Effects on Aerobic Processes at Silage Faces

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Summary

In animal nutrition, one of the most important and indispensable requirements is high quality of feed. Especially the staple feed, which builds the basic feed for ruminants' rations, has to fulfill high-quality criteria. In most cases, silage is used as staple feed for productive livestock. The conservation of forage as silage offers the opportunity of preserving nutrients and energy in an adequate way. The aim of silage production is to obtain a product which contains a maximum of nutrients and energy originating from the fresh plant. The production of high-quality staple feed is critically important because the conservation of plant material represents a central cost factor for agricultural businesses. Additionally, farmers have to spend more money on concentrated feed to avoid a lack of energy or nutrients compared to the animals' requirements. Not only from the nutritional point of view but also from the perspective of economically successful biogas production, dry matter and energy losses have to be prevented. Aerobic-induced silage reheating is responsible for energy and nutritional losses in this preserved staple fodder, potentially leading to a complete deterioration of the silage. If the plastic cover of the silage is damaged or opened for feed-out, silage gets in contact with oxygen from the ambient air, which endangers the nutritional value.

Since different methods for laboratory experiments with silage are used, the results are often neither comparable to each other nor to farm conditions. The amounts of losses caused by aerobic deterioration determined in laboratory experiments are different from losses observed on farms. One of the goals of the conducted research project was the development of a new optimized method for trials investigating silage under aerobic conditions. The developed test method was used to quantify the physically, biologically and chemically influencing factors on aerobic stability of silage. The test method has a practical orientation and simulates the circumstances of a clamp silo and is therefore similar to farm conditions. It includes temperature measurements, gas sampling and gas analyses, and laboratory analyses of silage samples. In the further course of the investigation, this method was used for the trials conducted. The objective of the first study implementing the new method was to investigate the effect of the physical factor 'bulk density' on temperature profiles, microbial respiration activities and DM as well as energy and nutritional losses during the reheating of maize silage. In conclusion, the results of this study showed that high density of plant material is an important physical factor supporting the aerobic stability at the open silage face. Furthermore, the compaction has a great impact on the reduction of the silage temperature during the feed-out period. Additionally, high density reduces microbial respiration activity in silage, can

potentially reduce mass losses, and it preserves DM, nutrients and energy during the aerobic feed-out period.

Based on these findings, the following study investigated the effects of different factors (biological, chemical and physical) on silage during aerobic conditions. As a physical factor, different bulk densities were adjusted again to get more insights concerning this factor. Additionally, two different biological inoculants were added to the silage, and a chemical additive was also used. The impacts of the different factors were compared to each other. The findings confirmed that high-bulk density improves the aerobic stability of maize silage. The chemical additive prevented silage from deterioration very effectively and inhibited microbial heat production even during a period of ten days of air exposure. Higher density had no additional positive effect on silage in aerobic conditions when using the chemical additive. In this case the high density offers the advantage of smaller volume of the silo stock, which may be positive if storage capacity is limited. The silages treated with biological inoculants also did not undergo reheating. Thus, the biological inoculants could also successfully prevent silage from aerobic reheating. Furthermore, higher density had no additional positive effect on silage in aerobic conditions when using the biological additives. The comparison of the different factors shows that the influence of the additive and inoculants used is high and the physical factor bulk density also had positive influence on aerobic stability.

To transfer results into practice as a final step, silage density was investigated on a farm in a clamp silo. Therefore, packing quality was precisely assessed by a penetrometer-based mapping system, which was specifically developed for measurement at the silo face. The experiment was conducted in a maize bunker silo. The density distribution of such silos shows great variation between different parts of the silo. The spoilage risk for a bunker silo, especially in the upper parts or in the side region with low density, is rather high. Developing a penetrometer-based mapping system was the major objective of the fourth study and was successfully met. The developed penetrometer mapping system offers the opportunity to represent the packing density and is thereby able to detect deficits in compaction. The mapping system may be beneficial for the rapid assessment of aerobic deterioration risks in bunker silos.

Zusammenfassung

Eine der wichtigsten und unabdingbaren Voraussetzungen für eine nachhaltige Tierernährung ist qualitativ hochwertiges Futter. Vor allem das Grundfutter als Basis der Ration für den Wiederkäuer, muss hohe Qualitätskriterien erfüllen. In den meisten Fällen stellt Silage das Grundfutter für Nutztiere dar. Die Konservierung von Futterpflanzen durch Silierung bietet die Möglichkeit, Energie und Nährstoffe in adäquater Weise bereitzustellen. Das Ziel der Silageproduktion ist es, ein Produkt zu erhalten, welches ein Maximum an Nährstoffen und Energie der Ausgangspflanze enthält. Die Produktion von qualitativ hochwertigem Grundfutter ist auch von großer Bedeutung, weil die Futterkonservierung einen zentralen Kostenfaktor in landwirtschaftlichen Betrieben darstellt. Außerdem kommt es im Falle eines Mangels an Energie oder Nährstoffen in der Silage zu einem erhöhten Kostenaufwand, da der Kauf zusätzlicher hochwertiger Futtermittel notwendig wird, um die Tiere bedarfsgerecht zu versorgen. Nicht nur aus tierernährungsphysiologischer Sicht, sondern auch vom Standpunkt ökonomischer Biogasproduktion betrachtet, sollten Trockenmasse- und Energieverluste bei der Lagerung auf ein Minimum reduziert werden. Sauerstoffinduzierte Nacherwärmung ist für Energie- und Nährstoffverluste verantwortlich und kann zu vollständigem Verderb und damit zur Unbrauchbarkeit der Silage führen. Wenn die Silofolie beschädigt oder für die Fütterung geöffnet wird, kommt die Silage mit Sauerstoff aus der Umgebungsluft in Kontakt, wodurch der ernährungsphysiologische Wert gefährdet werden kann.

Da für Silageversuche im Labormaßstab verschiedene Methoden angewendet werden, sind die Ergebnisse oft weder miteinander, noch mit der Praxis vergleichbar. Die Höhe der Verluste durch aeroben Verderb, die unter Laborbedingungen ermittelt werden, unterscheiden sich von jenen, die in der Praxis ermittelt werden. Darum war es ein Projektziel eine verbesserte Methode für Silageversuche unter aeroben Bedingungen zu entwickeln. Diese Methode wurde dann verwendet, um physikalische, biologische und chemische Einflussfaktoren auf die aerobe Stabilität von Silage zu untersuchen. Die Methode hat eine praktische Orientierung und simuliert die Gegebenheiten in einem Flachsilo und ist somit näher an den realen Praxisbedingungen. Sie umfasst Temperaturmessungen, Gasprobennahme und deren Analyse, sowie Laboranalysen von Silageproben. Im weiteren Verlauf der Untersuchungen, wurde die Methode für die folgenden Versuche angewendet. Ziel des ersten Versuchs war es, den Effekt des physikalischen Einflussfaktors "Materialdichte" auf die Temperaturentwicklung, die mikrobielle Atmungsaktivität sowie Trockenmasse-, Energie- und Nährstoffverluste während der Nacherwärmung von Maissilage zu untersuchen. Aus den Ergebnissen dieser

Untersuchung lässt sich schlussfolgern, dass eine hohe Dichte des pflanzlichen Materials ein wichtiger physikalischer Faktor ist, der die aerobe Stabilität an Silageanschnittflächen unterstützt. Des Weiteren hat eine hohe Dichte einen großen Einfluss auf das Nacherwärmungsrisiko während der Entnahmephase und reduziert außerdem die mikrobielle Atmungsaktivität. Somit hat sie das Potential, die Gesamtmasseverluste zu reduzieren und Trockenmasse, Nährstoffe und Energie während der aeroben Fütterungsphase zu erhalten.

Auf diesen Ergebnissen basierend, wurde in den darauffolgenden Versuchen der Einfluss verschiedener Faktoren (biologischer, chemischer und physikalischer) auf Silage unter aeroben Bedingungen untersucht. Neben den Dichteunterschieden wurden zwei verschiedene biologische Siliermittel zu Teilen der Silage hinzugefügt; ein chemisches Additiv wurde ebenfalls verwendet. Der Einfluss der verschiedenen Faktoren wurde miteinander verglichen. Die Ergebnisse bestätigen, dass hohe Materialdichte die aerobe Stabilität an Silageanschnittflächen der Maissilage erhöht. Das chemische Additiv schützte die Silage effektiv vor dem Verderb und verhinderte mikrobielle Wärmeproduktion auch während einer zehntägigen Periode der Luftzufuhr. Hohe Dichte hatte jedoch keinen zusätzlichen positiven Effekt auf Silage unter Lufteinfluss, wenn das chemische Additiv verwendet wurde. In diesem Fall bietet die höhere Dichte den Vorteil eines geringeren Volumens des Silos, was vor allem bei geringer Lagerkapazität vorteilhaft ist. Silagen, die mit den biologischen Siliermitteln behandelt wurden, zeigten ebenfalls keine Nacherwärmung. Hohe Dichte hatte auch in diesem Fall keinen zusätzlichen positiven Effekt auf die Silage unter aeroben Bedingungen. Der Vergleich der verschiedenen Faktoren zeigt, dass der Einfluss des chemischen Additives und der biologischen Siliermittel, die verwendet wurden, größer ist, als der Einfluss des physikalischen Faktors Dichte.

Um die Ergebnisse auf die Praxis übertragen zu können, wurde die Dichte im letzten Schritt auf einem landwirtschaftlichen Betrieb in einem Fahrsilo untersucht. Dazu wurde die Verdichtungsqualität mit einem penetrometerbasierten Visualisierungssystem, welches hierzu speziell für Siloanschnittflächen entwickelt wurde, präzise bestimmt. Der Versuch wurde an der Anschnittfläche eines mit Maissilage gefüllten Fahrsilos durchgeführt. Die Dichteverteilung solcher Silos ist sehr ungleichmäßig. Das Verderbsrisiko ist in den oberen Bereichen sowie in den Randbereichen, die in der Regel geringere Dichte aufweisen, relativ hoch. Das entwickelte Visualisierungssystem bietet die Möglichkeit, die Lagerungsdichte differenziert für die ganze Anschnittfläche darzustellen und kann somit Defizite in der Verdichtungsarbeit aufspüren. Dies kann für eine schnelle Beurteilung des Verderbsrisikos in der Entnahmephase vorteilhaft sein.

Table of Contents

Figures

Figures

Tables

Abbreviations

ADF   acid detergent fibre

ADF_{OM} ADF determined on an organic matter basis

CFU   colony forming unit

CP   crude protein

DM   dry matter

e.g.   (=exempli gratia) for example

FM   fresh matter

ME   metabolizable energy

NDF   neutral detergent fibre

NDF_{OM} NDF determined on an organic matter basis

NEL   net energy content for lactation

OM   organic matter

RNB   ruminal nitrogen balance

uCP   utilisable crude protein

1 General Introduction

1.1 Scope of the Thesis

High quality of feed is the most important requirement for animal nutrition. Especially silage as a feed for productive livestock is of particular importance (WOOLFORD, 1984). The conservation of forage as silage provides a substantial opportunity of conserving nutrients for livestock (WILKINSON & DAVIES, 2012). In all countries where there is a restricted vegetation period, such as winter or a dry season, conserved feeding material, like hay or silage, plays a significant role. In all those parts of the world, also here in Germany, conserved forage is an essential component of ruminant diets during times when fresh crops are unavailable (PAHLOW et al., 2003; JEROCH, 2008). This highlights the necessity of long-term stability and storage suitability (WAGNER et al., 2004; JÄNICKE, 2011). Corn silage has also become the most important substrate for biogas production in Germany (REINHOLD & PEYKER, 2007).

The aim of the ensiling process is to produce staple fodder which contains a maximum of nutrients originating from the fresh plant (JÄNICKE, 2011; SPIEKERS, 2011), has hygienically immaculate condition, and is palatable for the animals. Impeccable feeding material is indispensable to achieve health and high efficiency in livestock production, hence the nutritional value of silage and the animal´s feed intake are influencing the performance levels of animals (SPIEKERS, 2011; STAUDACHER, 2011).

Furthermore, the production of high quality staple fodder is crucially important because conservation of plant material represents a central cost factor in the cost structure of agricultural businesses. Therefore, the cost per produced unit of staple fodder should be minimized (PÖTSCH et al., 2014). GREIMEL (2002) characterises the costs for preservation of feed as a decisive factor for economic success.

Aerobic-induced silage reheating is responsible for energy and nutritional losses in preserved staple fodder, potentially leading to a complete deterioration of the silage (WAGNER et al., 2004; REINHOLD & PEYKER, 2007). After encountering damage to the plastic cover of the silage as well as during the time after opening the staple fodder, oxygen from the ambient air can deteriorate the nutritional value (PAHLOW & HÜNTING, 2011). In addition to aerobic-induced silage reheating endangering the quality and nutritional value of silage, it also decreases the feed intake of the animals (GERLACH et al., 2013).

The process of silage production is fully understood and therefore, the conditions that are needed to obtain high silage quality are well defined, and the risk of poor silage quality should be minimised (WOOLFORD, 1984). However, it appears to be difficult in agricultural practice to comply with these conditions and the aerobic deterioration of silage is a worldwide problem for feed quality and farm profitability (TOBACCO et al., 2011). In agricultural practice, low quality silage is often used (WICHERT et al., 1998). Considerable shortcomings concerning silage and roughage production can be observed, showing that the potential which arose by research activities is not exploited (PÖTSCH et al., 2014). Losses caused by moulds and faulty fermentation are visible in practice (LATSCH & SAUTER, 2014). Though the problems as well as the solutions are known, but their practical implementation is lacking (PÖTSCH et al., 2014). By reducing the energy and feed losses, the efficiency and sustainability of agricultural production can be improved (KÖHLER et al., 2013). Additionally, from the viewpoint of economically successful biogas production, dry matter and energy losses should also be prevented (REINHOLD & PEYKER, 2007).

1.2 Objectives

Based on the outset described above, it was the **main objective** of the project to investigate different influencing factors on the aerobic stability of silage. The overriding attention was paid to the processes at the open silo face. The project included trials which were planned to be close to practice. Finally, conclusions for advice for practical application should be possible.

The goal of the first main study (paper I) was to develop a new optimized method to test the physical and chemical influencing factors on aerobic stability of silage in a small scale.

The objective of the second study (paper II), which was conducted in the course of the project, was to investigate the effect of the physical factor 'bulk density' on the temperature profiles, microbial respiration activities and DM, energy and nutritional losses during the reheating of maize silage under controlled conditions.

The objective of the third study (paper III) was to investigate the effects of different factors (physical, biological and chemical) on silage during aerobic conditions. For this purpose, silage was ensiled with different densities. Two different biological inoculants were added to

parts of the silage and a chemical additive was also used. Another objective of the study was to compare the impact of these different factors to each other.

Because of the fact that the spoilage risk for a bunker silo packed with maize silage is rather high, especially because of the unequal density distribution within one clamp silo, practical investigations were conducted directly on a farm. The main aim of this study (paper IV) was the development of a penetrometer-based mapping system for maize silage in a bunker silo.

2 Literature Analysis

2.1 Process of Ensiling

The process called 'ensiling' means that a crop, without drying or with minimal drying, is stored under anaerobic conditions (MUCK, 1988), where lactic acid bacteria produce organic acids by fermentation of carbohydrates (PAHLOW & HÜNTING, 2011). The goals of the conservation system are maintaining the quality and feeding characteristics of the crop, as they have been before storage, and reducing dry matter and energy losses to a minimum (MUCK, 1988; MUCK et al., 2003).

Silage making involves harvesting forage crops by mechanical choppers, which usually reduces the size of plant particles at the same time. This chopping process accelerates the release of plant cell contents. The substrate produced is afterwards compacted in a silo or pressed into bales. The silo is sealed airtight to reach anaerobic conditions (WILKINSON, 1999). The conservation effect is based on bacterial fermentation of sugars, lowering the pH by the production of fermentation acids. These fermentation acids are mainly lactic acid and acetic acid. The course of fermentation is shown in figure 1. In addition, the anaerobic environment itself contributes to the conservation effect (MUCK, 1988).
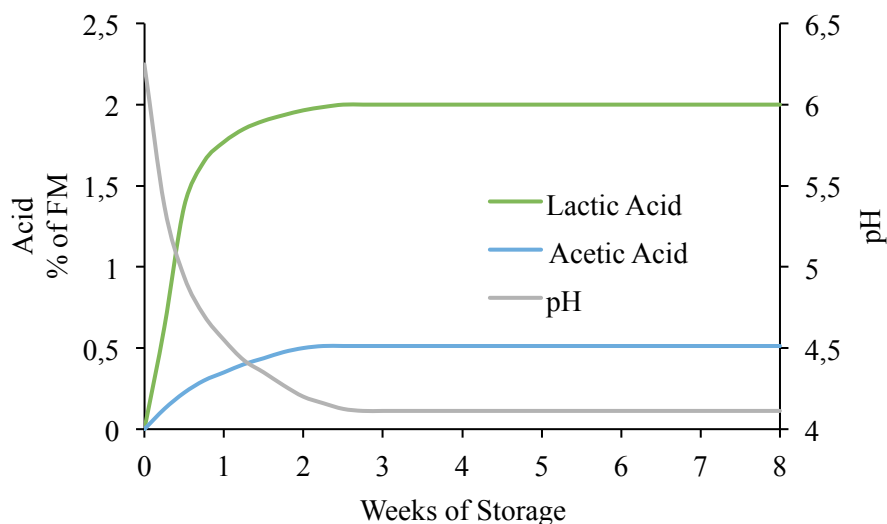
Figure 1   Typical Course of Fermentation during Ensiling (modified according to KASAL et al. (2003) and GROß (1974))

The ensiling process can be divided up into four phases. Each of these phases has different characteristics (WEINBERG & MUCK, 1996). The first phase of the ensiling process is the

aerobic phase (NISHINO, 2011). During this phase which begins after filling the silo, there is still air, including oxygen, between the plant particles and the pH averages 6.0-6.5 (WEINBERG & MUCK, 1996). Respiration activity of the plant using oxygen and sugars while producing carbon dioxide, water and heat is still possible under these conditions (MUCK, 1988). Besides, protease activity and activity of aerobic and facultative aerobic microorganisms takes place (WEINBERG & MUCK, 1996) and is accompanied by heat production (PAHLOW & HÜNTING, 2011). The respiration, which releases energy in terms of heat, takes place according to the following formula:

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 2835$ kJ (STEINHÖFEL, 2008)
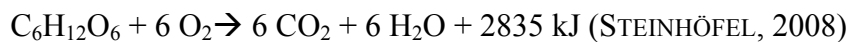
The process after oxygen is consumed and anaerobic conditions are reached can be divided up into two phases. The first of them is mainly characterized by fermentation and the second, in which silage should stay stable, is the storage phase (NISHINO, 2011). During fermentation, lactic acid and other acids are produced by bacteria, mainly lactic acid bacteria, which become the predominant species in this phase (WEINBERG & MUCK, 1996).

The homofermentative lactic acid fermentation follows the formula:

$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH + 197$ kJ

Energy losses of only 3% of energy from glucose characterize this way of fermentation as a very effective one. According to the type of fermentation, some other products can occur besides lactic acid. Ethanol, acetic acid, mannitol and CO_2 are products of heterofermentative fermentation according to the following equations:

$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2 + H_2O$

or

$3\ C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + CH_3COOH + 2\ C_6H_{14}O_6 + CO_2 + H_2O$ (STEINHÖFEL, 2008).
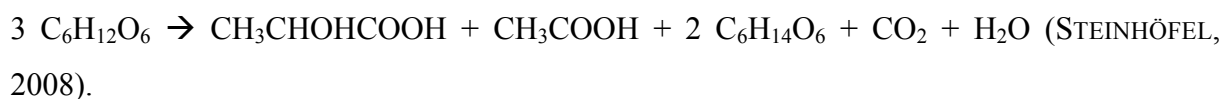
Due to fermentation, the pH decreases and reaches 3.8-5.0 (WEINBERG & MUCK, 1996). Thereby, the anaerobic bacterial concurrence of lactic acid bacteria is supressed. Besides, enzymes degrading proteins are also inhibited and consequently less buffering substances are produced (PAHLOW & HÜNTING, 2011). Finally, the low pH or the absence of fermentable carbohydrates terminates lactic acid fermentation. The activity of lactic acid bacteria ends if pH decreases under 3.0-3.6. If lactic acid fermentation is terminated because of a limited amount of fermentable carbohydrates, the pH may be higher. In this case, there is the risk of malfermentation by clostridia which break down valuable lactic acid and protein and thereby lead to rot and deterioration. The pH has to decrease under 4.2-4.4 to inactivate clostridia

(STEINHÖFEL, 2008). Figure 2 gives an overview of the anaerobic fermentation of carbohydrates.

In the feed-out phase, which takes place after several weeks or months, the silo is opened and the silage is removed for use as feed mainly for ruminant animals (WILKINSON, 1999). This entails an activation of aerobic microorganisms, e.g. yeasts, moulds, acetic acid bacteria and bacilli, which can lead to aerobic deterioration (WEINBERG & MUCK, 1996).
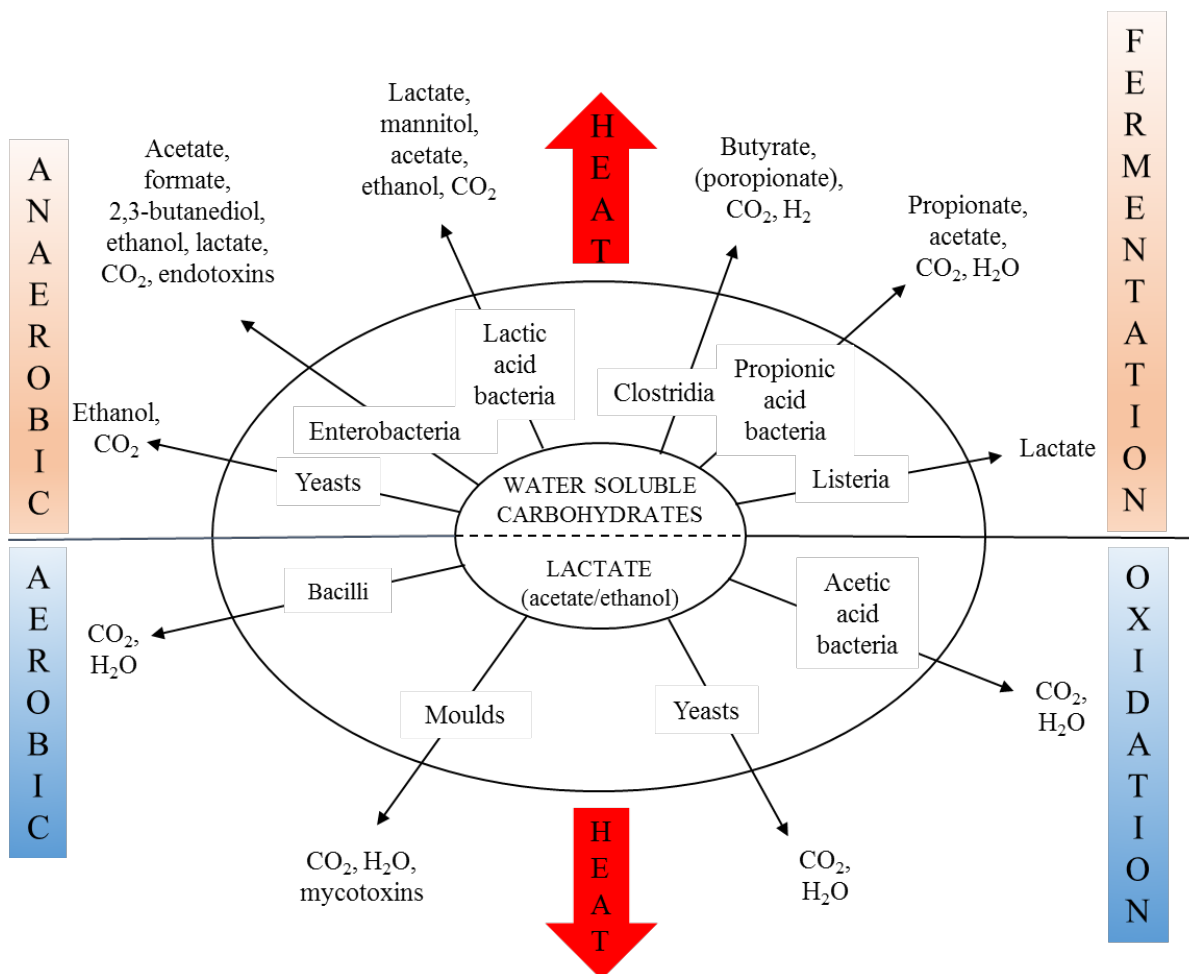
Figure 2   Scheme of the Anaerobic (Upper Part) and Aerobic (Lower Part) Metabolism of Carbohydrates and Fermentation Products by Microorganisms in Silage (modified according to MERRY & DAVIES, (1999); DAY & LISANSKY, (1987))

2.2 Quality Parameters of Silage

The quality features of silage are good palatability, high nutritive value and the ability of storage (ZHANG et al., 2015). Ensiling pursues the target of preserving crop quality, dry matter and energy in the silo. Therefore, respiration and proteolytic activity of the plant itself as well as aerobic microbial growth have to be restricted. To reach this aim, quick attainment and maintenance of anaerobic conditions are the keys of ensiling success (MUCK, 1988).

Table 1 shows the most important orientation values of silages which should be reached for silage as a feed for cattle or other animals, or even for silage as a substrate for the biogas production (SPIEKERS, 2011).

Table 1   Orientation Values for Grass and Maize Silage Appropriate as Feed for Dairy Cattle and Beef Cattle (modified according to SPIEKERS, 2011)

Parameter	Unit	Grass Silage	Maize Silage
Dry matter	g/kg FM	300-400	300-370
Crude ash	g/kg DM	<10	<4
Crude protein	g/kg DM	<17	< 9
NDF_{OM}	g/kg DM	40-48	35-40
ADF_{OM}	g/kg DM	23-27	21-25
Structure effective crude fiber	g/kg DM	23-25	18-22
Starch	g/kg DM	no	>30
ME	MJ/kg DM	≥10.5 (≥10.1*)	≥11.0
NEL	MJ/kg DM	≥6.4 (≥6.1*)	≥6.6
uCP	g/kg DM	>135	>132
RNB	g/kg DM	<6	-8--9

*Second or following cut

Fermentation quality and nutrient composition are influenced by the maturity of the plant at the time of harvest (ZHANG et al., 2015) and by the species (genotype) of plant chosen for ensiling (ZHAO et al., 2015). Also each phase of the ensiling procedure can affect the quality of the silage produced (NISHINO, 2011). Especially the fourth phase of the ensiling process, also called feed-out phase, got into the focus of interest because it has great impact on maintaining nutrients and good hygienic quality for animal nutrition (WILKINSON & DAVIES, 2012). It is important that the quality of silage stays stable until silage is consumed by the animal. Therefore, the target figures from Table 2 should be reached (SPIEKERS, 2011).

Table 2   Target Figures for Fermentation Quality (modified according to SPIEKERS, 2011)

Target Figure	Orientation Value
pH at 20-45% dry matter	4.0-5.0
Butyric acid (g/kg DM)	<3
Acetic and propionic acid (g/kg DM)	20-30
NH_3-N (% of total N)	<8
Aerobic stability (days)	>3

There are four processes potentially affecting silage quality: plant respiration, plant enzyme activity, clostridial activity and aerobic microbial activity. The last three of them have the greatest impact on reduction of quality. Plant respiration, aerobic activity and clostridia can additionally induce dry matter and energy losses (MUCK, 1988). Bad silage quality and malfermentation can reduce feed intake by the animal. Goats can detect slight changes due to spoilage even before temperature rise or chemical composition indicates deterioration (GERLACH et al., 2014).

2.3 Aerobic Stability and Deterioration

When the silo is opened for feed-out or after the removal of silage from the silo, silage is exposed to air. At that time fermentation acids and silage components are oxidized by aerobic bacteria, yeasts and moulds (WILKINSON & DAVIES, 2012; PAHLOW et al., 2003). This process is characterised by an increase of pH (PAHLOW & HÜNTING, 2011) as shown in figure 3.
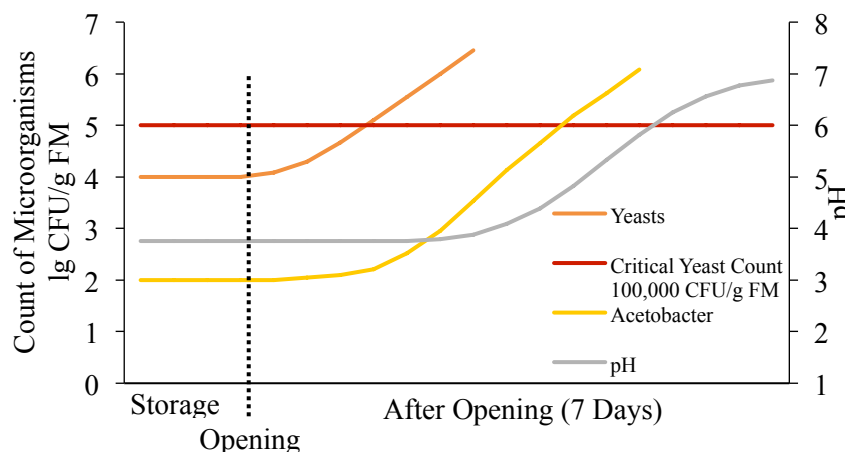
Figure 3   Course of Reheating Caused by Yeasts and Acetobacter (modified according to PAHLOW & HÜNTING (2011))

Silage rich in sugar and energy, free of butyric acid and including only low concentrations of acetic acid spoil as soon as oxygen enters into the material (WILKINSON & DAVIES, 2012). In general, maize silage is more endangered to be spoiled by aerobic deterioration than grass silages because they typically include higher amounts of lactic acid and lower amounts of acetic acid (SPIEKERS & POTTHAST, 2004).

According to ASHBELL and WEINBERG (1992), spoilage in silage is primarily caused by air. Because of that, the success of silage conservation and fermentation quality is mainly judged by aerobic stability (HONIG, 1990). To prevent spoilage and reduce top losses to a minimum, ASHBELL and WEINBERG (1992) highlight the importance of an intact plastic sheet. Even in well-sealed silos, the diffusion of small amounts of oxygen into the silage is unpreventable (ROTZ, 2003). Cracks or holes in the plastic cover of silos or the top of uncovered silos are the typical areas for aerobic induced spoilage. So, plastic covers should be used and repaired in case of damage (MUCK, 1988; ASHBELL & WEINBERG, 1992). Otherwise, the inflowing oxygen is utilised for microbial respiration, which occurs in conjunction with dry matter losses (ROTZ, 2003). Consequently, maintaining anaerobic conditions in the silo is the most successful and apparent possibility to prevent aerobic microbial activity (MUCK, 1988). Feeding well preserved silage, including valuable nutrients for the animal without high amounts of mould spores and toxins, requires long aerobic stability as a key factor (WILKINSON & DAVIES, 2012). Figure 2 gives an overview of the aerobic processes potentially happening in silages exposed to air.

The period in which silage stays aerobically stable should reach seven days, including the time when silage is offered to the animal in the feed trough (WILKINSON & DAVIES, 2012). Counts of bacteria, yeasts and moulds found by WICHERT et al. (1998) increased strongly due to aerobic deterioration. Bacterial counts in fresh silage were between $10^{4.6}$ CFU/g and $10^{6.8}$ CFU/g compared to bacterial counts in secondary fermented silage which were between $10^{6.7}$ CFU/g and $10^{8.04}$ CFU/g. Counts of yeasts rose from values between $10^{2.3}$ CFU/g and $10^{5.5}$ CFU/g to values between 10^{5} CFU/g and $10^{7.5}$ CFU/g due to secondary fermentation. Counts of moulds increased from values between $< 10^{2}$ CFU/g and 10^{5} CFU/g to values between $10^{3.6}$ CFU/g and $10^{7.5}$ CFU/g due to secondary fermentation.

Aerobic deterioration is affected by various factors. Biochemical factors affecting aerobic stability are the development of yeasts and moulds during plant growth, field wilting, storage and the concentration of undissociated acetic acid in silage (WILKINSON & DAVIES, 2012).

HONIG (1990) suggests a two-part approach for the determination of aerobic losses to approximate to in-silo-losses on a farm. Measurement of aerobic deterioration of a forage sample in a laboratory test with detailed description of the sample including filling conditions, fermentation pattern, air influence, microbial population and nutrient content is the first step of this method. In a second step, the conditions of gas exchange and air infusion in the silo are included and the duration and intensity of air influence in the feed-out period of the silo are derived. For the first step, the determination of aerobic deterioration in the laboratory, there are three main methods: The determination of CO_2-production, the measurement of O_2-consumption and the determination of temperature rise. CO_2-production and O_2-consumption are directly correlated to DM-losses because during microbial respiration, carbohydrates of the plant are degraded and O_2 is metabolized while CO_2 is produced. Temperature development expresses DM-losses because microbial respiration is an exothermic process.

In silos on a farm, the temperature of the front face can be used as an indicator for aerobic stability and can be easily assessed (ANDRIEU & DEMEY, 2015). HONIG (1990) recommends temperature measurement as a standard procedure for silage evaluation because it is simple to conduct and suitable for great numbers of samples. The results of GERLACH et al. (2013) show that this decision is still correct from today's point of view because temperature was the best indicator for feed intake by goats. To reduce the heat exchange with the surrounding air, small experimental buckets have to be insulated to simulate conditions on a farm, where heat accumulates caused by the insulation effect of forage (HONIG, 1990).

2.4 Influencing Factors on Silage Quality

2.4.1 Physical Influencing Factors

2.4.1.1 Dry Matter Content

Wilting of crops on field is an opportunity to reduce effluent losses and ensure good fermentation quality. Plants that are usually ensiled as whole crop, like maize, are not wilted before ensiling. They have to be harvested at beneficial conditions with dry matter contents above 300 g/kg (MUCK et al., 2003). To improve the conditions for lactic acid producing bacteria, RESCH (2008) recommends pre-wilting of plant material for green fodder to increase the concentration of sugar. Dry matter contents between 30% and 40% offer optimal conditions for ensiling. Dry matter contents lower than 28% entail the risk of effluent losses.

These preventable losses should be avoided. On the other hand, dry matter contents above 40% increase the risk of growth and activity of yeasts and moulds (RESCH, 2008). MUCK (1988) outlines that a rapid decline in pH is essential in wet crops. In crops wilted to dry matter contents above 55%, the influence of fermentation on silage quality is smaller.

In practical trials, GERLACH et al. (2014) found that low-DM forages are aerobically stable, but they were classified as badly fermented and therefore, feed intake by goats was lower than for high-DM forages. A strongly positive correlation between dry matter and dry matter intake was found. This might be an indirect effect of high silage quality resulting from higher dry matter concentration.

2.4.1.2 Chopping Length

Chopping is the last operation on the field a plant is subjected to. It influences silage density and fermentation dependent on type of machine and length of cut (MUCK et al., 2003). The recommended theoretical chopping length of grass is 2.5-4.0 cm dependent on dry matter content. With increasing dry matter, the chopping length should decrease. For maize a theoretical chopping length of 6-8 mm is recommended (SPIEKERS et al., 2009).

The theoretical cutting length has a significant influence on the material´s ability of compaction. Short cut particles are more easily compactable than larger particles (RESCH, 2008). Consequently, chopping length influences aerobic stability (MUCK et al., 2003). On the other hand, chopping to short length constitutes a compromise because it results in unfavourable forage structure for dairy cows. For animals with high amounts of maize in the ration, a cutting length between 15 and 20 mm is recommended (SPIEKERS et al., 2009). SALVATI et al. (2015) observed that farmers increase the theoretical length of cut to reach increased mean particle length with the aim of greater physically effective fibre. However, the mean particle length found in samples from field trials and surveys was not related to the theoretical length of cut that had been striven for. These results show that the effective mean particle length underlies influencing factors of the plant itself and the circumstances during harvest. Additionally, the mean particle length can affect effluent loss (MUCK et al., 2003).

The application of maize silage cut to a theoretical cutting length of 5 mm compared to 19 mm was investigated by PREIßINGER et al. (2006). Both silages were fed to 169 days old male Simmental cattle. A significant higher feed intake of the short cut material was found. Consequently, the intake of energy and nutrients was higher, resulting in higher means of

weight gain during the trial. On the other hand, short cut plant material has reduced structural impact, which is important for ruminant's rumen physiology. For this reason, larger particles are discussed for feed rations rich in energy and poor in structure.

A new procedure, which is a registered trademark, has been developed in the last years to produce whole-plant maize silage with a theoretical cutting length of 26 mm. The feeding material produced by this process is called shredlage and has a physical effective structure caused by the large particles (BEINTMANN et al., 2016). The peculiar feature of shredlage is a kernel processing step to improve starch digestibility (FERRARETTO et al., 2015). Different experiments showed that shredlage is lower compactable but nevertheless showed good aerobic stability and slightly increased feed intake at constant milk yield. For that reason, shredlage could potentially decrease the lack of energy at the beginning of lactation in dairy cows (BEINTMANN et al., 2016). FERRARETTO et al. (2015) found higher starch digestibility and greater lactation performance for shredlage used in dairy production compared to maize silage.

2.4.1.3 Density

For ensiled plant material, a high degree of compaction is necessary to reach a maximum of silage quality. In combination with airtight coverage high compaction is the primary factor influencing the prevention and reduction of energy losses (MAACK et al., 2007). Together with porosity, silage density is one of the main physical factors affecting the rate of oxygen ingress into the silage during feed-out (WILKINSON & DAVIES, 2012).

According to RUPPEL (1992) cited in HOLMES (2006), there is a relation between dry matter losses and dry matter density which can be described by the following formula:

$$\text{Dry matter losses (\%)} = 29.1 - 0.058 \times \text{dry matter density (kg DM/m}^3)$$

The variables that determine silage density are the liquid content, the solid matter and the void volume. During the process of compacting plant material, the void volume is removed by compression while the silage density increases (MUCK et al., 2003). To reach a maximum of aerobic stability, speed of harvest and weight of the packing tractor should be coordinated. By doing this, a minimum silage density of 210 kg DM/m^3 at time of feed-out and a maximum porosity of 0.4 can be reached (WILKINSON & DAVIES, 2012). ANDRIEU & DEMEY (2015) found that silos with higher density (238 ± 48 kg DM/m^3) had significantly lower average

temperatures than silos with lower density (209 ± 47 kg DM/m^3). This shows that high density of plant material reduces reheating. On the other hand, LATSCH & SAUTER (2014) mentioned that prolonged pressing with the compaction tractor cannot increase compaction unlimited. The exclusion of air results in the recovery of a large amount of dry matter but also increases effluent losses (MUCK et al., 2003).

According to HONIG (1987), the demanded silage density depends on the dry matter content of the harvested substrate. The compaction necessary to reduce the gas flow rate to 20 l/h/m^2, which is the airflow rate obtainable in well-compacted grass silage, is 225 kg DM/m^3 for maize with a dry matter content of 280 g/kg. The compaction necessary for maize with a dry matter content of 330 g/kg is 265 kg DM/m^3 (HONIG, 1987). Figure 4 shows orientation values for density recommendations at different dry matter contents.
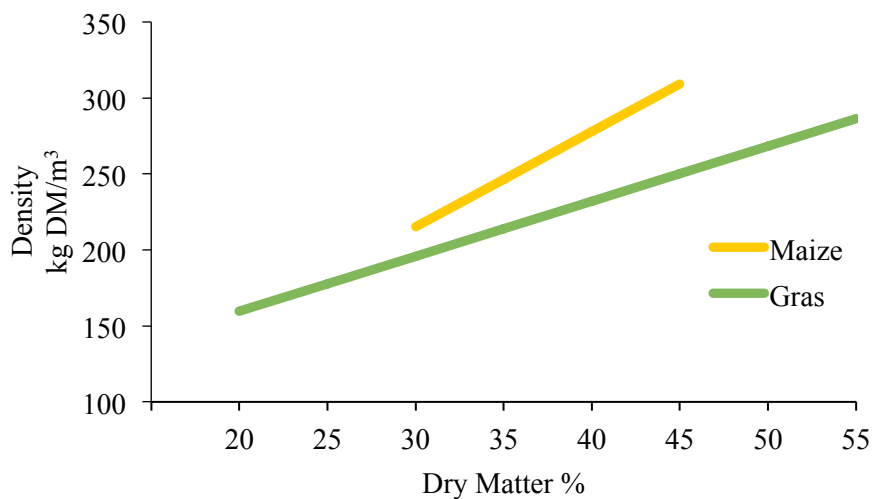
Figure 4   Target Range for Desirable Density of Grass and Maize Silage as a Function of Dry Matter Content (modified according to RICHTER et al. (2009) and SPIEKERS et al. (2009))

Within one clamp silo the density of silage varies a lot. Density is higher in the areas at the bottom of the silo and decreases towards the top of the silo (LATSCH & SAUTER, 2014). In addition to fermentation biology, bulk density plays an important role in farm management because it affects the volume of the silage, which is important concerning the storage capacity. Higher bulk density leads to decreased volume of silage at a given quantity of plant material and thereby reduces the costs to farmers for the storage of silage (MUCK et al., 2003).

Dry matter and crude fibre content are significant influencing factors affecting bulk density in the silo. By combining the parameters, a high crop density can be reached. A 1% increase of

dry matter predicts an increase of bulk density of 2 kg/m^3. A 1% increase of crude fibre predicts a decrease of bulk density of 3 kg/m^3. At dry matter contents of 40%, it is nearly impossible to reach the recommended density of 225-250 kg DM/m^3 (RESCH, 2008).

2.4.2 Biological Influencing Factors

2.4.2.1 Microflora of Ensiling

The microbial population of a silage is subjected to alterations which mainly depend on management factors and the composition of the ensiled crop (NISHINO, 2011). For a quick drop in pH, which is one of the most important requirements to reach high silage quality, an anaerobic environment, appropriate substrate and an adequate quantity of lactic acid producing bacteria are needed (MUCK, 1988). A fast drop in pH at the beginning of ensiling is the prerequisite for a minimum of losses (PAHLOW & HÜNTING, 2011; NISHINO, 2011). Therefore, at least 100,000 lactic acid bacteria per g silage are needed according to PAHLOW and HÜNTING (2011). According to MUCK (1988), even more (approximately 10^8 lactic acid bacteria per g of ensiled crop) lactic acid bacteria are needed. The amount of lactic acid bacteria available on material harvested for ensiling is nearly undetectable under typical conditions in practice. Additionally, the amount of lactic acid bacteria on freshly harvested material depends on the temperature at the time of harvesting (SPIEKERS et al., 2009).

During the phases of ensiling, the population of microorganisms in the silage changes and is influenced by many factors. Influencing factors on microorganisms can be the buffering capacity, the degree of anaerobiosis, the crop species and its dry matter content, the amount of water-soluble carbohydrates, or the amount of soil and manure contaminating the silage (NISHINO, 2011). Depending on these factors, the population and amount of microorganisms in the final product after ensiling differs strongly from the initial situation on the fresh plant (PAHLOW et al., 2003). Figure 2 mentions the most important microorganisms for the ensiling process. During the fermentation phase, lactic acid bacteria should oust other types of bacteria in their struggle for substrates because they are the most relevant microorganisms for the ensiling process (NISHINO, 2011).

Biological or chemical silage additives are used to improve the ensiling process if circumstances at the time of ensiling are not optimal. Biological additives, which are advantageous in some aspects compared to chemical additives, include enzymes or bacterial inoculants. The advantages of these natural products are based on their characteristic to be

non-hazardous, not corrosive to farm machinery, easy to use and based on the fact that they do not pollute the environment. Biological additives can reduce aerobic spoilage and improve animal performance (WEINBERG & MUCK, 1996). Table 3 gives an overview of the most common organisms used as silage inoculants.

Table 3   A List of Some Common Bacteria and Their Uses to Alter the Fermentation of Silage (KUNG et al., 2003)

Organism	Reason(s) for Addition	Pros (+) and Cons (-) of Use
Lactobacillus plantarum, acidophilus, brevis, bulgaricus, ceremoris, curvatus, xylosus, salivarus	Rapid and dominant producers of lactic acid	(+) improved energy and dry matter recovery (+) decreased proteolysis (-) low levels of acetic acid may result in worsened aerobic stability (-) some strains are slow in producing lactic acid until pH is below 5
Pediococcus acidilactici, cerevisiae, pentosaceus	Rapid and dominant producers of lactic acid	(+) grows rapidly at high pH (5-6,6) (can dominate during early fermentation) (-) low levels of acetic acid may result in worsened aerobic stability
Enterococcus faecium	Rapid grower and dominant producer of lactic acid	(+) grows rapidly at high pH (5-6,6) and when O_2 is present (can dominate during early fermentation) (-) low levels of acetic acid may result in worsened aerobic stability
Lactococcus lactis subsp. *ceremoris, diacetylactis*	Rapid and dominant producers of lactic acid	(+) grows rapidly at high pH (5-6,6) (can dominate during early fermentation) (-) low levels of acetic acid may result in worsened aerobic stability
Propionibacterium arabinosum, jensenii, shermanii	Can use lactic acid and glucose as energy sources to produce acetic and propionic acids	(+) acetic and propionic acid are highly fungistatic at low pH (-) organisms are slow growing, relatively acid intolerant, obligate anaerobes
Lactobacillus buchneri	Can anaerobically metabolize lactic acid to acetic acid. Sometimes also associated with fermentations higher in propionic acid	(+) acetic and propionic acid are highly fungistatic at low pH (-) small increases in dry matter loss during ensiling

Inoculants contain much smaller amounts of lactic acid bacteria as needed for the ensiling process, so the most important characteristic of an inoculant is the ability to reach great division rates during ensiling (MUCK, 1988). There are a lot of species of bacteria and yeasts which are facultative anaerobes and therefore capable of surviving in all phases of the ensiling process (NISHINO, 2011). The conservation success of a biological inoculant depends on more than only one factor and is defined by fast growth of the population in the silo. Two important influencing factors concerning an inoculants' success are adequate substrate and its population relative to the natural one (MUCK, 1988).

To reach a rapid decrease in pH caused by a great amount of lactic acid in a short time, inoculants containing only lactic acid bacteria are recommended. They use the plant´s water-soluble carbohydrates as substrates (WEINBERG & MUCK, 1996). When reviewing new trends and opportunities in the development and use of inoculants, WEINBERG and MUCK (1996) sum up that *Lactobacillus plantarum* was preferentially used as single strain inoculant in the first biological additives. Later, other strains were added which are more active at higher pH levels to support the beginning of fermentation. These inoculants were dispensed with 10^5-10^6 viable cells/g. Sometimes sugar was added to spend fermentable substrate and to make lactic acid bacteria the predominant strain in the silage.

RANJIT and KUNG (2000) as well as WEINBERG et al. (2009) found that different additives including *Lactobacillus buchneri*, *Lactobacillus plantarum* or a buffered product containing propionic acid, were able to prolong aerobic stability. ANDRIEU and DEMEY (2015) found significantly lower average temperature on the faces of silos treated with *Lactobacillus buchneri 40788* than on the faces of untreated silos. KRISTENSEN et al. (2010) also found increased aerobic stability by heterofermentative inoculation but could not observe this effect due to homofermentative inoculation. In fact, lactic acid producing bacteria used as inoculant have even been observed to decrease aerobic stability (MUCK, 2002; KUNG, 2010). MERRY and DAVIES (1999) explain that a high degree of lactic acid fermentation may lead to a quick drop in pH but exacerbates the risk for aerobic spoilage because the anti-mycotic effect of lactic acid is not satisfied. The addition of propionibacteria as silage additives offers the advantage of anti-mycotic properties of the propionic acid which is built.

Acetic acid also has a prolonging and improving effect on aerobic stability because it inhibits spoilage organisms. Heterofermentative microorganisms producing both acetic acid and lactic acid are e. g. *Lactobacillus rhamnosus* and *Lactobacillus plantarum* (DANNER et al., 2003). A heterofermentative *Lactobacillus* producing lactic acid and acetic acid, which is also associated with the production of propionic acid, is *Lactobacillus buchneri* (KUNG et al., 2003). From the viewpoint of aerobic stability, 20-30g acetic and propionic acid per kg DM are advantageous (SPIEKERS, 2011).

2.4.2.2 Spoilage Organisms

Aerobic spoilage organisms in silage are inhibited by anaerobic conditions. Anaerobic microorganisms which are endangering silage quality are inhibited by the low pH reached by

fermentation (MUCK et al., 2003). Figure 2 includes spoilage organisms and shows their fermentation products. Table 4 gives an overview of the most important spoilage organisms in silage and the main parameters influencing their activity. Because the focus of this thesis lays on the processes at the open silo face, yeasts and moulds, which are the most important aerobic spoilage organisms (GALLER, 2011), will be described below.

Table 4   Basic Demands of Different Spoilage Organisms and Lactic Acid Bacteria (modified according to GALLER, 2011 and KASAL et al., 2003)

Microorganism	Oxygen Demand			pH-Optimum					Temperature-Optimum (°C)			
	Yes	Facultative	No	3	4	5	6	7	10	20	30	40
Lactic Acid Bacteria			x	▭						▭		
Butyric Acid Bacteria			x		▭							▭
Acetic Acid Bacteria		x				▭					▭	
Yeast		x		▭						▭▭▭▭		
Putrid Bacteria	x					▭				▭▭▭		
Mould	x			▭▭▭						▭▭▭		

Yeasts are single-celled fungi which are able to build a bond. They prefer moist habitats and reproduce by cell division or budding (CAMPBELL & REECE, 2006). Yeasts make only few demands regarding their habitat. They are able to live under aerobic as well as under anaerobic conditions. For their growth, yeasts require an organic carbon source (FIEDLER, 2009). In anaerobic environment, they entail the disadvantage of fermenting sugar to ethanol and CO_2. The sugar, which is also the substrate for lactic acid fermentation, is thereby used in an unprofitable way of metabolism (NISHINO, 2011).

Yeasts are the main reason for aerobic deterioration (ASHBELL et al., 2002). The reheating process is characterized by degradation of fermentation acids to CO_2 and H_2O in conjunction with heat production entailing feed losses of up to 3% per day (PAHLOW & HÜNTING, 2011). This metabolism of yeasts is dependent on ambient temperatures. ASHBELL et al. (2002) found that yeasts had the highest aerobic spoilage effect after silo opening, when ambient temperature was between 20°C and 30°C. Despite the fact that the pH optimum of yeasts covers only a small range, as shown in table 4, their tolerance range for growth is between 1.5 and 8.5. Additionally, yeasts have small requirements concerning the availability of water.

They prefer A_w-values (activity of water) of 0.98 to 0.95 but some strains tolerate high osmotic pressure, which means low activity of water (FIEDLER, 2009).

Moulds are fast-growing fungi which reproduce asexual. Some moulds are spore-forming. They grow as parasites or saprobionts on different substrates (CAMPBELL & REECE, 2006). Moulds depend on oxygen. Therefore, they are not able to live in the closed silo although there are inclusions of air. Moulds endanger the silage after opening the silo (WILHELM & WURM, 1999; GALLER, 2011). They are independent of pH (GALLER, 2011).

UNDI and WITTENBERG (1996) found that calves given a choice between hay, including different amounts of fungal biomass, prefer hay with lower quantity of fungi and avoid consuming mouldy hay. WHITLOCK et al. (2000) found that higher amounts of surface spoiled silage in the rations of steers decreased their feed intake and the digestibility of DM, OM, CP, NDF and ADF. Moulds can build toxins and therefore mouldy silage should not be fed (GALLER, 2011; WILHELM & WURM, 1999).

2.4.3 Chemical Influencing Factors

2.4.3.1 Buffering Capacity

The buffering capacity is defined as resistance against acidification (SPIEKERS et al., 2009). There are more than only one substance contributing to resistance against acidification (JÄNICKE, 2011; GALLER, 2011). It is mainly determined by the protein and mineral content of the plant material and the dirt which unfortunately got into the silage stock. The buffering capacity is measured as gram of lactic acid per kilogram dry matter needed to reach a pH of 4.0. This requires an adequate amount of fermentable carbohydrates and lactic acid producing bacteria (SPIEKERS et al., 2009). The buffering capacity depends on crop species, nitrogen fertilizing, stage of development and degree of contamination with soil (JÄNICKE, 2011; GALLER, 2011).

The quick drop in pH is less important for silages with very high dry matter contents (>55%) because the low water activity inhibits the growth of clostridia. The final pH at the end of fermentation is not a guarantee for prevention of clostridia. Therefore, the time until the minimum pH is reached is decisive and has to be as short as possible (MUCK, 1988).

2.4.3.2 Water-Soluble Carbohydrates

To produce high quality silage, a minimum content of fermentable material is required. The most important substrate for lactic acid bacteria are water-soluble carbohydrates which are readily fermentable. Lactic acid bacteria can only metabolize simple sugars and the disaccharides sucrose and maltose. Starch and fructans, which are complex reserve carbohydrates, can be hydrolysed by the plants' enzymes to reach a sufficient amount of suitable carbohydrates (ROOKE & HATEFIELD, 2003). The sugar content, defined as total amount of water-soluble by lactic acid bacteria fermentable carbohydrates, determines a plants' ability to be ensiled (STEINHÖFEL, 2008). The amount of fermentable substrate needed for the fermentation process varies from crop to crop and depends on different factors. The bigger the buffering capacity and moisture content are, the bigger is the amount of fermentation substrate required (MUCK, 1988). On the other hand, crops with a high concentration of sugar and starch are potentially more endangered to be spoiled by yeasts (KUNG, 2010). Fermentable substrates are also used as silage additives. Molasses, feed sugar or beet slices are used for this purpose because they supply free sugar. Grain grist is used as a sugar releasing substrate. Substrates like this are often used in combination with enzymes for degradation of polysaccharides to monosaccharides or oligosaccharides (STEINHÖFEL, 2008).

As the plant for silage production matures, the amount of heavily digestible and indigestible structural substances increases to the disadvantage of water-soluble carbohydrates necessary for lactic acid production. For this reason, the time of harvest determines about fibre content and consequently about the quality of grass silage (RESCH, 2008).

Depending on the buffering capacity, an adequate amount of lactic acid and consequently sugar to produce lactic acid is needed (STEINHÖFEL, 2008). Therefore, the plants' ability to be ensiled is determined by the ratio of sugar to buffering capacity. Plants with a ratio of sugar to buffering capacity >2 are ensilable (SPIEKERS et al., 2009). This quotient describes the potential degree of biological acidification of the plant which is ensiled (STEINHÖFEL, 2008). The ratio should be ≥3 to characterize a well ensilable crop. Ratios of sugar to buffering capacity <2 characterize hardly ensilable crops and if the ratio is ≥8, the risk of aerobic deterioration is great because of the high residual sugar content (JÄNICKE, 2011). Table 5 sums up the most important parameters which are decisive for the ability to be ensiled.

Table 5 shows that there are many plants with a ratio of sugar to buffering capacity with an average of about 2. For this reason, it is even more important to pay attention to other influencing factors like the time of harvest. The time of harvest determines duration of

daylight and temperature a crop is subjected to and thereby determines the amount of water-soluble carbohydrates. All interventions should be done with the main aim to minimize the buffering capacity and reach a high amount of sugar in the crop (JÄNICKE, 2011).

The fermentability coefficients are also shown in table 5. They include the dry matter content of the plants because this is also an important influencing factor for the course of reheating (GALLER, 2011). Additionally, the dry matter content determines the concentrations of cell sap and thereby determines the osmotic conditions and water activity. Higher dry matter contents lead to deceleration of microbial metabolism and thereby lead to a shift of the critical pH limit for clostridial growth towards neutral pH values. Therefore, the dry matter content is the decisive factor for the amount of acidification necessary to inhibit clostridia. The fermentability coefficient (FC) is calculated by the formula $FC = \% DM + 8x\ S/BC$. On the other hand, a given ratio of sugar to buffering capacity requires wilting to get the necessary dry matter contents ($DM_{min} = 450 - 80 \times S/BC$) (STEINHÖFEL, 2008).

Table 5   Ensilability of Different Crops (modified according to STEINHÖFEL, 2008)

Crop	DM (g/kg FM)	Crude Protein (g/kg DM)	Sugar (S) (g/kg DM)	Buffering Capacity (g lactic acid/kg DM)	S/BC *	Fermentability Coefficients
Maize	280 (200-350)	75	230	35	6.6 (4.7-8.8)	81
Sugar Beat Leave	145 (120-180)	135	285	52	5.5 (1.9-10.8)	59
Green Oats	220 (145-265)	95	130	40	3.3 (2.7-4.7)	48
Field Peas	155 (130-165)	180	155	49	3.2 (2.4-3.6)	41
Field Bean	150 (110-165)	175	145	49	3.0 (1.6-3.2)	39
Sweet Lupine	150 (120-160)	180	115	46	2.5 (1.8-3.0)	35
Grasses	200 (140-270)	140	115	47	2.4 (0.8-4.6)	39
Green Rye	160 (155-210)	155	135	56	2.4 (1.6-3.3)	35
Red Clover	200 (165-250)	165	115	69	1.7 (0.9-1.8)	34
Alfalfa	200 (150-220)	190	65	74	0.9 (0.5-0.9)	27

* Ratio of sugar content (S) to buffering capacity (BC)

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3 Published Trials

3.1 Paper I

A new ex-situ method to investigate aerobic stability of maize silage faces

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ABSTRACT

A small scale method was developed to investigate the aerobic stability of silage during air exposure. 65 l-buckets were filled with maize. Three temperature sensors were inserted into each bucket at predefined positions. Cannulas were inserted to take gas samples from the buckets. The gas samples were analysed for CO_2, O_2, N_2O and CH_4 concentration. To quantify losses buckets were weighed before and after aerobic exposure. Silage samples were taken before and after aerobic exposure and analysed for pH and chemical composition.

The objective of the present study was to develop a new test method for the aerobic deterioration of silages under reproducible conditions. After validating the method with results from practical scale it can be concluded that the method is highly effective, close to practice, suitable to laboratory conditions, replicable and connectable to other experiments.

INTRODUCTION

The aerobic deterioration of silage is a worldwide problem for feed quality and farm profitability (Tobacco et al., 2011). Even in well-sealed silos, the diffusion of small amounts of oxygen into the silage is unpreventable. This inflowing oxygen is utilised for microbial respiration, which occurs in conjunction with dry matter losses. In time of open silo face, during the feed out, there is even more oxygen diffusing into the clamp, leading to silage heating and a further loss of dry matter (Rotz, 2003). This phase of the ensiling process got into the focus of interest because it is important for maintaining good hygienic quality until silage consumption by the animal (Wilkinson and Davies, 2012).

The amounts of losses caused by aerobic deterioration determined in laboratory experiments are different from losses calculated on farm (Honig, 1990). The standard test for aerobic stability of silages, which is the official testing method for silage additives used by the German Agricultural Society (DLG, 2013), usually takes place in 1.5 or 2-litre-tins. Another method used to determine aerobic stability developed and used by Ashbell et al. (1990) and Ashbell et al. (2002), takes place in 1.5-litre-bottles. All silage samples used in these methods are very small (250 to 300 g) and not compacted.

The experimental silos of Muck (2002) were larger in size (60 × 10 cm, h × d), but the silage samples had a weight of only 1.5 to 2 kg and were not compacted during the test. These circumstances do not have practical orientation, because the conditions are not directly comparable to agricultural practice, Kleinschmit et al. (2005) used laboratory silos with a capacity of 20 litres (92.7 × 38.8 cm, h × d) and achieved a final packing density of approximately 199 kg of DM/m3, which is much closer to practice, but the measurements for determination of aerobic stability were restricted to temperature measurement at one sampling point, Danner et al. (2002) used a successful method with a great amount of repetitions to investigate aerobic stability with compacted silage in 6.5-litre silos, but their measurements were also restricted to temperature at one measuring point. So the goal of the running project was the development of a new optimized method to test the physical and chemical influencing factors on aerobic stability of silage. The developed test method was used and tested for the first time and turned out to be suitable. The test method has a practical orientation and simulates the circumstances of a clamp silo. Honig (1990) recommends temperature measurement as a standard procedure for silage evaluation, because microbial respiration is an exothermic process. Besides it is simple to conduct and suitable for great numbers of samples.

In the method described below temperature measurements can be conducted in different distances to the silo face. Polyethylene buckets were used because they are movable, barely to handle and lockable airtight. Hussin et al. (2015) recommended them because of the high aerobic stability and small spoilage rate of silages produced with these buckets. Another advantageous aspect of the new test method is, that silage is produced in larger amounts. This offers the opportunity to hook up feeding trials, such as preference trials like it is done by Gerlach et al. (2013, 2014). By analysing gas samples for CO_2, O_2, N_2O and CH_4, data about the aerobic activity of the microorganisms in the silage and emission of climate relevant gas are produced. Other types of measurement methods have been tested by Sun et al. (2015) and by Shan et al. (2016) using oxygen sensors to investigate gas in silage, an important topic

MATERIAL AND METHODS

Material and experimental structure

The measurement trials were performed at the research facilities of the Institute of Agricultural Engineering, Bonn University, Germany. The method has been tested using maize silage, fresh maize, grass and alfalfa. All substrates had been produced at Frankenforst,

the practical agricultural education and research centre for animal production at Bonn University. The following explanations are focused on maize, but the trial can be conducted with other substrates in the same way. The whole procedure can be divided up into 3 phases. The first phase comprises the filling of buckets, which lasts approximate one day. The second phase includes the ensiling process, which takes four to six weeks, the preparation of buckets, which takes only a few hours and a resting period of one day. The third phase represents the experimental phase in fact, where 4 different measurements are made: Temperature measurement, gas analysis, analysis of silage samples and weighing of buckets. The experimental phase takes 7 to 10 days, dependent on crop and treatment. To minimize the environmental impact on temperature progression, the experiment was conducted in a hall with a nearly constant temperature (18 to 20°C), where air humidity in winter periods averaged 38.4% (calculated as a mean of values measured on 18 days, four measurements per minute) and without direct exposure to solar radiation. The experimental site in Bonn (Germany) is situated in a temperate climate zone, where the impact of coldness or heat is small and can be shielded by the experimental hall, which is a closed building with a heating system in winter. In 2015 the lowest outside temperature was -5°C in January and the highest temperature was 33°C in June. If the experimental procedure should be conducted in extremer climatic conditions, the use of a climatic chamber is recommended. The buckets were brought to the experimental location during the ensiling time for temperature equalisation to surrounding temperature.

Phase 1: Filling of buckets

Crops have been filled into the buckets per hand in layers and every layer has been compacted by a purpose-built hydraulic press (Figure 5). The pressing force of the hydraulic press can be adjusted and the density of the material can also be regulated and controlled by the filled crop mass and exactly known volume of the bucket. The buckets are sold with the product information, that they have a loading capacity of 60 l. Our investigation via volumetric measurement with water showed, that the exact filling volume is 65.3 l. All the buckets must have the same size for the experiment. Crop densities like in practice up to 300 kg/m^3 can be achieved. The same material was used, which was also used to fill the clamp silo on farm. After filling the buckets were closed immediately.

Phase 2: Ensiling process, preparation of buckets and resting period

Storage time of closed buckets, depends on the ensiled crop and should be at least 90 days.

Afterwards the buckets were prepared for measurements. Three temperature sensors (resistor-based sensors, Ahlborn Mess- und Regeltechnik GmbH, Holzkirchen, Germany) were inserted vertically into each horizontally lying bucket, as shown in Figure 5. Therefore holes with a diameter of 3 mm were drilled. The holes were placed at defined positions. The position for sensor 1 had a distance of 15 cm from the opening cover, the position for sensor 2 had a distance of 30 cm from the cover and the position for sensor 3 had a distance of 45 cm from the cover. These positions were chosen to represent the upper, middle and lower third of the bucket, which has a height of 60 cm. So the distance between sensor 1 and sensor 2 was 15 cm as well as the distance between sensor 2 and sensor 3. Each sensor formed the top end of a metal rod, which had a length of 200 mm. The space between the metal rod and the bucket wall was immediately closed by using sanitary silicone. The sensors were connected to data logger (ALMEMO®; Ahlborn Mess- und Regeltechnik GmbH, Holzkirchen, Germany).

To extract gas samples, two more holes were drilled into each bucket, where Blood Collection Sets (BD Vacutainer Safety-Lok™ Blood Collection Set, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA), composed of 2 syringe needles connected by a catheter tube were inserted. One Blood Collection Set was inserted near the opening of the bucket (sampling point A) and the second was inserted farther from the opening (sampling point B) (Figure 5). They were inserted by stinging one of the syringe needles through the whole. The openings around the syringe needles were closed with adhesive tape. As a result, the Blood Collection Sets were fixed at the same time. The catheter tubes were closed with catheter clamps to keep ambient air out of the buckets. After installation the buckets were stored sealed for three days to consume the oxygen that entered the buckets while insertion.
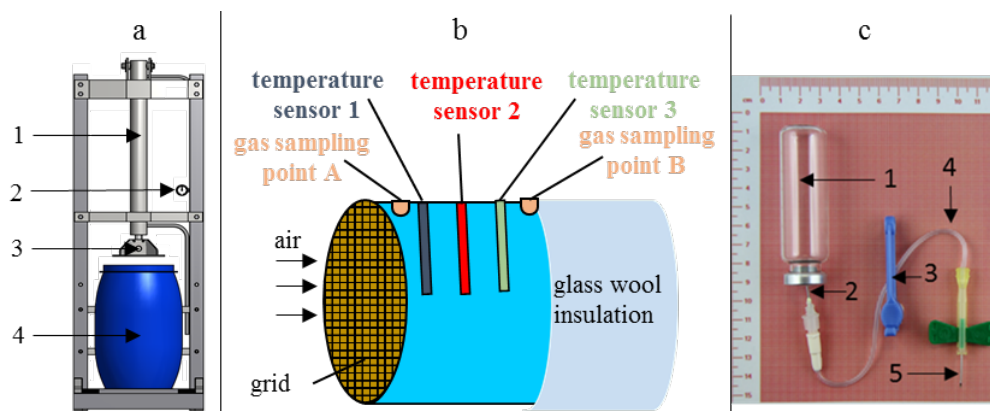

Figure 5   a) hydraulic press (1=hydraulic cylinder; 2=manometer; 3=extrusion punch; 4=bucket) b) Sketch of the experimental setup (in reality glass wool covered the whole bucket) c) Blood Collection Sets (1=injection headspace vial with puncturable stopper; 2=syringe needle to puncture the stopper; 3=catheter clamp; 4=catheter; 5= syringe needle to puncture the bucket)

Phase 3: Experimental phase in fact

To start the inflow of oxygen, the buckets were opened. So the air could diffuse into the buckets. The experiment has been conducted with the buckets in a lying position (Figure 5). To prevent silage from falling out of the bucket a grid (Figure 5) was used, which has the effect that the silage face stays a smooth surface but the ambient air can still enter the bucket. To inhibit resulting heat from dissipating, the whole buckets were thermally insulated with glass wool (100 mm, $\lambda = 0.04$ W K^{-1} m^{-1}). To reduce the heat exchange with the surrounding air Honig (1990) recommends to insulate experimental buckets to simulate farm conditions, where heat accumulates caused by the insulation effect of forage. After opening, one sample was taken at each open surface. The experimental phase took 7 days. At the end of the experiment, three samples were taken from every bucket: one from the upper third (15 cm distance from the open face), one from the middle third (30 cm distance from the open face) and one from the lower third (45 cm distance from the open face), each taken by drilling through the centre of the opened bucket with a boring rod. So the samples were taken from the same sampling points, where the temperature sensors were placed. All samples were analysed according to the German Handbook of Agricultural Research and Analytic Methods (VDLUFA, 2012) by an external laboratory (Landwirtschaftliche Kommunikations- und Servicegesellschaft mbH, Lichtenwalde, Germany), which is accredited in accordance to DIN EN ISO/IEC 17025 and certified according to DIN ISO 9001.). Dry matter, crude ash, crude protein, crude fibre, ether extract, starch, pH, aNDFom, ME and NEL were analysed.

The closed but movable buckets give the opportunity of weighing and calculation of the overall mass losses. The analyses of all samples and the bucket´s weights were used to calculate dry matter losses and finally energy losses can be determined by calculating. During the experimental period, gas samples were taken twice a day. The outer syringe needles of the Blood Collection Sets were used to puncture the stopper of an evacuated injection headspace vial, with the catheter clamp removed, to obtain a gas sample. The headspace vials had been evacuated to a pressure of less than 5 mbar. The suction caused by the vacuum (negative pressure) pulled the samples into the vials. The vials have a volume of 20 ml, and the concentrations of CO_2, O_2, N_2O and CH_4 in the gas samples were analysed using a gas chromatograph from SRI Instruments (8610 C, SRI Instruments, Torrance, USA) in an external laboratory. The analytic method is described by Wulf et al. (2002).

The data loggers were set to record temperature data of all the sensors every 15 minutes during the experimental phase. Thermography measurements are an additional opportunity to

visualize temperature distribution in the silage. Therefore a short opening of the insulation is necessary.

Statistical Analysis

The data were evaluated using IBM SPSS Statistics version 22. To investigate the significance of temperature differences between the three different temperature sensors on different experimental days, analysis of variance (ANOVA) was used. A single factor variance analysis was chosen with the experimental days as fixed factor and the sensors as dependent variables. Calculated daily mean temperature values were used and had been filtered to investigate different groups (for example one control group and one treated group) separately. The statistical significance of the mean differences was determined by the Tukey test. To investigate if the temperatures measured by the three temperature sensors differ on different experimental days between experimental groups, t-test was used. Previously Shapiro-Wilk normality test was used to verify if the data follows a normal distribution. Levene's Test was used to verify the equality of variances as a prerequisite for all tests. Differences of means < 0.05 ($P<0.05$) were accepted to be significant. Differences of means <0.001 ($p< 0.001$) were accepted to be highly significant. For statistical analysis of gas samples procedures were the same. Instead of the three sensors the two sampling points were used.

RESULTS AND DISCUSSION

The described method leads to manifold results to evaluate the technical and biochemical impacts on reheating. The measurements show the method being a suitable model to simulate a silo, where reheating starts at the silo face and moves into the silage mass over time.

Temperature increases due to oxygen infiltration and resulting microbial activity were recorded in different layers. Figure 6 shows means (n=4) of temperatures measured in maize silage (238 kg DM/m^3 vs. 297.5 kg DM/m^3; average dry matter content of 357 g/kg) as an example for the course of reheating. The average pH-value of this exemplary silage, which was analysed was 3.97 at the beginning of the experiment. At the end of the experiment the average pH-value of all the samples taken was 4.00. These results show, that the silage was well fermented and that there was no significant change in pH-values according to reheating.

A lag time (T_0-phase) of 24 to 62 hours between the opening of the buckets and the onset of temperature increase dependent on crop and treatment was observed.

In the T_0-phase, the microorganisms switch from anaerobic to aerobic metabolism. They are unable to immediately use the oxygen after opening. Thus, there was no significant difference regarding the daily mean temperatures between different treatments and between the different sensors during the T_0-phase ($p > 0.05$). In the T_0-phase the mean temperature of all the buckets and sensors was 19.67°C. For every bucket, sensor 1 reached higher ($p < 0.001$) temperatures than those recorded by sensor 2, and sensor 2 reached higher ($p < 0.001$) temperatures than those reached by sensor 3. Nussbaum (2006) defines, that silage has been reheated when different areas of the silo show a temperature difference of 5 K. The time until reheating is reached is called T_1-phase. During T_1-phase temperature averaged 21.03°C and ended in most buckets on day two or three and in some buckets on day four of the experiment, when temperature averaged 25.29°C and the following period begins, in which silage temperature rises on (T_2-phase). The T_2-phase ends with the maximum temperature (T_{max}), which averaged 37.55°C for sensor 1.

The results of the CO_2 measurements in maize silage are also graphically represented in Figure 6 as an example for the gas measurements. Concrete results of the other gases measured as well as results of weighing and silage analyses will be presented in pursuing papers focusing on microbial activity, emissions from silage and quantification of losses due to oxygen infiltration, because the present paper was especially designed to circumstantiate and establish the method.

The CO_2 concentrations were lower in the gas samples taken at sampling point A compared with those taken at sampling point B and were higher in the first samples taken at the beginning of the experiment than those measured 24 hours later. Subsequently, the CO_2 concentrations increased until they reached a plateau, which occurred at a level lower than the initial value. The CO_2 concentrations measured at sampling point A and B are both significantly higher ($p < 0.001$) in the high density variation. The CO_2 measurements indicate that the CO_2 inside the closed buckets flowed out after the buckets were opened because of a concentration gradient that was balanced by diffusion. Twenty-four hours after the buckets were opened, the CO_2 concentrations in the gas samples taken from the buckets reached their minimum. The minimum was followed by an increased CO_2 concentration in the gas samples. The CO_2 measured in the buckets originated from microbial respiration.

The measured increase of CO_2 concentration started at the same time or a few hours before the temperature increase started. This time course confirms the findings regarding the temperature progression initiated by respiration.
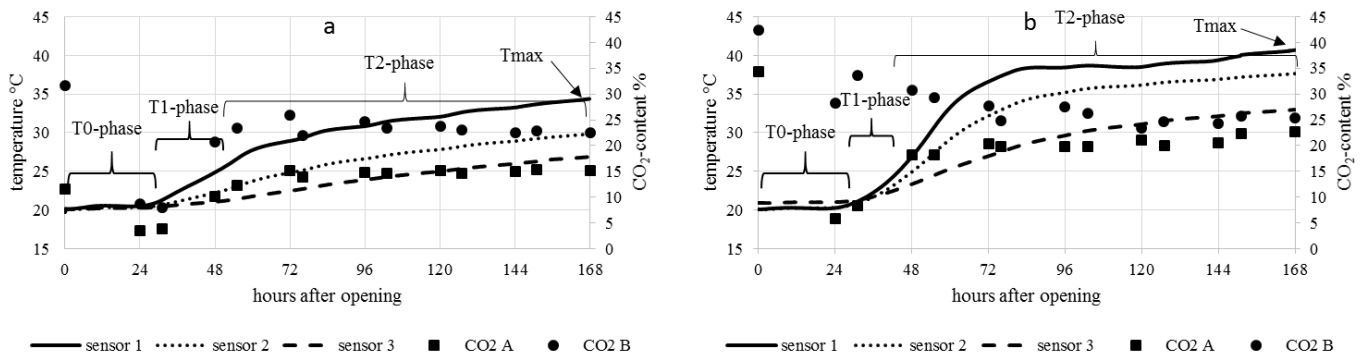
Figure 6   Means of temperatures measured by three temperature sensors in maize silage (average dry matter content=357 g/kg; a=297.5 kg DM/m^3; b=238 kg DM/m

Each type of ensilable crop could potentially be filled into the buckets. For further research it would be interesting to compare other plants like grass and alfalfa (unpublished data) to the results concerning maize. The results are close to practice and therefore transferable to farm conditions. On the other hand, the method is also suitable to laboratory conditions because it can be conducted under controlled conditions in an artificial environment to exclude the effects of influencing climate factors. Thereby the effect of one selected factor on silage aerobic stability can be tested against a control group and different treatments like additives, particle size and compaction. The experiment is easily repeatable and verifiable and also flexible if other parameters should be measured for example by inserting other or additional types of sensors. The method is connectable to other experiments, for example feeding experiments, which can be hooked up. The method also meets the requirements of Honig (1990), who outlined three main methods to determine aerobic deterioration, which are all combined in this method: Determination of CO_2-production, measurement of O_2-consumption and determination of temperature rise.

The option of gas analyses offers new opportunities for further research. They are relevant in the context of microbial fermentation but also regarding the topic of climate relevant gases, which are of great importance. Additionally to the measurements described, gas samples could be analysed for organic compounds. Montes et al. (2010) and Howard et al. (2010) show the importance of studying emissions of organic compounds, because of their impact on

ozone production. Furthermore organic volatile compounds have a negative effect on feed intake of dairy cattle (Weiß et al. 2009). The objective of the present study, which was to develop a new test method for aerobic deterioration of silages was successfully met. A highly effective method, which is close to practice, suitable to laboratory conditions and connectable to other experiments was developed.

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3.2 Paper II

Effects of different bulk densities on maize silage characteristics, temperature profiles, CO_2-and O_2-concentrations in small scale silos during aerobic exposure

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ABSTRACT

In this study, effects of different bulk densities on the Maize (Zea mays) silage characteristics, temperature, CO_2- and O_2- gases in small silos during the aerobic exposure were investigated. The method described in Jungbluth et al. (2016) was used. For this, 8 buckets (65.3 l) were filled with 40 kg FM (218.7 kg DM m^{-3}; n=4) or 50 kg FM (273.4 kg DM m^{-3}; n=4) of maize silage. Temperature was measured to observe heating resulting from microbial activity. Similarly, gas samples were taken and analysed by gas chromatography during reheating. Reheating was observed in every bucket. Temperature increases were higher (p=0.05) in the low-density treatment. Gas measurements showed CO_2 flowing out and O_2 diffusing into the buckets after opening. 24 h later, CO_2 concentrations reached their minimum when O_2 values reached their maximum. The CO_2 minimum was followed by an increase in concentration, whereas O_2 concentrations decreased. The reason for this change, happening immediately before reheating started, is microbial respiration, consuming O_2 and producing CO_2. The reheating process had no effect on the nutrient categories, crude ash, crude fibre, crude fat, neutral detergent fibre (aNDFom), and starch or on the pH value. Higher crude protein and metabolizable energy content(s) were found in the high-density treatment after reheating and dry matter losses between 0.58 and 4.38% were found and were tendentially higher in the low-density treatment. Therefore in agricultural practice it is recommended to reach high bulk densities in silage to preserve staple feed and it´s quality.

KEYWORDS

Maize (Zea mays) Silage, Oxygen Induced Deterioration, Density and Reheating

INTRODUCTION

The importance of silage as a livestock feed is tremendous and has continuously grown (Woolford, 1984). Today, apart from alfalfa (Medicago sativa) and various grasses, maize is the most important substrate for ensiling (Weinberg and Ashbell, 2002). Nowadays, the process of silage production is fully understood; therefore, the conditions needed to obtain high silage quality are well defined, and the risk of poor silage quality is thereby minimized (Woolford, 1984). However, in agricultural practice it seems to be difficult to meet these

requirements. The aerobic deterioration of silage is still a worldwide problem for quality of livestock´s feed and profitability of farms (Tobacco et al., 2011; Muck, 1988). Additionally, from the viewpoint of economically successful biogas production, dry matter (DM) and energy losses must be reduced to the minimum (Reinhold and Peyker, 2007). On farms, the diffusion of oxygen into silage is unpreventable. Even in well-sealed silos small amounts diffuse inside the material. Microorganisms metabolize this inflowing oxygen. A process, which proceeds along with DM losses. During the feed-out period, there is even more oxygen diffusing into the silage, leading to an increase in aerobic microbial metabolism. As a result heating of the silage and further losses of DM may occur (Rotz, 2003; Wilkinson and Davies, 2012; Pitt and Muck, 1993). The density and porosity of silage are the main physical factors affecting the amount of oxygen diffusing into the silage (Wilkinson and Davies, 2012).

In combination with airtight coverage, high compaction is the primary factor influencing the prevention and reduction of energy losses (Muck, 1988; Maack et al., 2007). By reducing the energy and feed losses, the efficiency and sustainability of agricultural production can be improved. It means that loses of the DM in maize silage can be reduced by a higher bulk density and feed-out rate (Köhler et al., 2013). In addition to fermentation biology, bulk density plays an important role in farm management because it affects the capacity of the silo and thereby the costs to farmers for the storage of a given quantity of plant material (Muck et al., 2003). A given size of a silo can include more silage if this material is higher compacted and new-built silos can be constructed to be smaller if there is the opportunity of high compaction. Therefore the main aim of the study was to investigate the effect of the physical factor 'bulk density' on silage under aerobic conditions. The silage characteristics investigated were the temperature development during oxygen influence (1), the concentrations of CO_2 and O_2 (2) and DM, energy and nutritional losses (3) during reheating of the maize silage. The hypothesis was that higher density leads to slower temperature rise and consequently lower losses. The concentrations of CO_2 and O_2 were expected to change due to microbial respiration expressed in a CO_2-increase and an O_2-decrease.

MATERIAL AND METHODS

The measurement trial was performed under laboratory conditions at the research facilities of the Institute of Agricultural Engineering of the University of Bonn, Germany in 2014. All the

experimental steps were done according Jungbluth et al. (2016). Four polyethylene buckets with a volume of 65 l were filled with 40 kg maize silage (low-density treatment, 218 kg DM m^{-3}) and another 4 with 50 kg (high-density treatment, 273 kg DM m^{-3}) maize silage, corresponding to densities slightly lower and higher, respectively, than those that are recommended by Honig (1987). The maize silage had been produced at Frankenforst, the research centre for animal production at Bonn University (Geographical coordinates: 7° 12' 22" E, 50° 42' 49" N). The cultivar used in the trials was Canon and had been harvested in autumn 2013. The samples were taken from a clamp silo that contained silage with DM contents varying between 356 g kg^{-1} and 358 g kg^{-1}, as found in the samples taken from the area of the silo used in the experiment. After filling, the buckets were resealed using an airtight cover with a rubber seal and clamping ring and were laid on their sides. During the experimental period, gas samples were taken twice per day and temperature was measured (resistor-based sensors and data logger ALMEMO®, Ahlborn Mess- und Regeltechnik GmbH, Holzkirchen, Germany) four times in each hour during the experiment. Gas analyses and temperature measurements were done according to Jungbluth et al. (2016). Each bucket had been weighed before and after the experimental period to quantify the weight losses that occurred during reheating. To start the inflow of oxygen, the buckets were opened, as shown in Figure 7, so that the air could diffuse into the unsealed buckets unhindered, which gives the microorganisms the opportunity to start aerobic metabolism. To prevent the resulting heat from dissipating, the buckets were thermally insulated with glass wool (100 mm, $\lambda = 0.04$ W K-1 m-1). The glass wool covered the whole bucket and is implied in Figure 7, which gives a schematic overview of the experimental setup.

After the buckets were opened, silage samples were taken through each open surface. After the entire experiment, three samples were taken from every bucket: one from the upper third, one from the middle third and one from the lower third. Each of these three samples was taken by drilling through the centre of the opened bucket with a drilling tube. All the samples were sent to an external laboratory (LKS Landwirtschaftliche Kommunikations– und Servicegesellschaft mbH, Lichtenwalde, Germany), which is accredited in accordance to DIN EN ISO/IEC 17025 and certified according to DIN ISO 9001 to analyse the feed components by near infrared spectroscopy (NIRS).

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temperature sensor 1   temperature sensor 2   temperature sensor 3

gas sampling point A   gas sampling point B

air

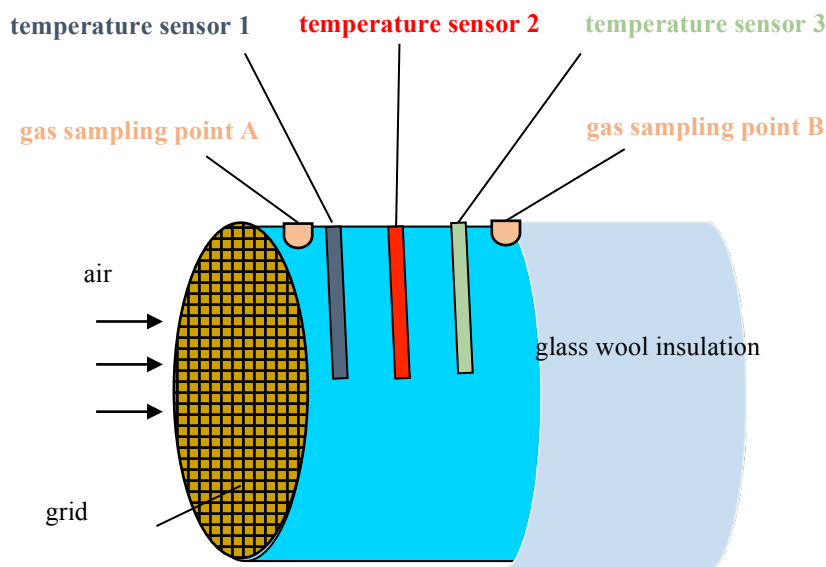glass wool insulation

grid

Figure 7   Schematic illustration of the experimental setup (modified according to Jungbluth et al., 2016)

The experiment was conducted twice at different times, each time using a group of four buckets: two of the high-density treatment and two of the low-density treatment to exclude the risk of random influences. At the end of the experiment the buckets were put in an upright position to take thermographic images using a thermal imaging camera (Variocam, InfratecnfraTec GmbH, Dresden Germany) and the IRBIS ® 3 software (Variocam, InfratecnfraTec GmbH, Dresden Germany). The data were evaluated using IBM SPSS Statistics version 22 as described in Jungbluth et al. (2016). First Kolmogorov-Smirnov-Test was conducted to examine if the measured data follows normal distribution. After this requirement was fulfilled, t-tests were used to compare the two different experimental groups (HD and LD) to each other and analysis of variance was used to compare the three different sensors to each other. The statistical significance was determined by Tukey test. Differences of means < 0.05 (P<0.05) were accepted to be significant. Differences of means <0.001 (p< 0.001) were accepted to be highly significant.

After the buckets were opened, silage samples were taken through each open surface. After the entire experiment, three samples were taken from every bucket: one from the upper third, one from the middle third and one from the lower third. Each of these three samples was taken by drilling through the centre of the opened bucket with a drilling tube. All the samples were sent to a certificated external laboratory to analyse the feed components by NIRS.

The experiment was conducted twice at different times, each time using a group of four buckets: two of the high-density treatment and two of the low-density treatment to exclude the risk of random influences.

The experiment has been conducted with the buckets in a lying position as shown in figure 7. At the end of the experiment the buckets were put in an upright position to take thermographic images using a thermal imaging camera (Variocam, InfratecnfraTec GmbH, Dresden Germany) and the IRBIS ® 3 software (Variocam, InfratecnfraTec GmbH, Dresden Germany).

RESULTS

Reheating was observed in each of the eight buckets. The course of reheating represented in Figure 8 shows a characteristically temperature development. It shows mean values for each hour of the experiment, calculated for each sensor of two buckets (one is low-density treatment and one is high-density treatment). Obtained temperature increase were significantly higher (p=0.05) in the buckets containing silage of low density compared with those containing silage of high density.
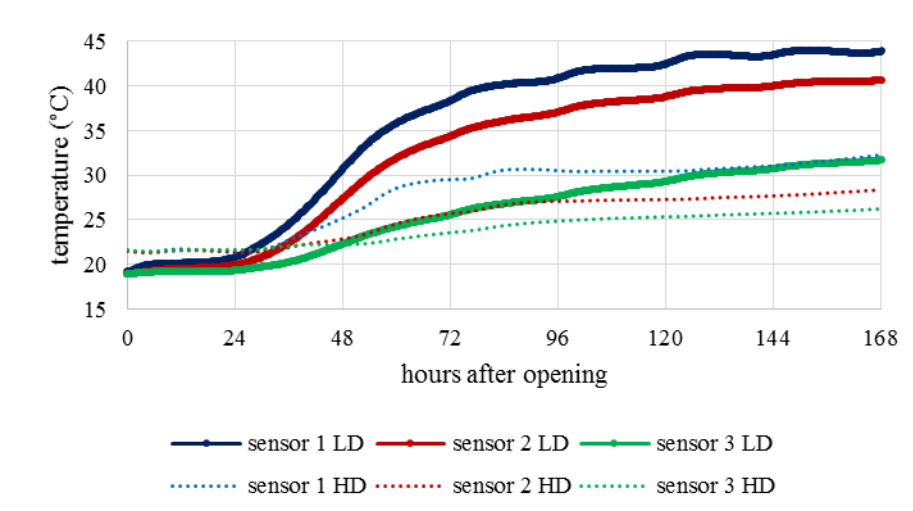

Figure 8   Courses of temperature measured in two buckets: One low-density (LD) and one high-density (HD) bucket, including three sensors each.

The calculated daily mean temperature values did not differ significantly between the high- and low-density treatments during the first two days of the experiment (T_0-phase). Starting on

the third day of the experiment, the calculated daily mean temperature values differed significantly between the high- and low-density treatments. On the 5th and 6th day of the experiment, the daily means of the temperatures measured by sensor 2 were significantly (p=0.001) different between the high- and low-density treatments. On the 6th and 7th day of the experiment, the daily means of the temperatures measured by sensor 3 were significantly (p=0.001) different between the high- and low-density treatments. The maximum temperature value was observed in a low-density treatment bucket, in which the temperature rose from 19.2°C to 44.0°C in 151.75 h (6th day of the experiment), as measured by sensor 1. The minimum temperature value was observed in a high-density treatment bucket, in which the temperature measured by sensor 1 increased from 21.4°C up to 32.2°C in 168 h (7th day of the experiment). The courses of temperature measured in these buckets are shown in Figure 8.

In most of the buckets of the low-density treatment, all sensors within single buckets recorded reheating on the same day or within a period of 24 h. In the high-density treatment, the temperature difference between the sensor positions within each single bucket was much greater. In every high-density treatment bucket, sensor 3 measured reheating two days later than the day indicated by sensor 1. Figure 9 shows the time in hours (T$_1$-phase) until multiple sensors measured a temperature difference of 5 K within each bucket, which is the time until reheating. Reheating according to this definition was reached in the low-density treatment buckets after 24 to 72 h of the experimental period. In comparison, the high-density treatment buckets were reheated after 24 to 96 h of the experimental period.
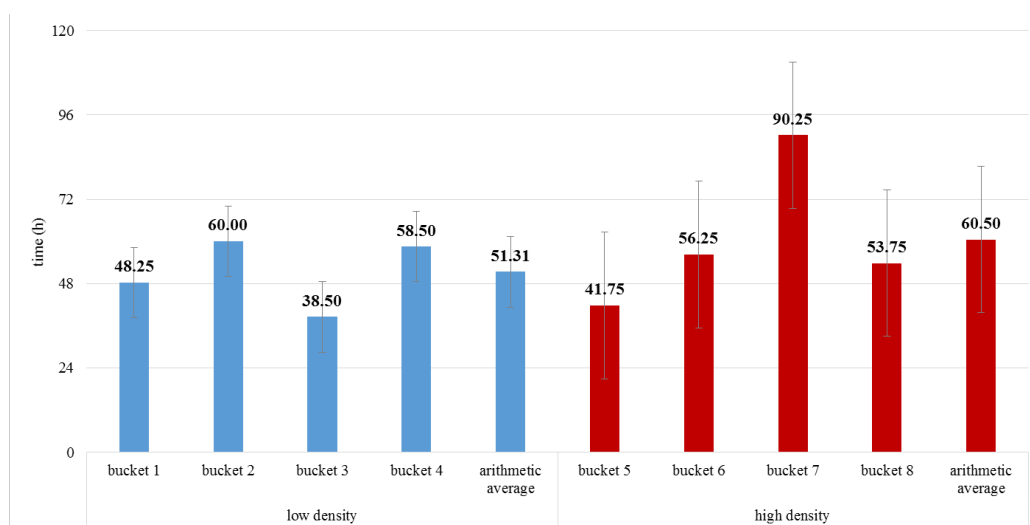

Figure 9   Length of T$_1$-phase (h) by silage density and bucket until reheating, measured as the time at which multiple sensors within a bucket of silage detected a temperature difference of 5 K

Figure 10 shows a thermographic representation of two buckets, one low-density treatment bucket and one high-density treatment bucket. The image has been taken at the end of the seven-day experiment to visualize the status of heat moving into the material. The figure illustrates the area and position of the hotspot, which had penetrated deeper into the material of lower density.
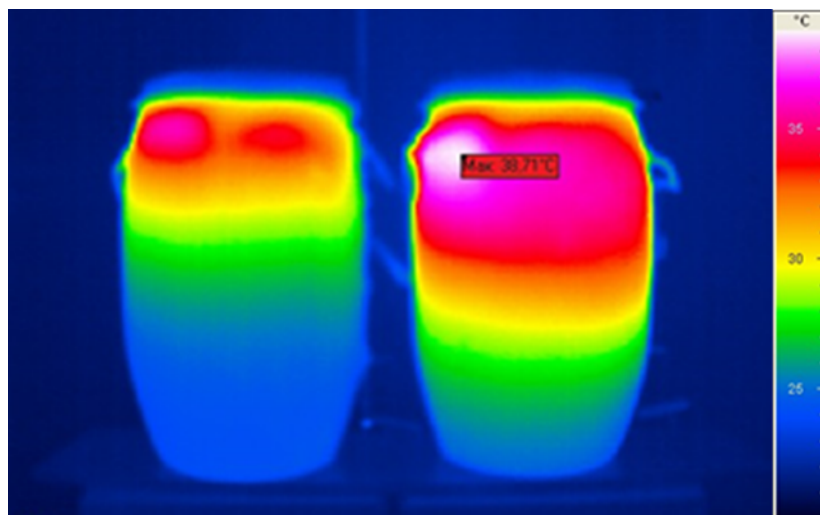
Figure 10   Thermographic image of one high-density treatment bucket (left) and one low-density treatment bucket (right) obtained on the last day of the experimental period (day 7)

The measured CO_2 concentrations are displayed in Figure 11a for the low-density treatment buckets and in Figure 11b for the high-density treatment buckets. Figure 11 also shows the measured O_2 concentrations, which increase after opening of the buckets and decrease again in the T_0-phase. After T_0-phase O_2-concentrations decreased below 5%, which is the lower level that can be analysed by the standard method. O_2 concentrations were higher in the samples taken at sampling point A than those taken at sampling point B.

In the high-density variation concentrations of O_2 at sampling point B could not be determined, because they fell below the lower level. CO_2 concentrations were lower in the samples originating from sampling point A compared with those originating from sampling point B. In the first samples taken at the beginning of the experiment, CO_2 concentrations were higher than those measured at the second day. Afterwards, the CO_2 concentrations rose until they reached level lower than the initial value, which persists for the rest of the experimental period.
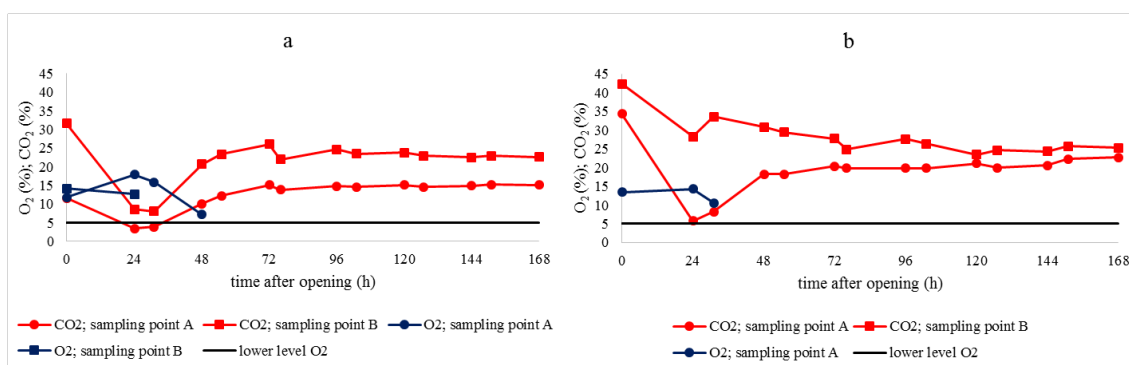
Figure 11   Mean CO_2 and O_2 concentrations measured in gas samples from a) low- and b) high-density treatment buckets (O2 concentrations below 5% (= lower level) could not be analysed)

The analyses of the silage samples, which are represented in table 6, showed that the bucket-ensiled material tended to dry after reensiling compared with silage from clamp silo, especially in the high-density treatment buckets, as shown by the analyses of the samples taken directly after opening the buckets before reheating started. Furthermore, the data indicated that none of the nutrient values, which included those for crude ash, crude protein, crude fibre, crude fat, starch and neutral detergent fibre determined on an organic matter basis (aNDFom), changed significantly as a result of the reensiling process. The pH was higher in the buckets after reensiling. The energy content (calculated as metabolisable energy and net energy content for lactation) was not changed significantly after reensiling.

Table 6 also shows the analytical state of the silage samples based on the DM before and after reheating. The analyses of the silage samples showed that the low-density buckets lost more moisture compared with the high-density treatment, as shown by the analyses of the samples taken after reheating.

The data also indicated that none of the nutrient concentrations in the crude ash, crude fibre, crude fat, aNDFom or starch categories changed significantly due to the reheating process. The pH value in the buckets did not change after reheating. In the high-density treatment buckets, significantly higher protein content was observed in the reheated samples compared with the samples taken before reheating. There was no similar protein increase in the low-density treatment buckets. For the high-density treatment, there was a significantly higher content of metabolizable energy in the reheated samples compared with the samples taken before reheating. There was no similar increase in the energy content for the low-density treatment.

Effects of different bulk densities on maize silage characteristics, temperature profiles, CO_2- and O_2-concentrations in small scale silos during aerobic exposure

Table 6   Analytical state based on dry matter for silage samples from the silo on farm before filling the buckets (sample 0), from the buckets after opening (sample 1) and from the buckets after reheating for silage originating from three different sampling depth as described in Jungbluth et al. (2016) (samples 2, 3 and 4); mean (standard deviation)

treatement	sample	dry matter [g/kg]	crude ash [g/kg DM]	crude protein [g/kg DM]	crude fibre [g/kg DM]	ether extract [g/kg DM]	starch [g/kg DM]	pH	aNDFom [g/kg DM]	ME [MJ/kg DM]	NEL [MJ/kg DM]
silo	0	357.0 (1.1)	38.4 (2.6)	76.4 (0.8)	184.0 (3.9)	33.0 (3.6)	331.6 (12.9)	(0.03)	372.1 (13.3)	11.4 (0.1)	7.0 (0.1)
low density	1	353.7 (12.5)	38.7 (0.7)	78.76 (2.7)	179.2 (9.0)	35.0 (3.1)	345.9 (16.9)	4.0 (0.2)	376.9 (24.2)	11.5 (0.2)	7.0 (0.1)
low density	2	353.2 (8.4)	39.1 (2.3)	75.8 (7.0)	184.3 (7.4)	34.9 (2.8)	334.6 (31.1)	4.0 (0.0)	384.8 (22.6)	11.4 (0.2)	7.0 (0.1)
low density	3	360.5 (11.6)	38.2 (1.9)	74.9 (3.3)	188.4 (9.9)	33.6 (1.8)	336.0 (26.7)	3.9 (0.2)	380.4 (21.0)	11.4 (0.1)	7.0 (0.1)
low density	4	353.8 (18.8)	38.2 (2.1)	75.4 (3.8)	179.3 (6.3)	33.4 (2.4)	370.4 (18.8)	4.0 (0.2)	370.4 (24.3)	11.4 (0.2)	7.0 (0.1)
high density	1	369.9 (8.8)	37.4 (3.4)	70.8 (6.1)	185.8 (12.6)	33.2 (2.6)	360.4 (47.5)	4.0 (0.1)	386.2 (32.3)	11.3 (0.2)	7.0 (0.1)
high density	2	367.3 (5.5)	40.0 (3.2)	80.7 (6.0)	178.7 (10.2)	38.5 (1.7)	347.7 (17.0)	4.1 (0.2)	384.8 (41.4)	11.6 (0.2)	7.1 (0.2)
high density	3	366.2 (10.2)	38.1 (1.9)	79.5 (4.1)	173.5 (6.6)	36.2 (3.8)	359.8 (32.2)	4.0 (0.2)	372.5 (5.9)	11.6 (0.1)	7.1 (0.0)
high density	4	369.9 (13.2)	39.3 (3.3)	77.7 (7.4)	185.5 (8.3)	34.8 (1.5)	334.9 (9.9)	4.0 (0.3)	389.9 (31.5)	11.4 (0.1)	7.0 (0.1)

Average DM losses of 2.8% were calculated based on the data from low-density treatment and average DM losses of 1.9% were calculated based on the data from high-density treatment for the reheating period of the experiment. The minimum loss was found in a bucket from the high-density treatment, and the maximum loss was found in a low-density treatment bucket. The total DM losses due to reheating were tendentially higher in the low-density treatment.

DISCUSSION

The reheating, which was observed in the buckets during the T_1-phase was caused by the microbial activity that was induced by the entrance of oxygen into the silage vessel during the T_0-phase. The CO_2 measurements showed that the CO_2, inside the closed buckets followed a concentration gradient and flew out after the buckets were opened and at the same time O_2 diffused into the buckets (T_0-phase). After opening but before the heating process started (T_0-phase), the microorganisms especially yeasts switch from an anaerobic to an aerobic metabolism. Most likely, the microorganisms were unable to immediately use the oxygen that diffused into the buckets after they were opened. As a result, there was no difference regarding the daily mean temperatures between the density treatments during the T_0-phase. This could be reasoned by the change in microbial metabolism (anaerobic → aerobic), which seemed to depend only on oxygen availability and not on the density of the silage in the buckets. The results of oxygen measurement during T_0-phase showed that oxygen was available in the first 36 to 48 h even in the high-density buckets and values even increased on

the first day after opening. In the high density-buckets O_2 did not reach sampling point B in concentrations higher than 5%. According to Muck et al. (2003), the exclusion of air results in the recovery of a large amount of DM.

The variables that determine silage density are the liquid content, solid matter and void volume. During the process of compacting plant material, the void volume is removed by compression while the silage density increases (Muck et al., 2003). The compaction necessary to reduce the gas flow rate to less than $20 \ l \ h^{-1} \ m^{-2}$, which is the airflow rate obtainable in well-compacted grass silage, is $225 \ kg \ DM \ m^{-3}$ for maize with a DM content of $280 \ g \ kg^{-1}$. The compaction necessary for maize with a DM content of $330 \ g \ kg^{-1}$ is $265 \ kg \ DM \ m^{-3}$ (Honig, 1987). Because of a greater void volume and resulting greater porosity of the silage in the low-density treatment, this treatment was expected to diffuse more air compared with the high-density treatment. This expectation is confirmed by the data of oxygen measurement. More oxygen entered the low-density buckets. In contrast, the dense compaction of the silage and lesser void volume in the high-density treatment represented a stronger barrier against the diffusion of incoming air. As a result, the oxygen entered the low-density buckets more easily compared with the high-density treatment. Thus, a higher temperature rise caused by the higher amounts of oxygen metabolized by microbial respiration was observed in the low-density compared with the high-density treatment. At the same time the microbial respiration is the reason for the decrease of oxygen measured during the T_1-phase. Twenty-four hours after the buckets were opened at the end of T_0-phase, the CO_2 concentrations in the gas samples taken from the buckets reached their minimum (Jungbluth et al., 2016), at the same time when O_2 values reached their maximum.

The CO_2 minimum was followed by an increase in CO_2 concentration in the gas samples during T_1-phase, whereas O_2 concentrations decreased until it was not possible to detect any more O_2 using the applied test method. The reason for this change which happened immediately before the heating process started was the respiration of microorganisms, which used O_2 and produced CO_2. The fact that less oxygen reached sampling point B compared to sampling point A means that less oxygen reached temperature sensor 3 compared to temperature sensor 1 in all of the buckets, apparently because the microorganisms utilized most of the oxygen before it could diffuse to the deeper position of sensor 3.

This oxygen gradient led to a greater temperature rise in the material surrounding sensor 1 compared to that in the material surrounding sensor 2 and 3. The recent findings concerning

temperature and oxygen concentrations confirm the calculated diffusion model of aerobic deterioration calculated by Pitt and Muck (1993). Likewise the temperature development as well as the course of oxygen concentrations measured by Sun et al. (2015) using oxygen sensors in silage underlines our results. The possibility of taking gas samples out of silage is also applicable on farm from clamp silos, whereas sensors are more expensive and not easy to applicate them in practice silos. The buckets in the high-density treatment showed slightly longer T_1-phases than those in the low-density treatment, whereas temperatures itself differed much stronger between the density variations. This fact indicates that high density has minor impact on delay of reheating. This is confirmed because T_0-phase was not significantly longer in the high density variation, but higher density had great impact on reduction of temperature during T_1-phase and thereby T_{max} was significantly lower in the high density treatment buckets.

The silage used in this experiment had been previously ensiled. Silage was used instead of fresh maize to make sure, that the material in the buckets has the same fermentation quality and properties to make the buckets comparable. The same experiment has been conducted with fresh shopped maize directly ensiled into buckets, to obtain information regarding changes in the material according to the influence of air using both fresh and previously ensiled silage (unpublished data). Results of this trial will be presented in prospective papers. During the process of transferring the silage from the silo to the buckets, the material lost moisture and the compaction process also led to moisture losses caused by squeezing fluid out of the silage. For these reasons, the material tended to be dryer in the high-density compared with the low-density treatment. Based on these findings, available results confirmed the prediction of Muck et al. (2003) that excessive densities increase effluent losses. The analyses of the silage samples showed that after reheating the silage in the buckets tended to be drier in the low-density than in the high-density treatment because the higher moisture content in the former treatment implies a steeper gradient in moisture content between the silage and the surrounding air. Obviously, this condition corresponds to a higher potential for moisture loss. A second and more important reason is that the evaporation rate was higher in the opened buckets in the low-density compared with the high-density treatment, as shown by the analyses of the samples taken after reheating (Table 6).

The amount of H_2O produced by respiration was inadequate to compensate for the losses. The increase in pH resulted from the conversion of acetic and lactic acid into CO_2 and H_2O by

yeasts, activated by the oxygen entering the buckets during silage transfer. The fact that none of the nutrient concentrations in the crude ash, crude fibre, crude fat, aNDFom or starch categories changed significantly due to the reheating process are in accordance with our expectations. The higher content of metabolizable energy calculated by the silage in the high-density treatment could be explained by the higher content of protein in this silage compared with that in the low-density treatment. The higher protein content observed in the high-density compared with the low-density treatment showed that the different nutrient categories were not degraded in equal amounts. As a result, the relation of the nutrients to one another was changed by reheating in the high-density treatment because there was relatively less protein degraded compared with the other nutrients. The fact that this phenomenon was not observed in the low-density treatment implies that the higher density preserves valuable protein in the silage and results in higher energy content. The fact that there were only small or nearly no changes in the analytical categories of the silages due to oxygen might be justified by the fact that the silage used was well ensiled and the circumstances chosen, as well as the crop itself were conducive for quality silage. Garcia et al. (1989) found much greater losses in quality parameters and larger changes in nutrient categories due to oxygen infiltration, when they used alfalfa silage under circumstances that were not beneficial for quality silage. These results showed that further research is needed using valuable crops, which are less easy to ensile such as alfalfa, or grass. Also other influencing factors like parameters at ensiling should be taken into account in further research. Another interesting topic to investigate in the future is the remain of nitrogen resulting from protein degradation. Therefore, in future studies gases containing nitrogen will be included and the focus of further research should be on emissions resulting from silage.

On farm scale, Köhler et al. (2013) found that DM losses in case of maize silage averaged 10%, as measured by the total-in versus total-out procedure. Compared with the current results, the DM losses found by Köhler et al. (2013) were higher, depending on the treatment. Compared with small-scale experiments, there are more sources of losses in agricultural practice or in farm-scale operations. Rotz (2003) quantified total silo losses to range from 6% for sealed structures up to more than 15% for bunker silos. The losses described by Rotz (2003) are higher than those found in the present study. A difference between the studies in the experimental duration might be a reason for this discrepancy. (Pitt, 1986) predicted that the long-term storage losses resulting from oxygen infiltration through the silo container and

into the silage mass would vary between 1 and 3% of the ensiled DM per month, as calculated with a mathematical model. Consistent with the present findings, the predicted losses by Pitt (1986) had similar magnitude. In contrast to the results obtained here with an opened system, Pitt (1986) assumed a closed silo, with oxygen infiltration occurring through the silo container into the silage mass. For that reason, the values calculated by Pitt (1986) are lower than the values reported here. According to the findings of Köhler et al. (2013), the DM losses in the low-density treatment exceeded those in the high-density treatment. The total DM losses due to reheating were tangentially higher in the low-density treatment of the present study. Contrary to the expectation, these losses were not significantly different but tended to be higher in the low-density compared with the high-density treatment. Dense compaction of plant material is one of the most important factors supporting the stability of silage by restraining the growth of microbial populations and their metabolism and thereby preserves DM, nutrients and energy during the aerobic exposure. However, dense compaction is only one factor influencing silage quality. High silage quality and aerobic stability is always a result of many factors issuing from crop, environment and management during harvest, filling, storage and feed out (Wilkinson and Davies, 2012).

CONCLUSIONS

The findings confirm that dense compaction of plant material is an important physical factor supporting the stability of silage. High density has great impact on reduction of temperature during feed out period (objective 1). Additionally high density reduces microbial respiration activity in silage and can potentially reduce total mass losses (objective 2). High silage density preserves DM, nutrients and energy during the aerobic feed-out period (objective 3).

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Effects of Three Different Additives and Two Different Bulk Densities on Maize Silage
Characteristics, Temperature Profiles, CO$_2$ and O$_2$-Dynamics in Small Scale Silos during
Aerobic Exposure

3.3 Paper III

Effects of Three Different Additives and Two Different Bulk Densities on Maize Silage Characteristics, Temperature Profiles, CO$_2$ and O$_2$–Dynamics in Small Scale Silos during Aerobic Exposure

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Effects of Three Different Additives and Two Different Bulk Densities on Maize Silage Characteristics, Temperature Profiles, CO_2 and O_2-Dynamics in Small Scale Silos during Aerobic Exposure

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ABSTRACT

Silage quality and aerobic stability are sometimes insufficient. If management requirements are not met or to improve silage quality, additives are often used. The objective of this study is to investigate the effects of different factors on silage during aerobic conditions. Whole-crop forage maize was harvested and 24 buckets (65 l) were filled with three silage treatments: A chemical additive (sodium benzoate, potassium sorbate, sodium acetate) was used. Two other treatments were made with biological inoculants (*Lactobacillus buchneri*, *L. plantarum*, *Pediococcus acidilacti* and the other one containing *L. buchneri*, *L. plantarum*, *L. rhamnosus)*. An untreated variation was also ensiled. Two different densities were adjusted during ensiling. After opening temperature was measured for seven days and O_2 and CO_2 concentration were analysed.

The findings show that the chemical additive prevented silage from reheating and deterioration very effectively. Aerobic reheating of silage was also successfully inhibited through biological additives and high density.

KEYWORDS

Inoculation; additives; silage quality; aerobic stability; reheating; microbial respiration

INTRODUCTION

Nowadays, it is increasingly important to save energy in the food production chain. The world's population is growing and the challenge of feeding all people presupposes efficiency in every step of food production [1]. The aims of feed conservation as silage are maintaining quality and feeding characteristics of the fresh crop, and reducing dry matter and energy losses to a minimum [2]. Silage, which is used as feed for milk and meat producing animals, undergoes spoilage when it is exposed to air [3]. The diffusion of oxygen into silage in bales or clamp silos is unpreventable. Even in well-sealed silos on farms small amounts of oxygen diffuse into the silage. This inflowing oxygen is used as a source for microbial respiration, a process, which proceeds along with DM losses. When the silo has been opened for feed-out, there is even more oxygen diffusing into the silage, leading to an increase in aerobic microbial

metabolism. As a result heating of the silage and further losses of DM may occur [4, 5]. Spoilage of silage means energy losses, which should be prevented. The aerobic deterioration of silage is a worldwide problem for feed quality and farm profitability [6].

Along with an anaerobic environment, appropriate substrate and an adequate quantity of lactic acid producing bacteria are needed to reach a quick drop in pH during ensiling, which is one of the most important requirements to reach high silage quality [2]. The quality of silage is often not optimal because the process of ensiling is dynamic and complex and can be influenced by many different factors [7]. Wilkinson and Davies [5] give the advice of using additives if there is the risk that management requirements are not met. The use of homofermentative lactic acid producing bacteria as silage inoculants has the main aim to direct fermentation towards lactic acid production. As a consequence, the pH drops fast [8]. Spoilage is caused by damaging microorganisms. Some of them are activated when coming in contact with oxygen, which leads to aerobic deterioration [3]. One of the common reasons for using additives is to inhibit aerobic microorganisms, especially those associated with aerobic stability [7]. Yeasts have been identified to be the primary initiator of aerobic spoilage. Lactic acid is not as effective in its antimycotical effect as propionic acid [8, 9]. In fact, lactic acid producing bacteria used as inoculant have even been observed to decrease aerobic stability [9, 10]. Bacteria producing both propionic and lactic acid have a great potential to be used as heterofermentative inoculants [8]. A heterofermentative *Lactobacillus*, producing lactic acid and acetic acid, which is also associated with the production of propionic acid, is *Lactobacillus buchneri* [7]. Muck [10] describes *Lactobacillus buchneri* to be the steadiest heterofermentative concerning the improvement of aerobic stability, compared to others commercially available. The same prolonging and improving effect on aerobic stability as propionic acid applies to acetic acid. Acetic acid is an inhibitor of spoilage organisms and thereby increases aerobic stability. Heterofermentative microorganisms producing both acetic acid and lactic acid are e. g. *Lactobacillus rhamnosus* and *Lactobacillus plantarum* [11].

The objective of the study was to investigate the effects of different factors (biological, chemical and physical) on silage during aerobic conditions. As a physical factor, different bulk densities were adjusted during ensiling. Silage density is one of the main physical factors, which affects the rate of oxygen inflow into the silage during feed-out [5]. There is an increasing desire to reach high density of silage because a low void volume means a low

initial air content. This can significantly reduce the risk of temperature rise and the loss of DM and energy [12]. Two different biological inoculants (biological factor) were added to parts of the silage and a chemical additive (chemical factor) was also used. Another objective of the study is to compare the impact of the different factors to each other.

Maize has become the most important feed in the world and due to the development in animal husbandry, the importance of maize silage is also on the rise [13]. For this reason and because of the great ensilability of maize, the trials in the present study are conducted with maize.

MATERIALS AND METHODS

Ensiling of material

Maize (*Zea mays*) has been harvested at Frankenforst, the education and research centre for animal production (longitude: 7° 12' 22" E, latitude: 50° 42' 49" N) at the University of Bonn, Germany. Maize of the variety "Canon" was used. It has been harvested in September 2014 by a single–row Pöttinger MEX GT chopper (PÖTTINGER Landtechnik GmbH, Grieskirchen, Austria) and chopped to a theoretical chopping length of 5 mm. The chopper included a grinding component which hit the corns of maize plants and led to good chopping quality. The maize contained dry matter with a mean of 355 g/kg (standard deviation=10 g) as found in the samples taken at the day of ensiling.

Four different silage treatments were produced for the trial: An untreated control (treatment CON) was ensiled. Two other treatments were ensiled with biological inoculants, one containing *Lactobacillus buchneri, Lactobacillus plantarum* and *Pediococcus acidilacti* (treatment B1; Bonsilage Twin MS, H. Wilhelm Schaumann GmbH, Pinneberg, Germany) and the other one containing *Lactobacillus buchneri, Lactobacillus plantarum* and *Lactobacillus rhamnosus* (treatment B2; Bonsilage Twin MF, H. Wilhelm Schaumann GmbH, Pinneberg, Germany). Treatment CHEM was produced with a chemical silage additive (Silostar Liquid HD, H. Wilhelm Schaumann GmbH, Pinneberg, Germany) containing sodium benzoate, potassium sorbate and sodium acetate. All additives are used in practice. The additives were applied manually with a pressurized air duster connected to a compressor.

Effects of Three Different Additives and Two Different Bulk Densities on Maize Silage Characteristics, Temperature Profiles, CO_2 and O_2-Dynamics in Small Scale Silos during Aerobic Exposure

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Twenty-four polyethylene buckets with a volume of 65 l were used for the experiment as they are recommended by Hussin et al. [14]. Twelve of these buckets (three of each treatment) were filled with 36 kg FM (196 kg DM/m^3) as a low–density variation (LD), and another twelve (three of each treatment) with 48 kg FM (261 kg DM

/m^3) as a high–density variation (HD).

After filling with a hydraulic press as described in Jungbluth et al. [15], the buckets were sealed for six months using an airtight cover with a rubber seal and clamping ring and were laid on their sides to avoid an enrichment of CO_2 inside the bucket and to simulate a silo on farm.

Preparation of buckets

All measurements and analyses have been conducted according to the method described in Jungbluth et al. [15]. Thus, after the six months of exclusion of oxygen, three temperature sensors (resistor-based sensors, Ahlborn Mess- und Regeltechnik GmbH, Holzkirchen, Germany) were inserted vertically into each horizontally lying bucket, as shown in Figure 12. The sensors were placed at a distance of 150 mm (sensor 1), 300 mm (sensor 2) and 450 mm (sensor 3) from the opening cover to represent the upper third, the middle third and the lower third of the bucket. Each sensor formed the top end of a metal rod, which had a length of 200 mm. The temperature sensors were connected to a data logger (ALMEMO®; Ahlborn Mess- und Regeltechnik GmbH, Holzkirchen, Germany) to register the temperature data every 15 minutes for the next seven days. The experimental period took ten days. The target for potential aerobic stability recommended by Wilkinson & Davies [5] is seven days. So the experimental period was calculated to cover the expected or potential period of reheating.
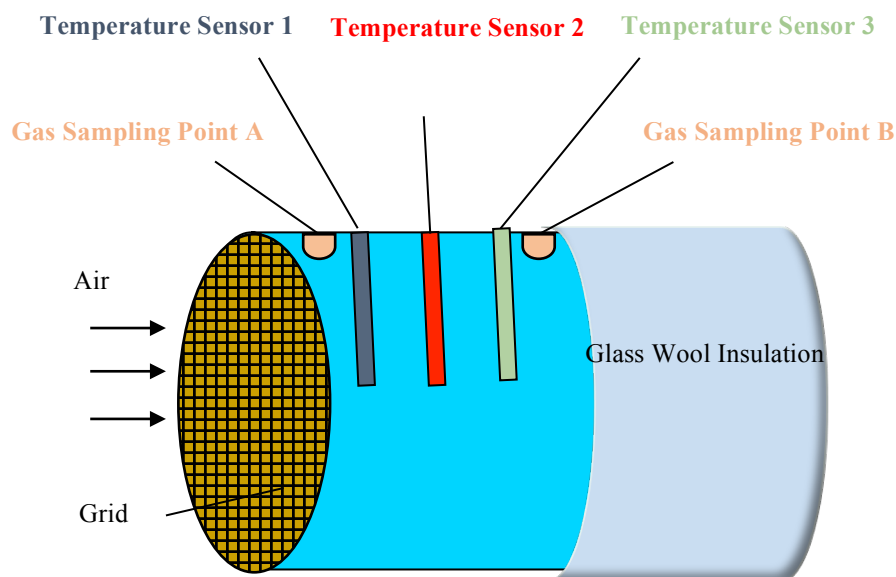
Figure 12: Schematic Illustration of the Experimental Setup (Modified According to Jungbluth et al.)

Gas samples were taken from the buckets to observe the courses of CO_2 and O_2 during aerobic exposure to gain insights into processes of microbial respiration and diffusion. To extract gas samples from the buckets, cannulas were inserted (BD Vacutainer Safety-Lok[TM] Blood Collection Set, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) and used to puncture the stopper of an evacuated 20 ml headspace vial. This method of gas sampling has also previously been described by Jungbluth et al. [15]. According to the method, each bucket received two gas sampling points. One near the opening of the bucket (sampling point A, 100 mm from the opening), and the second was inserted farther from the opening (sampling point B, 400 mm from the opening). The CO_2 and O_2 concentrations of the gas samples were analysed by gas chromatography in an external laboratory.

Experimental phase

To start the exposure of oxygen, the buckets were opened. To prevent the buckets from heat losses, the entire buckets were thermally insulated with glass wool (100 mm, $\lambda = 0.04$ W K^{-1} m^{-1}) as plotted in figure 12 which shows a schematic depiction where the glass wool insulation is only implied at the bottom of the buckets. To minimise the environmental impact on temperature progression, the experiment was conducted in a closed building with a nearly

constant temperature (mean = 20.1 °C; standard deviation = 1.15 °C) and no direct exposure to solar radiation.

After the buckets were opened, silage samples were taken through each open surface. At the end of the entire experiment, three samples were taken from every bucket: one from the upper third, one from the middle third and one from the lower third. Each of these three samples was taken by drilling through the centre of the opened bucket with a drilling tube as it is already described in Jungbluth et al. [15]. All samples were send to an external laboratory (LKS Landwirtschaftliche Kommunikations– und Servicegesellschaft mbH, Lichtenwalde, Germany), which is accredited in accordance to DIN EN ISO/IEC 17025 and certified according to DIN ISO 9001 to analyse the feed components and parameters dry matter, crude ash, crude protein, crude fibre, crude fat, starch, pH, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom), metabolisable energy (ME) and net energy lactation (NEL) and the parameters which additionally important to characterize fermentation success: lactic acid, acetic acid, propionic acid, ethanol, 1,2-propandiol and 1-propanol.

During the experimental period, gas samples were taken twice per day and analysed in an external laboratory using a gas chromatograph from SRI Instruments (8610 C, SRI Instruments, Torrance, USA). The analytic method is described by Wulf et al. [16].

The experiment has been conducted with the buckets in a lying position as shown in Figure 12. At the end of the experiment, the buckets were put in an upright position to take thermographic images using a thermal imaging camera (Variocam, InfraTec GmbH, Dresden Germany) and the IRBIS ® 3 software (Variocam, InfraTec GmbH, Dresden Germany).

Statistical Analysis

The data was evaluated using IBM SPSS Statistics version 23. To investigate if the data follows a normal distribution, the Kolmogorov-Smirnov test was used. According to the results of this test, the temperature data from the trial did not follow a normal distribution. Consequently, the Kruskal-Wallis-H-test was used to analyse if differences between temperatures were significant. Differences among means < 0.05 ($p < 0.05$) were accepted to be significant. Differences among means < 0.01 ($p < 0.01$) were accepted to be highly significant.

RESULTS AND DISCUSSION

The results of the temperature measurements are graphically represented in Figures 2 and 3. Figure 13 shows the temperature dynamics of the control without silage additive (treatment CON) and the temperature dynamics in the silage treated with chemical additive (treatment CHEM). Figure 13 also represents the buckets including silage treated with the biological additives (B1 and B2). Each graph shows the means calculated from the hourly average of the original data of three buckets per variation.
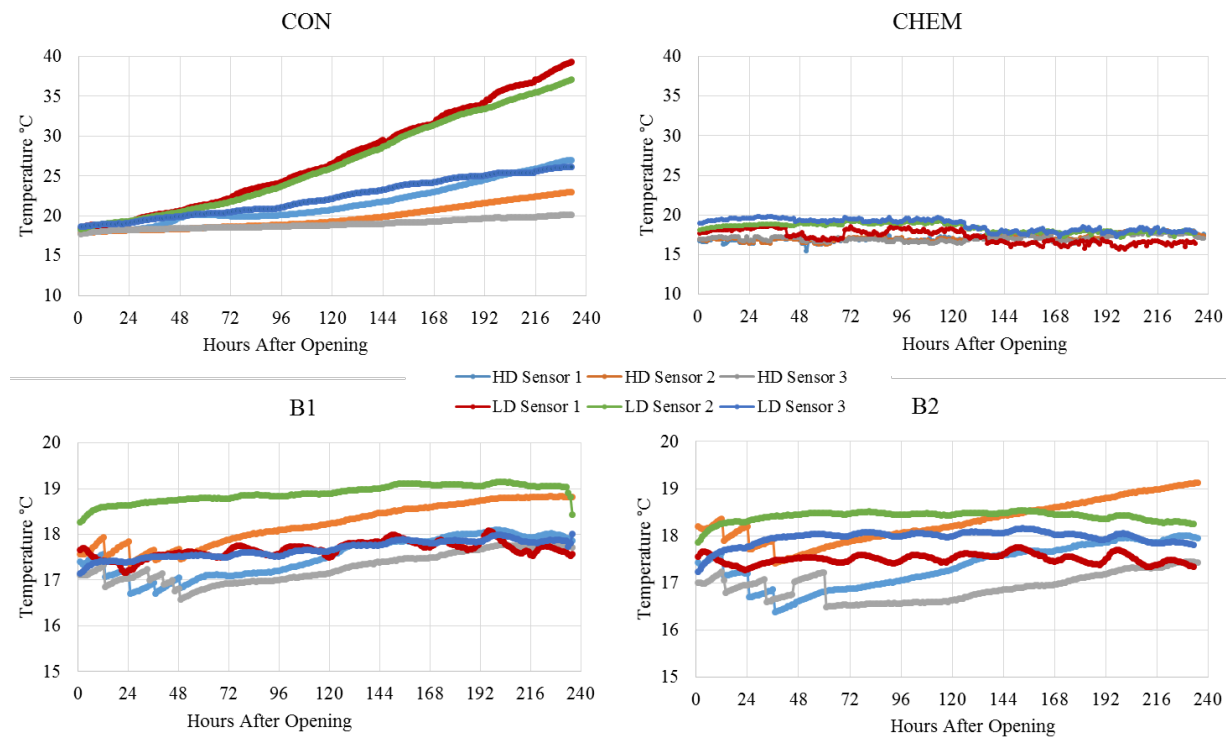
Figure 13: Temperature Means per Sensor, Obtained from Hourly Average of Temperature Data Measured in Different Treatments

At the beginning of the experimental period, a lag time (T0–phase; cf. [15]) of 24 hours can be observed, in which the temperature of the control does not rise significantly. Therefore, the statistical analyses did not show any significant differences between the temperatures of the sensors in the control on the first 48 hours. These findings are consistent with the data shown in Figure 13.

Figure 14 graphically visualises the gas concentrations and shows that there is nearly no oxygen inside the untreated silage at the time of opening but the CO_2 content is much higher than in the surrounding air. After opening, O_2 diffused into the buckets during the T0–phase while CO_2 flowed out. When the temperature started rising at the beginning of the T1–phase (cf. [15]), the oxygen content of the buckets decreased. This fact underlines that microorganisms start to change their metabolism and use oxygen as a direct response to an anaerobic phase. Once they have changed their metabolism from anaerobic to aerobic, the oxygen is consumed by microbial respiration which is accompanied by temperature rise.
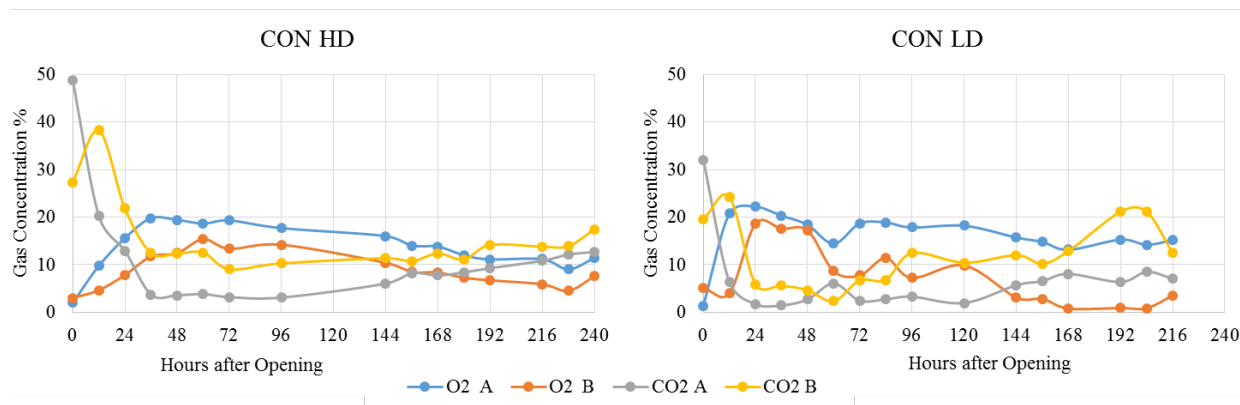
Figure 14: Means of Gas Concentrations of Samples Taken from Buckets of the Control Variation at Two Sampling Points (A and B)

The low-density variation of treatment CON became significantly warmer than the high-density variation. Figure 14 shows that oxygen diffused into the low-density buckets much faster than into high density buckets. The maximum O_2-Konzentration in the lower part of the LD buckets of treatment CON is reached after 14 hours, whereas the maximum O_2-Konzentration in the lower part of the HD buckets of treatment CON is reached after 60 hours. This can be reasoned by the fact that the low-density buckets include a much higher void volume holding a higher capacity for entering gas. In contrast to this, the higher density of HD buckets represents a stronger barrier against incoming air. Johnson et al. [17], observed a longer period of aerobic stability in mechanical processed corn silages compared to unprocessed variations. They justify this finding by the fact that the processed variation has a greater wet pack density and thereby excludes oxygen. These findings go along with the findings at hand.

Sensor 1 measured higher temperatures than sensor 2 which in turn measured higher temperatures than sensor 3 in the HD and LD variation. These results underline previous findings by Jungbluth et al. [15] and Figure 14 shows the reason for this fact: Less oxygen is reaching the deeper parts of the buckets (sampling point B) than the parts of the silo directly near the face. Additionally, more CO_2 accumulates in the deeper silo areas. This is underlined by the results of the statistical analysis which showed the temperature measured by sensor 1 to be highly significant (p<0,001) higher than the temperature measured by sensors 2 and 3 in the HD variation during the period beginning in hour 48 of the experiment. In the LD variation, the temperatures measured by all three sensors differ significantly (p<0.05) from each other in this period.

During the entire experimental period, the temperatures measured in both variations of treatment CON keep on rising while O_2 is metabolised and CO_2 is produced. Analysing the results of gas samples, it should be kept in mind that gas concentrations are a result of two processes: microbial metabolism and air exchange with the surrounding air. Even if the O_2 content of the gas samples does not decrease at any time of the experiment, there can be respiration because surrounding air including oxygen enters the buckets through the open face and balances the concentration gradient by diffusion. The same process can be observed on farm scale in clamp silos. Another aspect which may occur during the experimental phase and influence the measured gas concentrations, is the changing dissolvability of CO_2 in the plant water content dependent on temperature and pH.

The findings of treatment CON are not new. Similar results have already been shown by many studies, e. g. Muck et al. [18], Maack et al. [19], Köhler et al. [20], and Jungbluth et al. [15]. Nevertheless, the findings are important because on the one hand, they confirm previous findings and on the other hand, they show that the experiment functions properly and that the circumstances of the trial have been chosen adequately. Furthermore, the findings are significant for drawing a comparison between different treatments.

In contrast to treatment CON, treatment CHEM did not undergo reheating. The temperature stayed on the same level during the total experimental procedure. These observations were made in both the HD and LD variation. The findings show that the chemical additive can prevent silage from deterioration very effectively and can inhibit microbial heat production. Therefore, the silage gets colder if surrounding temperatures decrease. This observation is

obvious and can be proved by statistical significance. According to these results, the findings of Muck [10] also showed prolonged aerobic stability by using an additive containing sodium benzoate.

The results of gas measurement in samples from silage treated with the chemical additive are shown in Figure 15. At the time of opening, no O_2 but a high amount of CO_2 can be measured inside the buckets, like treatment CON also showed. The CO_2 inside the buckets had been built during the fermentation process and shows that fermentation worked well. The absence of oxygen indicates that the buckets are well suited because they are airtight. After opening, the CO_2 diffuses out of the buckets while surrounding air, including O_2, diffuses inside until the concentration gradient is balanced. No changes could be observed during the experimental period after this balance is reached 36 hours after opening. This shows that there is no microbial respiration activity in the silage treated with chemical additive during the time of the experiment.

Higher density has no additional positive effect regarding temperature development on silage in aerobic conditions when using the chemical additive. In this case, the high density offers the advantage of smaller volume of the silo stock which may be positive if storage capacity is limited. Additionally, the higher density restrains the gas exchange in the lower part of the bucket. For this reason, the CO_2 content in the HD buckets of treatment CHEM decreases slower and O_2 increases slower than in the LD variation.
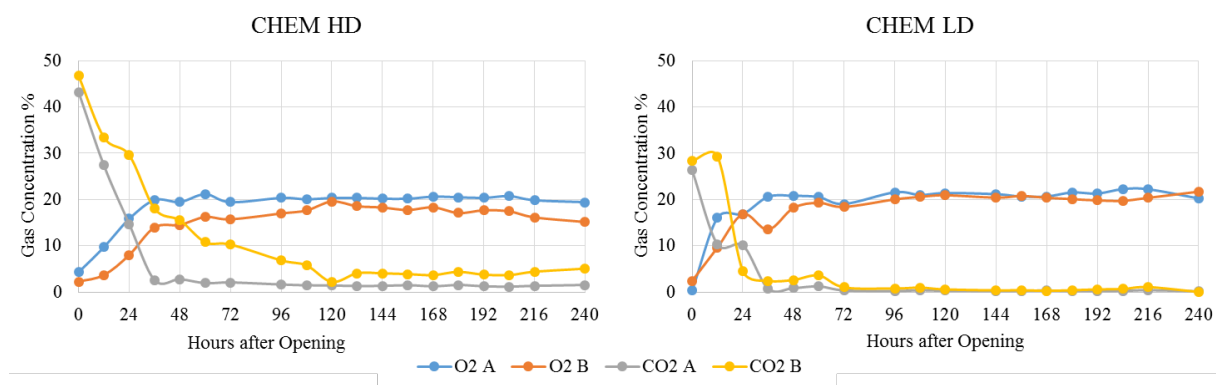

Figure 15: Means of Gas Concentrations of Samples Taken from Buckets of the Chemical Treatment at Two Sampling Points (A and B)

Effects of Three Different Additives and Two Different Bulk Densities on Maize Silage
Characteristics, Temperature Profiles, CO_2 and O_2-Dynamics in Small Scale Silos during
Aerobic Exposure

Ranjit and Kung [3] observed a prolonged aerobic stability of corn silage in their trial using a chemical additive with the ingredients calcium propionate, citric acid, sodium acetate and sodium aluminosilicate. This underlines the findings at hand. Kung [9] reviews that sorbate, benzoate and acetic acid are ingredients of commonly sold antifungal additives but are too expensive to be used in high concentrations as a pure additive.

Like treatment CHEM, the treatments B1 and B2 did not undergo reheating. The results of temperature measurements are graphically represented in the lower part of Figure 13 for the treatments B1 and B2. This means that the biological additives were also able to successfully prevent silage from aerobic reheating. These observations are also obvious and can be proved by statistical significance. Moreover, the findings of Muck [10] showed prolonged aerobic stability by using *Lactobacillus buchneri* as an inoculant. Just as in treatment CHEM, it could be observed in treatment B1 and B2 that higher bulk density had no additional positive effect on reduction of temperature. The results of the temperature measurement are graphically represented in Figure 13. An important particularity of treatments B1 and B2 is that the temperatures measured by sensor 2 in the LD and the HD variation and in both treatments treated with biological inoculant (B1 and B2) are higher than the temperatures measured by the other sensors within the same treatments. This means that an activity occurred in the middle part of the. Consequently, it can be assumed that an energy consuming process independent of air influence takes place in the buckets treated with biological silage additive. A possible explanation for this phenomenon is the particularity that more alcohol, especially 1,2-propandiol and 1-propanol, is in the silages treated with the biological additives (cf. Table 1). Results from the gas measurement shown in Figure 16 (treatment B1) and in Figure 17 (treatment B2) underline these outcomes.

Effects of Three Different Additives and Two Different Bulk Densities on Maize Silage Characteristics, Temperature Profiles, CO₂ and O₂-Dynamics in Small Scale Silos during Aerobic Exposure
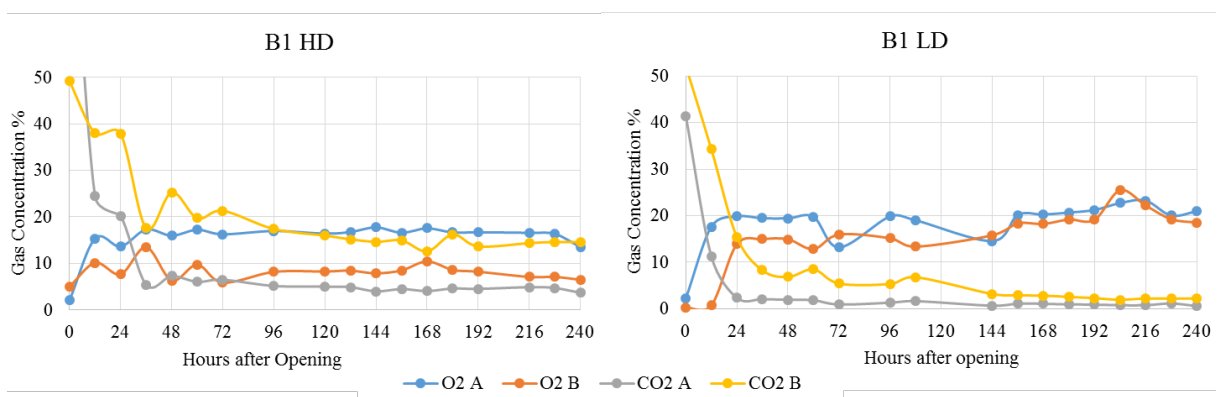
Figure 16: Means of Gas Concentrations of Samples Taken from Buckets of Treatment B1 at Two Sampling Points (A and B)
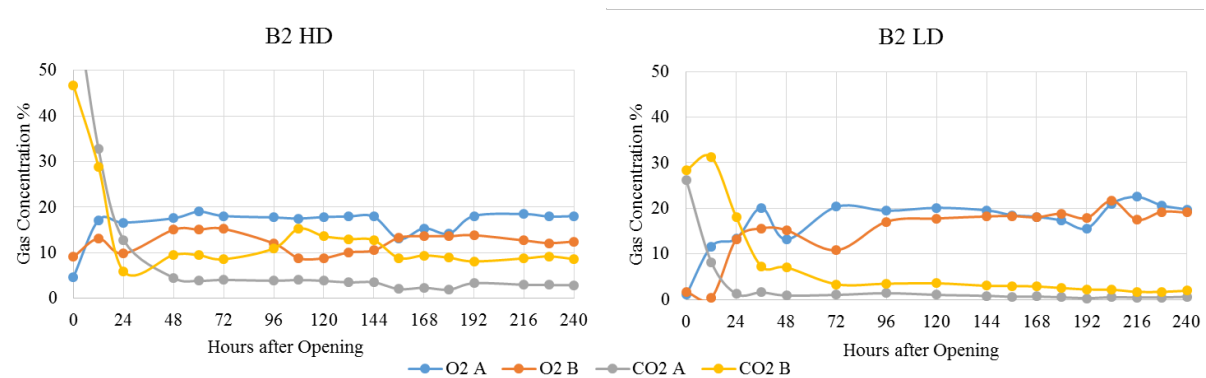
Figure 17: Means of Gas Concentrations of Samples Taken from Buckets of Treatment B2 at Two Sampling Points (A and B)

This comes along with the findings of Kristensen et al. [21], who investigated the effects of microbial inoculants on corn silage fermentation, microbial contents, aerobic stability, and milk production under field conditions. For their investigation, Kristensen et al. [21] used two different inoculants, one containing *Lactobacillus buchneri* as a heterofermentative strain, which was also included in treatments B1 and B2 of the present study. Kristensen et al. [21] found an increase in pH, acetic acid content, propionic acid, propanol, propyl acetate, 2-butanol propylene glycol, ammonia and free amino acids using this additive. Although not all of these parameters have been measured in the trial at hand, the results concerning propanol and acetic acid and an increase of aerobic stability fully correspond with Kristensen et al. [21].

In their study mentioned above, Ranjit and Kung [3] also used biological inoculants containing *Lactobacillus buchneri* and *Lactobacillus plantarum*. They found lower concentrations of lactic acid in all their treated silages compared to the untreated variation and higher concentrations of acetic acid in silages treated with a high concentration ($1x10^6$ cfu/g) of *Lactobacillus buchneri*. The findings of the present study support these results.

Driehuis et al. [22] used *Lactobacillus buchneri* alone or in combination with homofermentative lactic acid bacteria as an inoculant in their study. They also found enhanced aerobic stability and reduced yeast and mould counts, increased final pH and dry matter loss as well as increased acetic acid and 1,2-propanediol contents and decreased lactic acid content. Johnson et al. [17] found an improved aerobic stability for silages inoculated with *Lactobacillus plantarum* and *Enterococcus faecium*.

The higher amount of acetic acid in treatments B1 and B2, especially in the LD variation (cf. Table 1), compared to the control treatment and the chemical treatment shows the heterofermantative character of the biological additives. Acetic acid is known for its ability to improve aerobic stability of silages [11] and can be seen as main reason for the absence of deterioration of silages treated with biological additives in the present study. According to this, [11] also used *Lactobacillus buchneri* in trials associated with silages high in acetic acid concentrations connected to higher aerobic stability. The high initial CO_2 concentrations in all of the buckets used, confirms the circumstances of ensiling to be optimal and thereby underpins the findings of Hussin [14]. Ashbell and Weinberg [23] also refer to high CO_2 concentrations as an indicator for well-sealed silage.

CONCLUSION

High bulk density improves stability of maize silage. The chemical additive prevents silage from deterioration even during longer times of air exposure. The biological additives could prevent silage from reheating during the experimental period. When using chemical or biological additives, high density offers the advantage of smaller volume of the silo stock which may be positive if storage capacity is limited. The influence of the additive and inoculants and the influence of bulk density were great. To decide if an additive should be used, the farmer has to consider the circumstances at the time of ensiling.

Table 7   Analytical State of Maize Silage Samples from the Buckets Before (sample 0) and after Reheating for Silage Originating from Three Different Sampling Depth as Described in Jungbluth et al. (2016) (samples 2, 3 and 4)

Treatment	Position	Dry Matter g/kg	Crude Ash g/kg DM	Crude Protein g/kg DM	Crude Fibre g/kg DM	Crude Fat g/kg DM	Starch g/kg DM	pH	aNDFom g/kg DM	ME MJ/kg DM	NEL MJ/kg DM	Lactic Acid % of DM	Acetic Acid of DM	Propionic Acid % of DM	Ethanol % of DM	1,2-propandiol % of DM	1-propanol % of DM
K HD	0	379.6	34.4	82.3	171.0	30.6	375.8	4.1	374.4	11.4	6.9	3.4	1.1	0.0	0.8	0.0	0.0
	1	377.1	32.5	75.6	158.1	32.3	402.6	4.2	370.1	11.5	7.0	2.6	0.3	0.0	0.4	0.0	0.0
	2	371.9	31.4	72.2	168.7	31.8	384.4	4.0	371.2	11.4	7.0	3.9	0.8	0.0	0.6	0.0	0.0
	3	372.2	31.4	74.9	163.6	31.8	387.1	4.0	367.4	11.6	7.1	4.1	0.8	0.1	0.8	0.0	0.0
K LD	0	368.7	37.2	84.2	172.6	32.4	368.1	4.1	388.9	11.4	7.0	3.5	1.4	0.2	0.8	0.0	0.0
	1	406.6	34.5	78.3	175.2	31.0	393.1	4.6	388.4	11.3	6.9	1.9	0.4	0.0	0.3	0.0	0.0
	2	369.3	31.3	76.8	161.9	30.3	399.7	4.2	357.0	11.5	7.1	3.0	0.6	0.0	0.6	0.0	0.0
	3	356.6	30.0	72.4	162.3	29.9	398.7	4.0	349.6	11.5	7.1	4.3	1.5	0.0	0.7	0.0	0.0
B1 HD	0	371.4	37.3	81.7	175.7	34.7	392.7	4.3	416.3	11.3	6.8	0.9	2.8	0.0	0.7	1.4	0.2
	1	389.3	34.1	80.3	151.8	35.2	430.0	4.3	381.3	11.5	7.0	1.1	2.5	0.0	0.5	0.9	0.2
	2	371.2	34.0	82.0	158.9	31.4	423.4	4.1	390.2	11.4	7.0	0.9	3.0	0.0	0.5	0.5	0.4
	3	355.2	36.3	81.5	177.5	33.4	379.2	4.1	425.2	11.2	6.8	1.5	3.3	0.0	0.6	0.0	1.2
B1 LD	0	362.9	40.2	84.0	180.4	35.3	380.7	4.3	432.4	11.2	6.8	0.3	4.6	0.6	0.7	0.4	0.5
	1	364.1	34.6	74.5	158.4	35.0	447.3	4.5	376.1	11.5	7.1	0.9	4.8	0.5	0.6	0.4	0.7
	2	349.4	34.1	78.8	155.3	36.2	443.1	4.4	373.1	11.6	7.1	1.1	4.7	0.5	0.8	0.1	1.4
	3	356.6	34.6	76.6	163.8	36.0	429.9	4.4	383.5	11.4	7.0	0.8	5.0	0.6	0.7	0.3	1.3
B2 HD	0	364.2	36.8	80.3	169.4	37.1	410.7	4.3	402.3	11.4	7.0	0.8	3.0	0.0	0.7	1.5	0.4
	1	374.8	36.5	78.7	166.7	33.3	424.1	4.3	400.8	11.3	6.9	0.6	3.2	0.0	0.6	1.6	0.4
	2	366.1	34.8	81.1	166.6	35.1	411.5	4.3	407.7	11.4	6.9	1.2	3.2	0.2	0.6	0.9	0.4
	3	374.1	37.5	79.1	172.1	34.7	403.6	4.2	406.1	11.3	6.9	1.6	3.0	0.0	0.8	1.4	0.5
B2 LD	0	351.0	40.9	81.9	183.7	33.2	382.5	4.4	431.5	11.1	6.7	0.4	6.0	1.0	1.1	1.1	0.9
	1	346.1	37.1	76.6	172.8	37.1	410.5	4.5	415.9	11.3	6.9	0.5	6.0	0.7	0.8	1.1	0.9
	2	337.2	36.0	77.0	166.0	34.4	422.1	4.5	394.4	11.3	6.9	0.8	5.6	0.7	1.2	0.7	1.3
	3	334.6	33.7	74.2	153.0	37.5	454.5	4.4	361.4	11.7	7.2	0.7	5.5	0.7	1.1	1.0	1.1
C HD	0	392.3	35.7	81.0	168.2	33.1	380.0	3.9	379.8	11.5	7.0	3.4	1.3	0.1	0.3	0.0	0.1
	1	402.6	31.7	83.8	141.2	32.2	433.0	4.0	323.9	11.9	7.3	3.2	0.9	0.0	0.2	0.0	0.0
	2	385.4	31.1	77.5	159.5	32.3	405.6	3.8	353.8	11.6	7.1	4.1	1.1	0.0	0.4	0.0	0.1
	3	391.2	32.0	80.9	148.2	31.6	428.7	3.8	336.5	11.8	7.2	3.9	1.0	0.0	0.1	0.0	0.0
C LD	0	392.1	36.3	80.3	165.3	31.4	397.9	4.1	374.5	11.4	7.0	3.8	1.4	0.2	0.2	0.1	0.1
	1	397.1	32.9	77.8	159.4	32.3	413.8	4.0	358.6	11.5	7.1	4.5	1.3	0.0	0.2	0.0	0.1
	2	388.4	31.4	78.0	146.4	31.7	438.9	4.0	342.2	11.7	7.2	4.6	1.3	0.0	0.3	0.0	0.1
	3	379.0	31.2	77.1	154.5	33.0	425.3	3.9	336.6	11.7	7.2	5.0	1.6	0.0	0.4	0.0	0.1

Effects of Three Different Additives and Two Different Bulk Densities on Maize Silage Characteristics, Temperature Profiles, CO₂ and O₂-Dynamics in Small Scale Silos during Aerobic Exposure

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Aerobic Exposure

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3.4 Paper IV

Developing a Penetrometer-Based mapping System for Visualizing Silage Bulk Density from the Bunker Silo Face

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Developing a Penetrometer-Based mapping System for Visualizing Silage Bulk Density from the Bunker Silo Face

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ABSTRACT

For silage production, high bulk density (BD) is critical to minimize aerobic deterioration facilitated by oxygen intrusion. To precisely assess packing quality for bunker silos, there is a desire to visualize the BD distribution within the silage. In this study a penetrometer-based mapping system was developed. The data processing included filtering of the penetration friction component (PFC) out of the penetration resistance (PR), transfer of the corrected penetration resistance (PR_c) to BD, incorporation of Kriged interpolation for data expansion and map generation. The experiment was conducted in a maize bunker silo (width: 8 m, middle height: 3 m). The BD distributions near the bunker silo face were represented using two map groups, one related to horizontal- and the other to vertical-density distribution patterns. We also presented a comparison between the map-based BD results and core sampling data. Agreement between the two measurement approaches (RMSE= 19.175 kg m^{-3}) demonstrates that the developed penetrometer mapping system may be beneficial for rapid assessment of aerobic deterioration potential in bunker silos.

INTRODUCTION

Bunker silos are recommended for dairy-farm scales of 100 cows or more when the silo is unloaded at feeding rates above 100 mm d^{-1} in summer and 75 mm d^{-1} in winter. The merits of siloed feed include a relatively low storage cost, minimal loss of biomass and time-saving management [1, 2]. On the other hand, there is a high risk of silage spoilage near the zone of the exposure face when a bunker silo is opened for livestock feeding. In this situation, the silo face is exposed to air; facilitating rapid growth of microorganisms and leading to aerobic deterioration as oxygen rapidly diffuses into the silage. Thus, it is critical for bunker silo management to maintain an optimal face-removal-rate associated with aerobic stability in the silage [3].

High silage bulk density (BD) can significantly reduce aerobic deterioration because the high BD creates low porosity, thereby reducing O_2 diffusion into the silage [4−7]. Well-compacted

silage should not only exhibit a high BD, but a uniform BD distribution as well [7]. In reality the BD of maize silage can be highly variable at the farm scale in bunker silos. For instance, a previous study reported BD values that ranged from 125 to 378 kg m^{-3} dry matter (DM) content for maize silage based on the investigation from 81 commercial bunker silos [8].

To assess the silage packing quality, a simple method was used to calculate the mean BD from the known packed mass and its volume. However, this approach does not reveal the spatial BD distribution within the silage. For map-based BD measurements, a gamma ray scanner was tested in two studies [9, 10], where the relative measurement error was about ±1% after calibration. Despite the high accuracy, few producers would be able to effectively use gamma ray due to regulations and the potential danger of exposure to radiation. An improved penetrometer technique for map-based determination of BD in grass bale silage was developed [7]. Subsequently, a study verified that this novel technique can replace the gamma ray scanner for imaging silage BD distribution [11]. Considering that the spoilage risk for a bunker silo packed with maize silage is rather high [5], developing a penetrometer-based mapping system especially for maize silage in a bunker silo was the major objective of this study.

MATERIAL AND METHODS

Penetrometer-Based Measurement Platform

Figure 18 shows the measurement platform made by us, consisting of a motorized penetrometer, a y-axis shifter driven by a brush motor (24 V, 200 W, 5930 rev. min^{-1}, Maxon RE50, Swiss) through a planetary gear device (reduction ratio, 57:11, Maxon GP62, Germany), a relay-box, all installed on a green steel-frame that mounts to a forklift device and facilitates vertical movement of the penetrometer mechanism parallel with the silage face. A LabVIEW-based measurement interface was programmed to control the measurement process using a laptop. Figure 19 illustrates the mechanical principle of the penetrometer, where the black color represents the penetrometer structural support (i.e., rest components), the brown color shows dual screw-drive shafts (i.e., rotary components) and the blue color illustrates the slide, penetration shaft and cone with linear movement function. The penetrometer was powered by a permanent-magnet synchronous motor (model M63x60/I, Kählig Antriebstechnik GmbH, Hannover, Germany, 12 V, 99 W maximum output power).

Following the cone movement along x-axis, a potentiometer (ten-turn, 10 kΩ, ± 0.25% linearity) acted as a transducer to output the depth-specific signal. During the penetration process, when the cone reached the predetermined penetration depth (maximum measurement depth 1 m) or when the penetration resistance (PR) value exceeded 1000 N, the DC motor automatically reversed, causing the cone to retract to the original zero position. Based on Newton's law of action and reaction, a constant cone velocity is reqired because either acceleration or deceleration can cause uncertainty in the PR measurement [12–14]. To comply with American Society of Agricultural and Biological Engineers (ASABE) Standard S313.3 [15], the penetration velocity was controlled at 30 mm s^{-1}. Similarly, the dimension of penetration cone (diam. of the cone's base 12.83 mm; cone apex 30°) and the shaft (diam. 9.53 mm) are designed based on the ASABE Standards [15–16]. In addition, Figure 20 shows that the entire apparatus deployed at the silage face with a forklift, which controlled the vertical (z-axis) positioning over a height of 3 m in 0.5 m increments.

Figure 18   A photo of the penetrometer-based mapping system: 1) frame, 2) penetrometer, 3) motor for y-axis translation, 4) relay-box, 5) maize silo, and 6) the interface of the measurement system.
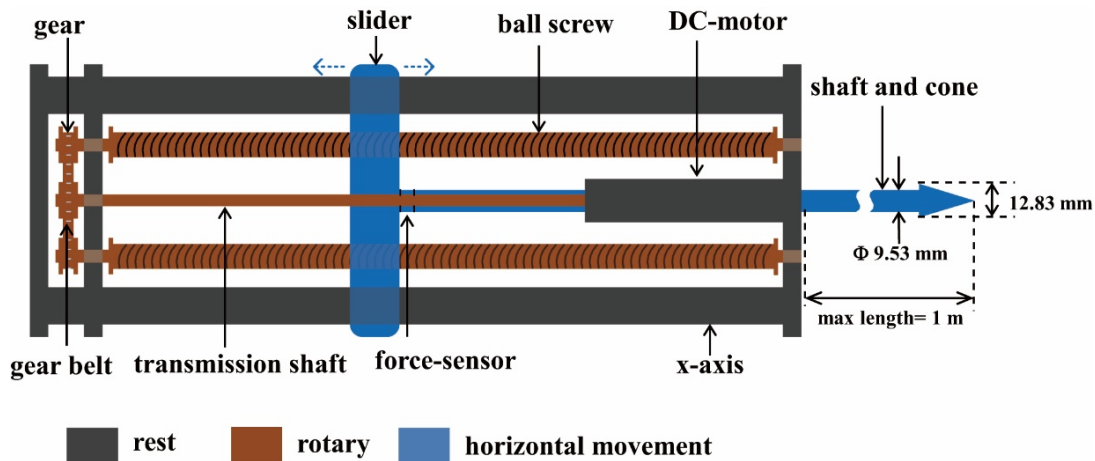
Figure 19 The mechanical structure and working principle of the penetrometer designed, where the black color refers to the rest part (frame), the brown color to the rotary part (crew-drive shafts), and blue color to the horizontal movement part (slide, penetration shaft and cone)

Figure 20 Using a forklift to position the frame prior to penetrating the face of the bunker silo at different heights

Control Unit and LabVIEW-Based Interface

The control unit had three functions: 1) accomplishing a control sequence, 2) logging measurement data and 3) displaying results. To simplify the hardware design, an electronic multifunction module (USB-6212, National Instruments) was chosen which had 16 analog inputs (16-bit, 400 kHz), 2 analog outputs (16-bit, 250 kHz), 32 digital input/output channels (I/Os), and two 32-bit counters. A group of control cables connected the I/Os to a relay-box (Figure 18). The module used was compatible with LabVIEW, ANSI C/C++, C#, Visual Basic.Net and Visual Basic 6.0 software. The software was programmed with LabVIEW 6.0

as a whole measurement process package following a logical sequence, except for the forklift positioning of the frame. Data acquired from each sensor were saved to a laptop as an EXCEL file and displayed graphically on interface. For instance, the PR results could be dynamically displayed as a curve or a hue bar associated with instant penetration depth on the relevant display panels as shown in Figure 18.

Data Processing Procedure

Five steps listed in Figure 21 illustrate the PR data collection and processing for map generation of the silo silage density. Step-1 includes acquisition of PR measurements (n= 60) assigned with the penetration network (Figure 22) relative to a silo face (length 8 m, height 3 m).
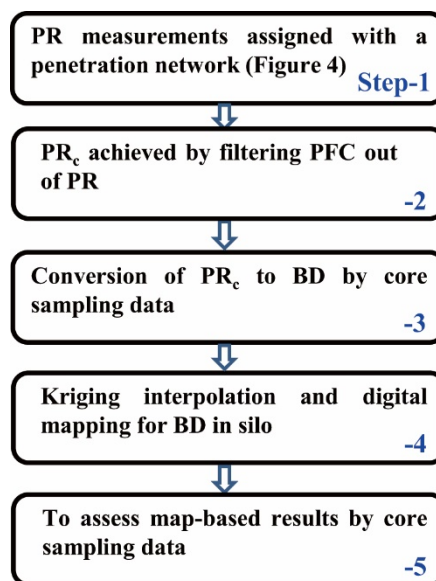

Figure 21   Flow chart of the penetration resistance data collection and processing procedures

Developing a Penetrometer-Based mapping System for Visualizing Silage Bulk Density from the Bunker Silo Face
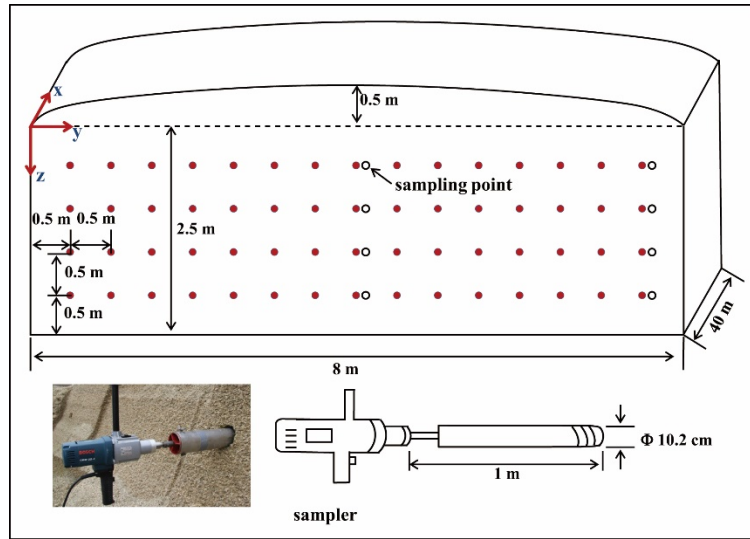

Figure 22 Measurement network showing core sampling location on the bunker silo face (solid dots) and the core sampler dimensions and locations (open circles).

Step-2 is to filter penetration friction out of the depth-related profile data. Previous studies have verified a substantial penetration friction force between the penetrometer shaft and maize silage being penetrated [18-19], creating uncertainty in how much of the PR should be translated as BD. The penetration friction component (PFC) was determined by penetrating a specific cylinder filled with maize silage at a known BD as illustrated in Figure 23. The cylinder (inside dia. 200 mm, height 500 mm) had two covers (dia. 200 mm, thickness 20 mm) and each cover had a hole (dia. 20 mm) at the center. Therefore, the penetration process included two phases. In phase-1 (Figure 23a), the PR measured was the sum of cone resistance (CR) and PFC. After the cone passed through the bottom of the cylinder (i.e., in phase-2; Figure 23b), the PR measured was only due to the PFC. As the literature stated [18], the PFC could be attributed to two factors: (1) it is directly proportional to the contact area of the shaft on the penetrating material, and (2) the overburden forces, and therefore the forces perpendicular to the shaft, increase as the penetration depth increases. Based on these, an approximate filter function (f_c) was suggested as:

$$f_C = \frac{C_1}{C_2 + S_{shaft}} = \frac{C_1}{C_2 + \pi D_{shaft} L_{depth}} \qquad (1)$$

where D_{shaft} denotes the contact area between the shaft and the maize silage, C_1 and C_2 are correction coefficients and are dependent on the elastic-plastic property of the measured

material (C_1 is a gain coefficient, and the initial filtering depends on the C_2/C_1 ratio), L_{depth} is a dynamic parameter of penetration depth, and D_{shaft} is the diameter of the shaft (9.53 mm). Thus, the corrected measurement value (PR_c) can be calculated as the product of the instantly measured PR and f_c:

$$PR_C(L_{depth}) = PR(L_{depth})f_c(L_{depth}) = \frac{PR(L_{depth})C_1}{C_2 + \pi D_{shaft}L_{depth}} \quad 100\, mm \leq L_{depth} \quad (2)$$
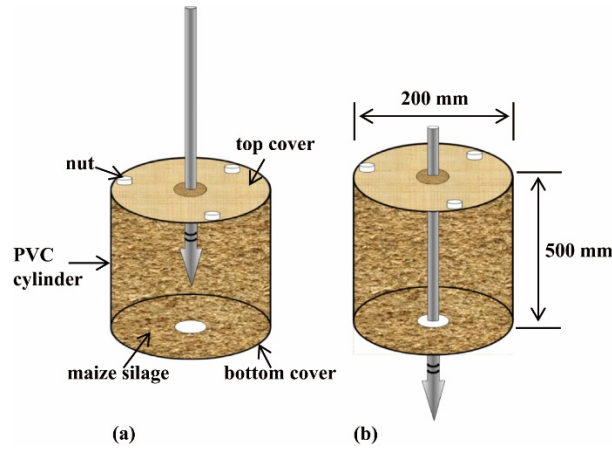
Figure 23   Determination of the penetration friction component (PFC) using a designed cylinder with two covers, each having a hole at the center. (a) the measurement for penetration resistance (PR) and, (b) the measurement for determining the penetration friction component (PFC)

Moreover, for m-number of penetration profiles, C_1 and C_2 can be found using a pair of optimal solutions:

$$\sigma(C_1, C_2)^2 = min \frac{1}{n}[\sum_{i=1}^{n}(PR_C - PR)_i^2] \quad (3)$$

and

$$\begin{cases} \dfrac{\partial \sigma(C_1,C_2)^2}{\partial C_1} = 0 \\ \dfrac{\partial \sigma(C_1,C_2)^2}{\partial C_2} = 0 \end{cases} \quad (4)$$

After the PFC was filtered out of the PR measurements, the next task (i.e., Step-3) was to convert the PR_c to BD values using a transfer equation. For this, a core sampler (shown on the bottom of Figure 22) was used to extract maize silage samples. For each sampling process,

two samples were extracted in 0.5 m increments of penetration depth. Here sampling data were randomly divided into two groups, half for determining the BD transfer equation and the other half for assessing map quality. The open circles in Figure 22 show the in situ BD sampling locations. All samples were weighed to determine the fresh/wet BD and then oven-dried for 24 h at 103 $^{\circ}$C to determine silage moisture content [17]. In Step-4, two of the basic functions in ArcGIS 9.2 software were employed, the data post-conditioning by ordinary Kriging interpolation and the digital mapping with the expanded data set. As an unbiased estimation method to generate high-resolution maps, Kriging interpolation can optimally predict unknown values from the data measured at known locations associated with the spatial correlation of these data and the predicted variance. Finally, the map-based results were assessed using half of the core sampling data (Step-5).

Experimental Condition

The bunker silo (40 m × 8 m × 3 m), located at a dairy farm in Haus Riswick in Kleve, Germany, was constructed of two concrete side-walls and a back-wall. The maize crop filling the silo was harvested in the fall of 2014. Figure 24 illustrates the distribution of the chopped maize particle length. For compacting the bunker silo, a 12 ton tractor was used (Fendt Vario 714). A layer depth was 20 cm and the total packing time of the bunker silo was 12 h. The sampling data (n= 16) showed a mean DM of 335 kg m^{-3}. The measurement was conducted on September 25, 2015 when the silo was being unloaded at a rate of approximately 0.5 m per day. For the 60 penetration measurements shown in Figure 22, it took about 3 hours.
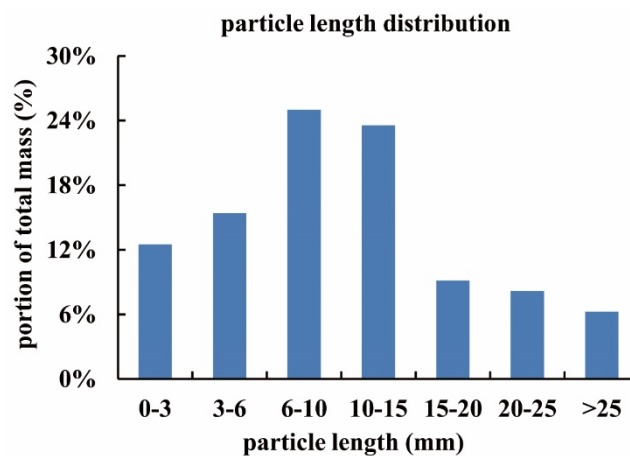

Figure 24   Chopped maize particle length distribution from the tested bunker silo

RESULTS AND DISCUSSION

Filtering PFC from PR

The three graphs in Figure 25 show the PR profiles measured in the maize silage in the cylinders at different levels of fresh BD, i.e., 900, 1000 and 1100 kg m^{-3}. Each graph has two traces associated with the penetration depth; solid dots referring to the PR measurements and the hollow squares to the PR$_c$ corrected by the filter (given in Eq.2). From these graphs, three observations can be clearly made. (i) All of the PR values exhibited a nearly linear relationship with the penetration depth within phase-1. This is because the contact area between the penetration shaft wall and the measured medium increased following the increase of penetration depth [18]. (ii) Within phase-2 the different PFC values became constants, reflecting the effect of BD. In this case the contact area also was constant so that the higher BD packing resulted in the larger PFC [19]. (iii) The optimal values of C$_1$ and C$_2$ are shown in relation to each BD.

Equation for Transferring PRc to BD

Figure 26 presents a linear regression equation between the values of PR$_c$ and the fresh BD values ranging from 820 kg m^{-3} to 1125 kg m^{-3} (samples: n= 8), which were obtained by the core sampler. The data showing somewhat deviation to the regression line is likely due to the fact that each sample cored in situ had a derivation to the adjacent penetration point as shown Figure 22. Despite this, the high R^2 (0.9393) suggested the regression equation to be acceptable for converting PR$_c$ to BD.
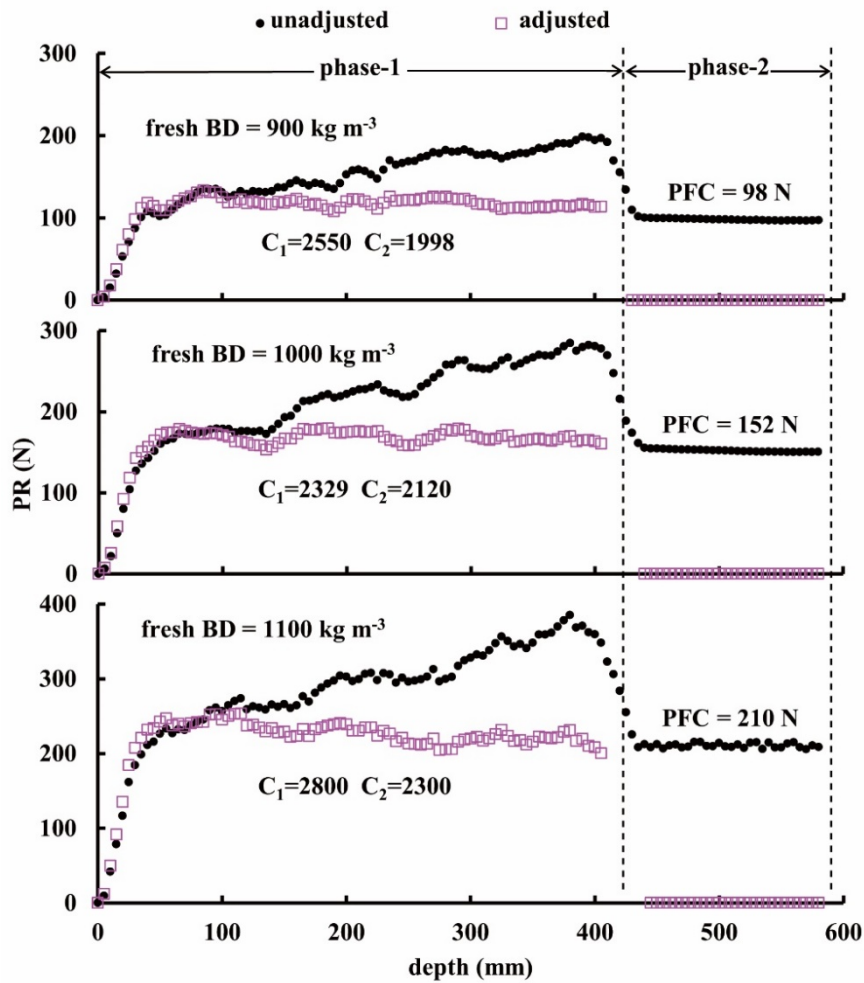
Figure 25   Results of penetrating chopped maize with different packed densities: (a) 900 kg m^{-3}, (b) 1000 kg m^{-3}, and (c) 1100 kg m^{-3}. Solid dots denote uncorrected PR data, hollow squares denote corrected PR data, i.e., PR$_c$
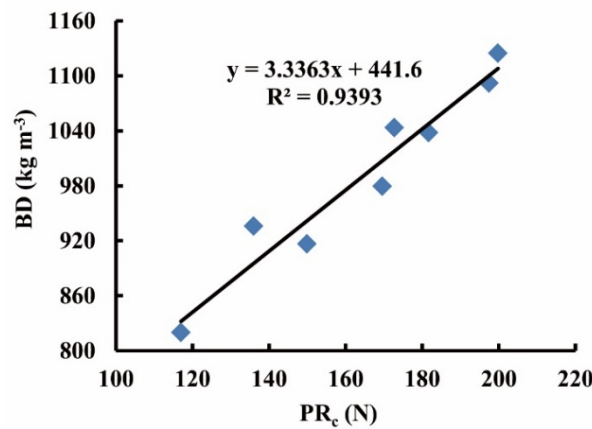

Figure 26   The converting equation between the PR$_c$ corrected from penetration resistance (PR) and silage fresh bulk density (BD)

82

Developing a Penetrometer-Based mapping System for Visualizing Silage Bulk Density from the Bunker Silo Face

Mapping Silage BD in the Bunker Silo

Figure 27 exhibits two groups of BD maps generated from the same volume of the bunker silo. The color bar represents a range of BD varying from 790 to 1120 kg m^{-3}. The upper group (Figure 27a) illustrates slices of the horizontal BD variations and the lower group (Figure 27b) shows vertical BD distributions. More importantly, from each two-dimensional (2D) array we can envision three-dimensional (3D) density distribution. Comparing horizontal with vertical arrays, we see that the horizontal BD exhibited smaller variation, but the vertical BD apparently increased with increasing the vertical depth of the bunker silo (z-axis). The average BD near the top layer was 880 kg m^{-3}, whereas that of the bottom was 1090 kg m^{-3}. The increasing gradient of BD along with vertical depth was observed in some previous studies. The literature [8] reported a statistical result surveyed with 175 bunker silos, showing that densities were generally higher in deeper zones. Similarly, another study[20] from 6 maize bunker silos found that cores taken near the top of the silo were always less dense than the samples taken near the floor by an average of 23%. This could be explained due to the effect of self-compaction [8, 21−22] or a combination of the self-compaction under silage weight and cumulative compression from the packing tractor [20]. Figure 28 provides the vertical gradient of BD measured from our core data, indicating that self-compaction occurred in this bunker silo as well. In terms of horizontal BD discrepancy, the study [20] reported that samples taken at the center were generally denser than samples taken near the wall by an average of 7%. This is also visible from all maps of Figure 27b. Figure 28 shows similar trends, where the circles denote the core data sampled in the center and the triangles denote core data sampled on the side. Figure 29 shows a comparison with 1:1 line between the map-based BD values (n= 8) and the corresponding core-sampled data. The low RMSE (19.175 kg m^{-3}) points to the accuracy of these BD maps, which were generated by the data processing procedures suggested in Figure 23.
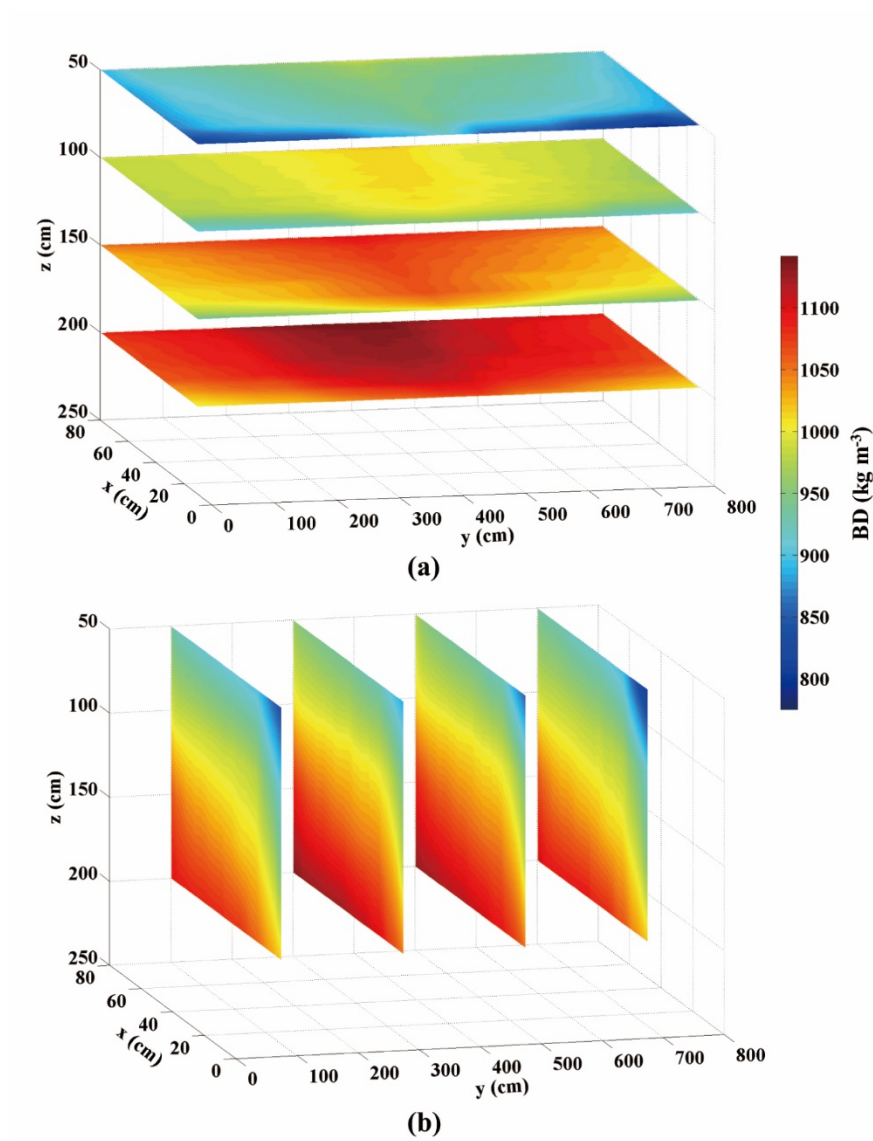
Figure 27   Silage BD maps generated for (a) horizontal- and (b) vertical-distributions

$$y = 1710 - 843e^{(-0.0013x)}$$
$$R^2 = 0.8529$$
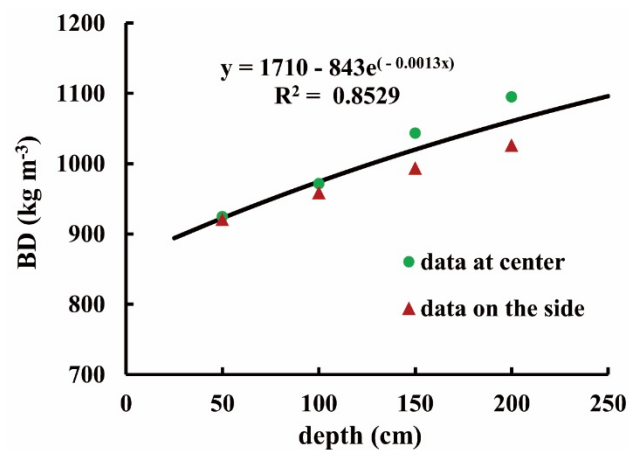
Figure 28   The vertical BD gradient within the bunker silo
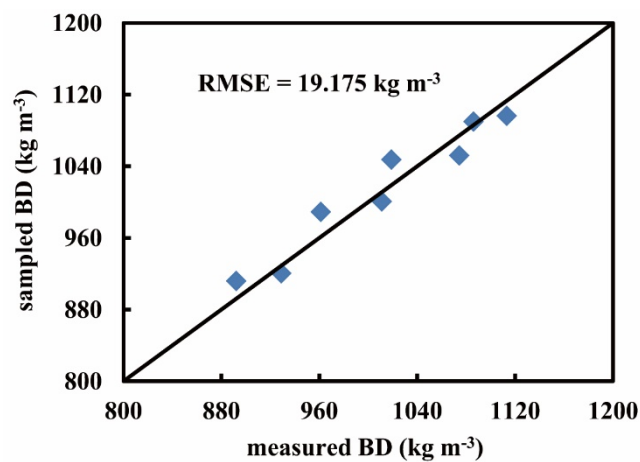
Figure 29   Evaluation of map-based results comparing the core-sampled data with the penetrometer-measured data.

CONCLUSIONS

The penetrometer-based bunker silo mapping system coupled with the presented PR data processing procedures, yielded digitally imaged silage BD distributions within the outer 1 m of the exposure face. These horizontal and vertical maps are informative and understandable in relation to the bunker silo and packing characteristics. The agreement between the core sampling data and the map-based results also confirmed the effectiveness of the PFC filter in minimizing the friction noise to the PR measurement. Therefore, the developed penetrometer-based mapping system can potentially contribute to not only detecting poor compaction management, but also in estimating the risk of aerobic deterioration of feeding materials for farm-scale bunker silos.

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Developing a Penetrometer-Based mapping System for Visualizing Silage Bulk Density from the Bunker Silo Face

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AUTHOR CONTRIBUTIONS

For this research article, Menghua Li, Kerstin H. Jungbluth and Yurui Sun conceived and designed the experiments; Menghua Li, Kerstin H. Jungbluth, Yurui Sun , Qiang Cheng, Haiyang Zhou, and Christian Maack, Wolfgang Buescher performed the experiments; Menghua Li, Kerstin H. Jungbluth and Qiang Cheng analyzed the data; Yurui Sun, Menghua Li, Kerstin H. Jungbluth, Christian Maack,Wolfgang Buescher and Zhongyi Wang contributed to the reagents/materials/analysis tools; Jianhui Lin made LabVIEW-based program, Yurui Sun Menghua Li, Kerstin H. Jungbluth and Qiang Cheng wrote the paper.

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4 General Discussion and Conclusions

In agriculture, crops and their products are converted into foods for human nutrition by domesticated livestock. However, many livestock diets include feeding material such as cereal grains, which could be eaten directly by humans. This leads to the widespread debate about the competition between livestock and humans for land and other resources. The climate in Northern Europe is appropriate for the production of grass and forage crops. These crops should be grown, harvested, and preserved as efficiently as possible to reach a minimum of spoilage and an innocuous feed for the animals. Consequently, they can be used in diets to meet a large portion of the animals' requirements. To reach this goal the improvements of efficient resource use is a key challenge for animal scientists and the animal feed industry (WILKINSON, 2011).

A lot of scientific work in the field of silage production has been done in the last few years (WILKINSON & DAVIES, 2012), and it seems to be easy to produce high-quality, highly nutritional and hygienic silage as long as all the best practice tips and framework conditions are considered. Nevertheless, there are various influencing factors affecting quality-related characteristics of silage in agricultural practice such as weather, time, availability of contractors for harvest, plant material, and many more. Therefore, requirements may sometimes be unrealisable or only partially executable, even if the demands are known and the person responsible is qualified and skilled. In these cases it can be very helpful to use assistive devices (WEINBERG & MUCK, 1996). These findings illustrate the importance of research in the topic of ensiling and silage production that should be put into practice.

The key factors for a successful silage production, leading to a well-fermented high quality product, are microorganisms because they are responsible for all phases of ensiling. Malfermented or reheated silage leads to the degradation of nutrients, decreased feed intake, bad taste, and low quality of milk (WILHELM & WURM, 1999). Knowing as much as possible about microorganisms offers the opportunity to optimize the conditions for desirable microorganisms as far as the circumstances allow this. If all the questions concerning silo management are answered with the importance of microorganisms in mind, ensiling will turn out well. Routine monitoring and responsible handling of the silo and the silage will lead to the best possible products. If the principles of good professional practice in agriculture are observed until silage is fed to the animal, best results can be reached.

The main objective of the project was to investigate different influencing factors on the aerobic stability of silage. The main attention was given to the processes at the open silo face. To reach this goal a new scientific measurement method was developed (paper I), which turned out to be suitable and close to practice. The project included trials in which the method was actively used. The method was successfully applicable and combined the parameters temperature, O_2-consumtion and CO_2-production in a meaningful way in one test. HONIG (1990) also recommended to combine these parameters, and the trials conducted during the present study confirm this suggestion to be favourable. Microbial respiration is characterised by O_2-production and CO_2-consumption while the temperature is rising (cf. paper II and III). Observing the courses of O_2 and CO_2 during reheating, it is important to recognize that gas concentrations are a result of more than only one process: Microbial metabolism, air exchange with the surrounding air and dissolvability of CO_2 in the plant water content.

Besides the measuring of gas samples from silage via gas chromatography, the technique was also used to analyse climate relevant gases arising during aerobic deterioration. These gases might contribute to the emissions originating from agriculture endangering our environment. The ex-situ method also turned out to be suitable for measurements like this. Discussing the results of these trials would go beyond the scope of this thesis but they might be an interesting starting point for further research.

The ex-situ method also included analyses of silage samples before and after silage was aerated to observe changes in the material due to penetration of air (paper I-III). A potentially useful addition to these analyses is the determination of bacterial counts concerning the topic of reheating especially the counts of yeasts and moulds. This is another important starting point for further research that has been included in some unpublished trials as part of the present study.

Table 8 shows the results of these analyses. Samples 1 A-1 C are taken from experimental silos (as described in paper I) filled with maize that had been ensiled for one year. These samples were taken directly after opening the silo. A, B and C are the markings for three repetitions of buckets ensiled with the same material. The samples 2 and 3 have been taken from the same experimental buckets one week later. The samples 2 A, 2 B and 2 C are repetitions taken from the upper part of the buckets close to the opened face (c.f. paper I) and the samples 3 A, 3 B and 3 C are repetitions taken from the lower part of the buckets with a greater distance to the opened face (c.f. paper I).

The yeast counts shown in table 8 underline the findings of LINDGREN et al. (1985), describing the increase of yeast counts due to oxygen infiltration. Moulds which were not detectable directly after silo opening germinate during the process of aerobic influence. The total bacterial counts show that other types of microorganisms are inactivated at the same time. Comparing results of samples 2 and 3 leads to the assumption that yeast counts are lower with greater distance to the open face. This underlines the oxygen dependence of yeasts.

Table 8   Microbial Analyses of Silage Samples before (1) and after (2 and 3*) Aerobic Exposure in three different (A, B and C) experimental buckets (unpublished data)

Sample	pH	Total Bacteria Count (detection limit 10 CFU/g)	Yeasts (detection limit 10^2 CFU/g)	Moulds (detection limit 10^2 CFU/g)
1 A	4.17	1.6×10^9 CFU/g	2.2×10^5 CFU/g	Not detectable
1 B	4.25	1.9×10^9 CFU/g	1.9×10^5 CFU/g	Not detectable
1 C	4.22	1.3×10^9 CFU/g	6.6×10^6 CFU/g	Not detectable
2 A	6.10	2.0×10^{10} CFU/g	5.9×10^8 CFU/g	$>10^7 <10^8$
2 B	6.18	2.9×10^{10} CFU/g	5.2×10^8 CFU/g	$>10^6 <10^7$
2 C	6.20	2.4×10^{10} CFU/g	3.3×10^8 CFU/g	$>10^6 <10^7$
3 A	3.87	6.9×10^8 CFU/g	1.5×10^5 CFU/g	$>10^4 <10^5$
3 B	3.82	2.3×10^7 CFU/g	1.0×10^3 CFU/g	$>10^3 <10^4$
3 C	3.89	6.6×10^7 CFU/g	5.4×10^4 CFU/g	$>10^3 <10^4$

* 2=sampling point near opened face; 3=sampling point with 45cm distance to the open face

The results of pH measurement shown in table 8 are consistent with the findings of microbial counts. The pH increases due to the metabolism degrading lactic acid, also described by BORREANI & TOBACCO (2010) and LINDGREN et al. (1985).

Based on these results it can be assumed that the continuous measurement of pH values during reheating would be an additional and informative parameter for further research. In that case the degradation of fermentation acids could be indirectly observed. This would be an indirect measuring technique for microbial activity and degree of spoilage, and could be integrated in the ex-situ method (paper I).

Since the open silo face, the area where silage is aerated, is the critical part of the silo concerning the topic of aerobic deterioration, mechanisms of silo face sealing are an interesting topic of research. An opportunity to seal the open face, and prevent silage from

spoilage with a product that might be consumable by the animals is a promising future prospect.

Although there are many influencing factors on silage quality, all silages produced for the trials conducted in this study were classified as well-fermented by an external laboratory (Landwirtschaftliche Kommunikations- und Servicegesellschaft mbH, Lichtenwalde, Germany). Therefore, one can conclude that it is not difficult to produce silage high in quality and nutritional value if all conditions are fulfilled. On the other hand, the production of silage for trials using the ex-situ method described above (paper I) excludes some of the problems that may arise in agricultural practice. If the quality of silages produced on farm is lower than those of experimental silages, aerobic stability may be restricted.

The silages produced for the trials were much cleaner than silages on farms might be, and the buckets used are much more airtight than a clamp silo can be. A higher degree of soiling determines higher values of crude ash, which in turn means higher buffering capacity. Thereby, aerobic stability could be shortened (SPIEKERS et al., 2009).

According to ROTZ (2003), slow diffusion of air through the silo walls or the silo cover occurs even in well-built and well-covered silos. In this context it is a factor if the construction and geometry of clamp silos is still as modern and as good as possible. By today, a lot of functional materials have been developed. Further research, not only from the viewpoint of airtightness and silage quality but also of durability and prevention of effluent outflow, regarding the topics of silo geometry and silo construction is needed. According to KRENTLER (2006), horizontal silos are the most damaged constructions in agriculture. Most of the damages noted result from mistakes concerning construction. Consequently, the durability of horizontal silos has frequently been discussed in the last years.

In addition, in clamp silos in agricultural practice the density distribution is unequal (c.f. paper IV). This disadvantageous condition supports air to enter the silage at the top area or at the side regions where density is less as shown in paper IV. This problem did not occur to this extent in the experimental buckets.

Furthermore, the filling of plant material into the buckets for the trials in the present study only took a few hours each time. Consequently, the period between harvesting and ensiling of the material was short (not longer than 5 hours). In agricultural practice, this span of time is often much longer. The time between beginning of harvest and covering of the silo should not be longer than 35 hours (SPIEKERS & POTTHAST, 2004).

An advantageous aspect of the experimental buckets used (described in paper I) is their size. Along with the production of a larger amount of silage for other potentially following experiments, there is a second and important benefit. In small buckets as mostly used, the ratio of surface to volume is disadvantageous compared to greater buckets. The impact of environmental conditions is smaller if buckets are bigger because their ratio of surface to volume is smaller. Therefore, conditions for silage experiments with smaller buckets have to be controlled much more strictly. Experiments with glasses of small volume, even if encased with a polystyrene cover, have to be conducted in climatic chambers as already recommended by HONIG (1986). Additionally, the conduction of experiments simulating a silo requires the opportunity to measure in different distances to face. This requirement is met in the method described (paper I).

A great number of silage experiments were conducted in the last years since the importance of silage has been increasing. Most of these experiments used different measurement techniques. Even if most of these measurement techniques were good, it would be better to define a standard procedure for silage trials in order to make results comparable to each other, independent of persons or places involved. Consequently, a greater number of results could be taken into account when designing a model showing the deterioration risk under different influencing factors. Large-scale tests would also be appropriate in the context of improved comparability.

A common method for the evaluation of silage is done by a sensory assessment of smell, colour and structure. Additionally, the dry matter content and the degree of soiling are estimated. Derived from that assessment, which is done by human senses, the quality and energy content of the silage is valued (DLG, 2004). Since this is a fast, inexpensive and practicable method, it should be recommended for use. On the other hand, the subjective estimation result may only be an assistive device, for which experienced persons are necessary, and all results should be backed up by laboratory analyses. The assessment does not require any information about parameters concerning reheating despite the fact that they are noticeable in smell, colour or structure. Our own experimental results (unpublished data) show that this method often rates the silage lower or higher in quality than the laboratory did. For this reason it should be recommended to conduct both laboratory analyses and sensory assessments, and to deduce a combined evaluation from both. ROß (2014) and GERLACH (2013) investigated different variants of silage qualities concerning taste, ensiling success, deterioration, and hygienic status. Therefore different methods for evaluation were used and

compared. ROß (2014) used a chemosensor-system, whereas GERLACH (2013) conducted preference trials with goats to detect deterioration. The comparison of both methods showed that the chemosensor-system was able to detect silage deterioration even before the feed intake by goats decreased. Even if the chemosensor-system is not a practicable method on farms yet, it shows the importance of sensory evaluation of silage.

The laboratory scale method for the investigation of aerobic stability recommended by HONIG (1990) is performed by continuously measuring the temperature of silage which is exposed to air for several days at constant ambient temperatures. Despite the fact that there are many potential parameters which could be used as an indicator for reheating, temperature is still the most common one. The measurement of temperature is not difficult to conduct, nor expensive, but fast and easy to handle. With modern technology temperatures can be measured and data can be stored without great expenditure. The problem concerning this topic is that a clear and consistent definition of the term 'reheating' is lacking. According to the definition of SPIEKERS and POTTHAST (2004), silage has been reheated once a temperature increase of 5 K has occurred. According to SPIEKERS et al. (2009) and GALLER (2011), silage is reheated if temperature reaches 10°C above ambient. NUSSBAUM (2006) as well as BORREANI and TOBACCO (2010) include a comparison between different areas of the silo in the definition of reheating. According to the definition of NUSSBAUM (2006), silage has been reheated when different areas of the silo show a temperature difference of 5 K. The definition for aerobic stability made by the German Agricultural Society (DLG, 2000) understands the term within narrow bounds. A temperature increase of 3 K above ambient is considered as reheating for laboratory experiments.

It might be reasonable to differentiate between definitions for reheating depending on conditions. For small-scale laboratory experiments the orientation value for reheating should be severely restricted compared to orientation values for silage in agricultural practice where the orientation value should grant a wider range.

The lack of an equal definition with precise orientation values and reference parameters is also discussed by GERLACH (2013), who points out the necessity of implementing objective control points for the silage management under practical conditions. According to GERLACH (2013), the 'Regulation of the European Parliament and of the council laying down requirements for feed hygiene' (ANONYMUS 2005) necessitates such a definition because it requires the transparency of compliance with hygienic regulations at all stages of food production. Silage as a basic feed for milk or meat producing ruminants is also affected by

this regulation. Thereby, GERLACH (2013) underlines monitoring units in silage management as necessary but missing instruments that could supply reliable information.

The T-phases defined (paper I) could potentially be used as generally valid definitions. They simplify the description of reheating and express the pattern of a material ensiled. The phases T_0 and T_1 are more related to practical conditions on a farm, whereas the phases T_2 and T_{max} are closer to laboratory conditions. Comparisons between the T-phases of two different silages portray the characteristics of these ensiled crops during reheating and potentially lead to an informative and conclusive rating.

Although the T_0-phases of different silages may differ in their length, aerobic bacteria, yeasts and molds will degrade lactic acid under aerobic conditions. As a consequence, the pH will rise and the silage will deteriorate inexorably (STEINHÖFEL, 2008). This fact and the implementations from chapter 2 show that none of the physically influencing factors, as well as those that are not considered in the present study (e.g. cutting length), can stop aerobic deterioration. Consequently, the silage has to be taken out of the silos faster than deterioration during the feed-out period. Physically influencing factors cannot do more than decelerate deterioration. The main advantage resulting from this deceleration is a gain of time.

Relating to the different ensilabilities of different crops already shown in chapter 2.4.3, for logical reasons the trials, and especially the first applications of the new method, were done with maize. Maize belongs to the well-fermentable crops (GALLER, 2011) and is therefore suitable for the trials. In the further course of the study, the trials have also been conducted with grass and alfalfa (unpublished data). The results showed that the ex-situ method was appropriate to be used for the substrates grass and alfalfa. The silages produced were high in quality. The results concluded from trials with maize silage are transferable to grass and alfalfa. An increase of bulk density as well as a decrease of dry matter content increased aerobic stability of grass silage. None of the alfalfa silages reheated during 10 days of aerobic exposure. This might be reasoned by the lower content of fermentable sugar in alfalfa silage.

A comparison of maize and grass silages (unpublished data) showed that grass silage reheated later than maize silage. These results go along with the statement of GALLER (2011) who mentions that the high content of available sugar and energy leads to a faster reheating of maize silage compared to grass silage.

As shown in papers II and III, high silage density is an important factor concerning aerobic stability. These results confirm the statement of KÖHLER et al. (2013) who points out that dry

matter losses in corn silage can be reduced by a high bulk density and greater feed-out rate. Paper IV shows the measurement of this factor in agricultural practice. An innovative topic for further research would be the online measurement of density during the filling of the silo. A technique like this would support farmers in reaching favourable densities suitable for the actual dry matter content of the plant being ensiled, and it would save superfluous crossing time for compaction after the preferable density is reached.

Paper III shows that silage additives and inoculants can significantly influence aerobic stability of silage. An important factor on the decision whether silage additives or inoculants should be used is the velocity of silage removal from the silo. If a silo is well-constructed in size in relation to the farm size (number of animals or size of biogas plant), the velocity of silage removal may be fast enough to exceed reheating even without the use of additives or inoculants. On the other hand, these products may grant some buffer, which means assurance for the farmer in unpredictable cases. Whether products like this should be used and which products should be chosen depends on the circumstances of ensiling, the actual situation on the farm, as well as prices of products in relation to economic conditions.

In her postdoctoral thesis WAGNER (2005) describes critical control points influencing the long-term stability of silage. The critical control points of cutting length, density, and removal from the silo are highlighted to be significantly important. This study confirms the control point density to improve the aerobic stability of silage. Neither the cutting length, nor the removals from the silo were investigated in this study but additional control points could be added to the model of WAGNER (2005). The parameters O_2 content and CO_2 content of gas samples used in this study are suitable to be used as control points for laboratory scale experiments but are not practicable for farm conditions. For farm conditions surface temperature is still the parameter of choice when it comes to control of the silo after opening. An important criterion a critical control point system has to fulfil is that actions are taken if control points are not met. As shown above (paper III) biological inoculants and chemical additives are adequate influencing factors to be used as such an action.

Aerobic deterioration as well as ensiling is characterized by an interaction of physics, chemistry, and biology. These factors cannot be considered separately from each other because they are mutually dependent. All the influencing parameters have to be optimized as far as possible but also underlie natural or operating limits. Therefore, each recommendation concerning ensiling has to be assessed in the context of given conditions and the current operating situation.

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Conference Contributions

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Jungbluth, K.H., G. Jia, M. Li, Q. Cheng, J. Lin, Y. Sun, G.C. Maack and W. Büscher: Risk and prediction of aerobic-induced silage bale deterioration (Oral Presentation and contribution in the conference transcript)

18th World Congress of CIGR, Beijing, China, September 16-19, 2014

Kerstin Helena Jungbluth: Model-based research for the risk and prediction of silage bale deterioration suffered from aerobic impact (Oral Presentation)

XVII International Silage Conference, Piracicaba, Brazil, July 1-3, 2015

Jungbluth, K., G. Jia, M. Li, Q. Cheng, J. Lin, Y. Sun, G.-C. Maack and W. Büscher: Effect of different bulk densities on temperature profiles and microbial respiration activities during reheating of corn silage (Poster Presentation and contribution in the conference transcript)

Publications

1)   Li, M., Y. Sun, Q. Cheng, K. Jungbluth, W. Buescher, C. Maack, H. Cheng, Z. Wang. (2016). Mapping oxygen-induced temperature patterns of round bale silage based on 3D stepwise-profiling measurement. Measurement 82, 115–122

2)   Shan, G., Y. Sun, M. Li, K. Jungbluth, C. Maack, W. Buescher, K. Schütt, P. Boeker, P. Schulze-Lammers, H. Zhou, Q. Cheng, D. Ma. (2016). An assessment of three different in situ oxygen sensors for monitoring silage production and storage. Sensors 91, 1–11

3)   Sun, Y., M.H. Li, Q. Cheng, K. Jungbluth, W. Buescher, D. Ma, H. Zhou, H. Cheng. (2015). Tracking oxygen and temperature dynamics in maize silage novel application of a Clack oxygen electrode. Biosyst. Eng. 139, 60–65.

4)   Jungbluth, K., C. Maack, W. Büscher, Y. Sun, Q. Cheng, L. Menghua, C. Hong. (2016). A new ex-situ method to investigate aerobic stability of maize silage faces. J. Agric. Sci. Food Technol. Vol. 2 (4), 49-54.

5)   Menghua Li, K. Jungbluth, Y. Sun, Q. Cheng,C. Maack, W. Buescher, J. Lin, H. Zhou, Z. Wang. (2016). Developing a Penetrometer-Based mapping System for Visualizing Silage Bulk Density from the Bunker Silo Face. Sensors. 16, 1038.

6)   Jungbluth, K., C. Maack, W. Büscher, L. Menghua, C. Hong, C. Qiang, Y. Sun. (2016). Effects of different bulk densities on Zea mays silage characteristics, temperature profiles, CO_2- and O_2-Concentrations in small scale silos during aerobic exposure. Journal of Agricultural Science and Food Technology. Vol.2 (11), 180-188

7)   Jungbluth, K., M. Trimborn, G. Maack, W. Büscher, M. Li, H. Cheng, Y. Sun. (2017). Effects of three different additives and two different bulk densities on maize silage characteristics, temperature profiles, CO_2 and O_2- dynamics in small scale silos during aerobic exposure. Applied Sciences. 2017;7(6):545

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Erklärung

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Wachtberg,12.04.2017

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