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Improving aerobic stability and biogas production of maize silage using silage additives

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HIGHLIGHTS

- Aerobic deterioration remarkably reduces the original methane potential of silage.
- 17% of the methane potential of maize silage was lost during 7 days air exposure.
- Air stress during storage reduced aerobic stability and increased methane losses.
- Additive treatment had little effects on methane yield after anaerobic storage.
- Additive treatment led to up to 29% higher methane yields after exposure to air.

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ABSTRACT

The effects of air stress during storage, exposure to air at feed-out, and treatment with silage additives to enhance aerobic stability on methane production from maize silage were investigated at laboratory scale. Up to 17% of the methane potential of maize without additive was lost during seven days exposure to air on feed-out. Air stress during storage reduced aerobic stability and further increased methane losses. A chemical additive containing salts of benzoate and propionate, and inoculants containing heterofermentative lactic acid bacteria were effective to increase aerobic stability and resulted in up to 29% higher methane yields after exposure to air. Exclusion of air to the best possible extent and high aerobic stabilities should be primary objectives when ensiling biogas feedstocks.

1. Introduction

The fermentative production of biogas via anaerobic digestion of biomass and the use of its major compound methane as a source for renewable electricity, heat or biofuel has largely evolved during the last two decades. Besides digestion of organic wastes, agricultural by-products and animal slurries, the co-digestion of energy crops with manure or other liquid feedstocks is common practice in several European countries such as Germany, Austria, Sweden, France and Finland (Murphy et al., 2011). Among biogas crops, maize is the most widely used crop species for methane production in farm-scale biogas plants (Murphy et al., 2011). Advantages of maize include its high biomass production potential, high methane yields, and easy integration into existing farming systems (Schittenhelm, 2008). For example, in Germany about 73% of the mass input of renewable raw materials to on-site energy generating biogas plants consists of maize (Multerer, 2014).

Maize grown under temperate climate conditions is usually harvested once a year in late summer or autumn. Seasonal harvest requires preservation and storage of feedstock material for continuous feeding to the digester throughout the year. Maize whole crop biomass used as biogas feedstock is commonly preserved by wet anaerobic storage via ensiling (Murphy et al., 2011). During the ensiling process soluble carbohydrates and proteins are fermented to organic acids, alcohols and soluble nitrogenous compounds. Formation of acids, mainly lactic acid, results in a drop in pH and inhibits activities of undesirable microorganism, such as clostridia and enterobacteria, leading to the conservation of dry matter (DM) and nutrients. However, the success of preservation depends on appropriate biological and chemical conditions that allow a rapid and sufficient decline in pH and stabilisation of a low pH within the silage. Losses of DM can easily reach more than 30% in poorly managed silage (Allen et al., 2003).

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Maize is a crop with exceptional good ensiling characteristics due to its relatively high DM content at harvest, its low buffering capacity and adequate level of water-soluble carbohydrates (WSC) (McDonald et al., 1991). However, carbohydrate-rich silages and well preserved silages with high lactic acid concentrations and low concentrations of higher volatile fatty acids have been reported to be particularly prone to aerobic deterioration (McDonald et al., 1991). Aerobic deterioration of silage is mainly related to the development of yeasts and moulds that remain dormant under anaerobic conditions and rapidly multiply after re-exposure to air. Undesirable aerobic microbial growth result from penetration of oxygen into the silo that can occur at different stages of the ensilage process. Firstly, air might infiltrate into the silage through inappropriate or damaged protecting cover, or by diffusion through the cover during the storage phase (Driehuis et al., 1999). Secondly, during the feed-out phase the silo is opened and silage is inevitably exposed to air. As emphasized by Wilkinson and Davies (2013), changes that occur during the feed-out phase are as important for preservation of nutrients as the silage fermentation process itself. Penetration of oxygen into the silo induces microbial oxidation of products of silage fermentation, such as lactic acid, and of remaining WSC to carbon dioxide and water (Wilkinson and Davies, 2013). This involves an increase in temperature above ambient and elevated mass and nutrient losses (Wilkinson and Davies, 2013).

While a number of studies on the effects of silage fermentation on methane yields exist already, studies on effects of aerobic deterioration on methane production are scarce. McEniry et al. (2014) analysed the methane yield of grass silage before and after exposure to air, however, silages were comparatively stable and no significant change in methane yield due to exposure to air was found. In contrast, results of studies on perennial ryegrass silage (Nussbaum, 2012) and maize silage (Plöchl et al., 2009) indicate that methane yields can considerably decrease under aerobic conditions.

The objective of the present study is to comprehensively analyse the effects of aerobic conditions and the use of silage additives designed to increase aerobic stability on methane production of maize silage at laboratory scale. This includes for:

- Evaluating the effects of air stress during storage on aerobic stability and methane production from maize silage.
- Analysing the effects of exposure to air at feed-out of silage on methane production.
- Evaluating the effects of 6 silage additives on storage losses, silage quality and methane production under anaerobic and aerobic storage conditions.

2. Methods

2.1. Description of raw materials

Maize (*Zea mays*), variety PR39R68 (PIONEER Hi-Bred Northern Europe Sales Division GmbH, Buxtehude, Germany) was gained as raw material from an experimental site located in the Teltow-Fläming district in North-East Germany (52°13′N, 13°12′E; 39 m a.s.l.). Maize was harvested at the beginning of October at the stage of physical maturity. Maize whole crops were chopped to a nominal particle size of 6 mm with a precision forage harvester (Big X 650, Bernhard Krone GmbH, Spelle, Germany).

An inoculum was used in order to ensure high methanogenic activity during the analyses of methane production. The inoculum (average chemical characteristics: pH 7.8, DM 3.6%, ODM 2.3%, N 2.8 g kg^{-1}, NH_4-N 1.4 g kg^{-1} and organic acids 1.2 g kg^{-1}) consisted of digestate of previous batch digestion tests conducted with crop feedstock.

2.2. Silage preparation

Ensiling was conducted subsequent to the harvest of maize using 1.5 L glass jars (J. WECK GmbH u. Co. KG, Wehr, Germany) as lab scale silos. Different additive treatments were applied prior to filling the lab scale silos. Six commercially available silage additives were dissolved or diluted in sterile tap water (chemical additive) or Ringer's solution (biological additives) at concentrations recommended by the suppliers. An equal volume of 15 mL liquid per kg treated biomass was sprayed onto the previously weighed and thoroughly mixed maize raw material using a commercial hand sprayer. Silage additive treatments included one chemical additive and four biological additives with hetero- or combined homo- and heterofermentative lactic acid bacteria (LAB) effective to increase the aerobic stability of silage. For comparison, one biological additive with homofermentative LAB only, facilitating the silage fermentation process as its mode of action, was further included in the study. Treatments were applied as follows:

(1) Control: Maize without silage additive.
(2) Chem: Chemical silage additive MAIS KOFASIL® Liquid (ADDCON Europe GmbH, Bonn, Germany) containing sodium benzoate and sodium propionate; applied at a concentration of 4.5 L t^{-1} raw material (1.16 g sodium benzoate per kg, 0.42 g sodium propionate per kg raw material).
(3) LAB-ho: Microbial inoculant BIO-SIL® (Dr. Pieper Technologie und Produktentwicklung GmbH, Neuruppin, Germany) containing homofermentative LAB (*Lactobacillus plantarum* DSM 8862, DSM 8866); applied to achieve a final concentration of 3×10^5 colony-forming units (CFU) g^{-1} raw material.
(4) LAB-he A: Microbial inoculant BioCool® (Lallemand Animal Nutrition SA, Blagnac Cedex, France) containing heterofermentative LAB (*Lactobacillus buchneri* NCIMB 40788) and enzymes (*Apergillus oryzae* β-glucanase and α-amylase, *Trichoderma longibrachiatum* xylanase); applied to achieve a final concentration of $>10^5$ CFU g^{-1} raw material.
(5) LAB-he B: Microbial inoculant PIONEER® 11CH4 (PIONEER Hi-Bred Northern Europe Sales Division GmbH, Buxtehude, Germany) containing heterofermentative LAB (*L. buchneri* LN 40177); selected strain LN 40177 produces the enzyme ferulate esterase which promotes decomposition of lignocellulosic compounds; applied to achieve a final concentration of 1.1×10^5 CFU g^{-1} raw material.
(6) LAB-ho+he A: Microbial inoculant PIONEER® 11CFT (PIONEER Hi-Bred Northern Europe Sales Division GmbH, Buxtehude, Germany) containing a combination of homofermentative LAB (*Lactobacillus casei* 32909) and heterofermentative LAB (*L. buchneri* LN 40177); selected strain LN 40177 produces the enzyme ferulate esterase which promotes decomposition of lignocellulosic compounds; applied to achieve a final concentration of 1.1×10^5 CFU g^{-1} raw material.
(7) LAB-ho+he B: Microbial inoculant SILASIL ENERGY® (Schaumann GmbH, Pinneberg, Germany) containing a combination of homofermentative LAB (*L. plantarum* NCIMB 30142) and heterofermentative LAB (*L. buchneri* NCIMB 30141); applied to achieve a final concentration of 2×10^5 CFU g^{-1} raw material.

Ensiling of maize after additive treatment was either conducted under anaerobic conditions or with simulation of air stress. For anaerobic conditions, a defined mass of maize chosen to give a pore volume of 4 L kg^{-1} (DLG, 2000) was weighed and filled into each silo. Maize samples were compressed using a manually operated plunger in such way that silos were filled completely and no headspace was left in the jars. This ensured equal conditions of com-

paction for all treatment variants. After filling, silos were sealed with a glass lid, rubber ring and four metal clamps. This allowed gas formed during ensiling to leave the silo, but prevented air to infiltrate into the silo.

In order to simulate suboptimal storage conditions and air stress during storage silos were equipped with two 6-mm diameter holes, one placed in the centre of the lid and one placed near the bottom. Silos were filled with two third of the mass of maize used for variants at anaerobic storage conditions. After filling and sealing the silos, holes were immediately closed with sterile rubber stoppers. Air stress was induced by removing the rubber stoppers after 4 and 6 weeks of storage (day 28 and 42) for 24 h, respectively.

For each additive treatment maize silage was stored under anaerobic conditions for a period of 49 and 90 days, and under air stress conditions for 49 days. Silos were stored at a constant temperature of 25 °C. Losses during ensiling were determined by weighing the silos after filling and after storage as described by Weißbach (2005). All treatments were conducted in triplicate.

2.3. Exposure to air and test of aerobic stability

After feed-out of maize silages, changes in chemical composition and methane production due to further aerobic storage for a period of 7 days were investigated. Degradation during exposure to air was monitored by analysing temperature changes within the silage according to Honig (1990). Maize silages were loosely filled into 1 L plastic jars directly after samples were taken out of the glass silos. 10 mm holes at the top and bottom of the jars allowed air to enter the silage. Jars were insulated with polystyrene foam and incubated in a tempered room at 20 °C. Temperature probes (Platinum 100 sensors) were used to measure changes in temperature in the centre of the silage samples. Temperatures were recorded every 2 h using a data logger system (PS-ES Electronics Service, Nieuwendijk, Netherlands). Aerobic stability of silages represents the time until temperature within the silage rises more than 3 °C above ambient temperature. Losses during exposure to air were calculated based on temperature rise as described by Honig (1990).

2.4. Chemical analyses

Samples of fresh and ensiled maize of different additive and storage treatments were stored at −18 °C prior to chemical analyses and analyses of methane production. DM content was measured by oven drying at 105 °C, and organic dry matter (ODM) content was determined by subsequent ashing of the samples in a muffle furnace at 550 °C (VDLUFA, 2006). DM content and all analytical parameters expressed on DM basis were corrected for losses of volatile organic compounds during oven drying as suggested by Weißbach and Strubelt (2008).

Samples dried at 60 °C for 48 h and milled to a particle size <1 mm were used for determination of buffering capacity (BC), crude protein (CP), crude fat (CL), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). BC (raw maize samples only) was measured by suspending 1 g of sample in 100 mL distilled water for 30 min, followed by titration to pH 4.0 with lactic acid (0.1 mol L^{-1}). CP, CL, NDF, ADF and ADL content were analysed as described previously (Herrmann et al., 2011).

Samples of silages taken prior to drying were analysed for pH, ammonia–nitrogen, organic acids and alcohols. pH values and NH_3-N content of silages were measured as described by Herrmann et al. (2011). Organic acids and alcohols were determined in cold water extracts of silages. Lactic acid was analysed using a high performance liquid chromatograph (Dionex, Sunnyvale, USA) equipped with an Eurokat H column (Knauer, Berlin, Germany) and refractive index detector. Volatile fatty acids (acetic, propionic, butyric, iso-butyric, valeric, iso-valeric and caproic acid) and alcohols (ethanol, propanol, 1,2-propanediol, 2,3-butanediol) were detected by gas chromatography (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a PERMABOND® FFAP capillary column (Machery-Nagel GmbH & Co KG, Düren, Germany) and a flame ionisation detector.

2.5. Batch anaerobic digestion test

Effects of additive and storage treatments on methane production of maize were analysed in batch anaerobic digestion tests in accordance with VDI 4630 (VDI, 2006). Batch tests were conducted in 2 L glass reactors, each filled with 1.5 L inoculum and 50 g of sample. This corresponded to a substrate-to-inoculum ratio on ODM basis of on average 0.53 (range 0.41–0.60). Reactors were incubated in a water bath at 35 °C and shaken manually once a day to resolve sediments and scum layers. Biogas was collected in wet gas meters. Volume of biogas was measured daily by applying the liquid displacement method (VDI, 2006) using acidified saturated NaCl solution as barrier solution. Measured biogas volume was corrected for the volume produced by the blank (inoculum without sample) and was normalised to standard conditions (dry gas, 0 °C, 1013 hPa). Biogas composition (methane and carbon dioxide) was detected using a portable gas analyser (GA94, Ansyco, Karlsruhe, Germany). Analyses of biogas composition required a certain volume of biogas and were performed on average 14 times throughout the test period of 30 days. Methane content was corrected for its dilution within the headspace of the experimental set-up (VDI, 2006). Methane yields are reported as sum of the methane volume produced during the test period on the basis of ODM added to the test (ODM_{added}), and on the basis of the corresponding ODM prior to ensiling (ODM_{orig}), the latter taking account of storage losses.

2.6. Statistical analyses

Chemical parameters, silage fermentation products and methane yields of maize silages were subjected to analysis of variance using the GLM procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Silage additive, storage treatment and additive × storage interactions were included as fixed effects in the statistical model. Parameters were compared separately for silages after anaerobic or semi-anaerobic storage and after exposure to air. The significance of differences of means in methane yield based on ODM_{added} and ODM_{orig} between silage additive treatments was tested by multiple comparisons applying the test procedure SIMULATE. Multiple comparisons were conducted separately for each storage variant due to significant additive × storage interactions. The level of significance α of the statistical analyses was set at 0.05.

3. Results and discussion

3.1. Freshly harvested maize

Chemical composition of freshly harvested maize without and with silage additives is presented in Table 1. Raw material was characterised by high ODM content, and low fat and fibre content as typically found for maize whole crop biomass (Schittenhelm, 2008). DM content within the optimal range for ensiling and a low buffering capacity suggest good conditions for lactic acid fermentation (McDonald et al., 1991). No considerable differences were found in chemical composition of freshly harvested maize before and after addition of silage additives (Table 1).

Table 1
Chemical characteristics of freshly harvested maize without (Control) and with addition of silage additives (mean ± standard deviation).

Silage additive	DM (%)	ODM (%$_{DM}$)	CP (%$_{DM}$)	CL (%$_{DM}$)	NDF (%$_{DM}$)	ADF (%$_{DM}$)	ADL (%$_{DM}$)	BC (g LA kg$_{DM}^{-1}$)
Control	37.0 ± 0.2	96.8 ± 0.1	8.3 ± 0.1	3.0 ± 0.3	31.4 ± 2.2	15.5 ± 0.6	1.4 ± 0.1	23 ± 1.4
Chem	36.7 ± 0.3	96.8 ± 0.1	8.1 ± 0.2	3.0 ± 0.3	30.4 ± 0.5	15.2 ± 1.2	1.5 ± 0.1	26 ± 0.9
LAB-ho	36.4 ± 0.2	97.0 ± 0.3	8.3 ± 0.3	3.6 ± 0.1	32.7 ± 0.3	16.8 ± 0.6	1.6 ± 0.1	22 ± 1.1
LAB-he A	36.1 ± 0.4	96.9 ± 0.4	8.4 ± 0.3	3.3 ± 0.2	30.8 ± 1.5	15.0 ± 1.0	1.4 ± 0.2	25 ± 1.7
LAB-he B	36.0 ± 0.5	96.8 ± 0.1	8.2 ± 0.3	3.0 ± 0.3	31.2 ± 2.3	14.9 ± 0.7	1.5 ± 0.1	22 ± 0.4
LAB-ho+he A	36.1 ± 0.3	96.9 ± 0.1	8.2 ± 0.5	3.4 ± 0.2	33.2 ± 0.2	17.3 ± 0.9	1.5 ± 0.1	22 ± 1.2
LAB-ho+he B	36.3 ± 0.4	96.8 ± 0.2	8.0 ± 0.5	3.0 ± 0.3	30.3 ± 0.3	16.0 ± 0.7	1.6 ± 0.0	23 ± 0.3

DM: dry matter; ODM: organic dry matter; CP: crude protein; CL: crude lipids; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; BC: buffering capacity; LA: lactic acid

3.2. Anaerobic storage and air stress during storage

3.2.1. Products of silage fermentation

Ensiling and anaerobic storage of maize resulted in well preserved silages with low pH values (<4.3) for all variants tested (Table 2). No butyric acid or only traces of butyric acid were found, implying absence of undesired clostridial activity during ensiling.

Treatment with additives led to contrasting pattern of fermentation products of silages with significant effects on pH-value ($P < 0.001$), lactic acid ($P < 0.001$), acetic acid ($P < 0.001$) and alcohol content ($P < 0.001$). Chemical treatment and addition of homofermentative LAB had only minor effects on lactic and acetic acid content compared to the control. In contrast, addition of heterofermentative LAB or mixtures of homo- and heterofermentative LAB resulted in significantly lower lactic acid and higher acetic acid concentration. Control silages and silages with chemical additive showed higher alcohol content than silages with other additives, though propanol dominated in these silages and ethanol dominated in silages with heterofermentative LAB treatment. Silage additives also significantly affected ODM losses during storage ($P < 0.001$, Table 2). Lowest losses occurred in silages with chemical additive, while highest losses occurred in silages with heterofermentative LAB. Thus, 0.3–2.5% (abs.) higher ODM losses were observed for silages with heterofermentative LAB treatment compared to control silages under anaerobic conditions.

Storage conditions during ensiling had significant effects on ODM losses ($P < 0.001$), pH ($P < 0.001$), and products of silage fermentation ($P < 0.01$) with exception of lactic acid, whereas significant interactions were found between storage conditions and silage additive treatments for all parameters analysed (Table 2). Prolonged storage duration of 90 days predominantly increased the sum of fermentation products and slightly increased ODM losses by on average 0.5% (abs.). For heterofermentative LAB treatments, the 90 day storage duration resulted in a rise in acetic acid content compared to the silages stored for 49 days. The NH_3-N concentration of the maize silages was mainly affected by silage additive and storage duration with an increase in NH_3-N concentration at prolonged storage and with addition of *L. buchneri* containing additives. Air stress during storage caused 0.1–1.9% higher ODM losses compared to anaerobic storage with largest effects found

Table 2
Organic dry matter losses during ensiling, pH of maize silages and products of silage fermentation (mean ± standard deviation).

Silage additive	Storage conditions	ODM loss (%)	pH	LA (%$_{DM}$)	AA[a] (%$_{DM}$)	BA[b] (%$_{DM}$)	ALC[c] (%$_{DM}$)	LA/FP	NH_3-N (% N_{tot})
Control	49 d; anaerobic	4.8 ± 0.4	3.9 ± 0.1	5.0 ± 0.1	0.9 ± 0.0	0.0 ± 0.0	2.6 ± 0.0	0.58 ± 0.01	6.2 ± 0.6
	49 d; air stress	5.5 ± 0.3	3.8 ± 0.0	5.2 ± 0.6	0.8 ± 0.1	0.0 ± 0.0	2.1 ± 0.4	0.64 ± 0.05	6.3 ± 0.2
	90 d; anaerobic	4.5 ± 0.2	3.8 ± 0.0	5.5 ± 0.4	0.9 ± 0.0	0.0 ± 0.0	2.5 ± 0.9	0.62 ± 0.01	7.4 ± 1.0
Chem	49 d; anaerobic	3.8 ± 0.2	3.8 ± 0.0	5.2 ± 0.2	0.8 ± 0.0	0.0 ± 0.0	2.2 ± 0.3	0.63 ± 0.03	5.2 ± 0.2
	49 d; air stress	5.0 ± 0.3	3.9 ± 0.1	4.4 ± 0.4	1.1 ± 0.2	0.0 ± 0.0	2.3 ± 0.5	0.56 ± 0.06	5.5 ± 0.2
	90 d; anaerobic	4.0 ± 0.1	3.8 ± 0.0	5.2 ± 0.2	0.9 ± 0.0	0.0 ± 0.0	2.0 ± 0.3	0.64 ± 0.02	7.1 ± 0.4
LAB-ho	49 d; anaerobic	5.0 ± 0.2	3.8 ± 0.0	4.8 ± 0.1	1.2 ± 0.2	0.0 ± 0.0	1.5 ± 0.2	0.64 ± 0.03	6.0 ± 0.4
	49 d; air stress	6.9 ± 0.1	3.9 ± 0.0	4.6 ± 0.1	0.8 ± 0.0	0.0 ± 0.0	1.4 ± 0.2	0.69 ± 0.02	6.5 ± 0.2
	90 d; anaerobic	5.3 ± 0.1	3.9 ± 0.0	5.1 ± 0.1	1.3 ± 0.1	0.0 ± 0.0	1.4 ± 0.0	0.66 ± 0.01	8.3 ± 0.6
LAB-he A	49 d; anaerobic	5.1 ± 0.0	3.8 ± 0.1	4.7 ± 0.6	1.0 ± 0.2	0.0 ± 0.0	2.5 ± 0.2	0.57 ± 0.05	7.5 ± 0.4
	49 d; air stress	6.3 ± 0.2	3.9 ± 0.0	3.6 ± 0.8	1.9 ± 0.4	0.0 ± 0.0	0.9 ± 0.1	0.56 ± 0.02	8.1 ± 0.5
	90 d; anaerobic	5.8 ± 0.1	4.0 ± 0.0	3.9 ± 0.2	2.2 ± 0.0	0.0 ± 0.0	1.0 ± 0.1	0.55 ± 0.01	9.7 ± 0.1
LAB-he B	49 d; anaerobic	6.1 ± 0.1	4.2 ± 0.0	2.3 ± 0.0	2.8 ± 0.1	0.0 ± 0.0	1.3 ± 0.0	0.36 ± 0.01	8.3 ± 0.2
	49 d; air stress	6.4 ± 0.3	4.0 ± 0.0	2.9 ± 0.1	3.1 ± 0.6	0.0 ± 0.0	1.1 ± 0.1	0.42 ± 0.04	8.1 ± 0.2
	90 d; anaerobic	7.0 ± 0.5	4.3 ± 0.0	2.0 ± 0.2	3.4 ± 0.2	0.0 ± 0.0	1.6 ± 0.1	0.28 ± 0.03	12.6 ± 0.5
LAB-ho+he A	49 d; anaerobic	5.7 ± 0.4	4.2 ± 0.1	2.5 ± 0.3	3.5 ± 0.7	0.0 ± 0.0	1.3 ± 0.1	0.34 ± 0.07	8.1 ± 0.4
	49 d; air stress	6.5 ± 0.2	4.1 ± 0.1	2.3 ± 0.8	4.5 ± 0.7	0.0 ± 0.0	1.3 ± 0.0	0.28 ± 0.09	8.4 ± 0.7
	90 d; anaerobic	6.4 ± 0.5	4.2 ± 0.1	2.6 ± 1.0	4.2 ± 0.9	0.0 ± 0.0	1.5 ± 0.2	0.31 ± 0.11	11.3 ± 1.7
LAB-ho+he B	49 d; anaerobic	6.5 ± 0.1	4.2 ± 0.0	2.1 ± 0.1	3.0 ± 0.1	0.0 ± 0.0	1.2 ± 0.0	0.34 ± 0.01	8.3 ± 0.5
	49 d; air stress	6.6 ± 0.3	4.0 ± 0.0	3.5 ± 0.1	4.2 ± 0.4	0.2 ± 0.0	1.3 ± 0.1	0.38 ± 0.02	7.8 ± 0.5
	90 d; anaerobic	7.0 ± 0.2	4.3 ± 0.0	1.5 ± 0.1	3.5 ± 0.1	0.0 ± 0.0	1.4 ± 0.0	0.24 ± 0.01	10.9 ± 0.2
Level of significance									
Silage additive ($n = 7$)		***	***	***	***	–	***	***	***
Storage conditions ($n = 3$)		***	***	ns	***	–	**	*	***
Additive × storage		***	***	***	**	–	***	**	***

DM: dry matter; ODM: organic dry matter; LA: lactic acid; AA: acetic acid; BA: butyric acid; ALC: alcohols; LA/FP: lactic acid as a portion of total fermentation products; ns: not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

[a] Sum of acetic and propionic acid.

[b] Sum of i-butyric, n-butyric, i-valeric, n-valeric and n-caproic acid.

[c] Sum of ethanol, propanol, 1,2-butanediol, 2,3-propanediol.

for silages with addition of homofermentative LAB. Silages treated with heterofermentative LAB revealed an increase in acetic acid content under air stress conditions.

Good fermentation qualities of silages in the present study reflect favourable initial conditions of maize as a carbohydrate-rich raw material for preservation by ensiling (McDonald et al., 1991). Without additive the ensilage process was dominated by lactic acid fermentation which could not be apparently improved by addition of homofermentative LAB. Homofermentative LAB ferment sugars almost exclusively to lactic acid while heterofermentative LAB are known to ferment sugars to lactic acid, acetic acid, alcohols and carbon dioxide (McDonald et al., 1991). An increased concentration of acetic acid in silages after inoculation with heterofermentative LAB is desired since it can inhibit yeasts and moulds and increase the aerobic stability (Danner et al., 2003). Inoculation with heterofermentative LAB in the present study resulted in higher acetic acid content, higher pH-value, higher ethanol and NH_3-N content and lower lactic acid content which is in accordance with findings in literature (Driehuis et al., 1999). These changes in fermentation products led to higher mass losses during anaerobic storage due to the formation of CO_2 on conversion of sugars and lactic acid to acetic acid and alcohols (McDonald et al., 1991). Additives containing a combination of homo- and heterofermentative LAB can be advantageous over the sole addition of heterofermentative LAB since they promote a more rapid initial lactic acid formation and pH reduction, and can decrease fermentation losses (Filya, 2003). However, such effects could not be detected in the present study.

Concentration of fermentation products slightly increased with prolonged storage from 49 to 90 days. This indicates that fermentation processes continued to a minor extent during this stable phase of silage fermentation. Effects of heterofermentative LAB were more pronounced with prolonged storage duration. *L. buchneri* is reported to increasingly convert lactic acid into acetic acid at decreasing pH of silages (Oude Elferink et al., 2001). After addition of heterofermentative LAB Driehuis et al. (1999) observed a shift of lactic acid towards acetic acid and alcohols with longer storage duration which supports results of the present study. The NH_3-N concentration in silages indicates the level of protein degradation (McDonald et al., 1991). Increased NH_3-N concentrations after inoculation with *L. buchneri* have been reported before and might be explained by an increase in pH during storage due to high metabolic activity of *L. buchneri* (Filya, 2003). Air stress conditions during storage likely slowed lactic acid production, and stimulated acetic acid production in silages with addition of *L. buchneri* which resulted in numerically increased mass losses.

3.2.2. Chemical characteristics of maize silages

DM content of maize slightly decreased during ensiling and was on average 0.8% lower compared with the maize raw material. Further, only minor changes in chemical composition were determined (Table 3) which support the finding that silages where well preserved. The effects of storage duration and air stress during storage on chemical characteristics of maize silages were low and were not found to be significant (Table 3). Treatment with silage additives significantly influenced the DM ($P < 0.001$) and ODM content ($P < 0.05$), and fibre fractions ($P < 0.001$) of the silages (Table 3). Changes in chemical composition appeared to be related to DM losses during ensiling. Highest DM and lowest fibre contents were found for silages with chemical additive, the treatment which resulted in lowest DM losses. Lowest DM and highest fibre contents were found for additive treatments which resulted in higher storage losses (LAB-he B, LAB ho+he B). WSC are the main compounds converted into organic acids during ensiling (McDonald et al., 1991). Storage losses can lead to a relative increase of components which are not degraded during silage fermentation such as the fibre fractions. Fibre fractions are reported to be negatively correlated to the methane yield (Herrmann et al., 2014; Rath et al., 2013), however, numerical differences in fibre content between the maize silages in the present study were comparatively low (maximum 0.8%$_{DM}$ and 1.8%$_{DM}$ for ADL and ADF, respectively) and no such effects were identified.

3.2.3. Methane yields of maize silages

Methane yields of maize ranged from 342–354 $L_N\ kg^{-1}$ - ODM_{added} prior to ensilage, and from 344–381 $L_N\ kg^{-1}\ ODM_{added}$ after ensiling. Changes during silage fermentation increased the methane yield based on ODM_{added} by up to 10%. However, consideration of storage losses compensated for differences and revealed methane yields of maize silages similar to those of the raw material (326–356 $L_N\ kg^{-1}\ ODM_{orig}$).

Treatments with silage additives showed little effects on methane yields of maize silages when stored for 49 and 90 days under anaerobic conditions (Fig. 1). A 2–3% lower methane yield was observed with chemical additive, and a 1–3% higher methane yield was measured with additives containing homo- and heterofermentative LAB compared with the control silages. The heterofermentative LAB treatments resulted in slightly lower methane yields after 49 days of storage, and in 2–4% higher methane yields after 90 days of storage. Consideration of storage losses further decreased differences between additive treatments. Methane yields were not found to be significantly different both without and with consideration of storage losses (Fig. 1).

When comparing silages after 49 and 90 days of storage, prolonged storage slightly increased methane yields of all variants ($P = 0.0002$) by on average 3.5%. An increase in methane yield with prolonged storage was still apparent when taking account of storage losses ($P = 0.001$).

Storage for 49 days under air stress conditions decreased the methane yield of the control silage by 4.5%, while no negative effects were found for silages treated with silage additives. Thus, a 3% higher methane yield was found for silages treated with chemical additive, and 4–9% higher methane yields were found for silages treated with heterofermentative or homo- and heterofermentative LAB when taking account of storage losses. Highest methane yields were analysed with addition of LAB-ho+he B. Under air stress conditions, treatment of maize with the inoculant LAB-ho+he B significantly increased the methane yield compared with the control both without and with consideration of storage losses (Fig. 1).

An enhancing effect of silage fermentation on methane yields based on ODM added to the anaerobic digestion process as compared with methane yields of biomass prior to ensiling is in accordance with findings in literature (e.g. Gao et al., 2012; Herrmann et al., 2014; Kafle and Kim, 2013) and was discussed in detail previously (Herrmann et al., 2011). It is suggested that products of silage fermentation, such as alcohols, with higher methane production potential than substrates of silage fermentation, such as glucose, increase the methane yield when storage losses are not taken into account (Herrmann et al., 2011). Hence, changes in chemical composition during ensiling can compensate or partly compensate for storage losses (Herrmann et al., 2011; Pakarinen et al., 2008).

Results on the impact of silage additives on biogas production reported in literature have not always been consistent. Chemical additives were found to predominantly decrease the methane yield of maize silage under anaerobic conditions in the range of 3–15% (Herrmann et al., 2011; Plöchl et al., 2009). Treatments with homofermentative LAB resulted in decreasing methane yields of up to 21% to increasing methane yields up to 3% (Neureiter et al., 2005; Plöchl et al., 2009), and treatments with combinations of homo- and heterofermentative LAB were reported to affect the

Table 3
Chemical characteristics of maize silages without and with addition of silage additives (mean ± standard deviation).

Silage additive	Storage conditions	DM (%)	ODM (%$_{DM}$)	CP (%$_{DM}$)	CL (%$_{DM}$)	NDF (%$_{DM}$)	ADF (%$_{DM}$)	ADL (%$_{DM}$)
Control	49 d; anaerobic	35.9 ± 0.1	96.7 ± 0.1	8.6 ± 0.5	3.1 ± 0.3	29.2 ± 0.5	14.9 ± 0.3	1.3 ± 0.2
	49 d; air stress	36.6 ± 0.1	96.9 ± 0.1	7.9 ± 0.3	3.3 ± 0.2	26.4 ± 0.4	13.5 ± 0.4	1.1 ± 0.2
	90 d; anaerobic	36.0 ± 1.0	96.9 ± 0.1	8.4 ± 0.1	3.2 ± 0.1	27.3 ± 1.4	13.7 ± 0.8	1.0 ± 0.1
Chem	49 d; anaerobic	36.7 ± 0.7	96.9 ± 0.0	7.9 ± 0.3	2.9 ± 0.3	26.8 ± 1.1	13.8 ± 0.7	1.2 ± 0.4
	49 d; air stress	35.9 ± 0.4	96.7 ± 0.2	8.0 ± 0.3	3.4 ± 0.1	26.8 ± 1.3	13.5 ± 0.7	0.9 ± 0.1
	90 d; anaerobic	36.5 ± 0.4	96.8 ± 0.1	7.8 ± 0.5	3.1 ± 0.4	26.2 ± 1.6	13.2 ± 1.0	1.0 ± 0.1
LAB-ho	49 d; anaerobic	35.0 ± 0.6	96.8 ± 0.1	8.4 ± 0.4	3.3 ± 0.1	28.5 ± 0.6	14.5 ± 0.4	1.6 ± 0.3
	49 d; air stress	34.0 ± 0.3	96.5 ± 0.1	8.2 ± 0.3	3.5 ± 0.4	30.0 ± 0.6	15.0 ± 0.5	1.6 ± 0.2
	90 d; anaerobic	34.8 ± 0.4	96.7 ± 0.1	8.1 ± 0.3	3.4 ± 0.1	29.2 ± 1.4	14.9 ± 0.7	1.6 ± 0.2
LAB-he A	49 d; anaerobic	36.0 ± 1.1	96.9 ± 0.1	8.1 ± 0.0	3.1 ± 0.1	28.7 ± 1.6	14.7 ± 0.9	1.4 ± 0.2
	49 d; air stress	34.7 ± 0.5	96.7 ± 0.2	8.2 ± 0.2	3.4 ± 0.0	29.5 ± 0.5	14.5 ± 0.3	1.8 ± 0.6
	90 d; anaerobic	36.0 ± 0.2	96.6 ± 0.0	8.1 ± 0.2	3.4 ± 0.4	28.5 ± 0.8	14.5 ± 0.5	1.4 ± 0.5
LAB-he B	49 d; anaerobic	34.9 ± 0.0	96.5 ± 0.1	7.8 ± 0.2	3.3 ± 0.2	29.6 ± 0.4	14.8 ± 0.3	1.8 ± 0.6
	49 d; air stress	35.3 ± 0.3	96.8 ± 0.1	8.0 ± 0.3	3.4 ± 0.2	28.6 ± 0.5	14.2 ± 0.4	1.3 ± 0.1
	90 d; anaerobic	35.7 ± 0.8	96.6 ± 0.2	8.2 ± 0.2	3.6 ± 0.1	30.1 ± 0.5	15.0 ± 0.1	1.4 ± 0.3
LAB-ho+he A	49 d; anaerobic	35.9 ± 0.5	96.9 ± 0.1	7.8 ± 0.3	3.5 ± 0.1	28.7 ± 0.9	14.2 ± 0.4	1.0 ± 0.2
	49 d; air stress	35.7 ± 0.5	96.7 ± 0.1	7.9 ± 0.2	3.2 ± 0.2	27.3 ± 1.4	13.6 ± 0.8	1.0 ± 0.1
	90 d; anaerobic	36.8 ± 0.4	96.6 ± 0.2	8.3 ± 0.4	3.3 ± 0.2	29.1 ± 0.9	14.9 ± 0.2	1.3 ± 0.2
LAB-ho+he B	49 d; anaerobic	34.8 ± 0.7	96.7 ± 0.0	8.5 ± 0.4	3.3 ± 0.1	29.1 ± 1.7	14.8 ± 1.0	1.7 ± 0.4
	49 d; air stress	36.0 ± 1.2	96.8 ± 0.2	8.4 ± 0.5	3.2 ± 0.1	29.0 ± 0.8	14.5 ± 0.4	1.5 ± 0.1
	90 d; anaerobic	35.1 ± 0.5	96.7 ± 0.1	8.3 ± 0.2	3.5 ± 0.2	29.1 ± 1.1	14.8 ± 0.6	1.5 ± 0.1
Level of significance								
Silage additive ($n = 7$)		***	*	ns	ns	***	***	***
Storage conditions ($n = 3$)		ns	ns	ns	ns	ns	ns	ns
Additive × storage		*	**	ns	ns	ns	ns	ns

DM: dry matter; ODM: organic dry matter; CP: crude protein; CL: crude lipids; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; ns: not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

methane yield of maize silage within a range of −13% to +22% (Neureiter et al., 2005; Vervaeren et al., 2010). Large effects of silage additive treatments under anaerobic storage conditions on methane production are contrary to small differences in methane yields between additive variants and the control silage found in the present study. Methane yields are usually reported based on ODM with the DM content being determined by oven-drying. One reason for contradictory findings could be losses of volatile organic acids and alcohols during oven-drying which are often not considered in literature. This can lead to an overestimation of methane yields of silages with larger amounts of volatile compounds (Kreuger et al., 2011). Furthermore, it is crucial to also analyse and take account for storage losses when evaluating silages for biogas production (Herrmann et al., 2011). Results of the present study suggest that effects of silage additives on methane yield under anaerobic storage conditions are rather low, especially when storage losses are taken into account. A positive effect of cell wall degrading enzymes added with the silage additive or produced by certain strains of the heterofermentative LAB *L. buchneri* could not be clearly proven.

As opposed to silage additive treatments under anaerobic storage, storage conditions during ensilage were found to affect methane yields of maize silages. Prolonged storage slightly increased the methane yield which is in accordance with findings of other studies (Neureiter et al., 2005). Small amounts of air infiltrating into the silage during storage which was simulated by air stress in the present study resulted in a decrease in methane yield of the control silage. This might be due to initiation of aerobic microbial processes, although the observed effects on the products of silage fermentation were low (Section 3.2.1). Silage additives, especially microbial inoculants containing acetic acid producing LAB, were effective to prevent losses in methane yield under these suboptimal storage conditions. This is in agreement with Nussbaum (2012) who reported a significantly higher methane yield (3%) of perennial ryegrass silage treated with heterofermentative LAB compared with untreated silage stored under air stress conditions. Although significant differences in methane yields were found in the present study, effects of air stress and the use of silage additives under air stress conditions on methane yields were still comparatively low. It is likely that these effects will depend on the degree of air that penetrates into the silo in large scale application.

3.3. Exposure to air on feed-out

3.3.1. Aerobic stability and products of silage fermentation after exposure to air

The aerobic stability of silage describes the time until a noticeable temperature increase above ambient temperature occurs within the silage when exposed to air after feed-out, which indicates considerable aerobic microbial activity. The control silage showed aerobic stability of 48 h and 154 h, respectively, when exposed to air after a 49 and 90 days storage period under anaerobic conditions (Table 4). Air stress during storage decreased the aerobic stability to 26 h. Addition of homofermentative LAB further reduced the aerobic stability while the chemical additive as well as inoculants with heterofermenative LAB or mixtures of homo- and heterofermenative LAB were effective to increase the aerobic stability beyond the duration of the aerobic stability test (182 h) for storage variants without air stress (Table 4). Air stress during storage reduced the aerobic stability after feed-out compared with anaerobic storage for four of the six silage additive treatments investigated (Table 4).

Aerobic instability resulted in considerable increase in mass and ODM losses. Total ODM losses increased to up to 20% within 7 days of exposure to air. In maize silages with aerobic stabilities below 60 h (control silages and silage with homofermentative LAB), lactic and acetic acid were almost completely degraded and the concentration of alcohols decreased. The degradation of organic acids was accompanied by increasing pH values of the silages up to a pH

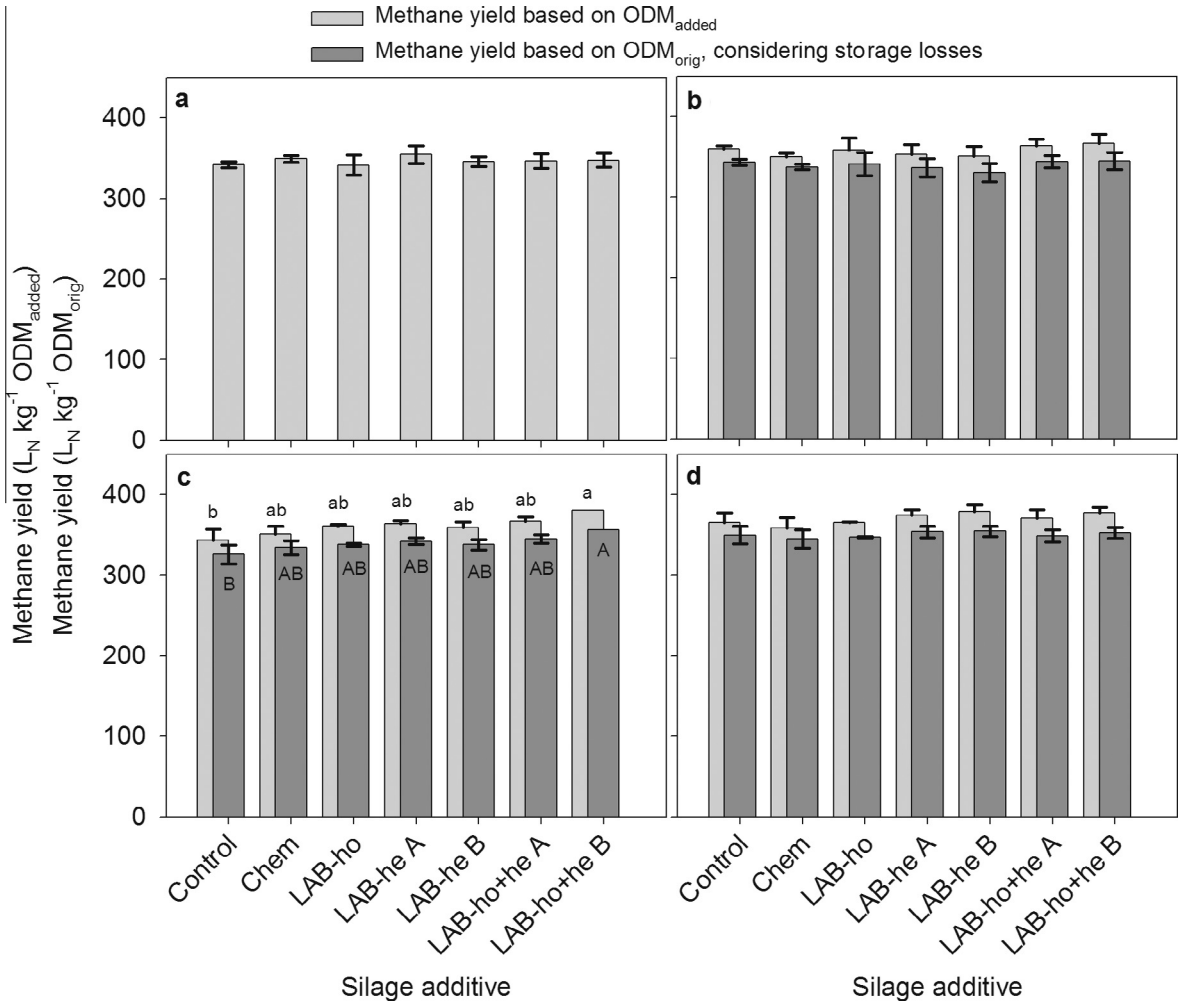

Fig. 1. Effects of silage additives on methane yields (means and standard deviation) without (based on ODM_{added}) and with consideration of storage losses (based on ODM_{orig}) of (a) fresh maize pre-ensiling, (b) maize silage after 49 days of anaerobic storage, (c) maize silage after 49 days of storage under air stress conditions, and (d) maize silage after 90 days anaerobic storage. [ab]Methane yields based on ODM_{added} with no lowercase letter in common differ significantly; [AB]Methane yields based on ODM_{orig} with no uppercase letter in common differ significantly ($p < 0.05$, Adjustment = SIMULATE).

value of 7 (Table 4). In contrast, only minor changes occurred in aerobically stable silages including a 0.7–1.4% increase in ODM losses, a slight increase in pH values and a slight reduction of the lactic acid content. In tendency, concentrations of acetic acid and alcohols increased. Smallest changes were observed for maize silages treated with the chemical additive. Butyric acid concentrations remained low for all variants.

Aerobic stability of the untreated maize silage can vary largely and values obtained in the present study lie within the range reported by others (e.g. Driehuis et al., 1999; Ranjit and Kung, 2000; Wilkinson and Davies, 2013). Salts of propionic and benzoic acid, which are active ingredients of the chemical additive, feature antimycotic properties and have been successfully used to reduce growth of yeasts and moulds (Kung et al., 2003). As found in the present study, they have few effects on silage fermentation but can result in a significant increase in aerobic stability of maize silage (Kung et al., 2003). Undissociated acetic acid, as well as other short-chain fatty acids, are also known to inhibit the growth of yeasts and moulds while lactic acid is largely ineffective against these initiators of the aerobic deterioration process (Danner et al., 2003; Wilkinson and Davies, 2013). Thus, the shift of lactic to acetic acid in maize silages treated with heterofermentative LAB or mixtures of homo- and heterofermentative LAB led to aerobically stable silages in the present study. As reviewed by Wilkinson and Davies (2013) and Kung et al. (2003), inhibition of the aerobic deterioration of maize silages through inoculants that contain strains of the heterofermentative LAB *L. buchneri* has been confirmed in many previous studies. In contrast, application of homofermentative LAB likely reduces the aerobic stability due to its stimulation of lactic acid fermentation which is usually associated with reduced formation of acetic acid (Wilkinson and Davies, 2013). A slightly negative effect of LAB-ho on the stability of maize silage during exposure to air was found in this study although homofermentative LAB failed to markedly change the pattern of fermentation products. In summary, the chemical additive as well as microbial inoculants containing strains of *L. buchneri* were successfully applied to increase the aerobic stability of maize silage after anaerobic storage beyond a period of 7 days of exposure to air which is recommended as target stability for farm-scale silages (Wilkinson and Davies, 2013).

Results of the present study further show that exclusion of air during storage is essential in order to prevent aerobic deterioration during feed-out. Air stress during the storage period likely promoted the growth or survival of yeasts and resulted in a more rapid multiplication of yeasts when aerobic conditions were available at feed-out. Under semi-anaerobic storage conditions, only the silage inoculants LAB-he B and LAB-ho+he B were effective to completely avoid aerobic deterioration during 7 days exposure to air. Findings

Table 4
Aerobic stability of maize silages, and total organic dry matter losses, pH and silage fermentation products in maize silages after 7 days exposure to air at feed-out (mean ± standard deviation).

Silage additive	Storage conditions	Aerobic stability[a] (h)	ODM loss[b] (%)	pH	LA (%$_{DM}$)	AA[c] (%$_{DM}$)	BA[d] (%$_{DM}$)	ALC[e] (%$_{DM}$)	NH_3-N (% N_{tot})
Control	49 d; anaerobic	48	14.2 ± 10.5	4.9 ± 1.5	2.5 ± 1.6	0.4 ± 0.3	0.0 ± 0.0	2.4 ± 0.8	4.9 ± 1.6
	49 d; air stress	26	20.2 ± 4.2	6.5 ± 0.3	0.4 ± 0.2	0.3 ± 0.0	0.0 ± 0.0	1.6 ± 0.2	3.1 ± 0.9
	90 d; anaerobic	154	6.7 ± 0.3	3.8 ± 0.0	6.0 ± 0.4	0.8 ± 0.1	0.0 ± 0.0	2.4 ± 0.0	9.2 ± 0.4
Chem	49 d; anaerobic	>182	4.6 ± 0.8	3.9 ± 0.0	5.1 ± 0.2	0.9 ± 0.0	0.0 ± 0.0	2.5 ± 0.2	5.8 ± 0.2
	49 d; air stress	167	6.3 ± 0.4	3.9 ± 0.1	4.7 ± 0.3	0.6 ± 0.3	0.0 ± 0.0	2.4 ± 0.5	5.4 ± 1.2
	90 d; anaerobic	>182	5.2 ± 0.2	3.8 ± 0.0	5.5 ± 0.1	0.9 ± 0.0	0.0 ± 0.0	2.7 ± 0.3	7.8 ± 0.8
LAB-ho	49 d; anaerobic	43	11.8 ± 1.7	6.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	7.4 ± 0.6
	49 d; air stress	19	16.2 ± 4.8	6.9 ± 0.3	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	5.9 ± 0.5
	90 d; anaerobic	57	16.3 ± 1.9	7.0 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	9.5 ± 1.9
LAB-he A	49 d; anaerobic	>182	6.5 ± 0.3	4.0 ± 0.0	3.9 ± 0.1	1.5 ± 0.0	0.0 ± 0.0	3.4 ± 0.4	8.7 ± 0.5
	49 d; air stress	165	7.5 ± 0.8	4.0 ± 0.1	3.9 ± 0.4	3.2 ± 1.1	0.1 ± 0.0	1.0 ± 0.3	6.7 ± 1.0
	90 d; anaerobic	>182	6.7 ± 0.3	4.0 ± 0.0	3.9 ± 0.2	2.9 ± 0.5	0.0 ± 0.0	1.3 ± 0.3	9.4 ± 2.1
LAB-he B	49 d; anaerobic	>182	6.7 ± 0.1	4.3 ± 0.0	1.0 ± 0.2	5.4 ± 0.4	0.1 ± 0.0	1.5 ± 0.4	11.0 ± 2.2
	49 d; air stress	>182	7.4 ± 0.3	4.1 ± 0.0	2.1 ± 0.1	5.1 ± 0.6	0.1 ± 0.0	1.4 ± 0.2	8.1 ± 0.6
	90 d; anaerobic	>182	8.0 ± 0.6	4.3 ± 0.0	1.4 ± 0.1	5.0 ± 0.1	0.1 ± 0.0	1.6 ± 0.1	12.8 ± 3.1
LAB-ho+he A	49 d; anaerobic	>182	6.9 ± 0.2	4.3 ± 0.1	1.6 ± 0.5	5.9 ± 0.8	0.1 ± 0.0	1.5 ± 0.1	11.5 ± 3.2
	49 d; air stress	169	7.6 ± 0.3	4.5 ± 0.5	1.2 ± 0.8	5.0 ± 2.1	0.1 ± 0.0	1.2 ± 0.6	6.8 ± 2.5
	90 d; anaerobic	>182	7.1 ± 0.6	4.2 ± 0.3	1.8 ± 1.4	5.4 ± 0.9	0.1 ± 0.0	1.6 ± 0.1	11.6 ± 2.7
LAB-ho+he B	49 d; anaerobic	>182	7.8 ± 0.3	4.3 ± 0.0	0.4 ± 0.2	4.0 ± 0.2	0.6 ± 1.1	6.5 ± 4.9	9.5 ± 0.0
	49 d; air stress	>182	7.7 ± 0.3	4.0 ± 0.0	3.3 ± 0.0	3.0 ± 0.0	0.0 ± 0.0	12.0 ± 0.0	8.6 ± 1.0
	90 d; anaerobic	>182	7.7 ± 0.2	4.2 ± 0.0	1.0 ± 0.1	3.3 ± 0.1	0.0 ± 0.0	1.5 ± 0.2	12.3 ± 0.4
Level of significance									
Silage additive (n = 7)		–	***	***	***	***	–	***	***
Storage conditions (n = 3)		–	**	*	**	ns	–	***	***
Additive × storage		–	*	***	***	ns	–	***	ns

DM: dry matter; ODM: organic dry matter; LA: lactic acid; AA: acetic acid; BA: butyric acid; ALC: alcohols; N_{tot}: total nitrogen content; ns: not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

[a] Time to temperature rise >3 °C.
[b] Total ODM loss of anaerobic and subsequent aerobic storage.
[c] Sum of acetic and propionic acid.
[d] Sum of i-butyric, butyric, i-valeric, valeric and caproic acid.
[e] Sum of ethanol, propanol, 1,2-butanediol, 2,3-propanediol.

Table 5
Chemical characteristics of maize silages after 7 days exposure to air at feed-out (mean ± standard deviation).

Silage additive	Storage conditions	DM (%)	ODM (%$_{DM}$)	CP (%$_{DM}$)	CL (%$_{DM}$)	NDF (%$_{DM}$)	ADF (%$_{DM}$)	ADL (%$_{DM}$)
Control	49 d; anaerobic	34.1 ± 3.1	96.5 ± 0.4	8.6 ± 0.9	3.4 ± 0.1	30.5 ± 4.2	15.8 ± 2.6	1.6 ± 0.5
	49 d; air stress	32.4 ± 1.4	96.0 ± 0.3	9.6 ± 0.2	2.9 ± 0.8	34.2 ± 1.5	17.8 ± 0.9	1.6 ± 0.1
	90 d; anaerobic	36.5 ± 0.8	96.7 ± 0.1	8.3 ± 0.2	3.6 ± 0.1	29.6 ± 1.3	15.0 ± 1.0	1.6 ± 0.2
Chem	49 d; anaerobic	37.0 ± 0.8	96.8 ± 0.1	8.1 ± 0.4	3.1 ± 0.1	27.4 ± 0.8	13.7 ± 0.6	1.4 ± 0.2
	49 d; air stress	36.1 ± 0.7	96.6 ± 0.1	8.1 ± 0.6	3.1 ± 0.5	28.8 ± 2.4	14.4 ± 1.2	1.5 ± 0.1
	90 d; anaerobic	36.1 ± 0.4	96.7 ± 0.0	8.4 ± 0.1	3.1 ± 0.1	27.1 ± 1.1	14.0 ± 0.6	1.2 ± 0.3
LAB-ho	49 d; anaerobic	33.9 ± 0.3	96.5 ± 0.1	8.4 ± 0.3	3.4 ± 0.2	30.7 ± 3.1	15.5 ± 1.5	1.3 ± 0.2
	49 d; air stress	32.0 ± 0.9	96.3 ± 0.2	8.6 ± 0.2	3.4 ± 0.4	32.9 ± 1.2	17.6 ± 1.0	1.7 ± 0.2
	90 d; anaerobic	33.7 ± 0.2	96.4 ± 0.2	8.1 ± 0.6	3.5 ± 0.2	31.1 ± 1.2	15.6 ± 0.5	1.4 ± 0.0
LAB-he A	49 d; anaerobic	35.8 ± 0.6	96.8 ± 0.0	8.1 ± 0.1	3.1 ± 0.1	29.5 ± 1.2	15.1 ± 1.0	1.6 ± 0.1
	49 d; air stress	35.7 ± 0.9	96.6 ± 0.2	9.0 ± 0.8	4.9 ± 0.3	37.5 ± 3.8	15.9 ± 1.4	2.3 ± 0.2
	90 d; anaerobic	35.7 ± 0.7	96.7 ± 0.1	10.0 ± 1.8	2.3 ± 0.1	34.3 ± 1.4	15.1 ± 0.7	2.1 ± 0.5
LAB-he B	49 d; anaerobic	37.3 ± 0.1	96.8 ± 0.1	7.5 ± 0.3	3.7 ± 0.3	33.9 ± 5.2	14.5 ± 0.7	1.8 ± 0.6
	49 d; air stress	36.6 ± 0.4	96.6 ± 0.2	7.7 ± 0.5	3.0 ± 0.4	30.5 ± 0.9	15.6 ± 0.8	2.6 ± 1.5
	90 d; anaerobic	37.5 ± 0.7	96.8 ± 0.1	7.4 ± 0.3	3.2 ± 0.3	27.1 ± 3.0	13.6 ± 1.5	2.2 ± 0.9
LAB-ho+he A	49 d; anaerobic	36.6 ± 0.9	96.9 ± 0.1	7.5 ± 0.1	2.1 ± 0.1	29.1 ± 1.8	14.7 ± 1.0	2.1 ± 0.3
	49 d; air stress	37.1 ± 0.8	96.8 ± 0.2	8.3 ± 0.3	3.0 ± 0.1	28.6 ± 1.3	14.3 ± 0.4	2.4 ± 1.6
	90 d; anaerobic	37.0 ± 0.7	96.9 ± 0.1	7.8 ± 0.3	1.9 ± 0.2	27.8 ± 1.1	14.1 ± 0.7	1.8 ± 1.2
LAB-ho+he B	49 d; anaerobic	37.5 ± 1.5	96.9 ± 0.3	7.4 ± 0.5	3.2 ± 0.2	26.3 ± 1.6	13.0 ± 0.7	1.0 ± 0.1
	49 d; air stress	35.4 ± 0.3	96.7 ± 0.0	7.9 ± 0.6	3.3 ± 0.1	31.4 ± 2.2	15.8 ± 1.3	1.6 ± 0.2
	90 d; anaerobic	38.3 ± 0.3	96.9 ± 0.0	7.2 ± 0.2	3.3 ± 0.1	26.3 ± 0.9	13.3 ± 0.4	1.1 ± 0.1
Level of significance								
Silage additive (n = 7)		***	***	***	***	***	***	*
Storage conditions (n = 3)		***	***	***	***	***	***	ns
Additive × storage		*	ns	***	***	**	ns	ns

DM: dry matter; ODM: organic dry matter; CP: crude protein; CL: crude lipid; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; ns: not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

of a more rapid heating of silage when stored under air stress conditions agree with results reported by Nussbaum (2012) who found a decrease in aerobic stability of perennial ryegrass silage by 98–104 h due to air stress conditions during storage.

Main changes that occur in aerobically deteriorating silages can be attributed to microbial oxidation of organic acids and WSC to carbon dioxide and water by yeasts (Wilkinson and Davies, 2013). A rapid reduction in lactic acid, alcohol and WSC content, an increase in pH-value and rapidly increasing DM losses are typically found in aerobically deteriorating silages (Honig, 1975; Ranjit and Kung, 2000). An increase in acetic acid content can occur during the initial stage of aerobic deterioration, but acetic acid is degraded with proceeding aerobic deterioration (Honig, 1975). A markedly increase in acetic acid during exposure to air in silages inoculated with *L. buchneri* strains have been reported previously and can be attributed to a continuing acetic acid production by *L. buchneri* under aerobic storage conditions (Ranjit and Kung, 2000). The loss of organic acids, alcohols and easily degradable carbohydrates in aerobically deteriorating silages which are valuable substrates for the biomethanation process with high methane production potential (Herrmann et al., 2011) can be expected to considerably decrease methane yields.

3.3.2. Chemical characteristics of maize silages after exposure to air

Silage additives and storage conditions influenced the DM and ODM content of the maize silages after 7 days exposure to air (Table 5). Lowest DM and ODM contents were found in maize silages with homofermentative LAB and in control silages after 49 days of storage without air stress and under air stress conditions. In general, air stress during storage reduced the DM and ODM content for all additive variants except LAB-ho+he A. Furthermore, the crude protein content as well as the fibre fractions, mainly NDF and ADF content, increased with air stress compared with the corresponding maize silages stored without air stress (Table 5). As already found for maize silages after anaerobic storage, changes in the chemical composition of the silages after exposure to air were related to storage losses. Aerobic deterioration associated with the degradation of organic acids and WSC resulted in a decrease in DM and ODM content, and in increasing portions of components that are less easily degradable such as crude protein and fibre fractions. This is in agreement with Honig (1975) who stated that in early stages of aerobic deterioration fibre, protein and ash fractions are unaffected and, therefore, tend to increase in the DM. Largest effects were observed for silages with lowest aerobic stability, hence, for silages without additive and with addition of homofermentative LAB, and for silages stored under air stress conditions. Besides the loss of easily degradable substrates, the relative increase of hardly degradable fibre fractions in aerobically deteriorating maize silages can also be expected to negatively influence the methane production.

3.3.3. Methane yields of maize silages after exposure to air

Methane yields of maize silages after 7 days exposure to air varied largely between 319 and 376 $L_N\ kg^{-1}$ ODM_{added}, and between 266 and 351 $L_N\ kg^{-1}$ ODM_{orig} when considering total storage losses (Fig. 2). Aerobic deterioration resulted in a decrease in methane yield and in high ODM losses. Methane yields based on ODM_{added} of the control silages and the silages with addition of homofermentative LAB *L. plantarum* which revealed low aerobic stability decreased by 3–12% compared with corresponding methane yields before exposure to air. Considering storage losses, methane yields based on ODM_{orig} of these silages decreased by 5–19%. On the other hand, methane yields of variants which were aerobically stable during the 7 days exposure to air were almost entirely preserved (Fig. 2). After 49 days of anaerobic storage and subsequent exposure to air a significantly higher methane yield (up to 20%) was found for maize silages treated with mixtures of homo- and heterofermentative LAB (LAB-ho+he A, LAB-ho+he B) compared with the control silage. After 49 days of storage under air stress conditions and subsequent exposure to air, treatment with the chemical additive and with all additives containing *L. buchneri* resulted in significantly higher methane yields (up to 29%) compared with the control silage, when considering ODM losses. Differences between these additive variants were not found to be significant (Fig. 2). After 90 days of anaerobic storage the control silage was aerobically more stable and exposure to air significantly

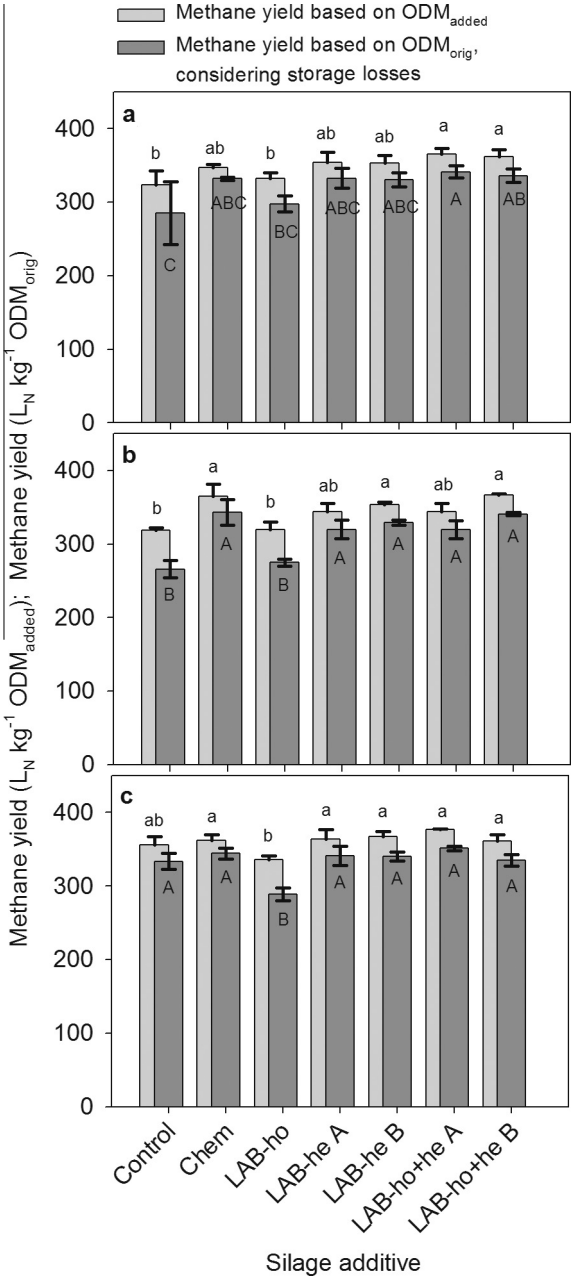

Fig. 2. Effects of silage additives on methane yields (means and standard deviation) without (based on ODM_{added}) and with consideration of total storage losses (based on ODM_{orig}) of maize silage exposed to air for 182 h after previous (a) 49 days of anaerobic storage, (b) 49 days of storage under air stress conditions, and (c) 90 days of anaerobic storage. $^{a–c}$Methane yields based on ODM_{added} with no lowercase letter in common differ significantly; $^{A–C}$Methane yields based on ODM_{orig} with no uppercase letter in common differ significantly ($p < 0.05$, Adjustment = SIMULATE).

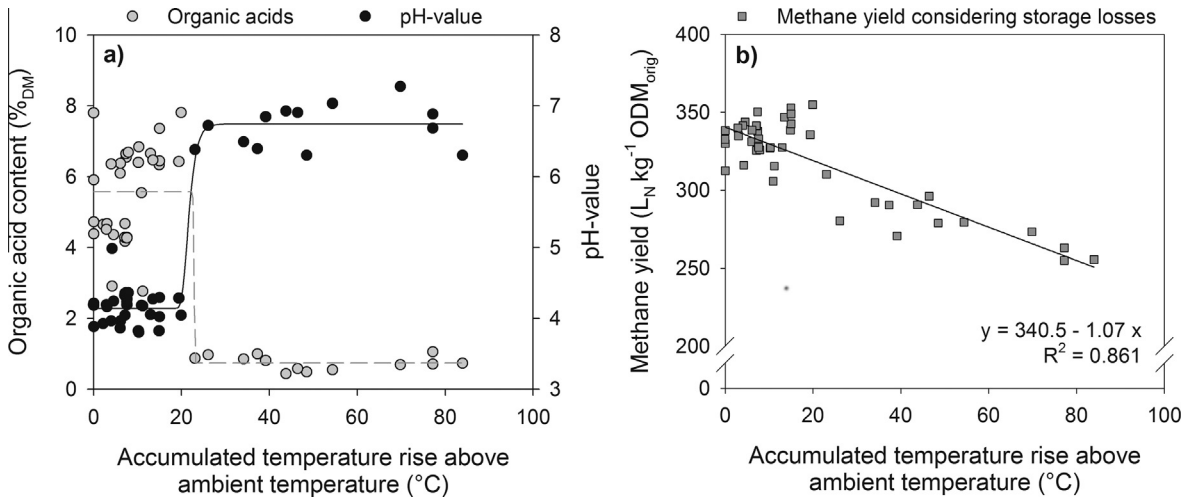

Fig. 3. Effects of the accumulated temperature rise above ambient temperature in maize silage during exposure to air on (a) organic acid content and pH value, and (b) methane yield considering total storage losses.

decreased the methane yield only for the maize silage treated with homofermentative LAB (LAB-ho).

Results indicate that aerobic deterioration has a largely negative effect on methane yield and leads to high losses in methane production potential within a short period of time. Changes during aerobic heating of silage on organic acids, pH value of silages and the methane yield potential considering ODM losses are summarised in Fig. 3. Per 1 °C increase in silage temperature above ambient and day, a loss in methane yield of maize silage of 1.1 L_N - kg^{-1} ODM_{orig} was observed (Fig. 3). It can be assumed that the changes in chemical composition due to aerobic deterioration, mainly the loss in organic acids, alcohols and WSC decrease the amount of available substrates for anaerobic digestion, and thus decrease the methane yield. Hence, aerobic stability of silages used for biogas production requires special attention. It is essential to target sufficient aerobic stability and avoid conditions that foster aerobic deterioration when preparing biogas silages. Suboptimal conditions that allow air to enter the silage during storage, such as low compaction and inappropriate or damaged sealing, decrease aerobic stability and substantially increase the risk for high losses in methane production. Furthermore, prolonged exposure to air during feed-out, e.g. induced by low feed-out rates (Wilkinson and Davies, 2013) or interim storage of silages after feed-out and before feeding to the digester, can lead to extensive methane losses. If a considerable risk for aerobic deterioration exists, silage additives effective to increase the aerobic stability can postpone aerobic deterioration and aid in preventing high methane losses. Acetic acid can increase aerobic stability and can directly be converted into methane during the anaerobic digestion process. The concern of potential depressions in animal intake due to elevated acetic acid contents of silages (Kung et al., 2003) does not apply to biogas silages. Thus, increasing the acetic acid content of silages by addition of heterofermentative LAB appears as a useful strategy to enhance the preservation of the methane production potential of silages prone to aerobic deterioration.

4. Conclusions

Exclusion of air is a fundamental requirement for successful preservation of the methane potential of silage feedstock for biogas production. Aerobic deterioration causes high biomass losses and additionally decreases the methane yield. Air stress during storage reduces the aerobic stability at feed-out and dramatically increases the risk of aerobic spoilage. Thus, it is essential to avoid conditions that promote aerobic deterioration such as delayed or inappropriate sealing, low feed-out rates or interim storage of biogas silages. Under suboptimal conditions chemical additives and heterofermentative LAB effective to enhance the aerobic stability can prevent losses and assist in preserving energy-rich biogas feedstocks.

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