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Effects of ensiling treatments on lactic acid production and supplementary methane formation of maize and amaranth – An advanced green biorefining approach

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HIGHLIGHTS

- Maize and amaranth were treated with different ensiling techniques.
- Biomethanation was observed from silage and separated residue in digestion tests.
- Lactic acid was increased about 91.9% in maize silage.
- Specific methane yield of silage compared to solid residue was similar for maize.
- Lactic acid and biogas were produced to a remarkable extent.

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ABSTRACT

A green biorefinery enables the material and energetic use of biomass via lactic acid and methane production. Different ensiling techniques were applied to maize and amaranth with the aim to increase the amount of lactic acid in the silage. In addition the methane formation potential of the ensiled samples and the remaining solid residues after separating the organic juice were assessed. Treating maize with homofermentative lactic acid bacteria in combination with carbonated lime increased the amount of lactic acid about 91.9%. For amaranth no additional lactic acid production was obtained by treating the raw material. Specific methane yields for the solid residues of amaranth were significantly lower in comparison to the corresponding silages. The most promising treatment resulted in a production of 127.9 ± 4.1 g kg^{-1} DM lactic acid and a specific methane yield for the solid residue of 349.5 ± 6.6 l_N kg^{-1} ODM.

1. Introduction

Times of accelerating climate change, growing energy needs of the world's population and the exhaustion of fossil fuels, raises greater awareness to maintain a healthy living environment. To achieve a sustainable industry for the production of goods and energy, safe resources of raw materials as well as entirely new approaches in research, development and fabrication are required (Kamm and Kamm, 2004). One possibility is the development of biorefinery concepts to use biomass as a continually renewable source for chemicals, materials and fuels as a substitute for petroleum (Kamm, 2007). Principles, classification approaches and working modes of biorefineries have been well described by Kamm and Kamm (2004, 2006) and Cherubini et al. (2009). One of those concepts is the green biorefinery. An approach to utilize green (grassland) biomass as a raw material for the production of bio-based products like proteins, lactic acids, fibers and energy via biogas (Kromus et al., 2004). In this process, grasses or ensiled grasses are fractionated into an organic juice and a remaining solid residue. The solid residue can then be used for fiber products, biogas or fertilizer production, while the organic juice is used to produce chemicals, e.g., lactic acid or amino acids (Cherubini et al., 2009). The refurbishment of lactic acid from the organic juice, which contains the organic acids, sugars, minerals and other nutrients, was investigated by Kromus et al. (2003) and Kromus et al. (2004). A fractionation efficiency of up to 85.0% from the silage into the juice was reached. Approximately 90.0% of the lactic acid in the

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organic juice can be extracted afterwards (Kromus et al., 2003). Pure lactic acid can be used to produce poly lactic acid, which is an essential building block for bio polymers. Furthermore a mixture of lactic acid, acetic acid and other ingredients can be produced to ferment the lactic acid with the application of genetically engineered *Corynebacterium glutamicum* into L-lysine (Neuner et al., 2013). In contrast to the utilization of the organic juice, literature still lacks information about the biogas potential and digestibility of the remaining solid residue.

To ensure a constant supply of raw material over the year in the northern hemisphere, harvested biomass has to be ensiled. By storing the fresh plant material under anaerobic conditions, a preservation process occurs. After a short aerobic phase in which the remaining oxygen is respired, lactic acid bacteria (LAB) convert water soluble carbohydrates into lactic acid. Consequently, the pH is lowered to values around 4.0 and prevents detrimental microorganisms, mainly clostridia and coliform bacteria, from spoiling the crop (Bolsen et al., 1996; McDonald et al., 1991). A stable phase, conserving nutrients and energy, is reached when the fermentation processes decline and may last until re-exposure to air. Although the ensiling process mainly depends on the epiphytic microflora, silage additives like additional LAB can be supplied to affect the course of the process (Herrmann et al., 2011). Homofermentative LAB produce lactic acid as a main product, whereas heterofermentative LAB produce lactic acid, acetic acid, ethanol (E) and carbon dioxide with the disadvantage of creating higher dry matter (DM) losses. Next to biological silage additives, which stimulate the fermentation, chemical additives inhibit or restrict undesirable fermentation or aerobic deterioration (McDonald et al., 1991). In addition the use of carbonated lime can increase the amount of lactic acid in silage due to buffering the pH value and hence preventing the *lactobacilli* from being inhibited (Huenting et al., 2012). Particle size is also affecting the ensiling process. With focus to the produced amount of lactic during the ensiling process, shorter chopping length can increase or decrease the amount of lactic acid in different raw substrates. Herrmann et al., 2012 showed that the amount of lactic acid in maize silage can be increased by reducing the nominal chopping length.

In this work, an advanced green biorefinery concept was pursued. The aim was to produce platform chemicals and energy, not only from green biomass but also from other plants which seemed to be auspicious for high yields in carboxylic acids and biogas, to improve the value added chain and create an additional source of income. Additionally, the ensiling process was directly regulated to produce lactic acid, as precursor for biopolymers, in high concentrations. It was expected that the amount of lactic acid during the ensiling process can be increased by treatments and working modes explained above. Lactic acid would then be the most promising platform chemical which can be increased to highest levels, while having a stable silage. Therefore maize and amaranth were treated with different silage additives and the development of ingredients during the ensiling process was observed. After separating the organic juice, the specific methane yields of the solid residue and the silage samples were determined in batch anaerobic digestion tests to evaluate potential methane formation losses. It was expected that the solid residue will have less organic acids than the fresh silage and hence will produce lower methane yields.

2. Methods

2.1. Raw materials

Both maize (*Zea mays*) and amaranth (*Amaranthus*) were obtained from the University of Hohenheim (Agricultural Experiment Station: Location Ihinger Hof, Germany). The whole crop was harvested at the dough-ripe stage with a precision forage harvester (Jaguar Speedstar 870, Claas, Germany) and chopped down to 8 mm theoretical length of cut.

2.2. Silage preparation

All silages were prepared in 1.5 l laboratory scale glass jars (Weck, Germany). To treat the material different silage additives were used. Biological silage additives were dissolved in sterile tap water and applied (6.7 ml kg^{-1} fresh material) with a precision air brush nozzle (SATAjet 1000, SATA, Germany). Carbonated Lime ($CaCO_3$) was filled directly into the jar and manually mixed with the silage. Afterwards, the whole plant material, including silage additives, was compacted with a pneumatic compacting device to ensure a constant density. The density was adjusted according

Table 1
Treatment variations for maize and amaranth with different silage additives and cutting device.

Material treatment	$CaCO_3$ [g kg^{-1}]	Ho 1 [g kg^{-1}]	Ho 2 [g kg^{-1}]	He [g kg^{-1}]	Thermomix [1 mm]
Maize					
Control	–	–	–	–	–
Chopped	–	–	–	–	+
pH 1	13.8	–	–	–	–
pH 2	27.6	–	–	–	–
Ho 1	–	0.001	–	–	–
He	–	–	–	0.002	–
Ho 1 + pH 1	13.8	0.001	–	–	–
Ho 1 + pH 2	27.6	0.001	–	–	–
He + pH 1	13.8	–	–	0.002	–
He + pH 2	27.6	–	–	0.002	–
Amaranth					
Control	–	–	–	–	–
Chopped	–	–	–	–	+
pH 1	7.5	–	–	–	–
pH 2	15.0	–	–	–	–
Ho 2	–	–	0.002	–	–
He	–	–	–	0.002	–
Ho 2 + pH 1	7.5	–	0.002	–	–
Ho 2 + pH 2	15.0	–	0.002	–	–
He + pH 1	7.5	–	–	0.002	–
He + pH 2	15.0	–	–	0.002	–