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A New Ex-Situ Method to Investigate Aerobic Stability of Maize Silage Faces

Jungbluth K[1*], Maack C[1], Büscher W[1], Sun Y[1], Cheng Q[1], Menghua L[2] and Hong C[2]

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[1]Institute of Agricultural Engineering, University of Bonn, Nussallee 5, 53115 Bonn, Germany.
[2]Department of Information and Electrical Engineering, China Agricultural University, Qing hua dong lu 17, 100083 Beijing, China.

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. Samples were 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, very suitable to laboratory conditions, replicable and connectable to other experiments.

Key words: Feed quality, Silage quality, Aerobic stability, Reheating, Deterioration and Maize silage.

*Corresponding author: E-mail: kjungblu@uni-bonn.de. Tel: +49228733042. Fax: +49228732596.

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/m^3, 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. Furthermore 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 concerning microbial respiration.

MATERIALS 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 1). 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

The 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 1. 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 2

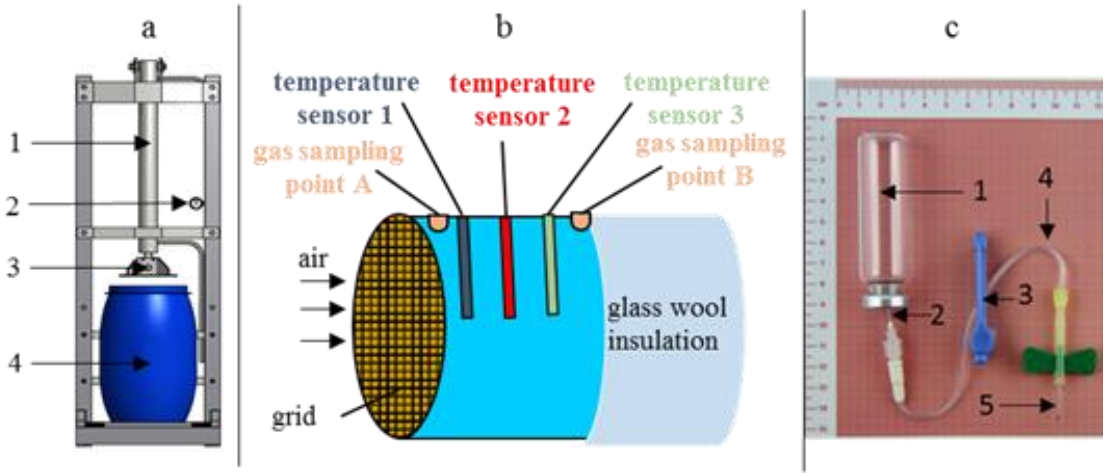

Figure 1. (a) Hydraulic press (1=hydraulic cylinder; 2=manometer; 3=extrusion punch; 4=bucket) (b) Sketch of the experimental setup (in reality glass wool covered the whole bucket) (c) Blood collection sets (1=injection headspace vial with puncturable stopper; 2=syringe needle to puncture the stopper;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

was 15 cm as well as the distance between sensor 2 and 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^{TM} Blood Collection Set, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA), composed of 2 syringe needles connected by a catheter tube were inserted (Figure 1). 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 1). 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 small amounts of oxygen, that entered the buckets while insertion.

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 1). To prevent silage from falling out of the bucket a grid (Figure 1) 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, λ = 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 min 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 2 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 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 (T0-phase) of 24 to 62 h between the opening of the buckets and the onset of temperature increase dependent on crop and treatment was observed.

In the T0-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 T0-phase (p>0.05). In the T0-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 T1-phase. During T1-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 (T2-phase). The T2-phase ends with the maximum temperature (Tmax), which averaged 37.55°C for sensor 1. The different phases during aerobic exposure are shown in Figure 2.

The results of the CO_2 measurements in maize silage are also graphically represented in Figure 2 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 h 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

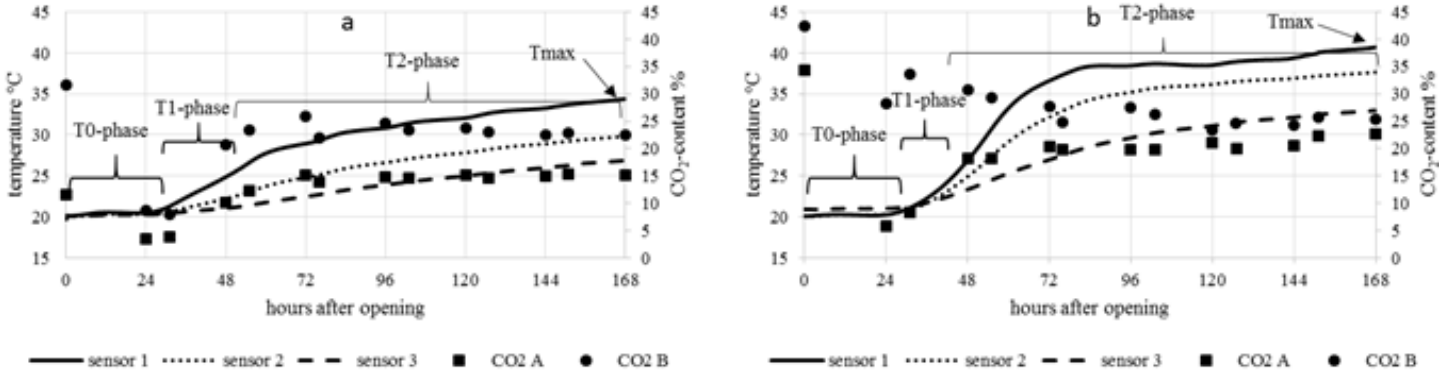

Figure 2. Means of temperatures measured by three temperature sensors in maize silage (average dry matter content=357 g/kg; a=297.5 kg DM/m^3; b=238 kg DM/m^3).

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. 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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