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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-