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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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Featured Application: Silage management on farm.

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 and assigned to one of four treatment groups: (1) control (no treatment); (2) chemical additive (sodium benzoate, potassium sorbate, sodium acetate); (3) a mixed biological inoculant containing *Lactobacillus buchneri*, *L. plantarum*, and *Pediococcus acidilacti*; and (4) a mixed biological inoculant containing *L. buchneri*, *L. plantarum*, and *L. rhamnosus*. An untreated variation was also ensiled. Two different densities were adjusted during ensiling. After opening, the temperature was measured for seven days and O_2 and CO_2 concentrations were analysed. The findings show that the chemical additive very effectively prevented silage from reheating and deteriorating. Aerobic reheating of silage was also successfully inhibited through biological additives and high density.

Keywords: inoculation; additives; silage quality; aerobic stability; reheating; microbial respiration

1. Introduction

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 minimizing dry matter and energy losses [2]. Silage, which is used as feed for milk- and meat-producing animals, undergoes aerobic deterioration 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 dry matter losses. When the silo has been opened for feed-out the feeding surface of the silage is exposed to oxygen, leading to an

increase in aerobic microbial metabolism. As a result heating of the silage and further losses of dry matter 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 aim to produce mostly lactic acid and fewer other fermentation products. As a consequence, the pH drops quickly [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 inoculants have even been observed to decrease aerobic stability [9,10]. Bacteria producing both propionic and lactic acids have 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 most consistent heterofermentative concerning the improvement of aerobic stability, compared to others that are commercially available. The same prolonging and improving effects 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 this 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 a high density of silage because a low void volume means a low initial air content. This can significantly reduce the risk of a temperature rise and the loss of dry matter and energy [12]. Two different biological inoculants (biological factors) 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].

2. Materials and Methods

2.1. 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 was 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 kernels of the 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; 1 g per ton silage; min. 2.5×10^{11} lactic

acid-producing bacteria per g Bonsilage Twin MS), and the other one containing *Lactobacillus buchneri*, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus* (treatment B2; Bonsilage Twin MF, H. Wilhelm Schaumann GmbH, Pinneberg, Germany; 1 g per ton silage; min. 2.5×10^{11} lactic acid-producing bacteria per g Bonsilage Twin MF). 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, which has been dispensed with 2 L per ton of silage. All additives are used in practice. The additives were applied manually with a pressurized air duster connected to a compressor.

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 fresh matter (FM) (196 kg dry matter/m^3) as a low–density variation (LD), and another twelve (three of each treatment) with 48 kg FM (261 kg dry matter/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 a clamping ring and were laid on their sides to avoid an enrichment of CO_2 inside the bucket and to simulate a silo on a farm.

2.2. 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 1. 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 min for the next seven days. The experimental period took ten days. The target for potential aerobic stability recommended by Wilkinson and Davies [5] is seven days. Thus, the experimental period was calculated to cover the expected or potential period of reheating.

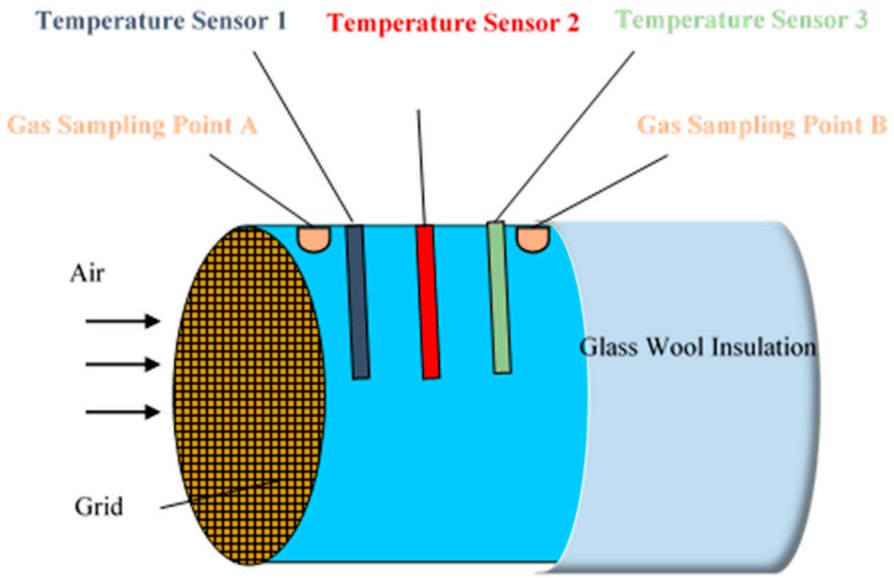

Figure 1. Schematic illustration of the experimental setup (modified according to Jungbluth et al.).

Gas samples were taken from the buckets to observe the courses of CO_2 and O_2 during aerobic exposure to gain insights into the processes of microbial respiration and diffusion. To extract gas

samples from the buckets, cannulas were inserted (BD Vacutainer Safety-Lok™ Blood Collection Set, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and used to puncture the stopper of an evacuated 20 mL headspace vial. This method of gas sampling has also been previously 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 with a gas chromatograph from SRI Instruments (8610 C, SRI Instruments, Torrance, CA, USA) in an external laboratory, as mentioned in Jungbluth et al. [15].

2.3. Experimental Phase

To start the exposure to 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 shown in Figure 1, 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 was already described in Jungbluth et al. [15]. All 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 and parameters, including 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), net energy lactation (NEL), and the parameters which are 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, CA, 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 1. 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 IRBIS® 3 software (Variocam, InfraTec GmbH, Dresden, Germany).

2.4. Statistical Analysis

The data was evaluated using IBM SPSS Statistics versions 23 and 24 (International Business Machines Corporation, Armonk, NY, USA). 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. Data from silage analyses shown in Table 1 followed a normal distribution. Analysis of variance was used to compare the different treatments to each other. Differences among means <0.05 ($p < 0.05$) were accepted to be significant.

Table 1. 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 2–4); DM = dry matter, aNDFom = neutral detergent fibre assayed with a heat-stable amylase and expressed exclusive of residual ash, ME = metabolisable energy, NEL = net energy lactation; [a,b] Mean values within columns having different superscripts differ ($p < 0.05$).

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
CON HD	0	379.6 [a,b]	34.4 [a]	82.3 [a]	171.0 [a]	30.6 [a]	375.8 [a]	4.1 [a]	374.4 [a,b]	11.4 [a,b]	6.9 [a,b]	3.4 [a,b]	1.1 [a]	0.0 [a]	0.8 [a]	0.0 [a]	0.0 [a]
	1	377.1 [a,b]	32.5 [a]	75.6 [a]	158.2 [a]	32.3 [a]	402.6 [a]	4.2 [a]	370.1 [a,b]	11.5 [a,b]	7.0 [a,b]	2.6 [a,b]	0.3 [a]	0.0 [a]	0.4 [a]	0.0 [a]	0.0 [a]
	2	371.9 [a,b]	31.4 [a]	72.2 [a]	168.7 [a]	31.8 [a]	384.4 [a]	4.0 [a]	371.2 [a,b]	11.4 [a,b]	7.0 [a,b]	3.9 [a,b]	0.8 [a]	0.0 [a]	0.6 [a]	0.0 [a]	0.0 [a]
	3	372.2 [a,b]	31.4 [a]	74.9 [a]	163.6 [a]	31.8 [a]	387.1 [a]	4.0 [a]	367.4 [a,b]	11.6 [a,b]	7.1 [a,b]	4.1 [a,b]	0.8 [a]	0.1 [a]	0.8 [a]	0.0 [a]	0.0 [a]
CON LD	0	368.7 [a,b]	37.2 [a]	84.2 [a]	172.7 [a]	32.4 [a]	368.1 [a]	4.1 [a]	388.9 [a,b]	11.4 [a,b]	7.0 [a,b]	3.5 [a]	1.4 [a]	0.2 [a]	0.8 [a,b]	0.0 [a]	0.0 [a]
	1	406.6 [a,b]	34.5 [a]	78.3 [a]	175.2 [a]	31.0 [a]	393.1 [a]	4.6 [a]	388.4 [a,b]	11.3 [a,b]	6.9 [a,b]	1.9 [a]	0.4 [a]	0.0 [a]	0.3 [a,b]	0.0 [a]	0.0 [a]
	2	369.3 [a,b]	31.3 [a]	76.8 [a]	161.9 [a]	30.3 [a]	399.7 [a]	4.2 [a]	357.0 [a,b]	11.5 [a,b]	7.1 [a,b]	3.0 [a]	0.6 [a]	0.0 [a]	0.6 [a,b]	0.0 [a]	0.0 [a]
	3	356.6 [a,b]	30.0 [a]	72.4 [a]	162.3 [a]	29.9 [a]	398.7 [a]	4.0 [a]	349.6 [a,b]	11.5 [a,b]	7.1 [a,b]	4.3 [a]	1.5 [a]	0.0 [a]	0.7 [a,b]	0.0 [a]	0.0 [a]
B1 HD	0	371.4 [a,b]	37.3 [a,b]	81.7 [a]	175.7 [a]	34.7 [b]	392.7 [a]	4.3 [a]	416.3 [b,c]	11.3 [a]	6.8 [a]	0.9 [c]	2.8 [b]	0.0 [a]	0.7 [a,b,c]	1.4 [b,c]	0.2 [a,b,c]
	1	389.3 [a,b]	34.1 [a,b]	80.3 [a]	151.8 [a]	35.2 [b]	430.0 [a]	4.3 [a]	381.3 [b,c]	11.5 [a]	7.0 [a]	1.1 [c]	2.5 [b]	0.0 [a]	0.5 [a,b,c]	0.9 [b,c]	0.2 [a,b,c]
	2	371.2 [a,b]	34.0 [a,b]	81.9 [a]	158.9 [a]	31.4 [b]	423.4 [a]	4.1 [a]	390.2 [b,c]	11.4 [a]	7.0 [a]	0.9 [c]	3.0 [b]	0.0 [a]	0.5 [a,b,c]	0.5 [b,c]	0.4 [a,b,c]
	3	355.2 [a,b]	36.3 [a,b]	81.5 [a]	177.5 [a]	33.4 [b]	379.2 [a]	4.1 [a]	425.2 [b,c]	11.2 [a]	6.8 [a]	1.5 [c]	3.3 [b]	0.0 [a]	0.6 [a,b,c]	0.0 [b,c]	1.2 [a,b,c]
B1 LD	0	362.9 [a,c]	40.2 [a,b]	84.0 [a]	180.4 [a]	35.3 [b]	380.7 [a]	4.3 [a]	432.4 [b,c]	11.2 [a]	6.8 [a]	0.3 [c]	4.6 [c]	0.6 [b]	0.7 [a]	0.4 [a,b]	0.5 [b,c]
	1	364.1 [a,c]	34.6 [a,b]	74.5 [a]	158.4 [a]	35.0 [b]	447.3 [a]	4.5 [a]	376.1 [b,c]	11.5 [a]	7.1 [a]	0.9 [c]	4.8 [c]	0.5 [b]	0.6 [a]	0.4 [a,b]	0.7 [b,c]
	2	349.4 [a,c]	34.1 [a,b]	78.8 [a]	155.3 [a]	36.2 [b]	443.1 [a]	4.4 [a]	373.1 [b,c]	11.6 [a]	7.1 [a]	1.1 [c]	4.7 [c]	0.5 [b]	0.8 [a]	0.1 [a,b]	1.4 [b,c]
	3	356.6 [a,c]	34.6 [a,b]	76.6 [a]	163.8 [a]	36.0 [b]	429.9 [a]	4.4 [a]	383.5 [b,c]	11.4 [a]	7.0 [a]	0.9 [c]	5.0 [c]	0.6 [b]	0.7 [a]	0.3 [a,b]	1.3 [b,c]
B2 HD	0	364.2 [a,b]	36.8 [b]	80.3 [a]	169.4 [a]	37.1 [b]	410.7 [a]	4.3 [a]	402.3 [c]	11.4 [a]	7.0 [a]	0.9 [c]	3.0 [b]	0.0 [a]	0.7 [a]	1.5 [d]	0.4 [a,b]
	1	374.8 [a,b]	36.5 [b]	78.7 [a]	166.7 [a]	33.3 [b]	424.1 [a]	4.3 [a]	400.8 [c]	11.3 [a]	6.9 [a]	0.6 [c]	3.2 [b]	0.0 [a]	0.6 [a]	1.7 [d]	0.4 [a,b]
	2	366.1 [a,b]	34.8 [b]	81.1 [a]	166.6 [a]	35.1 [b]	411.5 [a]	4.3 [a]	407.7 [c]	11.4 [a]	6.9 [a]	1.2 [c]	3.2 [b]	0.2 [a]	0.6 [a]	0.9 [d]	0.4 [a,b]
	3	374.1 [a,b]	37.5 [b]	79.1 [a]	172.1 [a]	34.7 [b]	403.6 [a]	4.2 [a]	406.1 [c]	11.3 [a]	6.9 [a]	1.6 [c]	3.0 [b]	0.0 [a]	0.8 [a]	1.4 [d]	0.5 [a,b]
B2 LD	0	351.0 [c]	40.9 [b]	81.9 [a]	183.7 [a]	33.2 [b]	382.5 [a]	4.4 [a]	431.5 [c]	11.1 [a]	6.7 [a]	0.6 [c]	6.0 [d]	1.0 [c]	1.1 [d]	1.1 [c,d]	0.9 [c]
	1	346.1 [c]	37.1 [b]	76.6 [a]	172.8 [a]	37.1 [b]	410.5a	4.5 [a]	415.9 [c]	11.3 [a]	6.9 [a]	0.5 [c]	6.0 [d]	0.7 [c]	0.8 [d]	1.1 [c,d]	0.9 [c]
	2	337.2 [c]	36.0 [b]	77.0 [a]	166.0 [a]	34.4 [b]	422.1 [a]	4.5 [a]	394.4 [c]	11.3 [a]	6.9 [a]	0.8 [c]	5.6 [d]	0.7 [c]	1.2 [d]	0.7 [c,d]	1.3 [c]
	3	334.6 [c]	33.7 [b]	74.2 [a]	153.0 [a]	37.5 [b]	454.5 [a]	4.4 [a]	361.4 [c]	11.7 [a]	7.2 [a]	0.7 [c]	5.5 [d]	0.7 [c]	1.1 [d]	1.0 [c,d]	1.1 [c]
CHEM HD	0	392.3 [b]	35.7 [a]	81.0 [a]	168.2 [a]	33.1 [a]	380.0 [a]	3.9 [b]	379.8 [a]	11.5 [b]	7.0 [b]	3.4 [a,b]	1.3 [a]	0.1 [a]	0.3 [c]	0.0 [a]	0.1 [a]
	1	402.6 [b]	31.7 [a]	83.8 [a]	141.2 [a]	32.2 [a]	433.0 [a]	4.0 [b]	323.9 [a]	11.9 [b]	7.3 [b]	3.2 [a,b]	0.9 [a]	0.0 [a]	0.2 [c]	0.0 [a]	0.0 [a]
	2	385.4 [b]	31.1 [a]	77.5 [a]	159.5 [a]	32.3 [a]	405.6 [a]	3.8 [b]	353.8 [a]	11.6 [b]	7.1 [b]	4.1 [a,b]	1.1 [a]	0.0 [a]	0.4 [c]	0.0 [a]	0.1 [a]
	3	391.2 [b]	32.0 [a]	80.9 [a]	148.2 [a]	31.6 [a]	428.7 [a]	3.8 [b]	336.5 [a]	11.8 [b]	7.2 [b]	3.9 [a,b]	1.0 [a]	0.0 [a]	0.1 [c]	0.0 [a]	0.0 [a]
CHEM LD	0	392.1 [b]	36.3 [a]	80.3 [a]	165.3 [a]	31.4 [a]	397.9 [a]	4.1 [b]	374.5 [a]	11.4 [b]	7.0 [b]	3.8 [b]	1.4 [a]	0.2 [a]	0.2 [b,c]	0.1 [a]	0.1 [a]
	1	397.1 [b]	32.9 [a]	77.8 [a]	159.4 [a]	32.3 [a]	413.8 [a]	4.0 [b]	358.6 [a]	11.5 [b]	7.1 [b]	4.5 [b]	1.3 [a]	0.0 [a]	0.2 [b,c]	0.0 [a]	0.1 [a]
	2	388.4 [b]	31.4 [a]	78.0 [a]	146.4 [a]	31.7 [a]	438.9 [a]	4.0 [b]	342.2 [a]	11.7 [b]	7.2 [b]	4.6 [b]	1.3 [a]	0.0 [a]	0.3 [b,c]	0.0 [a]	0.1 [a]
	3	379.0 [b]	31.2 [a]	77.1 [a]	154.5 [a]	33.0 [a]	425.3 [a]	3.9 [b]	336.6 [a]	11.7 [b]	7.2 [b]	5.0 [b]	1.6 [a]	0.0 [a]	0.4 [b,c]	0.0 [a]	0.1 [a]

3. Results and Discussion

The results of the temperature measurements are graphically represented in Figures 2 and 3. Figure 2 shows the temperature dynamics of the control without silage additive (treatment CON) and the temperature dynamics in the silage treated with the chemical additive (treatment CHEM). Figure 2 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.

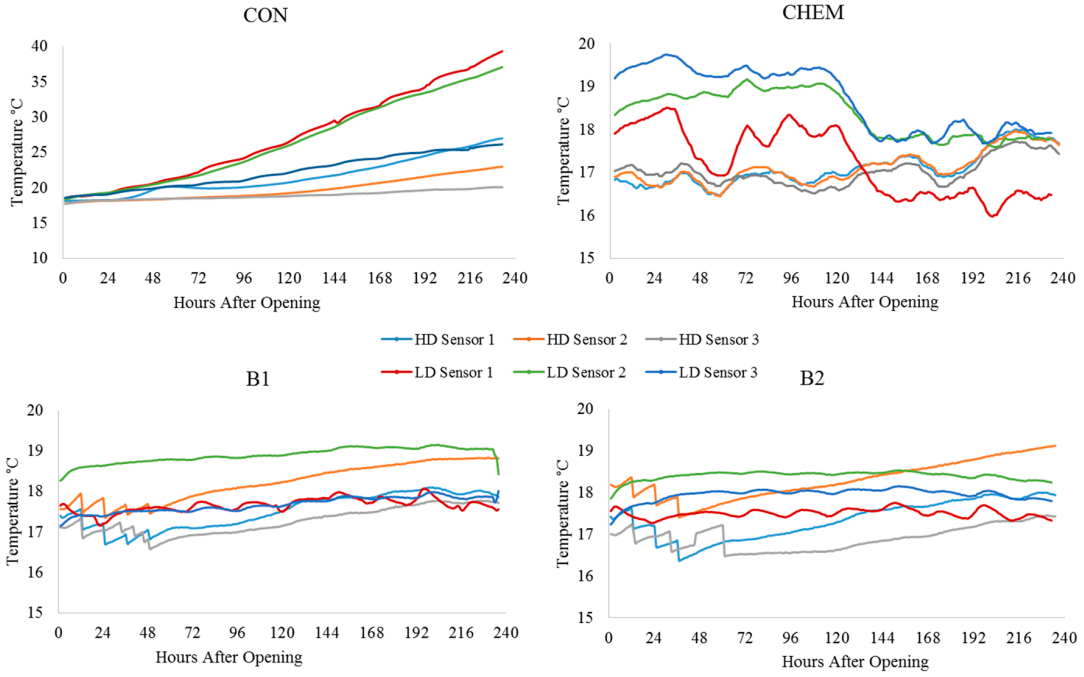

Figure 2. Temperature means per sensor, obtained from hourly average of temperature data measured in different treatments; CON = control group, CHEM = silage treated with the chemical additive, B1 = silage treated with biological additive 1, B2 = silage treated with biological additive 2, HD = high density, LD=low density.

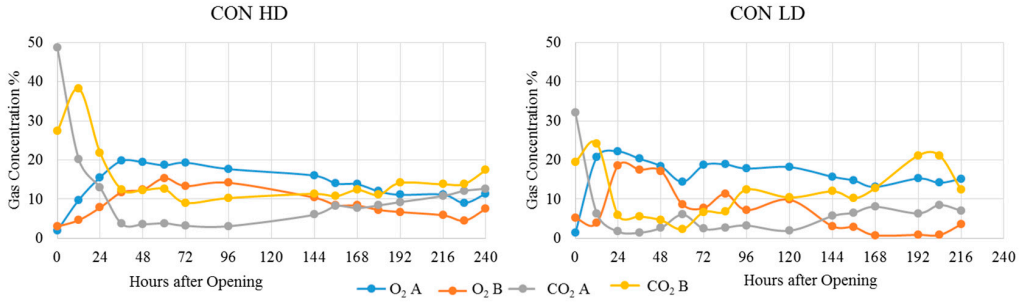

Figure 3. Means of gas concentrations of samples taken from buckets of the control variation at two sampling points (A and B); CON = control group, HD = high density, LD = low density.

At the beginning of the experimental period, a lag time (T0–phase; cf. [15]) of 24 h can be observed in which the temperature of the control does not rise significantly. Afterwards, the temperature starts rising slowly. Therefore, the statistical analyses did not show any significant differences between the temperatures of the sensors in the control in the first 48 h. These findings are consistent with the data shown in Figure 2.

Figure 3 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, 24 h after opening (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 a temperature rise.

The low-density variation of treatment CON became significantly warmer than the high-density variation. Figure 3 shows that oxygen diffused into the low-density buckets much faster than into the high-density buckets. The maximum O_2-Konzentration in the lower part of the LD buckets of treatment CON is reached after 14 h, whereas the maximum O_2 concentration in the lower part of the HD buckets of treatment CON is reached after 60 h. 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 the HD buckets represents a stronger barrier against incoming air. Johnson et al. [17], observed a longer period of aerobic stability in mechanically-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, therefore, excludes oxygen. These findings are consistent with the findings at hand.

As the data of treatment CON shows, 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 3 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 higher ($p < 0.001$) 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 the CON treatment keep on rising while O_2 is metabolised and CO_2 is produced. Analysing the results of the 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 the surrounding air includes oxygen entering the buckets through the open face and balances the concentration gradient by diffusion. The same process can be observed at the 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 the CON a treatment re not new. Similar results have already been shown by Muck et al. [18], Maack et al. [19], Köhler et al. [20], and Jungbluth et al. [15], and confirm previous findings which demonstrate that experimental conditions are consistent with those studies. 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 the CON treatment, the CHEM treatment did not undergo reheating. The temperature stayed at the same level throughout the experimental procedure. These observations were made in both the HD and LD variations. 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 the 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 4. At the time of opening, no O_2, but a high amount of CO_2, can be measured inside the buckets, like the CON treatment also showed. The CO_2 inside the buckets had built up 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 the 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 h after opening. This shows that there is no microbial respiration activity in the silage treated with the chemical additive during the time of the experiment.

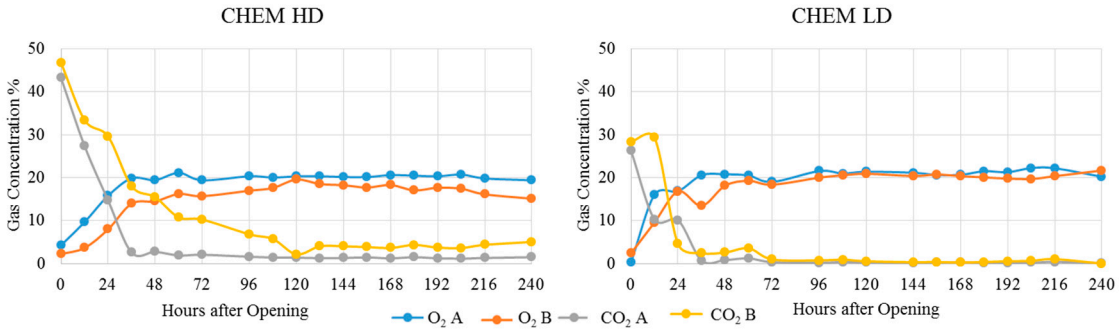

Figure 4. Means of gas concentrations of samples taken from buckets of the chemical treatment at two sampling points (A and B); CHEM = silage treated with chemical additive, HD = high density, LD = low density.

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 a 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 the CHEM treatment decreases slower and O_2 increases slower than in the LD variation.

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 the CHEM treatment, treatments B1 and B2 did not undergo reheating. 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 the CHEM treatment, it could be observed in treatment B1 and B2 that higher bulk density had no additional positive effect on the reduction of temperature. The results of the temperature measurement are graphically represented in the lower part of Figure 2 for the treatments B1 and B2. 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 buckets treated with biological inoculants (B1 and B2). Consequently, it can be assumed that an energy-consuming process independent of air influence takes place in the buckets treated with biological silage additives. A possible explanation for

this phenomenon is the particularity that there is significantly more alcohol, especially 1,2-propandiol and 1-propanol, in the silages treated with the biological additives (cf. Table 1). Table 1 also shows that the ethanol concentrations are higher in silages treated with the biological inoculants. The parameter ethanol concentration is additionally influenced by density. Results from the gas measurement shown in Figure 5 (treatment B1) and in Figure 6 (treatment B2) underline these outcomes. 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], as can be seen in Table 1.

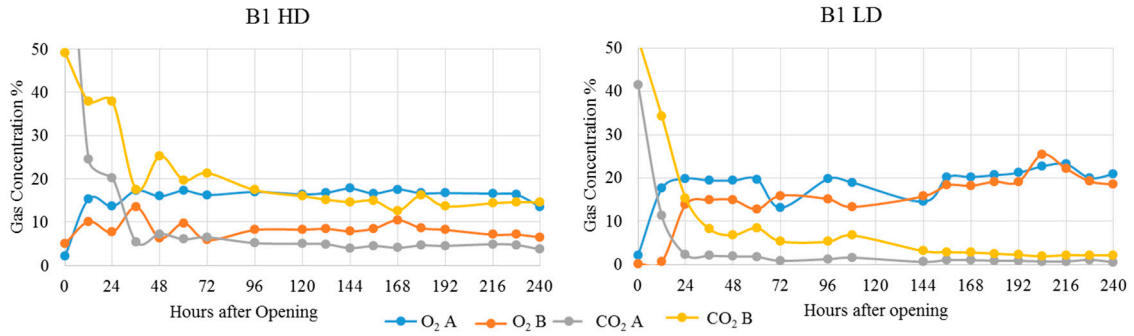

Figure 5. Means of gas concentrations of samples taken from buckets of treatment B1 at two sampling points (A and B); B1 = silage treated with biological inoculant 1, HD = high density, LD = low density.

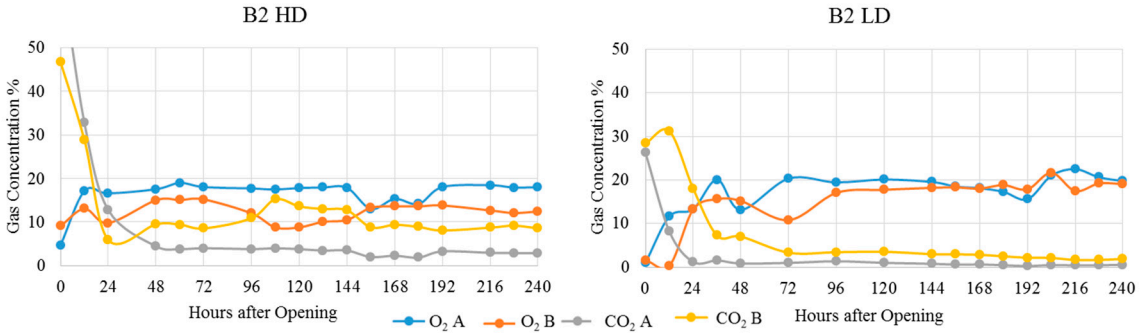

Figure 6. Means of gas concentrations of samples taken from buckets of treatment B2 at two sampling points (A and B); B2 = silage treated with biological inoculant 2, HD = high density, LD = low density.

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 of their treated silages compared to the untreated variation, as is also shown by Table 1 in the present study. Ranjit and Kung [3] also found higher concentrations of acetic acid in silages treated with a high concentration (1×10^6 cfu/g) of *Lactobacillus buchneri*. The findings of the present study support these results (cf. Table 1). As Table 1 show, the amount of acetic acid in silage treated with the biological additives is additionally influenced by density. Lower density increased the content of acetic acid significantly (cf. Table 1).

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 as also shown in the present study 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. The findings concerning acetic acid, 1,2-propanediol, and lactic acid fully correspond with the findings at hand, as shown in Table 1.

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, Danner et al. [11] also used *Lactobacillus buchneri* in trials associated with silages high in acetic acid concentrations and found higher aerobic stability.

Another parameter, which is changed by chemical inoculation is the pH (cf. Table 1). The pH is lower in the CHEM treatment than in the other treatments because there is less acid built by fermentation.

The high initial CO_2 concentrations in all of the buckets used confirms the circumstances of ensiling to be optimal and, therefore, underpins the findings of Hussin [14]. Ashbell and Weinberg [23] also refer to high CO_2 concentrations as an indicator for well-sealed silage.

4. Conclusions

The results underline that high bulk density improves the stability of maize silage. Therefore, it is recommended for practice to focus on this aim. The chemical additive prevents silage from deterioration very effectively, even during longer times of air exposure. If size or geometry of silos on farms are not constructed in a manner suitable to the amount of silage needed, or if other conditions lead to a slow feed-out rate from the silage, chemical additives can prevent aerobic deterioration. The biological additives were also able to prevent silage from reheating during the experimental period. When using chemical or biological additives, high density offers the additional 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. For a decision of whether to use, or not to use, additives, the financial circumstances of each particular business and the actual biological conditions in each particular year should be included.

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Conflicts of Interest: The authors declare no conflict of interest.

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