these losses. The results show that ensiling of different sugar beet varieties can be achieved quickly and safely. Also, alcoholic fermentation after acidification of the silage leads to considerable fluctuations in the level of losses, both the dry substance and the methane forming potential.

**Keywords**
Biogas, sugar beet pulp, ensilage, renewable raw material, losses, substrate storage

Due to its high dry matter yield and the resulting methane yield (e.g. 4163 $m^3 \cdot ha^{-1}$ at a yield of 65 t $FM \cdot ha^{-1}$) (KTBL 2012) and the equally rapid degradability in the fermenter, sugar beet is increasingly used in biogas plants in classical beet cultivation areas. The possibility to break up the crop rotation – especially in regions with high maize ratios – is a positive side effect of cultivating sugar beet (Thaysen 2011). Sugar beet can only compete with maize under optimal conditions. However, by improving sugar beet processing and increasing yields by breeding, supply costs for sugar beet can be substantially reduced. This way sugar beet can be a competitive alternative to maize (Hartmann and Döhler 2011).

Generally there is no difference between the cultivation of sugar beet for the production of biogas and the cultivation for sugar production (FNR 2012a). Only during the harvest the processes differ. For biogas production it is sufficient to defoliate the sugar beet while for sugar production the crown is topped (Thaysen 2011). This is possible since the quality requirements, which are valid for the production of sugar (amount of potassium, sodium and amino-nitrogen), are not important for the biogas

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production. At the same time, a higher yield of 3–4% can be achieved simply by defoliateing the beet (Schulze Lammers and Roller 2010). For usage in biogas plants the sugar beet has to be cleaned. Depending on the location, stones have to be eliminated, too. Before adding sugar beet into the fermenter it has to be crushed. Depending on the process, crushing occurs before or after ensiling (Schaffer et al. 2011). Since the fermentation process in biogas plants is sensitive to disturbances, the supply of a constant substrate mixture is of great importance for the continuous and smooth operation of a biogas plant (FNR 2012b). Therefore, substrates of the same quantity and quality are used throughout the year. If sugar beets are to be integrated into the substrate mixture, the challenge is to make them available all through the year. Due to their low dry mass content of approximately 23%, various methods of ensiling are established in practice (KTBL 2012). Ensiling whole beets in a bunker, covered with a silage film, as well as crushed beets (pulp) in a lagoon or a tower silo, are the prefered methods (Beeck et al. 2014).

Throughout the ensiling process under anaerobic conditions, sugar is transformed into organic acids. This is mainly done by anaerobic milk and acetic acid bacteria. As a result, the pH value drops from about 6.5 to less than 4. Due to this effect, microorganisms such as (butyric acid) bacteria, yeasts and molds, which are undesirable in the silencing process, are suppressed. Soilings of the ensilage material can lead to an increased intake of butyric acid bacteria, which can result in an incorrect fermentation. (Kaltschmitt et al. 2009)

At the beginning of the ensiling the existing residual oxygen is depleted by the microorganisms naturally occurring on the silage material. When oxygen is blocked by silo closure, these microorganisms consume the oxygen within a few hours and replace it with carbon dioxide ($CO_2$). From this point onward the main fermentation phase begins and the plant tissue dies within a few days. As a result, the cell contents are free and can be used by the silage microflora (lactic and acetic acid bacteria). The silage is acidified. Until the pH value is reduced, the unwanted microbial groups, which can multiply without oxygen are also active. These include clostridia, listeria, bacillus species and yeast fungi. (Pahlow and Hünting 2011)

When a pH value lower than 3.5 has been reached, lactic acid fermentation is terminated. At this stage, bacteria are no longer metabolically active. Yeasts on the other hand, are still active even at low pH values and ferment the remaining sugar to ethanol. (Weissbach et al. 2013)

## Problem and task description

There is still little information about the losses occurring during the ensiling of sugar beet. Furthermore, previous studies focused on the determination of losses occuring due to the respective storage method. The influences of the variety and its sugar content on the process of ensiling as well as the occurring losses are unknown so far. The main fermenting phase for maize and grass silage has been described by Pahlow and Hünting (2011), while the fermenting phase for sugar beet has not yet been described. This shall be documented in a first experiment with twelve sugar beet varieties using the pH value.

Since sugar beets have favorable silage properties due to their high sugar content (Augustin et al. 2010), it can be assumed that no difference in the process of ensiling can be observed, even in case of different sugar contents and epiphytic lactic acid bacterial stocks.

The following hypothesis was set up: Despite of different initial sugar contents, the acidification of the material proceeds at a similar rate and intensity at the ensiling of sugar beet.

Previous studies to determine the ensiling losses have shown that in sugar beet silage it is mainly sugar, which is converted into lactic acid, acetic acid and the alcohols methanol, ethanol and propanol (Weissbach et al. 2013, Beeck et al 2014). Because of their different energy content, the quantity of these products is important for the calculation of the methane forming potential (Weissbach 2009).

The range of losses within a storage method, e.g. due to different sugar beet varieties with different sugar contents, has not been taken into account in previous studies. Whether this leads to different levels of alcohols and acids in silage has neither been subject to previous investigations. In order to follow up on this, the following hypothesis was set up: Ensiling of sugar beets under anaerobic conditions leads to varying losses depending on the variety, its sugar content and the resulting fermentation products.

## Material and methods

### Process of ensiling

Twelve sugar beet varieties from the plant breeders KWS SAAT SE, SES VanderHave, Strube GmbH & Co. KG and Syngenta were tested. The investigations were carried out as part of the project „Conditioning and conservation of sugar beets for use in NawaRo-biogas plants". The varieties were selected by the involved breeders and included varieties which are intended for the production of biogas.

In 2013 investigations about the development of the pH value during the ensiling of the varieties were made. In this year, three varieties of each breeder were cultivated on one site, which results in a range of twelve variants. The variation of varieties, locations and harvesting times was deliberately chosen to create initial conditions, which are heterogeneous (Table 1). The varieties were cultivated in stripes in a fourfold repetition. Sowing, fertilization and plant protection were carried out according to good professional practice. The sugar beets were harvested with a sugar beet lifter and were thereby defoliated.

Table 1: Labeling of the varieties, location and harvest date used in the experiment in 2013

| Breeder | Variety | Location | Soil type | Soil quality | Harvest date |
|---|---|---|---|---|---|
| Syngenta | Syngenta 1<br>Syngenta 2<br>Syngenta 3 | Bad Salzuflen | loess | 76–80 | 14.10.2013 |
| SES VanderHave | SES 1<br>SES 2<br>SES 3 | Euerfeld (Würzburg) | silty loam | 82 | 07.10.2013 |
| KWS SAAT SE | KWS 1<br>KWS 2<br>KWS 3 | Einbeck | loess/loam | 82 | 01.11.2013 |
| Strube GmbH & Co. KG | Strube 1<br>Strube 2<br>Strube 3 | Söllingen | loam | 85 | 25.10.2013 |

The sugar beets were stored in big bags after harvesting and processed approximately two weeks later. This should correspond to the usual period between harvest and ensiling in practice. Since there was only a small amount of adhering soil (3–5%), the beets have not been cleaned.

In each case, 100 sugar beets per variety were crushed with a beet crusher (BISO), designed for animal feeding. In order to achieve a fine structure of the pulp, the beet material was fed three times to the beet crusher. After crushing the beets, approximately 80% of the particles were smaller than 5 mm and 20% smaller than 15 mm. A sample of about one kilogram was taken from the composite sample of the 100 beets, which had been crushed. To suppress microbiological activity, the sample was packed in an airtight freezer bag and immediately frozen. The pH value was measured immediately in the fresh beet pulp with a pH measuring instrument (BlueLine 21). The silage tests were carried out in preserving jars (1.5 L) in accordance to the DLG guideline for the testing of silage additives for DLG quality label (DLG 2013). The glasses were each filled with 1,000 g of beet pulp and then closed by a preserving jar cover. The filling level was approx. 75% of the jar volume in order to avoid any losses of silage by foaming. The gas produced during the ensiling escaped by excess pressure. The silage was stored for 90 days in a climatic chamber (BINDER) at 15°C. The potential influence of environmental effects on the results has not been taken into account. For each variant, the experiment was carried out in a fourfold repetition (4 glasses, each containing one kilo of sugar beet pulp) from the pulp of the 100 crushed sugar beets.

During the first seven days after the beginning of the experiment, the glasses were opened once a day and the pH value was measured. The glasses were opened consecutively and only for the time of the measurement, then immediately closed again. Since the pH value settled at a constant level after approximately seven days, the glasses were not opened again in order to prevent any further influence by entering oxygen. After a storage period of 90 days, the glasses were opened and the pH value in the completely ensiled sugar beet pulp was recorded. The results of the pH value are calculated as the mean of the fourfold repetition.

For the analysis of the silages in the laboratory, a composite sample was formed from the fourfold repetition of each variety. The samples were packed in an airtight freezer bag and immediately frozen to suppress microbiological activity, which generally occurs when the sample gets in contact with oxygen. Both the samples of the fresh material as well as the composite samples of the ensiled material were then handed over to an external laboratory for analysis. The analysis was carried out by the laboratory for agriculture and environment „Blgg Deutschland GmbH“, since this laboratory had already gained experience with the analysis of sugar beet silage from previous projects. The analysis included the determination of the sugar content according to Luff-Schoorl in fresh sugar beet, as well as residual sugar content and organic acids by photometry and gas chromatography in the silage. The investigations on the development of the pH value were not repeated in the second experimental period, because of the high similarity of the gained results.

## Determination of losses

To determine the conservation losses, a calculation of the methane forming potential in fresh and ensiled sugar beet pulp was performed in 2015. In addition, the weight of the sugar beet pulp was measured at the beginning and at the end of the experiment. The same varieties were used as in 2013. Also the experimental design corresponded with the one from 2013. Cultivation of the varieties took place in one location (Bad Salzuflen) in 2015. The sugar beets were sown on 18.04.2015. Fertilization and plant protection were carried out according to good professional practice. The beets were harvested with a beet lifter on 25.10.2015. Thereby the beets were defoliated. The soil type at the designated location was loess. Until the processing on 04.11.2015 the sugar beets were stored outside in big bags as they were in 2013. All steps of preparation and ensiling were carried out similarily to the experiment from the year 2013.

The material filled in the preserving jars was weighed with a precision of one decimal (in g). After closing the preserving jars they were not opened until the end of the experiment. The jars were stored in a climatic chamber (Binder GmbH) under constant conditions at 15 °C. When the jars were opened after 90 days, the pH value was immediately determined and the material had been weighed. A composite sample from the ensiled Material of the fourfold repetition was taken. The samples were packed in an airtight freezer bag and immediately frozen. Since the analysis of the samples involves high costs, an analysis of all repetitions could not be carried out. Thus, the laboratory analysis were carried out from the composite samples of the fresh and the ensiled material only. Table 2 gives an overview, which components were analyzed in fresh and ensiled sugar beet pulp.

Table 2: Analyzed parameters in fresh and ensiled sugar beet pulp in 2015

| **Analyzed parameters** | **Fresh sugar beet pulp** | **Ensiled sugar beet pulp** |
|---|---|---|
| Dry matter ($DM/DM_c$ [1)]) | x | x |
| Crude ash (XA) | x | x |
| Sugar according to VDLUFA (Luff Schoorl) | x | x |
| $ADF_{org}$ [2)] | x | x |
| Fermentation acids [3)] | | x |
| Alcohols (methanol, ethanol, propanol, butanol, 1,2-propanediol, 2,3-butanediol) | | x |

[1)] $DM_c$= corrected dry matter.
[2)] $ADF_{org}$= Acid Detergent $Fiber_{organic}$.
[3)] Fermentation acids = lactic acid; acetic acid, propionic acid, butyric acid.

On the basis of the recorded weight of the fresh and the ensiled material a determination of the losses occurred according to Weissbach (2009).

The fermentable organic dry matter (FoDM) serves as the basis of calculation. Weissbach (2009) assumes a digestibility of the organic substances of more than 90%. The FoDM is calculated using Equation 1:

$$FoDM = 991 - XA - 0{,}50\ ADF_{org} \qquad \text{(Eq. 1)}$$

The potential gas yield for fresh sugar beet is around 375 liters of methane or 750 liters of biogas per kg of FoDM (Weissbach 2009). For this reason, the specific methane volume for fresh beet is calculated using the following Equation 2:

$$V_{CH4} = 0{,}375\ FoDM \qquad \text{(Eq. 2)}$$

In case of silage, supplements for the contained alcohol have to be made. Ethanol yields about 86% more methane per unit than sucrose (Weissbach 2009). Therefore (Equation 3):

$$V_{CH4} = 0{,}375\ (FoDM + 0{,}86\ AL) \quad \text{(Eq. 3)}$$

Disolving the brackets leads to Equation 4:

$$V_{CH4} = 0{,}375\ FoDM + 0{,}32\ AL \quad \text{(Eq. 4)}$$

$V_{CH4}$ = specific methane volume at standard conditions in l $kg^{-1}$ DM
l = liter
AL = content of all alcohols in g $kg^{-1}$ $DM_c$
FoDM = fermentable organic dry matter in g $kg^{-1}$ DM

In order to calculate the losses, the respective dry matter of the glasses before and after ensiling had to be included. Alcohols and acids contained in the silage had to be considered, too. For this purpose, it was necessary to correct the dry matter in the silage. The dry matter content ($DM_c$) was corrected by the method of Weissbach and Strubelt (2008).

When determining the DM content of silages, volatile organic substances such as acids and alcohols must be taken into account. Since the organic dry matter of sugar beet silage can consist of up to 50% of volatile fermentation products the DM content has to be corrected. The volatility rate of lower fatty acids is 95%, as in maize silage. Furthermore, a volatility coefficient of lactic acid of 8% can also be applied to sugar beet silages. The volatility rate for monohydric alcohols is estimated to be 100%, just as it is with maize and grass silage. Since alcohols with two hydroxyl groups occur only in small amounts in sugar beet silages, a complete volatilization can be assumed also for all alcohols in total (Weissbach and Strubelt 2008).

All contents are inserted into equation 5 in g per kg FM (Weissbach and Strubelt 2008).

$$DM_c = DM_n + 0{,}95\ FA + 0{,}8\ LA + 1{,}00\ AL \quad \text{(Eq. 5)}$$

FA = total content of lower fatty acids
LA = lactic acid
AL = total content of alcohols

From the results of the analysis, a comparison was made between the fermentation products of the different varieties. In addition, a mass-related assessment of the methane forming potential losses, which occurs during the ensiling, could be established due to the laboratory results and the weights determined at the beginning and at the end of the experiment. The statistical evaluation of the results was done in Microsoft Excel and IBM SPSS Statistics 23.0.

## Results

### Process of ensiling

The measurement of the pH values in 2013 showed an approximately parallel course for all twelve tested variants (Figure 1). Starting with a pH value between 6.2 and 6.7, it decreases to a value below 4.5 during the first 48 hours. The initial differences in the pH values can be explained by the fact that the first measurement was carried out for practical reasons between thirty minutes and two hours after the silage was applied. Thus, acidification had already begun. After a period of severe pH reduction within the first 48 hours, the pH value developed in a markedly reduced intensity and decreased to a level slightly above 4.0. After four to five days, the pH value of all silages decreased below 4.0 and settled at a level between 3.5 and 3.9 from the seventh to eighth day. At the end of the experiment (90 days), the pH values were only slightly below the level reached after 8 days. They ranged between 3.4 (KWS 3) and 3.7 (Syngenta 3). The absolute differences were at maximum of 0.3 after 90 days between the silages of the different varieties. After one week of ensiling the acidification of the beet pulp of all varieties was almost complete.

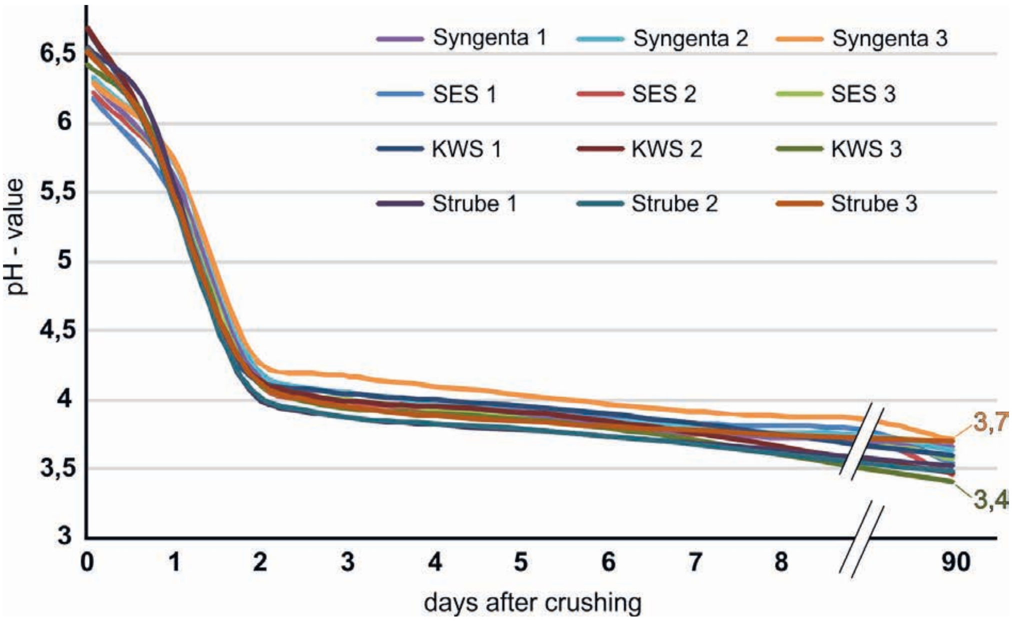


Figure 1: Development of the pH value in sugar beet pulp of twelve different sugar beet varieties in 2013. Ensiling over 90 days in preserving jars under anaerobic conditions, n = 4.

However, the statistical evaluation of the pH values showed significant differences between the variants. The significance of the differences increased over storage time. After 90 days, significant differences could be observed in 9 of the 12 variants. The standard deviations between the four repetitions of each measurement vary between 0.00 and 0.08 over all measurement dates. The mean standard deviation of all varieties at all measuring dates was 0.02.

The sugar contents contained in fresh sugar beet and the resulting acids in silage are shown in Table 3.

Table 3: Sugar content of fresh sugar beets and the contents of acetic acid (AA) and lactic acid (LA) resulting from the ensiling in 2013. Ensiling in preserving jars for 90 days at 15 °C in a climatic chamber.

| | Sugar content (fresh beets)[1)] | | AA | LA | ∑ Acid |
|---|---|---|---|---|---|
| Variety | in g $kg^{-1}$ FM | in g $kg^{-1}$ DM | in g $kg^{-1}$ FM | in g $kg^{-1}$ FM | in g $kg^{-1}$ FM |
| SES 1 | 151 | 725 | 9,78 | 6,47 | 16,25 |
| SES 2 | 161 | 754 | 12,60 | 7,52 | 20,12 |
| SES 3 | 159 | 725 | 7,76 | 6,60 | 14,36 |
| Syngenta 1 | 152 | 726 | 7,16 | 6,34 | 13,50 |
| Syngenta 2 | 151 | 725 | 6,97 | 5,94 | 12,91 |
| Syngenta 3 | 151 | 717 | 6,95 | 6,47 | 13,42 |
| KWS 1 | 176 | 738 | 7,38 | 4,75 | 12,13 |
| KWS 2 | 175 | 718 | 7,36 | 5,15 | 12,51 |
| KWS 3 | 165 | 719 | 9,83 | 9,77 | 19,60 |
| Strube 1 | 159 | 726 | 7,37 | 11,60 | 18,97 |
| Strube 2 | 159 | 727 | 9,81 | 8,84 | 18,65 |
| Strube 3 | 162 | 731 | 5,74 | 4,49 | 10,23 |

[1)] Sugar content according to Luff-Schoorl.

The sugar content in fresh matter (FM) was between 15.1% (Syngenta 2 & 3, SES 1) and 17.6% (KWS 1) at the beginning of the experiment. In comparison, the pH values of these four varieties were 3.63 (Syngenta 2), 3.71 (Syngenta 3), 3.54 (SES 1) and 3.59 (KWS 1) after 90 days. The contents of acetic acid varied between 5.74 g $kg^{-1}$ FM (SES 2) and 12.6 g $kg^{-1}$ FM (Strube 3). In case of lactic acid, the contents were between 4.49 g $kg^{-1}$ FM (Strube 3) and 11.60 g $kg^{-1}$ FM (Strube 1). It should be noted that, with exception of Strube 1, the content of acetic acid in silage had always been slightly higher than that of lactic acid. The contents of both propionic acid (PA) and butyric acid (BA) in the silage were in each case below 0.1 g $kg^{-1}$ FM. That's why they are not listed in Table 3.

The correlation between sugar content in fresh matter and the amount of acids in silage were calculated to determine whether there is a connection. These are shown in Table 4.

Table 4: Summary of the correlations between sugar content in fresh matter of sugar beet and the content of acetic acid (AA) and lactic acid (LA) in silage after 90 days of storage in 2013.

| | AA in g $kg^{-1}$ FM | LA in g $kg^{-1}$ FM | ∑ Acid in g $kg^{-1}$ FM |
|---|---|---|---|
| Sugar content in g $kg^{-1}$ FM | -0,01 | -0,18 | -0,12 |

In case of acetic acid with a correlation coefficient of -0.01, no correlation could be established within the sugar content of fresh sugar beet. In case of lactic acid and the total content of the acids, with correlation coefficients of -0.18 and -0.12, only weak negative correlations exist.

A correlation between the sugar content of fresh sugar beet and the pH value of the silage has not been proven with a coefficient of determination of $R^2$ = 0.0037 (Figure 2).

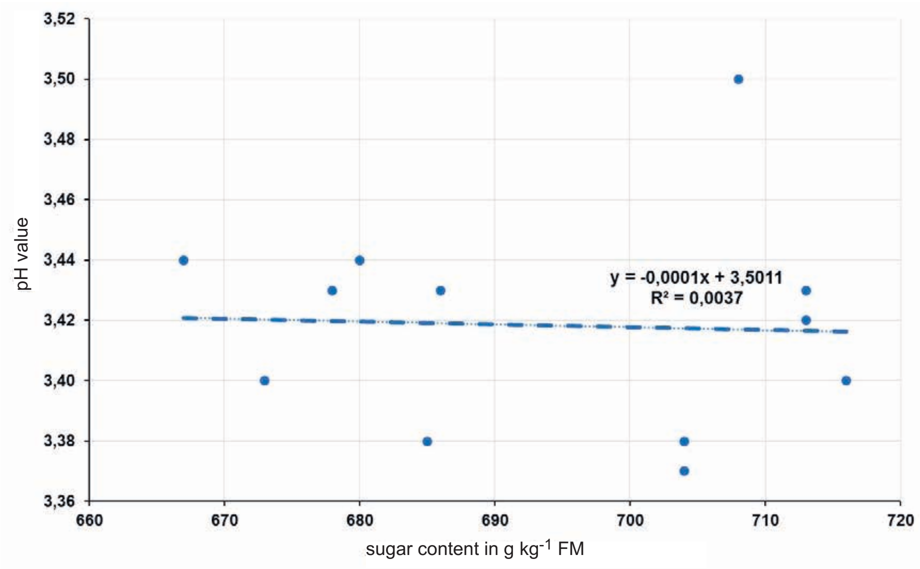


Figure 2: Relationship between sugar content in fresh beet pulp and the pH value after ensiling for 90 days under anaerobic conditions in preserving jars (Jahr: 2013, n =12)

Thus, it was found that the ensiling process of different sugar beet varieties with different initial sugar contents is approximately parallel throughout the first 48 hours. However, the pH values in the finished silage differ significantly from each other. Nonetheless, an influence on the pH value in the silage by the initial sugar content could not be detected.

## Determination of losses

The losses, both of dry matter (DM) and of methane forming potential (MFP) calculated in 2015 by means of laboratory analysis in combination with mass balancing differed significantly between the varieties (Table 5). The losses of methane forming potential (MFP) were obtained as the arithmetic mean of the four repetitions. They were calculated with the laboratory result from the respective composite sample.

Table 5: Dry matter content (DM) in fresh sugar beet and corrected dry matter content ($DM_c$) according to Weissbach and Strubelt (2008) in sugar beet silage after 90 days and resulting losses of dry matter and methane forming potential (MFP) according to Weissbach (2009) (measurement: 2015, s = standard deviation, n = 4)

| Variety | DM (fresh sugar beet) | $DM_c$ (Silage) | DM loss | | MFP loss | | pH |
|---|---|---|---|---|---|---|---|
| | in g kg$^{-1}$ | in g kg$^{-1}$ | in g kg$^{-1}$ | in % | in % | s | |
| SES 1 | 236 | 188 | 48 | 20,34 | 19,58 | 0,31 | 3,42 |
| SES 2 | 246 | 207 | 39 | 15,85 | 14,93 | 0,07 | 3,44 |
| SES 3 | 241 | 210 | 31 | 12,86 | 13,42 | 0,17 | 3,43 |
| Syngenta 1 | 224 | 205 | 19 | 8,48 | 10,35 | 0,02 | 3,40 |
| Syngenta 2 | 228 | 202 | 26 | 11,40 | 11,15 | 0,10 | 3,42 |
| Syngenta 3 | 237 | 209 | 28 | 11,81 | 14,23 | 0,02 | 3,50 |
| KWS 1 | 240 | 215 | 25 | 10,42 | 11,77 | 0,05 | 3,44 |

| Variety | DM (fresh sugar beet) | $DM_c$ (Silage) | DM loss | | MFP loss | | pH |
|---|---|---|---|---|---|---|---|
| | in g $kg^{-1}$ | in g $kg^{-1}$ | in g $kg^{-1}$ | in % | in % | s | |
| KWS 2 | 241 | 216 | 25 | 10,37 | 12,33 | 0,12 | 3,38 |
| KWS 3 | 236 | 195 | 41 | 17,37 | 16,44 | 0,30 | 3,40 |
| Strube 1 | 226 | 207 | 19 | 8,41 | 10,10 | 0,09 | 3,38 |
| Strube 2 | 226 | 199 | 27 | 11,95 | 13,13 | 0,27 | 3,38 |
| Strube 3 | 236 | 218 | 18 | 7,63 | 10,16 | 0,08 | 3,43 |

The dry matter of the fresh sugar beets ranged between 224 and 246 g $kg^{-1}$ FM. The ensiling caused a loss of DM for all varieties within the 90 days. Thus the corrected dry matter contents in silages ranged between 188 and 218 g $kg^{-1}$ FM. This corresponded to relative losses in a range of 7.63% (Strube 3) to 20.34% (SES 1). In four of the tested varieties the losses of methane forming potential, which is decisive for the assessment of silage as biogas substrate, were lower than the losses of dry matter. The maximum loss of methane forming potential was 19.58% for variety SES 1. The lowest loss was found at 10.10% for Strube 1. The standard deviation of methane forming potential losses was between 0.02 and 0.31% across all varieties. In order to be able to explain the amount of losses, the influencing parameters must be considered. For this reason, Figure 3 shows the correlation between the levels of alcohol, acetic acid and lactic acid and the DM losses in the silage.

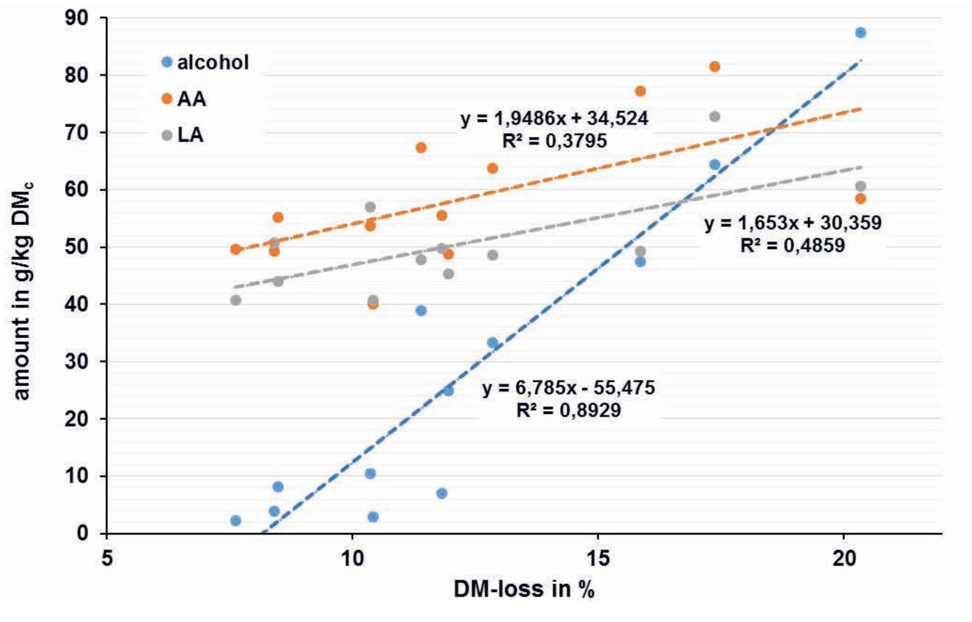


Figure 3: Correlation between the levels of alcohol, acetic acid (AA) and lactic acid (LA) and the dry matter losses (TS) in silage of twelve sugar beet varieties. Silage was stored for 90 days under anaerobic conditions in preserving jars in 2015.

The total amount of alcohol (methanol, ethanol, propanol, butanol), determined in the silages, varied to a great deal between the different varieties. Between 2.16 and 87.39 g $kg^{-1}$ $DM_c$ of alcohol were determined. The contents of acetic acid and lactic acid differed within a smaller range. Between 40.05 and 81.54 g $kg^{-1}$ $DM_c$ acetic acid and between 40.70 and 72.82 g $kg^{-1}$ $DM_c$ lactic acid were determined. In order to determine possible correlations the coefficient of determination ($R^2$) between the alcohol content and the acids as well as the DM losses were calculated. A coefficient of determination ($R^2$) of 0.38 was found between the content of acetic acid and the DM losses. For lactic acid the coefficient of determination was 0.49. The amount of alcohol, however, showed a close correlation to the amount of DM losses with a coefficient of determination ($R^2$) of 0.89.

Due to the high financial expenditure it was only possible to analyse one sample from the four replicates of one variety. Thus, the correlation of these values (DM losses) with the mean values of the FM losses carried out in fourfold repetition was additionally determined in order to check reliability. With a correlation coefficient of 0.96 a strong linear correlation was found. Due to the low standard deviations between 0.02 and 0.22 in FM losses, this can be interpreted as a confirmation for the usability of the laboratory results, which were obtained from a composite sample only.

The contents of ADF and raw ash (XA) in fresh sugar beet pulp as well as in the silage were in a narrow range for all varieties. Therefore they are not listed here. Butyric acid has not been detected in any of the silages. Also, other organic acids were detected in small amounts of below 1 g $kg^{-1}$ $DM_c$ only. The pH value, which differed within a very narrow range between 3.38 and 3.5 can be seen as an indication of the successful ensiling of all varieties. This confirms the results from 2013. The results show that despite a successful ensiling under the same conditions, significant differences in the occurring losses can be observed.

## Discussion

The lowering of the pH value, which occurs approximately parallel in the first 48 hours in all preserving jars, can be explained by the prevailing conditions in sugar beet pulp. According to Pahlow and Hünting (2011), the conversion of plant carbohydrates to organic acids spontaneously occurs, „when lactic acid bacteria meet a silage crop with sufficient fermentable sugars and a sufficient moisture content in an oxygen-free environment. These conditions were met in this experiment. Above all, the large amount of sugar contained in fresh beet pulp offers a favorable basis for the existence of lactic acid bacteria to produce organic acids, which lead to a lowering of the pH value. However, with the exception of one variant, more acetic acid than lactic acid was identified. Thus it has to be assumed that acetic acid plays an important role in the storage of sugar beet under anaerobic conditions. This is consistent with the statement by Jeroch et al. (1993), who found that ensiling of sugar beet by heterofermentative bacteria leads to the formation of lactic and acetic acid. By storing the sugar beet pulp in closed preserving jars, the state of a nearly oxygen-free environment could be achieved within a short time after consumption of the oxygen initially contained in the glasses. The described heterogeneous conditions have been established in order to check whether a uniformous and fast ensiling takes place under different conditions (varieties, location, harvesting time, sugar content). The hypothesis that the acidification of the silage is similar in speed and intensity during the ensiling of different sugar beet varieties can not be fully confirmed by the statistical evaluation. Although the acidification of the silages was found at almost the same rate in all varieties, significant differences in pH value were observed with increasing storage time. Since the pH values in all variants were below 3.7, despite a daily opening of the glasses, it can be assumed that all silages were completely conserved. In this way, they fulfilled the requirement formulated by Galler (2011) for a wet silage, which should have a pH value below 4 at a DM content of below 20% (higher in this investigation). In case of strictly anaerobic storage without an opening of the glasses, it is to be assumed that the pH values of the silages would have been even lower. This assumption was confirmed in the experiment from 2015. Apart from the storage method, sugar beets provide good conditions for fast and safe ensiling considering the sufficient moisture content and the large amount of available sugar. The amount of sugar contained in fresh sugar beet did not influence the intensity of the lowering of pH value. This suggests that the sugar content, normally contained in sugar beet at the regular harvesting time, forms a sufficient basis for an effective and rapid lowering of the pH value. This is confirmed by the fact that the fluctuations of sugar contents, which were in range between 15.1% (for example Syngenta 1) and 17.6% (KWS 1) (due to different harvesting times) in fresh sugar beet, had no influence on the lowering of the pH value. The different initial stocks of lactic acid bacteria, which can be assumed by cultivation of the varieties at different locations, had no influence on the success of ensiling.

The range of losses of methane forming potential, found in 2015, appears to be relatively high and deviated positively and negatively from the expectations of previous investigations. Investigations of Beeck et al. (2014) resulted in a reduction of methane forming potential of 10.8% over a storage period of almost nine months in air-tight covered 1000 liters containers. By means of the chosen test arrangement, which also ensured storage without the influence of oxygen, losses of similar dimensions could have been expected. However, the assumption formulated in the hypothesis has been confirmed. It has been proven, that losses vary greatly according to the respective storage method. An explicit influence of the variety has not been proven. The FM losses, determined gravimetrically in fourfold repetition, were confirmed by the amount of DM losses determined in the laboratory. A high correlation was detected. This indicates the usability of the laboratory analyzes, even if these have only been carried out as a composite sample of the fourfold repetition. These DM losses are responsible for the reduction of methane forming potential.

Particularly striking was the close correlation between the alcohol contents in silages and the DM losses. These were subject to strong fluctuations. Even if alcohol contained in the silage has a high energy density and is taken into account in the calculation of the methane forming potential, any kind of microbial conversion is a source of losses. Thus, all silages with high alcohol contents exhibited high DM losses and also high losses of methane forming potential.

There was no correlation between sugar content in fresh sugar beet pulp and the amount of alcohol in silage. Different amounts of yeasts, which are responsible for the formation of alcohol in the silages, can be a reason for different alcohol contents. Since no yeast stock analysis was carried out on the fresh sugar beet, this parameter cannot be conclusively assessed. However, this could be a possible explanation for the different alcohol contents. Since under anaerobic conditions the growth of yeasts is significantly reduced compared to aerobic conditions. The optimum temperature, which is between 20 °C to 25 °C for the growth of yeasts (Fiedler 2009), was not reached at a storage temperature of only 15 °C. Varying yeast populations could be a reason for the production of corresponding contents of alcohol. This is based on the assumption that a low yeast population has not been able to reproduce adequately in silage.

For an interpretation of the results it must be taken into account that they have only been determined by means of an estimation formula and are not based on direct measurements of the actual methane yield according to VDI guideline 4630. It should be noted that the production of alcohols during the ensiling has a decisive influence on the amount of the occurring losses. In process of anaerobic storage considerable differences between different batches can occur. However, this could not be explicitly attributed to the used variety or its sugar content.

## Conclusions

This investigation has demonstrated the process of ensiling crushed sugar beet under anaerobic conditions. It was found that the ensiling of different varieties from different locations result in significant differences in the pH value of the silage. But these differences are not important, since the pH value is sufficiently low at all times.

Despite the lack of repetition of the laboratory results, the results obtained in 2015 show, that the losses occurring during the conserved storage of sugar beet under anaerobic conditions can be subject to considerable fluctuations. In case of completely anaerobic storage, a reduction of methane forming potential between 10.10 and 19.58% was found. An influence of slow or non-uniformous ensiling on the amount of loss can be excluded by the results of the investigation of pH value development. Also, no link between the variety and its sugar content could be established. The amount of losses rather depended on the amount of alcohol produced by the yeasts. In the future it is necessary to investigate, whether the amount of losses can be reduced by the use of silage additives to inhibit alcoholic fermentation.

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